

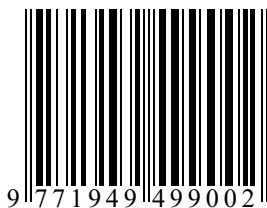
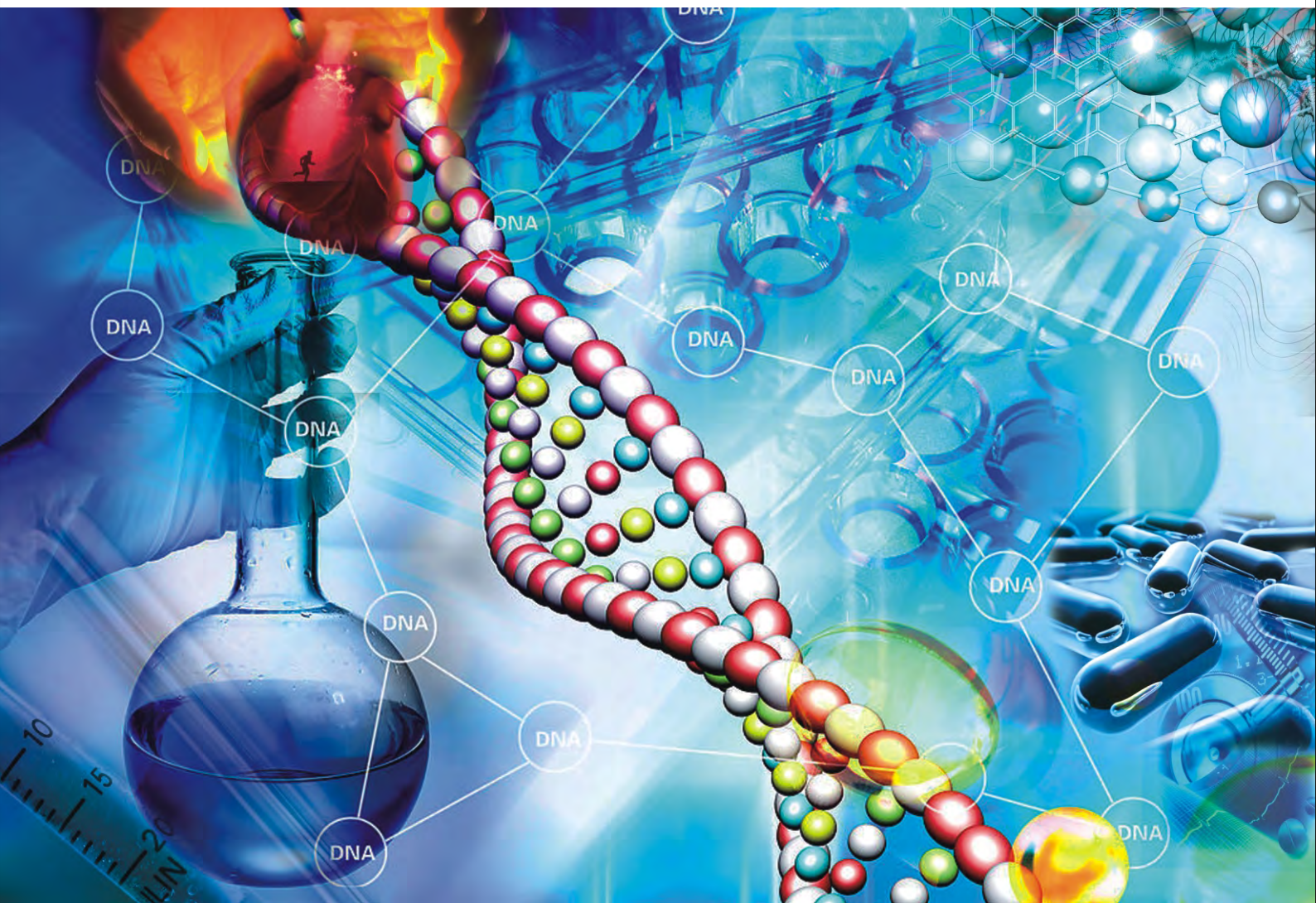
ISSN: 1949-4998 (Print), 1949-5005 (Online)

Volume 2, Number 4, April 2010



Scientific
Research

Health



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ISSN 1949-4998 (Print) ISSN 1949-5005 (Online)

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Using mutual information to evaluate performance of medical imaging systems

Eri Matsuyama¹, Du-Yih Tsai^{1*}, Yongbum Lee¹, Katsuyuki Kojima²

¹Department of Radiological Technology, Graduate School of Health Sciences, Niigata University, Niigata, Japan; tsai@clg.niigata-u.ac.jp

²Department of Information Networks, Faculty of Administration and Informatics, University of Hamamatsu, Hamamatsu, Japan

Received 6 December 2009; revised 6 January 2010; accepted 14 January 2010.

ABSTRACT

Information on physical image quality of medical images is important for imaging system assessment in order to promote and stimulate the development of state-of-the-art imaging systems. In this paper, we present a method for evaluating physical performance of medical imaging systems. In this method, mutual information (MI) which is a concept from information theory was used to measure combined properties of image noise and resolution of an imaging system. In our study, the MI was used as a measure to express the amount of information that an output image contains about an input object. The more the MI value provides, the better the image quality is. To validate the proposed method, computer simulations were performed to investigate the effects of noise and resolution degradation on the MI, followed by measuring and comparing the performance of two imaging systems. Our simulation and experimental results confirmed that the combined effect of deteriorated blur and noise on the images can be measured and analyzed using the MI metric. The results demonstrate the potential usefulness of the proposed method for evaluating physical quality of medical imaging systems.

Keywords: Image Quality; Medical Imaging; Mutual Information

1. INTRODUCTION

An important criterion for accepting any type of medical imaging system is the quality of the images produced by the imaging systems. The most fundamental quality-related factors in medical imaging systems are contrast, spatial resolution and noise. It is customary to describe contrast by the characteristic curve of the system, spatial

resolution by the modulation transfer function (MTF), and noise by the noise power spectrum (NPS, also referred to as the Wiener spectrum) [1,2]. One of the current dilemmas in digital radiography is the extent to which these parameters such as, resolution and noise affect physical or clinical image quality. An imaging system may only be superior in one metric while being inferior to another in the other metric.

In this study we present an information-entropy-based approach for evaluating overall image quality (including image noise and spatial resolution in this study) in medical imaging systems. The approach uses mutual information (MI) in information theory [3,4] as an image quality criterion. Differing from the MTF and NPS measures, this information-entropy-based metric is described in the spatial domain. The concept of MI has been applied in medical imaging processing, in particular for image registration tasks and computer-assisted detection schemes [5-7]. However, the application of MI as an overall quality metric has been rather limited so far [8,9]. The primary motivation behind this study was to use the MI to express the amount of information that an output image contains about an input object (subject). The basic idea is that when the amount of the uncertainty associated with an object before and after imaging is reduced, the difference of the uncertainty is equal to the value of MI. The more the MI value provides, the better the image quality is. Therefore, we can quantitatively evaluate the overall quality of an image by measuring the MI. The present work is an extension of the aforementioned studies [8,9]. The focus of this paper is to investigate and characterize the combined effect of noise and blur on the images obtained from medical imaging systems using the proposed metric. The advantages of our proposed method are: 1) simplicity of computation, 2) simplicity of experimentation, and 3) combined assessment of image noise and resolution.

In the present study, simulation studies were first carried out to investigate the relationship between noise and the MI, as well as that between spatial resolution and the MI. To validate the proposed method, two experiments were then performed. The first experiment was con-

ducted for verifying the effect of noise on the MI value. The second experiment was carried out for analyzing the effect of image blurring on the MI value. Furthermore, in order to compare the proposed method with the conventionally used metrics, the presampling MTF and NPS were also calculated and discussed. In addition, two imaging plates, a high resolution (HR) type detector and a standard resolution (ST) type detector, for computed radiography were used for verification of the potential usefulness of the MI metric. The verification was made by showing two real images with detailed discussion. Results show that the proposed method is simple to implement, and has potential usefulness for evaluation of overall image quality.

2. MUTUAL INFORMATION

Mutual information (MI) is a basic concept in information theory. It has been introduced for the registration of multimodality medical images. The definition of the term has been presented in various ways in the literature [10]. We will briefly describe the MI used for measurement of image quality.

Given events s_1, \dots, s_n occurring with probabilities p_1, p_2, \dots, p_n , the Shannon entropy H is defined as

$$H(p_1, p_2, \dots, p_n) = -\sum_{i=1}^n p_i \log_2 p_i. \quad (1)$$

Considering x and y as two random variables corresponding to an input variable and an output variable, the entropy for the input and that for the output are denoted as $H(x)$ and $H(y)$, respectively. For this case, the MI can be defined as

$$\begin{aligned} MI(x; y) &= H(x) - H_y(x) = H(y) - H_x(y) \\ &= H(x) + H(y) - H(x, y), \end{aligned} \quad (2)$$

where $H(x, y)$ is the joint entropy, and $H_x(y)$ and $H_y(x)$ are conditional entropies. The relationship among these entropies is shown in **Figure 1**.

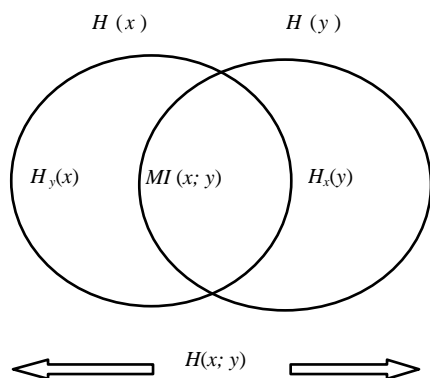


Figure 1. Relationship among $H(x)$, $H(y)$, $H(x, y)$, $H_x(y)$, $H_y(x)$, and $MI(x; y)$.

Consider an experiment in which every input has a unique output belonging to one of the various output categories. In this study, for simplicity, the inputs may be considered to be a set of subjects (for example, a test sample object with steps of various thickness, while the outputs may be their corresponding images varying in optical density or gray level. A method of occurrence-frequency-based computation is employed in the present study for calculating the entropies of input, output, and their joint entropies [11]. With this orderly system, the amount of MI is easily computed. The MI conveys the amount of information that output y has about input x .

3. METHODS AND MATERIALS

3.1. Computer Simulation

A simulation was designed and its framework is as follows. In mathematical terms, a simulation image $g(x, y)$ is the convolution of a uniformly-distributed signal (an object) $f(x, y)$ and the blurring function B . If the noise $u(x, y)$ is also taken into consideration, the resulting image may be represented by the following formula:

$$g(x, y) = \sum_{k=1}^5 \{ [k \times f(x, y)] * B + u(x, y) \times W \}, \quad (3)$$

where the symbol $*$ represents the convolution operation, B is a blurring function, and k is an integer representing the number of steps of the simulated image. In this simulation study, the input image $f(x, y)$ is a five-step wedge with a specific intensity or pixel value on each step. The term of W is a weighting coefficient used to adjust the extent of noise, and $u(x, y)$ is a zero-mean Gaussian noise with a standard deviation of 0.5.

Two simulations were performed separately. The first simulation was carried out to investigate the relationship between image noise and the MI. We employed signal-to-noise ratio (SNR) to describe the extent of noise level. The signal and noise used for SNR calculation were $[f(x, y)] * B$ and $u(x, y) \times W$, respectively, as given in (3). As a blurring function, we used a neighborhood averaging filter with a size of $m \times m$ (m is an odd integer). The extent of blurring was adjusted by varying the filter size. The reason for choosing neighborhood averaging filter was due to its commonality and simplicity of operation. The second simulation was conducted to investigate the relationship between the blurring (spatial resolution) and the MI.

An image of a simulated step wedge is shown in **Figure 2(a)**. Five regions of interests (ROIs) indicated with rectangles near the boundaries of two adjacent steps were chosen for calculation of the MI. The five steps of the step-wedge image are numbered from the right side as step 5, step 4, ..., and step 1. The right band without a rectangular box is the background of the image. The corresponding pixel-value distributions measured from

the ROIs are given in **Figure 2(b)**. The area of each ROI used in this study was 50×200 pixels. As a result, a total of 10×10^3 data for each step was obtained. As shown in **Figure 2(a)**, the number of inputs is five, and the number of outputs is the range of gray levels shown on the horizontal axis of the pixel-value distributions (see **Figure 2(b)**).

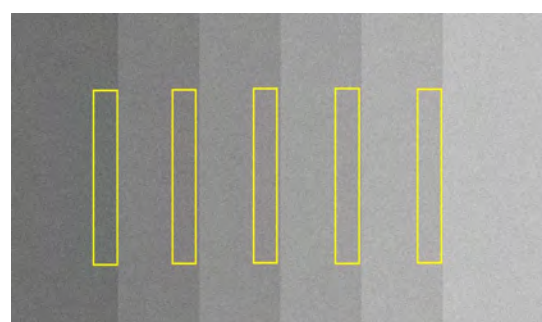
3.2. Experiments

An acrylic step wedge 0-1-2-3-4-5 mm in thickness was used as a test sample object for experiments. The specified exposure factors were kept at 42 kV and 10 mA, and the focus-imaging distance was taken as 185 cm, but the exposure time was varied from 0.1 sec to 0.4 sec. A tube voltage of 50 kV was also employed for comparison. An imaging plate (standard resolution type, ST, Fuji Film Japan, Inc.) was used as a detector to record X-ray intensities.

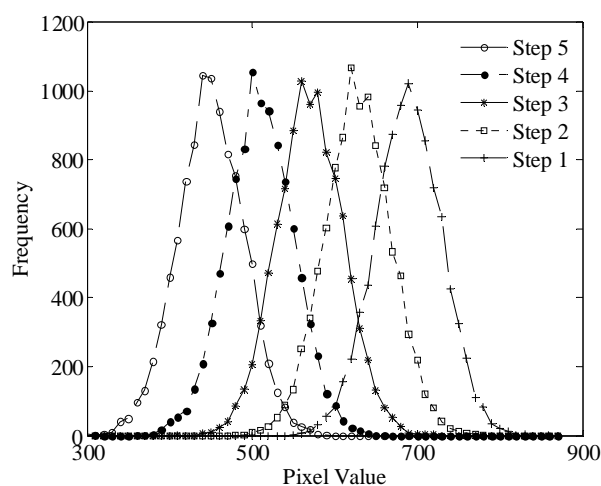
In this study, two experiments were performed. The first experiment was conducted for verifying the effect of noise properties on the measured MI value, and the second experiment was performed for analyzing the effect of resolution (blur) properties on the MI value. The experiments were carried out by varying of exposure levels and by use of various effective focal spot sizes of the X-ray tube, respectively. The latter experiment was achieved by shifting of the step wedge away from the center of the X-ray beam area toward the cathode end when imaging was performed. The effective focal spot size changes with position in the field. It becomes larger for points toward the cathode end of the field [12]. The increase in the effective focal spot size results in the degradation of resolution (blur). In addition, a high resolution type imaging plate HR for computed radiography was also used for evaluation and comparison. Moreover, two real images (the distal femur and the tarsal bone) were shown and compared for experimental validation of the advantages of the proposed method.

4. RESULTS AND DISCUSSION

Simulations were performed to investigate individual effects of noise and spatial resolution on MI. **Figure 3** illustrates the MI as a function of SNR for various levels of blurring at image contrast of 20. The results indicate that MI value increases with the increase of SNR (decrease in noise level). **Figure 4** shows the MI as a function of filter size of blurring function for various levels of SNR at image contrast of 20. The results indicate that MI value decreases when filter size of the blurring function increases (degradation of resolution). It was noted that the decline of the MI value is relatively small. It means that the effect of the level of blur on the MI is not so obvious in comparison to noise.



(a)



(b)

Figure 2. (a) Computer-generated step wedge. A region of interest (ROI) shown with a rectangle at each step of the step wedge was chosen for entropy computation. (b) The corresponding pixel-value distributions measured from the ROIs shown in (a).

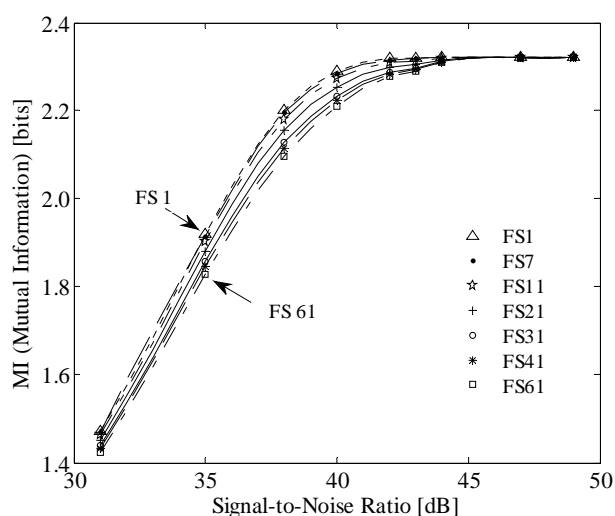


Figure 3. Relationship between the SNR and the MI for various levels of blur at an image contrast of 20.

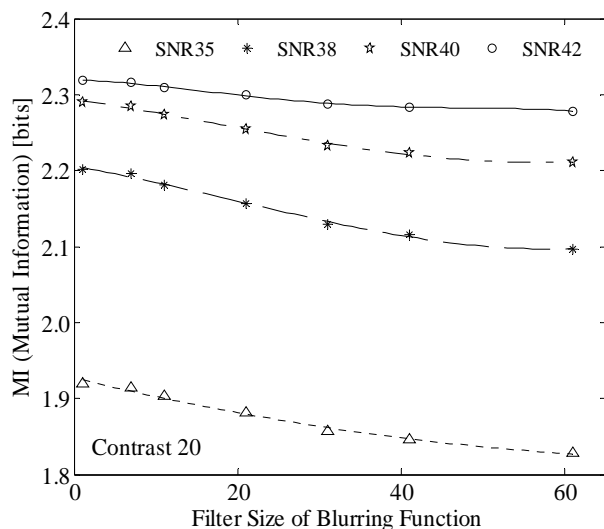


Figure 4. Relationship between the filter size of blurring function and the MI for various levels of SNR at an image contrast of 20.

Figure 5 illustrates the MI value as a function of the relative exposure level for tube voltages of 42 kV and 50 kV. The result shows that the MI value increases with the increase in the exposure level. The increase of the MI value is considered to be mainly due to the decrease of noise. **Figure 6** illustrates the NPS of the imaging system used in this study as a function of the relative exposure level at 42 kV for spatial frequencies of 0.5, 1.0, and 1.5 cycles/mm. The figure indicates that the NPS decreases with increasing exposure levels.

Figure 7 shows the presampling MTF as a function of spatial frequency for three effective focal spot sizes, obtained by shifting of the step wedge 15 cm and 30 cm away from the center of the X-ray beam area toward the cathode end. The MTF was measured with an angled-edge method. Theoretically, the amount of geometric blurring increases when the size of effective focal spot increases. As shown in **Figure 7**, the MTF was degraded with the increase of the effective focal spot size. **Figure 8** provides the one-dimensional NPS as a function of relative exposure for the three effective focal spot sizes at spatial frequency of 0.5 cycle/mm. The difference of NPSs is moderately small. **Figure 9** is a plot of the MI values for the three effective focal spot sizes. The measured results show that the MI value becomes lower when the off-center distance is greater. In other words, the MI value decreases when the effective focal spot size increases. This means that the MI value decreases when blur is deteriorated. It is noted that the decrease of the MI is mainly due to the image blurring resulting from the increase of the effective focal spot size. Therefore, the MI is also closely correlated with the resolution (blur) of imaging systems.

Figure 10 shows the relation between the exposure dose and the MI for the images obtained with ST and HR imaging plates. The results illustrate that the MI increases with the increase of exposure dose. The rise of

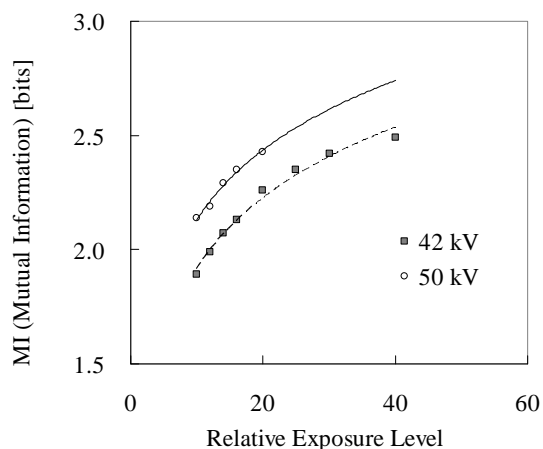


Figure 5. Mutual information as a function of relative exposure level for tube voltages of 42 kV and 50 kV.

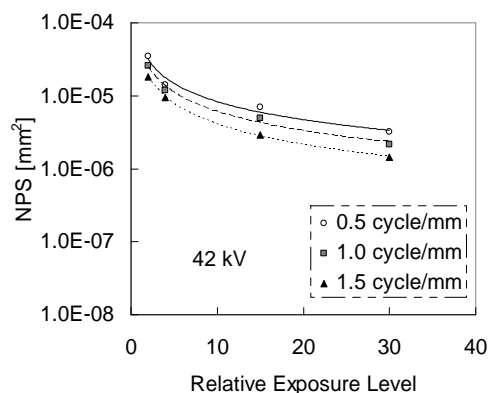


Figure 6. Noise power spectra (NPS) as a function of relative exposure level for three spatial frequencies at 42 kV.

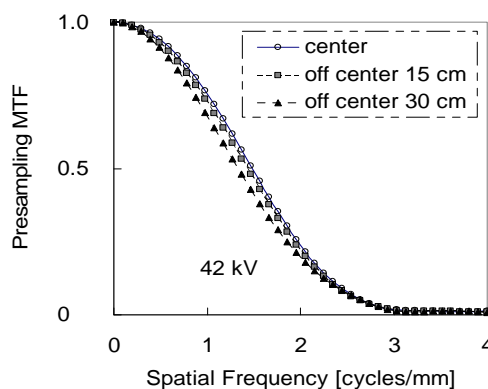


Figure 7. Presampling MTF as a function of spatial frequency for three effective focal spot sizes, obtained by shifting of the step wedge 15 cm and 30 cm away from the center of the X-ray beam area toward the cathode end.

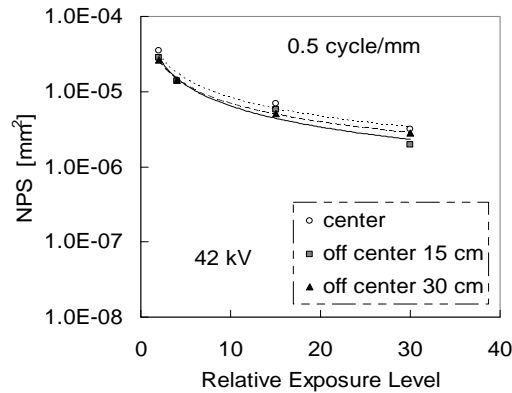


Figure 8. Noise power spectra (NPS) as a function of relative exposure level for three effective focal spot sizes for 42 kV at the spatial frequency of 0.5 cycle/mm.

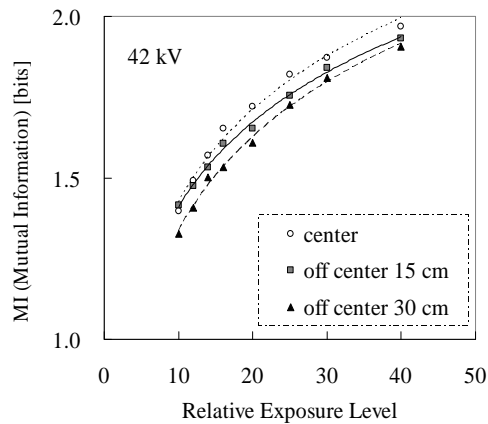


Figure 9. Mutual information as a function of relative exposure level for three different exposure positions of the step wedge at 42 kV.

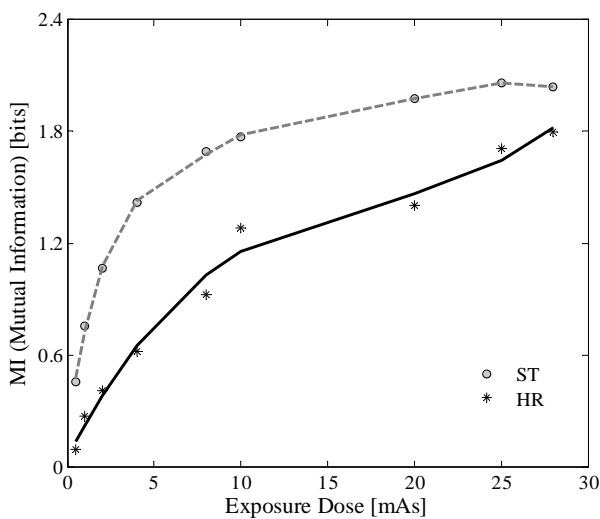


Figure 10. Mutual information as a function of relative exposure level for ST and HR imaging plates.

MI is considered due to the decrease of noise resulting from the increase of radiation dose. As shown in the figure, the MI value for the ST plate is higher than that for the HR plate at the same exposure dose. This can be explained by the fact that combined effects of the blur and noise lead to a higher MI value for the ST plate. **Figure 11** shows the presampling MTFs of the ST and HR imaging plates. The MTF of HR imaging plate is higher than that of ST imaging plate. This means that the spatial resolution (blur) of HR plate is higher than that of ST plate. **Figure 12** illustrates the NPS of the ST and HR imaging plates used in this study. The results show that the NPS of the HR imaging plate is higher than that of the ST plate. This means that ST imaging plate has better noise properties.

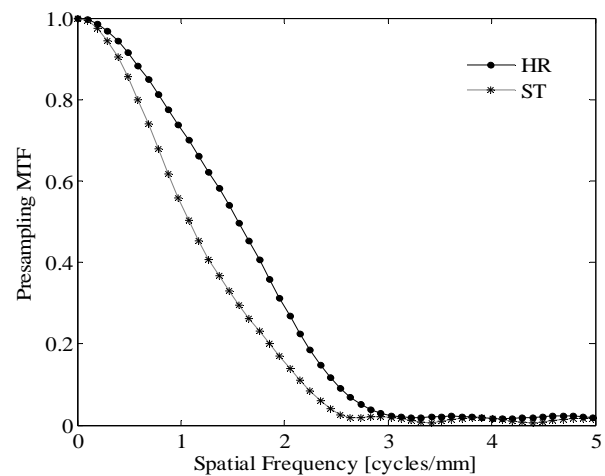


Figure 11. Presampling MTF as a function of spatial frequency obtained with the ST and HR imaging plates.

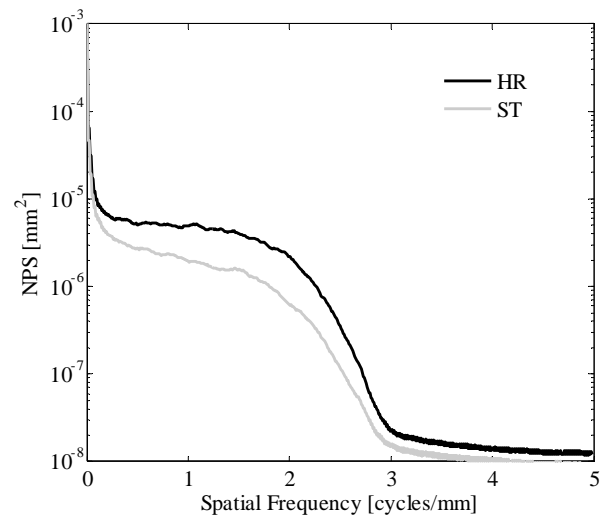


Figure 12. NPS as a function of spatial frequency obtained with the ST (standard resolution) and HR (high resolution) imaging plates.

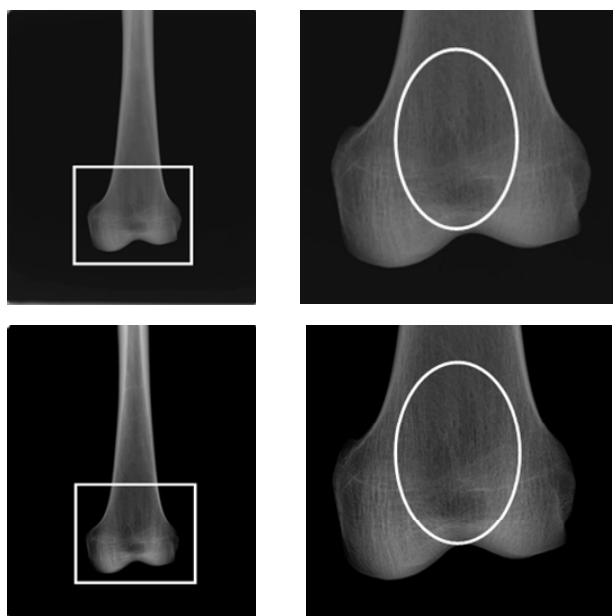


Figure 13. Real images of the distal femur acquired with ST (top row) and HR (bottom row) imaging plates.

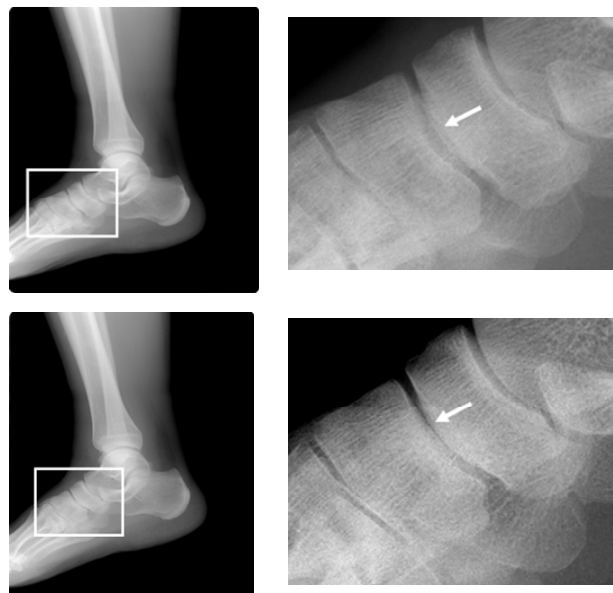


Figure 14. Real images of the tarsal bone acquired with ST (top row) and HR (bottom row) imaging plates.

In **Figures 13** and **14**, we display the real images of the distal femur (**Figure 13**) and tarsal bone (**Figure 14**) acquired with ST and HR imaging plates under the same exposure conditions. In the two figures, the left column illustrates the original images; while on the right are the magnified images of the white rectangles indicated in the original images. It is seen from the magnified images (**Figure 13**) that the patellofemoral joint (with a white circle) obtained with the HR plate shows better resolution

as compared to ST plate. Similarly, the magnified images of **Figure 14** (the cuneiform and navicular regions indicated by a white arrow) obtained with HR plate shows better resolution as compared to ST plate. The experimental validation provides confirming evidence for the MTF results presented in **Figure 11**. As regarding image noise, it can be seen from the magnified images of **Figures 13** and **14** that the images acquired with HR plates show higher noise levels. The perceptual results correctly reflect the outcome of the NPS shown in **Figure 12**.

5. CONCLUSIONS

In this study, we have presented a method for evaluating physical performance of medical imaging systems. In this method, mutual information was used to measure combined properties of image noise and resolution of an imaging system. To validate the proposed method, computer simulations were first performed to investigate the effects of noise and resolution degradation on mutual information. Then experiments were conducted to measure the physical performance of an imaging plate in terms of the proposed metric. Our simulation and experimental results confirmed that the combined effect of deteriorated blur and noise on the images can be measured and analyzed using the mutual-information metric. The method is expected to be useful for evaluating overall image quality of medical imaging systems.

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Coexistence of Duarte 1 and Duarte 2 variants of galactosemia with extrahepatic biliary atresia*

Ramandeep Singh¹, Ketan Kulkarni², Gurjit Kaur³, Babu Ram Thapa², Rajendra Prasad¹

¹Department of Biochemistry, Postgraduate Institute of Medical Education and Research, Chandigarh, India

²Division of Pediatric Gastroenterology, Postgraduate Institute of Medical Education and Research, Chandigarh, India

³Department of Physiology, Government Medical College and Hospital, Chandigarh, India; fateh1977@yahoo.com

Received 3 December 2009; revised 11 January 2010; accepted 15 January 2010.

ABSTRACT

Galactosemia is an autosomal recessive disorder caused by deficient or absent activities of one of the three enzymes involved in the galactose metabolic pathway. The predominant form is classic type galactosemia caused by severe reduction or absence of the galactose-1-phosphate uridyl transferase (GALT) enzyme. Coexistence of extrahepatic biliary atresia (EHBA) with Duarte 1 and 2 variants of galactosemia has not been described earlier. Here we report a case of EHBA with concordant Duarte 1 and 2 variants of galactosemia in an infant with cholestasis. Genetic analysis of the index patient for galactosemia revealed presence of Duarte 1/Duarte 2 variants of galactosemia with genotype N314D-L218L/N314D-G1105C-G1391A-G1323A-5'UTR-119delGTCA. Clinical evaluation of the patient showed the presence of EHBA. Henceforth, it may be hypothesized that EHBA may have a genetic basis with simultaneous involvement of the GALT gene.

Keywords: Duarte 1; Duarte 2; Extrahepatic Biliary Atresia; Galactosemia; Galactose-1-Phosphate Uridyl Transferase Gene; Los Angeles

1. INTRODUCTION

Galactosemia is an autosomal recessive disorder of galactose metabolism caused by deficient or absent activities of one of the three enzymes involved in the galactose metabolism. The deficiency of galactose-1-phosphate uridyl transferase (GALT; MIM# 230400) is most common and presents as neonatal hepatitis and liver failure. In addition to the predominant classic form, there are two clinical variants: Duarte-1 and Duarte-2 [1]. Although coexistence of Duarte 1 and 2 variant of galacto-

*This study was supported by Department of Biotechnology, Ministry of Science and Technology, Government of India.

sema has been reported in literature, synchronous presence of these 2 variants with extrahepatic biliary atresia (EHBA) has not been documented [1,2]. In this communication, we report a case of EHBA coexisting with Duarte 1 and Duarte 2 variants of galactosemia detected at our center during evaluation of cholestatic infants for galactosemia.

2. MATERIAL AND METHODS

2.1. Patients

A cohort of 215 infants (age range: 5 days - 10.5 months), admitted in Pediatric Gastroenterology ward of Postgraduate Institute of Medical Education and Research (PGIMER) over a period of 30 months (from January, 2007 to June, 2009) with cholestasis were evaluated for galactosemia. This study was approved by Institute's ethical committee.

2.2. Red Cell (Galactose-1-Phosphate Uridyl Transferase) GALT Assay

GALT activity was measured using a Perkin-Elmer neonatal GALT kit (Perkin Elmer Wallac Victor 2D fluorometer, Finland) which measured the uridylphosphoglucose (UDPG) consumption on samples of dried blood eluted from the filter.

2.3. DNA Isolation and Polymerase Chain Reaction (PCR)

Genomic DNA was isolated from whole blood by method of Daly *et al.* [3]. Polymerase Chain Reaction (PCR) was performed in order to amplify the required sequences by using specific primers mentioned in Table 1 [4,5].

2.4. Restriction Fragment Length Polymorphism (RFLP) Analysis

A rapid PCR-based DNA analysis was performed to confirm or identify the sequence variations that create or abolish the given natural and amplification-created re-

striction sites as mentioned in **Table 1** [4,5].

2.5. Case

The index case presented as follows. A 3.5 kilogram, term, appropriate for gestational age female child was born to a primigravida mother by lower segment cesarean section. The baby developed jaundice since day 3 of life. She received phototherapy for the same. Jaundice persisted and worsened over the next 2 weeks. Baby was passing high colored urine and clay colored stools. Hence, the baby was referred at 3 weeks of age to our institute for further evaluation and management. She was being exclusively breast fed. There was no lethargy, refusal to feed, fever, excessive cry, irritability, bleeding, altered sensorium or seizures. On enquiry, mother denied any history of rash, joint pains, fever or per vaginal discharge during antenatal period. There was no history of previous abortions or significant family history of any illnesses.

On examination, weight was 3.6 kg, length was 53 cm and occipitofrontal circumference was 35.7 cm. There were no congenital malformations or stigmata of TORCH group of infections. Eye examination did not show any cataracts. The baby had distended abdomen with dilated visible veins and everted umbilicus. There was hepatomegaly (liver was 5 cm below right costal margin with a span of 10 cm) and splenomegaly (4 cm under left costal margin). The liver was firm in consistency with well defined margins and coarse surface. The rest of the systemic examination was noncontributory and the baby was neurologically appropriate for age.

In view of the history and physical examination, neonatal cholestasis syndrome was considered and the patient was further evaluated. Hemoglobin was 10.5 gm/dL,

total leukocyte count was $15.1 \times 10^9/L$ with neutrophilic leucocytosis (86% neutrophils). The platelet count was within normal limits ($3.7 \times 10^9/L$). Liver Function tests showed aspartate-transaminase and alanine transaminase of 28 U/L and 53 U/L respectively (normal range: 15-45 U/L). The total serum bilirubin was 8.0 with conjugated fraction of 4.5 mg/dL. There was hypoalbuminemia (serum albumin of 1.9 with total serum protein of 5.0 g/dL). The serum alkaline phosphatase was 565 IU/L (range: 20-250 IU/L). The coagulation profile revealed a prothrombin time and activated partial thromboplastin time of 14 (control of 12 seconds) and 37 (control of 35 seconds) respectively with an international normalized ratio of 1.05. The C-reactive protein was negative. Blood and urine cultures were sterile. Urine for non-glucose reducing substances was negative. Toxoplasma, retroviral, rubella, cytomegalovirus and herpes virus serologies were negative.

Ultrasonography (along with colour Doppler evaluation) of the abdomen showed liver span of 10.5 cm, coarsened echotexture, normal outline of liver, normal portal vein and hepatic artery, small and atretic gall bladder, non-visualized common bile duct. The spleen spanned 7 cm. The overall features were suggestive of biliary atresia. The findings of mebrofenin scan were corroborative with the ultrasonography findings. Thus a diagnosis of (EHBA) was established.

In view of cholestasis, as per the study protocol, the patient was evaluated for galactosemia. GALT activity of 63% was observed. Genetic analysis revealed the presence of N314D mutation in exon 10 of the GALT gene. Interestingly, further work-up revealed that N314D mutation was present in two different alleles of the GALT gene: Duarte 1 (D1) and Duarte 2 (D2). D1 allele was

Table 1. Mutations or sequence variations, primers used for PCR and appropriate restriction enzymes used in detection of normal and mutant alleles.

Mutations/ Polymorphisms	Primers	Restriction Enzyme	Detection	
			Normal Allele [bp]	Mutant allele [bp]
N314D	5'-GGGTCGACGAGATGCTGGGACTGAGGGTGGAGCA-3' 5'-GGGGTCGACGCCTGCACATACTGCATGTGA-3'	Ava II	349 + 81	247 + 102 + 81
L218L	5'-ATGTGGAGGCTTGGAGGTA-3' 5'-TTCACCTCTAGCTTTCTCCT-3'	Mse I	272	147 + 125
IVS4nt-27g→c (G1105C)	5'-ACAGCCAAGCCCTACCTCCCG-3' 5'-ACTCCCTCCTGACCACACCC-3'	Msp I (ACRS)	171 + 93 + 19	171 + 112
IVS5nt+62g→a (G1323A)	5'-TGGATGGGCAGGGAGGGGGT 3' 5'-TGTC AAGGGGCAAAAGCAGA-3'	Dde I	222 + 120 + 6	186 + 120 + 36 + 6
IVS5nt-24g→a (G1391A)	5'-TGGATGGGCAGGGAGGGGGT -3' 5'-TGTC AAGGGGCAAAAGCAGA -3'	Sac I	239 + 109	348
5'UTR-119delGT CA	5'-CAGGGCAGCCAGTCACTCA- 3' 5'-GCGTTGCTGAGGATCGGTTTC-3'	Dde I (ACRS)	145 + 17 + 10	158 + 10

found to carry a silent mutation L218L in exon 7 in addition to the N314D in exon 10 of GALT gene (**Figure 1**). In contrast, D2 allele was found to co-exist with 3 different polymorphisms: IVS4nt-27g→c (G1105C), IVS5nt+62g→a (G1323A) and IVS5nt-24g→a (G1391A) and promoter region deletion of 4 nucleotides (5'UTR-119del GTCA) (**Figure 2**).

The patient was managed with appropriate supportive and nutritional care along with cessation of breast feeding whilst soya-milk formula was initiated. Subsequently Kasai's porto-enterostomy was performed at 3 months of age. Per operatively, the liver was greenish, hypertrophied; biliary channels were fibrotic although right hepatic duct was visible. Per-operative cholangiogram confirmed the diagnosis of EHBA.

The patient was discharged on appropriate dietary advice and multivitamin supplementation. At 4 months of follow-up, the infant is passing normal colored stools and is anicteric and gaining weight, although at a slower pace. In view of established biliary cirrhosis the family has been advised regular medical follow-up and has been counseled regarding the possible need of liver transplantation in future.

3. DISCUSSION

The report presents a peculiar case of coexistence of 2 variants of galactosemia together (Duarte 1 and Duarte 2 together) with EHBA in a solitary case amongst a cohort

of 215 patients with neonatal cholestasis.

The patient was found to carry the L218L transition along with N314D on one allele which is known as Duarte1 (D1) or Los Angeles variant (LA) of galactosemia [6,7]. This variant is known to be associated with the increased GALT activity [1]. A molecular mechanism involving an increased rate of translation through codon bias has been proposed to be the possible mechanism [8]. In addition, three different transitions were also found: IVS4nt -27 g→c (G1105C) in intron 4 and IVS5nt -24 g→a (G1391A) and IVS5nt +62 g→a (G1323A) in intron 5, all in a heterozygous state. Besides, a deletion of 4 nucleotides (5'UTR-119delGTCA) was detected in the promoter region of the GALT gene. This type of genotype, that is: N314D-G1105C-G1391A-G1323A-5'UTR-119delGTCA is known to constitute another galactosemia variant called Duarte 2 (D2) variant which is associated with the decrease GALT activity [6,7]. Investigators have suggested that these genetic alterations might be regulatory mutations which may be responsible for the decrease in GALT activity in D2 variants [7]. So the genotype of the patient was N314D-L218L/N314D-G1105C-G1391A-G1323A-5'UTR-119delGTCA or D1/D2. This unique genotype causing coexistence of D1 and D2 variant galactosemia along with EHBA has not been earlier reported in literature.

The coexistence of EHBA alongwith galactosemia made us ponder that although EHBA is not believed to be an inherited disease; genetic factors have been re-

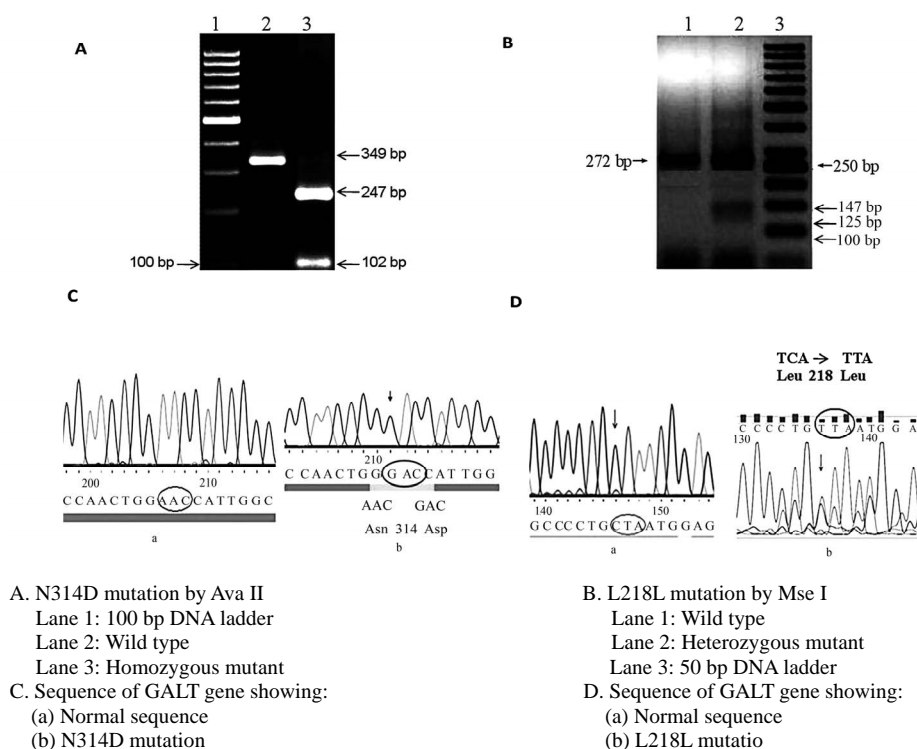


Figure 1. Restriction digestion analysis and electropherogram of N314D and L218L mutation.

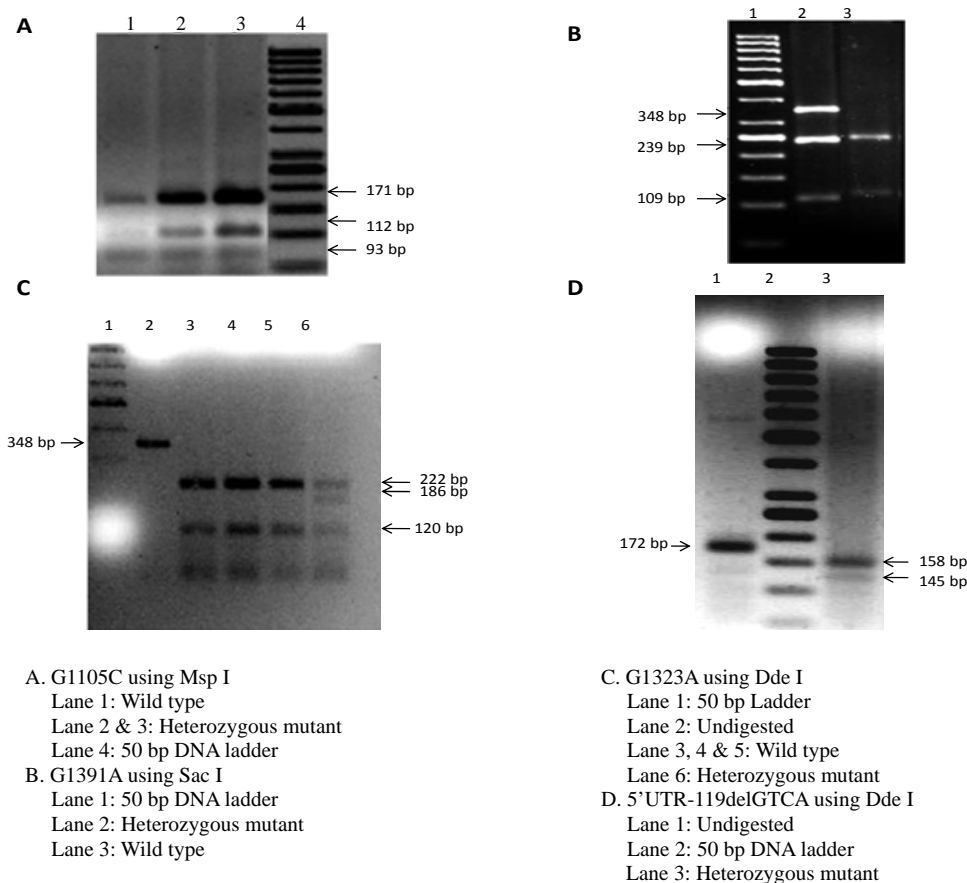


Figure 2. Restriction digestion analysis of different polymorphisms associated with Duarte 2.

ported to be involved in its pathogenesis. Reports of familial cases provide evidence in favor of this hypothesis [9,10]. Worldover, the most widely investigated genes are those which are related to laterality (inversin) and to the development of bile ducts [11]. In an animal study on mice, authors have observed a spontaneous mutation in the inversin (*inv*) gene, on chromosome 4, resulting in situs inversus, obstructive jaundice and death within the first week of life [12]. Analysis of the hepatobiliary system of these *inv* mice revealed EHBA and intrahepatic ductular proliferation [13]. However, when compared to histopathological findings in infantile EHBA, inconsistent features have been observed [11].

Further, the human inversin gene has been mapped on chromosome 9 [11], same as that of the gene for the GALT [14]. Despite being a non-syndromic variant of EHBA, our patient might have contemporaneous involvement of inversin and GALT genes on chromosome 9 leading to such peculiar coexistence of disorders. Another gene that may play a role in the genesis of EHBA is Jag-1, although its influence on the development of atresia has not been definitively confirmed [15]. Even though a genetic basis is likely to be contributory, the exact mechanism of this co-existence is open to speculation.

The clinical implication of such concordance remains to be conceived. The index patient is currently well and on active follow up. Long term follow up would be able to show the clinical impact of this association on the natural history of EHBA or that of EHBA on these variants of galactosemia. Moreover, identification of the genetic basis of such a peculiar concordance could have important connotation on prenatal diagnosis of these disorders.

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The role of fine-wire localization breast biopsy in the management of BI-RADS category 3-5 non-palpable breast lesions in northeastern Chinese women

Bo Chen¹, Cai-Gang Liu¹, Feng Jin¹, Ya-Nan Sun¹, Ting-Ting Zhao¹, Li-Na Zhang²,
Shu Li², Sai-Ching J. Yeung³

¹Breast Surgery Unit, Department of Surgical Oncology, First Hospital, China Medical University, Shenyang, China; angel-s205@163.com

²Department of Radiology, First Hospital, China Medical University, Shenyang, China

³Department of General Internal Medicine, Ambulatory Treatment and Emergency Care, The University of Texas M. D. Anderson Cancer Center, Houston, USA

Received 14 October 2009; revised 25 December 2009; accepted 28 December 2009.

ABSTRACT

A blinded retrospective validation study was performed in a university-based hospital in northeastern China to determine whether the breast imaging reporting and data system (BI-RADS) defines a group of patients with non-palpable breast lesions (NPBLs) in whom fine-wire localization biopsy (FWLB) is appropriate. We reviewed 182 consecutive patients with NPBLs who underwent FWLB. The patients' preoperative mammograms were categorized according BI-RADS by 2 radiologists blinded to the pathological findings. The positive predictive values of BI-RADS categories 3-5 were 3.4%, 42.1%, and 76.9%, respectively. For category 4 NPBLs, the percentage of cancer for those aged < 40 years was significantly lower than those aged ≥ 40 years. For category 3 NPBLs, the percentage of precancerous lesions for those aged < 40 years was significantly lower than those aged ≥ 40 years. Chinese NPBL patients aged ≥ 40 years with category 4 mammographic findings, and all patients with category 5 findings should undergo FWLB. FWLB should be offered as a treatment option for Chinese NPBL patients aged < 40 years with category 4 findings or aged ≥ 40 years with category 3 findings.

Keywords: Non-Palpable Breast Lesions; Mammography; Fine-Wire Localization Biopsy

1. INTRODUCTION

Screening mammography has been shown to reduce breast cancer mortality [1-3]. Unfortunately, traditional

mammography has a low positive predictive value (PPV) for breast cancer [4], and thus many women with benign breast lesions undergo open surgical biopsy. In this context, the Breast Imaging Reporting and Data System (BI-RADS) classification scheme was developed by the American College of Radiology to improve mammographic reporting through the use of standardized descriptive terms [5]. An important goal of BI-RADS is to provide a clear management recommendation for women with nonpalpable breast lesions (NPBLs). BI-RADS also offers specific PPV for each category of mammographic lesions and therefore is useful not only for discriminating many benign from malignant lesions but also for potentially reducing the number of unnecessary open breast biopsies performed. We retrospectively evaluated the mammograms of 182 consecutive women who underwent fine-wire localization breast biopsy (FWLB) of NPBLs in a tertiary referral cancer center in northeastern China to investigate whether the fourth edition of BI-RADS [5] is appropriate for patients in northeastern China with NPBLs and identify the BI-RADS-identified patient subgroups appropriate for FWLB.

2. MATERIALS AND METHODS

In adherence to a research protocol approved by the Institutional Review Board and institutional guidelines for ethical human research, we retrospectively reviewed the mammograms, histopathology and medical records of 182 consecutive women with NPBL who underwent FWLB between January 1, 2005 and March 1, 2008 at an affiliated hospital of China Medical University in Liaoning Province of northeastern China. Fine wire localization was guided mammographically. Two trained radiologists [L.Z. and S.L.], who were blinded to the patients' histopathologic diagnoses, categorized the patients' lesions using the fourth edition of BI-RADS. [5]

Histopathological results of FWLB were used as the gold standard to calculate the positive predictive values (PPVs) of malignancy. The PPV for each BI-RADS category was calculated by dividing the number of malignant cases on histopathological examination by the total number of patients who underwent FWLB in that BI-RADS category. All data were analyzed with SPSS statistics software (Version 13.0, SPSS, Inc., Chicago, IL, USA). We used Fisher's exact test for to compare ratios. We considered a *P* value less than 0.05 to be statistically significant.

3. RESULTS

182 consecutive cases were reviewed. The patients' mean age was 45.6 years [range, 24-76 years; standard deviation (SD) = 9.5 years]. According to the histopathologic diagnosis, 40 patients (22%) had malignant lesions and 142 (78%) had benign lesions (**Table 1**). The mean age of patients with malignant lesions was 48.3 years (range, 30-76 years; SD = 8.8 years), and the mean age of the patients with benign lesions was 45.6 years (range, 24-67 years; SD = 9.5 years). The radiologists identified 118 BI-RADS category 3 lesions (65%), 38 category 4 lesions (21%), and 26 category 5 lesions (14%). Four category 3 lesions, 16 category 4 lesions, and 20 category 5 lesions were malignant based on histopathology. The PPVs were 3.4%, 42.1% and 76.9% for category 3, 4, and 5 lesions, respectively (**Table 2**). Among patients with category 4 lesions, the cancer PPV for those aged < 40 years was significantly lower than that for those aged ≥ 40 years (*P* = 0.02; **Table 3**). Among patients with category 3 lesions, 20 (*i.e.*, 17%) had precancerous lesions on histopathology, and when these patients were subdivided by age < 40 years and ≥ 40 years, the difference in the rate of precancerous lesions between the two age groups was significant (*P* = 0.002; **Table 4**).

Table 1. Histopathologic diagnoses for 182 patients who underwent fine-wire localization biopsy for nonpalpable breast lesions.

Diagnosis	Number of patients (%)
Benign (n = 142)	
Mastopathy	96 (67.6)
Fibroadenoma	20 (14.1)
Sclerosing adenosis	2 (1.4)
Lymph node	2 (1.4)
Atypical ductal hyperplasia (precancerous)	20 (14.1)
Malignant (n = 40)	
Ductal carcinoma in situ	22 (55)
Infiltrating ductal carcinoma	18 (45)

Table 2. Positive predictive values for cancer of BI-RADS categories in the diagnosis of nonpalpable breast lesions.

BI-RADS category	Histopathologic diagnosis*		PPV
	Malignant	Benign	
3	4	114	3.4%
4	16	22	42.1%
5	20	6	76.9%
Total	40	142	22.0%

* Data are in numbers of patients.

Table 3. Relationship between the patients' age and the percentage of malignancy in BI-RADS category 4 nonpalpable breast lesions.

Age	Ratio of cancer to the total number of cases in the subgroup	Percentage of cancer *
< 40 years	2/16	12.5%
≥ 40 years	14/22	63.6%
Total	16/38	42.1%

* *P* = 0.002, Fisher's exact test

Table 4. Relationship between the patients' age and the percentage of precancerous lesions in BI-RADS category 3 nonpalpable breast lesions.

Age	Ratio of precancerous lesions to the total number of cases in the subgroup	Percentage of precancerous lesions *
< 40 years	2/48	4.2%
≥ 40 years	18/70	25.7%
Total	20/118	16.9%

* *P* = 0.02, Fisher's exact test

4. DISCUSSION

In our study of patients with NPBLs who underwent FWLB, the PPVs for category 3, 4 and 5 lesions were 3.4%, 42.1%, and 76.9%, respectively. For comparison, a retrospective Pakistani study reported PPVs of 5% for category 3 lesions, 34% for category 4 lesions and 83% for category 5 lesions [6]. A retrospective Singaporean study reported PPVs of 27% for category 4 lesions and 84% for category 5 lesions [7]. In a U.S. prospective study of mammographically detected NPBLs that were biopsied surgically, carcinomas were present in 34% of category 4 lesions and in 81% of category 5 lesions [8]. Therefore, our results are in agreement with published results from various geographic and ethnic backgrounds, and the BI-RADS classification is appropriate for women with NPBLs in northeastern China.

As a screening tool, traditional mammography provides high-sensitivity and low specificity for identifying breast cancer [9]. By providing distinct PPVs for each final assessment category, BI-RADS [5] offers a probability of carcinoma for each category of mammographic

findings. Category 5 lesions must be biopsied and treated. Categories 1 and 2 lesions do not require biopsy or surgical intervention. Category 3 and 4 lesions are in the grey zone where the ideal management strategy is controversial. Given the low probability of malignancy in category 3 and 4 and the imperative to limit the morbidity associated with cancer screening, a conservative and minimally invasive approach in certain subgroups within these 2 BI-RADS categories may be appropriate. For discrete lesions with category 3 mammographic findings, a stepwise approach of fine needle aspiration biopsy (FNAB) followed by core biopsy if FNAB is nondiagnostic would reduce the proportion of cases requiring surgical biopsy [9]. Core needle biopsy is highly accurate and safe in the diagnosis of mammographically detected breast lesions [10]. Close periodic mammographic surveillance is also an alternative way to manage NPBLs that are probably benign based on mammographic findings [11]. Among patients with category 4 lesions in our Asian population, the cancer PPV for those aged < 40 years was 12.5% compared with 63.6% for those aged ≥ 40 years (**Table 3**). A Canadian study recommended that stereotactic core needle biopsy should be applied to BI-RADS categories 3 and 4 patients who are < 50 years of age, and FWLB should be reserved for category 4 (> 50 years of age) and category 5 cases. However, this recommendation was partially based on an unusually low rate of malignancy in category 3 lesions (0%) which might be due to a small sample size of category 3 patients (10 cases) in this study [12].

FWLB is more invasive than FNAB and core biopsy, but FWLB is an accurate technique for diagnosis of mammographically-detected NPBLs, and may also be therapeutic for the NPBLs that are malignant or precancerous. In our study of women in northeastern China, there was a significantly higher incidence of precancerous lesions in category 3 patients aged ≥ 40 years than in those aged < 40 years. Taking into account other factors, such as patient anxiety, patient preference, and future breast cancer risk, surgeons may recommend for patients aged ≥ 40 years with category 3 lesions to undergo FWLB and for patients aged < 40 years with category 3 lesions to undergo stereotactic core needle biopsy.

There are many differences between breast cancer patients in Asia and those in Western countries. Compared with Caucasians, the incidence of breast cancer and the mortality from breast cancer are lower, and the age of peak incidence is younger in Asian women. The median age at diagnosis is 62 years for Swedish patients while the median age at diagnosis is 50 years for Singaporean patients [13]. With striking similarity to the Singaporean patients, the median age at diagnosis of breast cancer is 49.5 years for Taiwanese women [14]. These differences in incidence, mortality and the age of peak incidence may be due to differences in certain modifiable risk fac-

tors (e.g., diet, life style and environmental factors), differences in the biology of breast cancer (e.g., the prevalence of BRCA1/BRCA2 mutations) or differences in breast cancer screening and treatment. The age of peak breast cancer incidence is the most important difference that would change the positive predictive value of BI-RADS for Asian women. Since there is a difference of about 10 years between the age of peak incidence, we subdivided our patients with category 3 lesions into two groups using a cutoff at 40 years of age instead of 50 [12], *i.e.*, age < 40 years vs. ≥ 40 years. We found only two malignant lesions in patients aged < 40 years, and the difference in PPV between the two groups was significant (**Table 3**). Except for the cutoff used in age grouping, our findings are similar to those reported by Ball *et al.* [15] who found that the PPV of category 4 was 4.5% for women aged < 50 years.

5. CONCLUSIONS

Therefore, BI-RADS categories appropriately predict the risk of malignancy of NPBLs and aid in the decision-making process for biopsy in patients in northeastern China. We recommend that Asian NPBL patients aged ≥ 40 years with category 4 mammographic findings, and all patients with category 5 findings should undergo FWLB. FWLB should be offered as a treatment option, the risks and benefits of which to be discussed with the patient for Asian NPBL patients aged < 40 years with category 4 and aged ≥ 40 years with category 3 mammographic findings.

6. ACKNOWLEDGEMENTS

We would like to acknowledge grant support from the following finding sources: U. S. Department of Defense Breast Cancer Research Program of the Office of the Congressionally Directed Medical Research Programs (DOD SIDA BC062166 P.I.: Yeung S J), Susan G. Komen Foundation for Breast Cancer Research (Promise Grant 2008 KG081048, P.I.: Yeung S J), China Foundation of Science and Technology Bureau of Liaoning Province of China (20061038, Chen B), and Foundation of Educational Commission of Liaoning Province of China (2008762, Chen B).

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Predicting maximum voluntary ventilation in normal healthy individuals using indirect inspiratory muscle strength measurements: a correlation study

Rohit Sontakke¹, Mangesh Deore², Dhara Kothari³

¹Masterskill University College of Health, Cheras, Malaysia; rohitrohits@yahoo.com

²Sancheti Institute of Orthopedics, Pune, India

³Nivara Physiotherapy Center, Pune, India

Received 17 November 2009; revised 7 December 2009; accepted 15 December 2009.

ABSTRACT

Maximum Voluntary Ventilation (MVV), one of the components of Pulmonary Function Testing (PFT), has multiple uses. Various factors including the inspiratory muscle strength (IMS) influence its magnitude. Our aim was to quantify the IMS indirectly using an economical and non invasive bedside assessment tool, determine its association with MVV and then develop a predictive equation for MVV. 41 healthy non-athletic physical therapy students participated in the study. IMS measurement was performed with a sphygmomanometer. Average of the three net deflections in sphygmomanometer following deepest possible breaths was taken as indirect measurement of IMS in mm of Hg. MVV was measured according to ATS guidelines using a spirometer. Results from the data analysis revealed a significant correlation between IMS and MVV ($r = 0.83$, $p < 0.001$) and the coefficient of determination = 0.68. So, we developed a regression equation: $Y = 1.9669(X) + 49.838$ with SEE: 13.02L/min and ANOVA for the equation was ($F=68.9$, $p < 0.001$). Hence, it can be concluded that a strong correlation between the indirect IMS and MVV was established and a predictive equation to estimate MVV was developed. This equation proved to have a high predictive value with a small error of estimation. This indicates that the value of the indirect IMS measurement obtained using the sphygmomanometer can be used to estimate MVV in normal healthy individuals without the use of a conventional spirometer.

Keywords: Healthy Physical Therapy Students; Maximum Voluntary Ventilation; Inspiratory Muscle Strength; Regression Equation; Sphygmomanometer

1. INTRODUCTION

Evidence-based support for pulmonary rehabilitation in the management of patients with chronic respiratory dysfunction has grown tremendously, and this comprehensive intervention has clearly demonstrated to reduce dyspnea and health care costs, increase exercise performance, and improve health-related quality of life (HRQL) [1-6].

One of the factors responsible for exercise limitations and reduced HRQL in patients with respiratory disorders is dyspnea [7,8]. Weakness or mechanical inefficiency of the respiratory muscles results in a mismatch between central respiratory motor output and achieved ventilation. This mismatch can also explain the dyspnea experienced by patients with neuromuscular diseases affecting the respiratory musculature [9] and in patients with respiratory muscle fatigue [10]. As the pressure-generating capacity of the respiratory muscles falls and as the ratio of the pressures produced by the respiratory muscles to the maximum pressure that can be achieved increases dyspnea progressively worsens [11]. Since there is an established association between respiratory muscle dysfunction and dyspnea, an improvement in respiratory muscle function with inspiratory muscle training (IMT) could lead to a reduction in dyspnea [12,13]. A meta-analysis of IMT in 17 clinical trials found limited support for its use in terms of improving pulmonary function, respiratory muscle strength and endurance, exercise capacity, and functional status in patients with COPD [14-16]. A consistent improvement in baseline dyspnea index (BDI) and transitional dyspnea index was shown following IMT [17]. Also, fewer dyspnea was reported by patients who used IMT with a threshold loading device at 30% of Pimax nevertheless, those who used either a very light load or sham treatments also reported less dyspnea [18]. Inspiratory muscle training has also been found to reduce dyspnea during exertion not only in patients with respiratory ailments but also in normal healthy individu-

als [19].

There are many individuals with chronic cardiopulmonary disorders for which the underlying pathophysiology cannot be corrected and this, in turn, frequently results in long-term disability for the patient [20]. A pulmonary rehabilitation programme is essential for these patients. For a successful rehabilitation program, accurate assessment is very important. There are variety of measures to assess the functioning of respiratory system which include tests of flows and volumes [21], tests of respiratory muscle strength [22], endurance [23], fatigue [24], and chest wall function analysis [25]. Routine measurements of respiratory function (*i.e.*, volumes, flows and indices of gas exchange) are non specific in relation to diagnosis but give useful indirect information about respiratory muscle performance. More frequently, these measurements are of use in assessing the severity, functional consequences and progress of patients with recognized respiratory muscle weakness [21]. Amongst the various tests for flows and volumes, maximum voluntary ventilation (MVV) is a parameter that reflects lung volume changes, respiratory muscle functioning, compliance of the thorax lung complex and airway resistance [26]. MVV is defined as the maximum amount of air that a subject can breathe over a specified period of time (12 seconds for normal subjects) and is expressed in L/min [27]. It can be used as a tool for assessment of respiratory muscle weakness [21]. The accurate estimation of MVV is critical for interpretation of maximal sustainable ventilation (MSV). MSV is a measure of endurance of ventilatory muscles and is expressed as a fraction of MVV [23].

Direct measurements of respiratory muscle strength are conducted using invasive as well as noninvasive techniques. Both types of techniques require sophisticated instrumentations. Measurement of Pi max (Peak Inspiratory Pressure) is one of the most commonly used techniques for quantification of IMS. Measurement of Pi max, though is a non-invasive method, the device to measure Pi Max (**Manovacuumeter**) is not routinely available in Indian Physiotherapy set ups as it is not cost effective. Accurate estimation of MVV value according to ATS/ERS guidelines [27] requires a sophisticated machine and the maneuver itself requires coordination, motivation, understanding and may induce fatigue, giddiness and bronchospasm in the candidate who undergoes the test. Also, this maneuver is not recommended in the individuals either having or suspected respiratory muscle weakness [21]. In order to overcome the aforementioned limitations of various measurement techniques, we measured the IMS using a sphygmomanometer. This device is easily available in any physical therapy set-up, and is inexpensive. The purpose of our study was to explore the relationship between the values of IMS obtained using the sphygmomanometer and MVV measured using the spirometer. If a strong relationship exists, then

a regression equation to estimate MVV from the indirectly measured IMS values can be obtained. This equation will serve the purpose of guiding clinical decision making without the need of a sophisticated instrumentation and/or causing discomfort to our subjects.

2. MATERIALS AND METHODS

2.1. Ethical Approval

Institutional ethical committee approval was obtained from Sancheti Institute for orthopedics and rehabilitation. All the subjects were given a thorough explanation of the procedure and a written informed consent was obtained before participating in the study.

2.2. Participants

The study population consisted of normal healthy physiotherapy students from Sancheti Institute College of Physiotherapy, Pune. All the subjects were non- athletes, non smokers and healthy. A total number of 41 subjects participated in the study. Demographics are presented in **Table 1**.

2.3. Methods

A gap of at least four hours was given between the food intake and actual procedure to minimize the hindrance in the diaphragm excursion. The subjects were also instructed to empty the bladder before the procedure.

2.4. Estimation of Indirect Inspiratory Muscle Strength (IMS)

The equipments used for this measurement were a pediatric size blood pressure cuff, a leather belt and an aneroid sphygmomanometer. The subject was positioned supine on a plinth with hip knee flexion. Velcro straps of the blood pressure cuff were removed and it was secured to the candidates chest two cm below the xiphoid process. The leather belt was used for this purpose. The blood pressure cuff was inflated to a baseline pressure of 20 mmHg which was maintained. The candidate was then instructed to take the deepest possible breath starting from functional residual capacity (FRC) and hold it for 1 second until we noted the net deflection in manometer. The cuff was deflated and the entire procedure was repeated two more times with a rest of ten minutes in between each measurements. The average of the three

Table 1. Demographic data.

Variable	Mean (\pm SD)
Age (yrs)	21 (\pm 3)
Males(n)	16
Females(n)	25
IMS (mmHg)	49 (\pm 9)
MVV (L/min)	110 (\pm 21)

net deflections was taken as an indirect measurement of inspiratory muscle strength, expressed in mm Hg.

2.5. Estimation of Maximum Voluntary Ventilation

A device RMS-Respirator[®] was used for measuring MVV. The device consisted of a flow sensor and a central port attached to a desk top computer. The device was calibrated using the standard procedure before the commencement of the study. The calibration was ensured periodically during the data collection. After the IMS was measured, the candidate was given a rest of 30 min. The candidate underwent the slow vital capacity (SVC) maneuver and the ratio of total inspiratory time to total respiratory cycle time (Ti/Ttot) ratio was measured. The Ti/Ttot ratio between 0.35 and 0.4 ruled out the inspiratory muscle fatigue. The candidate was given a demonstration of the MVV maneuver to make sure that he/she had understood the procedure. The MVV was measured for 12 seconds using the ATS/ERS standard procedure [27].

2.6. Data Analysis

Microsoft Excel analysis tool pack was used for analyzing the data. Pearson correlation coefficient was calculated to study the strength of the association between indirect IMS and MVV. Regression equation was developed by manual calculation. In order to evaluate the accuracy of prediction, coefficient of determination and standard error of estimation (SEE) were calculated. Analysis of variance for regression equation (ANOVA) was performed. Level of significance was set at 0.05. Manual calculation of the statistical tests was performed as per the available guidelines [28].

3. RESULTS

3.1. Regression Equation

The Pearson correlation coefficient (r) obtained was 0.83 ($p < 0.001$). This showed a strong correlation exists between MVV and indirect IMS. This has been depicted in **Figure 1**. As the strong correlation was established, we developed a regression equation. It was as follows. $Y = 1.9669(X) + 49.838$. In this equation Y denotes the MVV and X denotes the indirect IMS.

3.2. Analysis of Residuals

Residual is the difference between the actual MVV value as obtained using the spirometer and the predicted MVV value as obtained from the regression equation. Actual MVV values plotted against the residuals (the difference between actual and predicted MVV values) demonstrated that they were evenly and randomly distributed

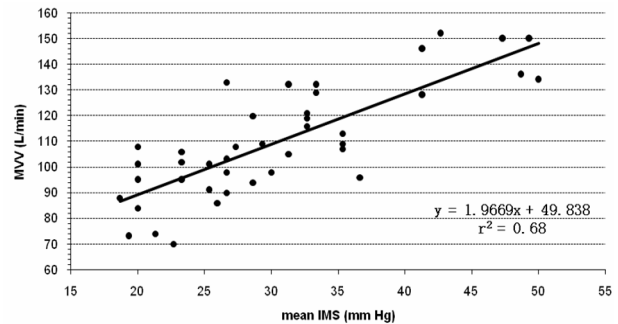


Figure 1. Regression equation and XY scatter showing the association between indirect IMS and MVV.

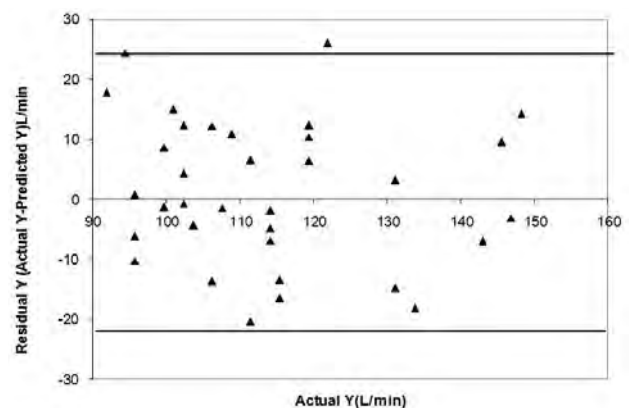


Figure 2. Analysis of residuals: Actual Y Vs residual Y value. As the actual value of the MVV increases, the error associated with the predicted MVV value does not increase.

about the regression line confirming the assumptions of the regression equation obtained as shown in **Figure 2**. As depicted in **Figure 2**, the residuals obtained in our study were evenly and randomly distributed about the regression line. This confirms that the assumptions of regression equation were met.

3.3. Accuracy of Prediction

The coefficient of determination r^2 was 0.68. This indicates that 68% change in the Y variable is been explained by X variable. Standard error of estimation (SEE) for the regression equation was 13.02 L/min. If we take into account the normal values of the MVV in healthy individuals, this is a very negligible value suggesting that our equation has a good accuracy of prediction. ANOVA (analysis of variance) was done for the regression equation which came to be significant (Calculated $F = 68.9$, critical value of (0.05) $F(1, 39) = 4.08$). This denotes that the obtained correlation between X and Y is not due to a chance.

4. DISCUSSION

The results of the study demonstrate that MVV is

strongly correlated with the indirect IMS and MVV can be predicted with a good accuracy using the indirect IMS in a group of normal healthy college students. In addition, the prediction equation has proved to have excellent validity when ANOVA and analysis of the residuals was done. Coefficient of determination of 0.68 suggests that 68% of the variance in MVV can be explained by variance in IMS. This is because in addition to IMS, expiratory muscle strength, compliance of lungs and chest wall, airway resistance also contributes to MVV [26].

Peak inspiratory pressure (Pi Max) is one of the simplest and commonly used tool that is used for quantification of IMS. But, this device is not routinely available in physical therapy set ups in India. Due to this reason we chose to quantify the IMS indirectly instead of using Pi max.

The reasons for trying to quantify the indirect IMS and then predicting MVV from it are multiple. Patients could possibly save time, expenses, and invasive procedures if an accurate prediction of their maximum voluntary ventilation could be made from their indirect IMS. A common equation used for MVV estimation is $MVV = 35.0 \times FEV1$. The usefulness of this equation has been established in predicting MVV, in American African girls [29]. This method may underestimate the maximum exercise ventilation in COPD patients [31]. Also, this equation requires the actual value of FEV1 to be substituted which can be obtained only with the use of a spirometer. In contrast to, these limitations, our study enables the clinicians to estimate the MVV using the regression equation which requires IMS value which can be obtained without the need of a spirometer or any other complicated device.

Applicability of Caucasian regression equations was also studied on Indian population for prediction of forced vital capacity (FVC), forced expiratory volume in first second (FEV 1) [31]. The conclusion of this study was that the commonly used Caucasian prediction equations, or a fixed percentage of their predicted values, were leading to the improper interpretation of the data obtained; that is, there was a significant difference between the values obtained using the equation and the values obtained by actual performing the maneuver. This happened in a significant proportion of patients and the study reflected that there is a need to assess performance of more than one regression equation before choosing any single prediction equation in Indian population as the most important step in diagnosing abnormality of lung function in individuals is to define whether they are within or out side the healthy subjects range. Though in this study it was the vital capacity (VC) which was studied, we can still expect that we can apply the conclusions from this study to the MVV also as VC is been found to have proportionate reductions to MVV [21].

None of these studies which studied the predictive

equations for MVV had focused on the direct or indirect IMS measurement. Also the studies which focus on IMS have used the devices which are still expensive in India and not routinely available in Indian Physiotherapy set ups. Our technique of estimating IMS was inexpensive, non invasive, uncomplicated and can be used in any small clinical set up and also in ambulatory care set up. Thus, our study overcomes the previously mentioned drawbacks of the techniques for quantification of IMS and MVV in Indian set up. Here, it was the chest expansion that is, overall chest wall movement that was used for assessment of IMS. Throughout the study it was taken into account that the changes in the intrathoracic blood volume could have resulted in the difference between actual lung volume change and change in the volume of the thorax or simply the chest expansion. But this difference being negligible has not influenced our results [25]. This means that the chest wall motion has reflected the volume changes occurring in the lungs during breathing as there is no disparity between volume changes in the lungs and volume changes of the thoracic wall. One more confounding factor can be the elasticity of the chest wall tissues. But, it has been shown that the tissues of the chest wall being essentially incompressible, volume changes of the chest wall surface are nearly equal to volume changes of the lungs and can be used for indirect measure of IMS [25]. Regression equation will be useful only when the IMS value lies between 18.67 mm Hg to 50 mm Hg and for the age group of 18 to 24 yrs. The quantification indirect inspiratory muscle strength needs to be studied in wider age range and diseased population to increase its clinical applicability. Also, the contribution of expiratory muscle strength to MVV was not taken into account. Along with this, reliability and validity of this new tool for evaluating IMS needs to be studied.

5. CONCLUSIONS

A strong correlation between the indirect IMS and MVV was established. A regression equation was developed. This equation was tested for its ability to accurately predict the MVV and it proved to have a high predictive value with a small error of estimation. This signifies that by substituting the value of the indirect IMS in the obtained equation, we can estimate MVV in normal healthy individuals. This prediction will have a good accuracy.

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Stress cardiomyopathy: clinical features and outcomes

Lilian Hamity¹, Franco Ramello¹, Leandro Marani¹, Alejandro Moyano¹, Eduardo Moreyra²,
Luis E. Alday¹

¹Hospital Aeronáutico Córdoba, Córdoba, Argentina; lealday@arnet.com.ar

²Professor of Medicine, Universidad Nacional de Córdoba, Córdoba, Argentina

Received 6 January 2010; revised 25 January 2010; accepted 29 January 2010.

ABSTRACT

Objectives: To establish the prevalence, clinical features, and outcomes of the recently recognized stress cardiomyopathy whose physiopathology is still not completely clarified. **Material and methods:** The prevalence and clinical findings of stress cardiomyopathy were assessed in a group of 378 patients undergoing cinecoronariography for acute coronary syndromes during a 7-year period. The inclusion criteria were severe chest pain, ischemic electrocardiographic changes, reversible left ventricular dys-hypokinesia, and normal coronary arteries. Eight patients, 7 female (2.1% of all patients and 5.0% of the women), with a mean age of 65.3 ± 8.5 years fulfilled the requirements. **Results:** The precipitating factor was severe stress in all of them. Cardiac enzymes were slightly raised. There was apical left ventricular dyskinesia in 6 patients, midventricular in another, and diffuse hypokinesia in the remaining. One patient showed moderate mitral regurgitation. The response to conventional treatment and patient outcomes were favorable in all cases with prompt reversal of the left ventricular dyskinesia as assessed by echocardiography. There were 4 recurrences, 2 requiring readmission to hospital, despite continuous treatment with combined alfa and beta adrenergic blockers and calcium antagonists. **Conclusions:** In our hospital, stress cardiomyopathy had a prevalence of 2.1% in all patients with acute coronary syndromes and 5.1% in women and should be considered in their differential diagnosis, especially in middle aged female patients with a history of severe previous stress. There was a favorable outcome but recurrences may occur despite uninterrupted medical treatment following discharge.

Keywords: Stress Cardiomyopathy; Takotsubo Syndrome; Myocardial Ischemia; Cardiomyopathy

1. INTRODUCTION

The recently described stress cardiomyopathy (SCM), resembles an acute coronary syndrome (ACS) presenting with severe chest pain, left ventricular dys- or hypokinesia not following a determined pattern corresponding to a single involved artery as it occurs in acute myocardial infarction.

It was first described in Japan in the early 90's as "takotsubo syndrome" (from tako: octopus, tsubo: trap) for the similarity with the narrow necked and large rounded bottom fishing pots used in Japan to trap octopus, with the angiographic left ventricular systolic appearance in affected patients [1]. Other names proposed for this syndrome are "apical ballooning syndrome", "broken heart", "transient myocardial stunning", and "transient apical dyskinesia" [2-8]. The syndrome occurs almost exclusively in middle aged women with a clinical onset usually following an episode of severe emotional stress although it may occur during the course of an illness, after some kind of surgery, or even without any provoking factor [3,9]. There is a varying severity, with risk of arrhythmias, congestive heart failure, shock and even death. The left ventricular kinetic abnormalities are transient with full recovery of the contractility in about 2 months along with normalization of the electrocardiographic ischemic changes in a variable period of time. The incidence of recurrences has been estimated around 11% in a group of 100 patients in a 4-year follow-up period [10].

In this study we analyze the features and frequency of SCM in a group of consecutive patients with ACS requiring selective coronarographic study (SCG) during a 7-year period in a general hospital.

2. METHODS

We performed a retrospective-prospective, observational study to determine the frequency of SCM, as already defined, in all patients studied by SCG for ACS from March 2002 to February 2009. Inclusion criteria were: a)

severe, oppressive, chest pain, b) ischemic ECG changes, c) creatin phosphokinase (CPK) enzyme elevation, d) normal or no significant lesions in the coronary arteries, and e) left ventricular dys- or hypokinesia. There were 378 consecutive patients with ACS undergoing SCG, of which 238 (63%) were males. The mean age was 67.8 ± 9.6 years. Eight patients, 7 of them women, fulfilled the inclusion criteria. Serial laboratory studies, EKG's, and transthoracic color Doppler echocardiograms were performed while admitted at the coronary care unit. They were treated according to current guidelines for the management of ACS's. Following discharge, patients continued tailored medical treatment in keeping with the accompanying risk factors. The left ventricular contractility was assessed by echocardiography. In case of recurrences, patients were studied and treated as in the first admission. The mean follow-up period was 3.8 ± 2.1 years. The continuous variables were expressed as mean \pm SD or median with its range.

3. RESULTS

The 8 affected patients made up 2.1% of all patients with ACS with an incidence for women of 5.0%, while the mean age was 65.3 ± 8.5 years. All of them presented to the Emergency Department with severe oppressive chest pain lasting more than 10 minutes. The precipitating factor in all patients was always a severe episode of emotional stress as a result of diverse causes. All patients had electrocardiographic signs of acute ischemia. In 7 of them, there were anterior wall negative T waves and in only one there was infero-lateral ST segment elevation. (**Figure 1**) The QTc interval was transiently prolonged in all cases with a median of 475 msec (range 464-490).

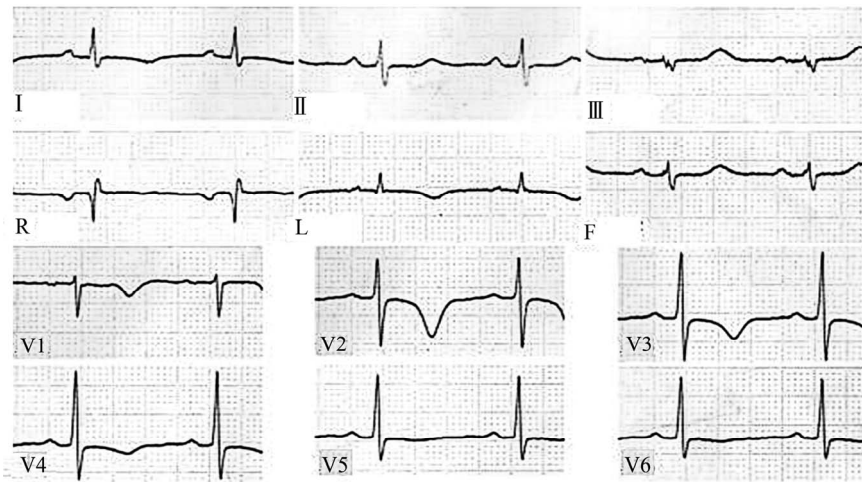
The CPK was also mildly elevated in all patients with a median of 290 u/100 ml (range 220-390). Echocardiographic studies showed segmental contractile left ventricular parietal abnormalities in 7 cases and generalized hypokinesia in the remaining. SCG was always performed in the acute phase, no longer than 48 hours from the onset of symptoms but for the patient with ST elevation that was initially treated with thrombolysis, and had the study done for recurrence of chest pain on the 5th day after admission. No patient showed significant coronary artery obstructions. Nevertheless, the left ventricular angiographies showed myocardial dys- or hypokinesia with decreased ejection fraction. There was apical dyskinesia in 6 (**Figure 2**), mid-ventricular involvement in one, associated with mild mitral incompetence, and the last patient had generalized hypokinesia. A summary of all clinical and laboratory findings is shown in **Table 1**.

The median length of stay in hospital was 6 days (range 4 to 10). As already mentioned, treatment was according to the guidelines for ACS's with nitrites, heparin, aspirin, alpha and beta adrenergic blockers agents, vasodilators, and analgesics as required. Following discharge, they were put on adrenergic blockers or calcium antagonists as decided by the personal physician. Besides, they received aspirin, statins, and associated risk factors were addressed (**Table 1**).

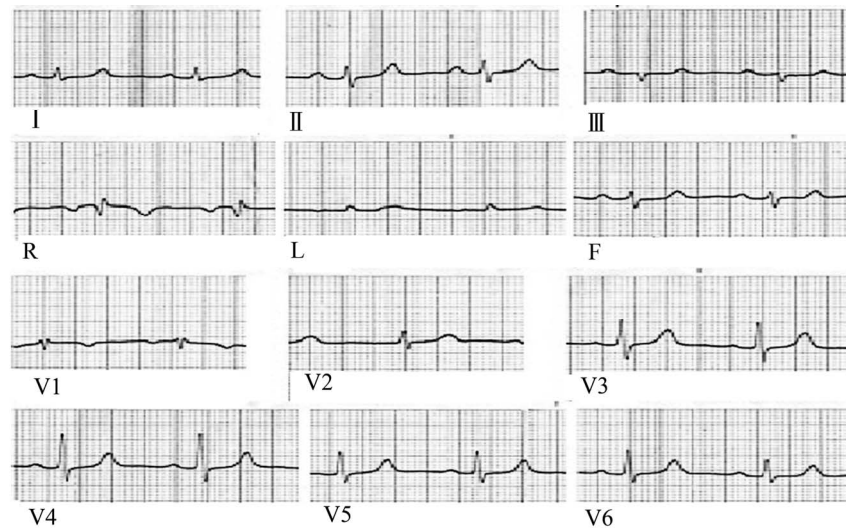
There were 4 recurrences but only 2 required readmission 8 and 18 months following the first episode. All had inadequate control of anxiety disorders. Severe stress was the precipitating factor. The 2 readmitted patients reproduced the initial symptomatology undergoing the same diagnostic and therapeutic measures, again with favorable outcomes. The SCG showed similar findings to the initial study.

Table 1. Clinical and ancillary findings. (AW: anterior wall; BMI: body mass index; ILW: infero-lateral wall; LV: left ventricular; D: dyslipidemia; EF: ejection fraction; SH: systemic hypertension)

Patient	Age	Sex	Stress trigger	EKG ST & T changes	QTc (msec)	CPK (u/100 ml)	Associated risk factors	LV EF & dysfunction
A	46	Female	Conjugal quarrel	Negative T waves AW	484	271	BMI > 25 SH - D	0.36, global
B	49	Female	Laboral stress	ILW ST elevation	480	311	BMI > 25 Diabetes	0.57, apical
C	64	Female	Family argument	Negative T waves AW	478	264	BMI > 25 SH - D	0.56, apical
D	62	Female	Infidelity	Negative T waves AW	469	220	BMI > 25 SH - D	0.58, mid-ventricular
E	65	Female	Spouse's terminal illness	Negative T waves AW	468	253	SH - D	0.55, apical
F	56	Female	Job loss	Negative T waves AW	464	276	Smoking	0.51, apical
G	59	Male	Laboral problems	Negative T waves AW	470	340	Smoking	0.45, apical
H	73	Female	Distressful disease	Negative T waves AW	490	390	SH - D Smoking	0.50, apical



(a) Admission EKG (patient E)



(b) EKG of same patient at discharge

Figure 1. (a) Admission EKG of patient E showing deeply negative T waves in the anterior wall and prolonged QT interval; (b) EKG of same patient at discharge with normalization of repolarization abnormalities.

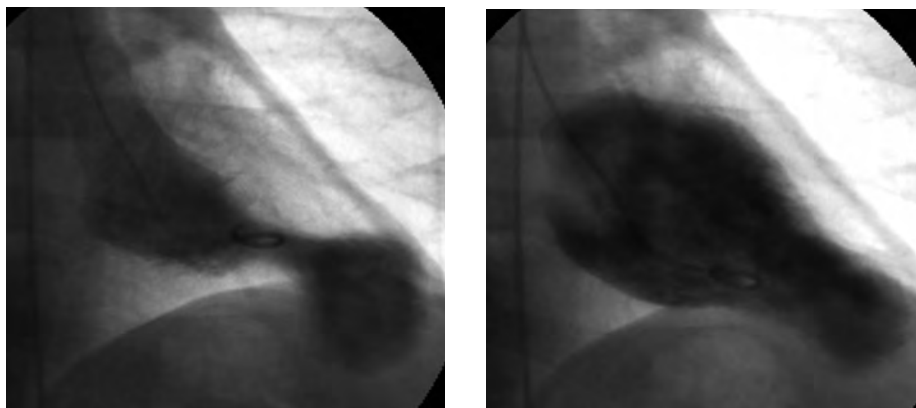


Figure 2. Left ventriculography of patient G in systole and diastole showing typical apical ballooning.

4. DISCUSSION

The findings in our study in patients with SCM mirrored those described in papers following the original description of the syndrome in Japan in the early 90's [1]. The involved population was mainly middle aged females [2]. The chest pain at presentation was indistinguishable from that occurring in ACS and always precipitated by a severe stress episode [9]. The EKG showed acute ischemic changes, usually anterior wall T wave inversion with lengthening of the QTc interval, accompanied by small rise of the myocardial markers (the CPK enzyme in our study) [2,10]. We did not find abnormal Q waves on the EKG though they may also transiently happen [2].

SCG ruled out the presence of significant coronary stenoses while apical left ventricular dyskinesia occurred in 7 patients with extension to the midventricular region in one of them. In the remaining patient there was generalized hypokinesia [12]. One patient also had moderate mitral regurgitation, which with the left ventricular contraction abnormalities were transient [13].

Response to ACS treatment was favorable without major complications resulting in short length of stay. Among them, arrhythmias, heart failure, shock, and very rarely in-hospital death have been described [2,9,12]. Several risk factors were present in all cases and were addressed according to current recommendations. Despite continuous medical treatment following discharge, there were 4 mid-term recurrences, 2 requiring readmission to hospital, with similar course to the initial presentation. Anxiety disorders were more difficult to control in patients with recurrences as compared to those without them. In one study, the frequency of recurrences was around 11%. Patients at risk could not be identified and adrenergic beta blocker therapy did not prevent them [10].

Prevalence of SCM has been estimated in about 2% of patients presenting with ACS and 3 times higher if just women are considered. We had a somewhat similar occurrence (2.1 and 5.0%, respectively). According to these figures the diagnosis of SCM should be borne in mind in patients with ACS in any of both situations. [5,9,14]. Increased recognition of this syndrome is reflected in the higher number of publications in recent years.

Since the physiopathogenesis of SCM has still not been elucidated several theories have been proposed. There is widespread agreement that an excess of circulating catecholamines would cause the observed derangements through different mechanisms. [4] The occurrence of spasm of the epicardial coronary arteries was initially proposed and then microcirculatory dysfunction. [1,9] However, both were discarded since the dyskinetic territory was more extensive than that corresponding to the perfusion of just one artery and it was not clear whe-

ther the microcirculatory dysfunction was the cause or the consequence of SCM. [16] On the contrary, other authors suggested, based on laboratory studies showing higher levels of catecholamines in SCM than in similar control patients with myocardial infarction, a direct myocardial damage. Furthermore, patients undergoing endomyocardial biopsy, showed monocyte inflammatory infiltrates and myocytolysis similar to those occurring in excess catecholamine myocardial damage. [17] More recently, it has been proposed that high level circulating catecholamines would trigger an intracellular signaling pathway that would change the stimulating protein G function to inhibiting protein G in beta 2 receptors. Although this change would serve as a protecting mechanism against cell apoptosis mediated by intensive activation of beta 1 receptors, it would also produce a negative inotropic effect in cardiomyocytes. The higher apical left ventricular adrenergic receptor density would explain why this region is the most frequently affected in SCM while the basal segments are usually spared. Adreno-receptor distribution individual phenotypes in SCM patients would explain the different left ventricular regional involvement. [7] Accordingly, less frequent left ventricular hypo-dyskinesias like the midventricular, generalized, and the inverted "takotsubo" affecting basal contractility but sparing the apex might occur [18,19].

On the other hand, in a recent review of all types of SCM, Bybee and Prasad proposed on the basis of experimental studies, that excessive stress would activate the right cortical insula and the ipsilateral cerebral amigdala stimulating the autonomic nervous system, producing a local excess of catecholamines. At their turn, they would activate the calcium channels increasing the cytosolic and mitochondrial calcium levels with release of free radicals and lipid membrane peroxidation., resulting in cell death and band contraction necrosis producing EKG abnormalities and arrhythmias [12].

Some authors, though accepting the role of increased catecholamine secretion as a triggering factor, suggest that SCM would be instead an aborted "myocardial infarction". It is hypothesized that coronary artery lesions, unidentified by SCG because of their very small size, would produce plaque accidents with spontaneous resolution through endogenous fibrinolysis preventing angiographic recognition due to the time elapsed since onset of symptoms and performance of the study. SCM would then be the consequence of previous myocardial ischemia. Radioisotope studies using metabolic markers have shown altered fatty acid handling and glucose transportation probably caused by catecholamine excess and calcium intracellular overload resulting in "myocardial metabolic stunning" [20-22].

The cause of the more frequent occurrence of SCM in menopausal women has not been elucidated. A gender difference has been suggested or else, microcirculatory

endothelial dysfunction facilitated by a decrease of estrogenic stimulation [5].

Dynamic left ventricular outflow tract obstruction has also been described in SCM patients. [23] It is still not clear if the gradient is the cause or a consequence of the syndrome [16].

Finally, Akashi *et al.*, based on experimental studies in rats, propose that sudden, unexpected, stress, would activate autonomic nervous system neurons with estrogenic receptors causing higher release of catecholamines that would stimulate vascular and cardiac adrenoreceptors resulting in systemic hypertension and increased inotropism. The aforementioned higher expression of apical adrenoreceptors, would be responsible of the dyskinesia at that level through the toxic effect on the cardiomyocytes. The dynamic left ventricular outflow tract obstruction present in some patients with SCM would be caused by the basal myocardial hypercontractility. The loss of estrogenic protection in the nervous system and myocardium following menopause would exaggerate all these disarrangements [15].

The previous discussion shows that the origin and facilitating triggering factors of SCM are still under debate. Since SCM description and better identification are recent, it is still not clear which is the ideal treatment for these patients in the acute phase and after discharge to prevent recurrences. Nevertheless, supported by laboratory animal experimental studies, Akashi *et al.*, recommend combined alfa and beta adrenergic blockers instead of individual administration. Calcium channel antagonists and nitrites would also be effective [15].

5. CONCLUSIONS

The prevalence of SCM in a 7-year period in our hospital was 2.1% of all patients with ACS requiring SCG and 5.0% if just women were considered. There was an initial favorable outcome with satisfactory response to conventional medical treatment though recurrences occurred in patients with inadequate control in anxiety disorders and 2 of them required readmission to hospital.

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Titanium allergy or not? “Impurity” of titanium implant materials

Thomas Harloff¹, Wolfgang Hönle², Ulrich Holzwarth³, Rainer Bader⁴, Peter Thomas⁵, Alexander Schuh¹

¹Research Unit, Neumarkt Clinical Center, Neumarkt, Germany

²Orthopedic Clinic Neumarkt, Neumarkt, Germany

³MedTitan, Erlangen, Germany

⁴Orthopedic Clinic and Polyclinic, University of Rostock, Rostock, Germany

⁵Clinic and Polyclinic for Dermatology and Allergology of the Ludwig-Maximilians-University of Munich, Munich, Germany;
Alexander.Schuh@klinikum.neumarkt.de

Received 25 December 2009; revised 26 January 2010; accepted 30 January 2010.

ABSTRACT

For patients suffering from allergies to nickel, chrome and cobalt, titanium implants are the implants of choice. Nevertheless, titanium implant sensitivity has been reported in the form of “allergies” and an increasing number of patients are confused. This paper aims to use spectral analysis as a diagnostic tool for analyzing different titanium implant alloys in order to determine the percentage of the alloy components and additions that are known to cause allergies. Different materials, such as sponge titanium, TiAl6Nb7, Ti21SRx, TiAl6V4 [forged alloy], TiAl6V4 [cast alloy], TMZF, pure titanium [c. p. 1] and iodide titanium were analyzed for the presence of the elements that have been associated with allergic reactions using spectral analysis. All the implant material samples contained traceable amounts of Be, Cd, Co, up to a maximum of 0.001 percent by weight [wt.%], Cr up to 0.033 wt.%, Cu up to 0.007 wt.%, Hf up to 0.035 wt.%, Mn up to 0.007 wt.%, Ni up to 0.031 wt.%, and Pd up to 0.001 wt.%. This paper demonstrates that all the investigated implant material samples contained a low but consistent percentage of components that have been associated with allergies. For example, low nickel contents are related to the manufacturing process and are completely dissolved in the titanium grid. Therefore, they can virtually be classified as “impurities”. Under certain circumstances, these small amounts may be sufficient to trigger allergic reactions in patients suffering from the corresponding allergies, such as a nickel, palladium or chrome allergy.

Keywords: Allergy; Implant; Nickel; Spectral

Analysis; Titanium

1. INTRODUCTION

Numerous studies on allergic reactions to synthetic materials have been carried out, in particular on allergic reactions to metallic components that are also used in orthopedic surgery. In case histories, localized or generalized eczemas, urticaria, persistent swelling, sterile osteomyelitis and cases of aseptic implant loosening are described as examples of allergic reactions to metal implants [1-28]. Nickel, cobalt and chrome are the classic contact allergens [1,2,11,29-32]. However, in contrast to the sensitization ratio of up to 12 percent of the general population to nickel and of up to 5 percent to cobalt and chrome [24,32], only a few cases of allergies to implant materials have been documented. Precise details on the frequency of such reactions are presently not available. Furthermore, up to now, the frequency of allergic reactions occurring in the peri-implant region, without any prior patch test reactions, has not been established. For example, inflammatory infiltrations of the peri-implant region displaying characteristics of late-type allergic reactions were found in a number of patients undergoing revision operations related to complications [2]. Thomas [24] and Willert [27] published cases of endoprosthesis loosening with accompanying T-lymphocyte-dominated immune reactions in the peri-implant region. In the 1970s, obvious allergic reactions to the cobalt-chrome alloy components of the McKee-Farrar prosthesis underwent scrutiny for the first time [3,11]. In case of a nickel allergy, individual responsiveness can be very diverse, with even minute quantities of nickel causing contact eczemas in sensitive patients [3,11,32]. Their high resistance to corrosion, the absence of any carcinogenic risk, their excellent bio-compatibility and their

lack of sensitization make titanium implants or titanium alloy implants the recommended alternative for patients with nickel, cobalt or chrome allergies [33,34]. Admittedly, there are also reports of incompatibility reactions to titanium materials [10,25,35-42]. In his study, Walsh [42] found several eyeglass frames made of a titanium alloy to contain nickel traces. Likewise, Suhonen [41] documented allergic contact dermatitis caused by titanium eyeglass frames. However, in Suhonen's case, palladium was established as the causative factor.

In his comparative histological and immuno-histochemical analysis of tissues surrounding titanium implants [$n = 23$] and implant steel [$n = 8$], Thewes [23] documented the presence of peri-vascular infiltrations, Langerhans cells, T helper cells, T suppressor cells, monocytes, macrophages and memory cells, and did not find any statistically significant difference between both groups of implants. Thewes concluded that a metal sensitization to both steel implants and titanium implants is possible. Yamauchi [43] described an eczema reaction in connection with a pacemaker made of titanium. Lalor *et al.* [38] analyzed the granuloma tissue of five patients that had undergone a revision operation following an aseptic prosthesis loosening. The granuloma tissue was found to contain primarily titanium. Each of the five patients subjected to scratch testing using diluted solutions of titanium salts yielded negative results. However, two of the patients displayed a positive skin reaction to titanium-containing ointments.

These above mentioned reports led to more and more confused patients. This paper aims to examine different titanium implant alloys in respect to impurity with components that are known to potentially cause allergies.

2. MATERIAL AND METHOD

A Spectrolab spectral analysis unit from the Spectro company [Kleve, Germany] was used to study the titanium materials [listed in **Table 1** with their respective producers]. Prior to the test, the optical analysis unit was calibrated using calibrated samples, the chemical com-

position of which was determined via optical spectral analysis by sparking sample slices [with a diameter of 6-60 mm and a thickness of 6 mm] under argon atmosphere using a 6 mm ceramic aperture. In this particular case, the measuring depth obtained by sparking is 0.5 mm, making the thickness of the examined samples irrelevant. The described method pertains to a material analysis and not to a layer analysis. A detailed analysis was performed on pure titanium slices with a diameter of 6 and 12 mm.

TiAl6V4 slices with a diameter of 10, 16, 22, 35 and 60 mm, respectively, and TiAl6Nb7 slices with a diameter of 14.5, 22 and 28 mm, respectively. Samples of rods with different diameters were analyzed because the various titanium alloys of the individual manufacturers are available with different diameters. The analyses were performed according to the established and [statistically] recognized measuring methods used in material science for determining alloy components. Since it has to be assumed that the material is homogeneous over the entire length of the respective [titanium or titanium alloy] rod, only a 6 mm thick sample slice was analyzed in each individual case. Three measurements and a final verification measurement were conducted. Each of the results indicated corresponds to the average value obtained from the three measurements, with the standard deviation being less than 0.01 percent by weight.

3. RESULTS

The results of the spectral analysis are shown in **Table 2**. All the implant material samples contained traceable amounts of Be, Cd, Co, up to a maximum of 0.001 percent by weight, Cr, up to a maximum of 0.033 percent by weight, Cu, up to a maximum of 0.007 percent by weight, Hf, up to a maximum of 0.035 percent by weight, Mn, up to a maximum of 0.007 percent by weight, Ni, up to a maximum of 0.031 percent by weight, and Pd, up to a maximum of 0.001 percent by weight [**Table 2**].

4. DISCUSSION

There is an increasing number of reports of incompatibility reactions to titanium materials [10,25,35-42]. All the titanium materials examined in the present study clearly showed consistently traceable amounts of additional components, such as nickel. Although contents between 0.01 and 0.034 percent by weight are considered to be insignificant from a metallurgic perspective, they are subject to discussion in the context of the high nickel sensitization rate present in the general population. The levels of additions found in iodide titanium correspond to the expected levels and demonstrate that, in this context, the absolutely lowest traces of nickel that are technologically possible can be adhered to, namely close

Table 1. Materials analyzed.

Materials	Producer/Supplier
Sponge titanium	Source Japan
Sponge titanium	Source Russia
TiAl6Nb7	TIMET USA
Ti21SRx	TIMET Laboratories, Henderson, USA
TiAl6V4	Allvac Teledyne, Monroe, USA
FG-TiAl6V4	ASTM F 1108
TMZF	Stryker
Pure titanium rod, Ti-2	TIMET
Pure titanium plate, Ti-1	Deutsche Titan
Iodide titanium	Metallgesellschaft Ff/M

Table 2. Analysis results (n.t.: not traceable).

Material	Analysis values in % by weight												
	Al	Be	Cd	Co	Cr	Cu	Fe	Hf	Mn	Mo	Ni	Pd	V
Sponge titanium (Japan)	0,001	0,001	0,001	0,001	0,002	0,007	0,001	0,001	0,001	0,001	0,008	0,001	0,001
Sponge titanium (Russia)	0,001	0,001	0,001	0,001	0,001	0,001	0,002	0,001	0,007	0,001	0,001	0,001	0,001
TiAl6Nb7	5,980	0,001	0,001	0,001	0,011	0,001	0,150	0,001	0,002	0,001	0,014	0,001	0,001
Ti21SRx	0,005	0,001	0,001	0,001	0,005	n n.	0,037	0,001	0,002	15,00	0,017	0,001	0,001
TiAl6V4	5,930	0,001	0,001	0,001	0,033	0,001	0,160	0,001	0,004	0,002	0,031	0,001	3,880
FG-TiAl6V4 ASTM F 1108	6,20	0,0001	0,0001	0,001	0,012	0,001	0,170	0,001	0,001	0,001	0,011	0,001	4,15
TMZF	0,005	0,001	0,001	0,001	0,008	0,003	2,090	0,035	0,001	12,00	0,013	0,001	0,002
Pure titanium rod, Ti-2, Timet	0,021	0,001	0,001	0,001	0,014	0,001	0,041	0,001	0,002	0,001	0,013	0,001	0,012
Pure titanium Ti-1, Plate (Deutsche Titan)	0,004	0,001	0,001	0,001	0,012	0,001	0,028	0,001	0,001	0,001	0,012	0,001	0,001
Iodide titanium	0,003	0,001	0,001	0,001	0,001	0,001	0,010	0,013	0,001	0,001	0,001	0,001	0,002

to the detection limit of less than 0.001 percent by weight. All the other samples, independent of the producer, were always found to contain a consistently low percentage of additions, such as nickel, following their further processing into rods of different sizes [diameters between 6 and 60 mm were analyzed]. Numerous publications deal with hypersensitivity reactions to osteosynthesis materials used in the treatment of fractures, the majority of these materials being stainless steel implants [7,19,32].

An immunological response to metals [partly as an exaggerated allergic reaction] is discussed to be the cause of impaired wound healing or the delayed healing of fractures [19]. Allergic reactions to orthopedic implants can thus also necessitate the removal of the implant [24]. Lymphocyte infiltration was discovered in the peri-prosthetic tissue, indicating T-lymphocyte-related inflammation components [25-28]. This lymphocyte infiltration can be considered a component of a delayed hypersensitivity reaction [DTH, Delayed Type Hypersensitivity] [2,26,27]. Vasculitis with lymphocyte infiltration of the vascular walls and substantial fibrin exudation have been described [11,27,32]. Nickel, cobalt and chrome can cause allergic reactions in humans [2,19,31], with nickel being one of the most common contact allergens. The average sensitization ratio in the general population lies between 2 percent and 12 percent, depending on age, gender and living conditions. In addition to the typical findings, such as hand eczema, uncommon manifestations, such as pseudo-lymphomas or implant-associated intolerance reactions, are also known to occur [24]. Many aspects of skin allergies have already been analyzed, such as thresholds above which allergens, such as nickel, chrome or cobalt trigger skin reactions, the use of standardized provocation testing for the detection of an allergy [patch test], [immuno-] histological characteristics of such reactions, tracking elements, such as CLA [cutaneous leukocyte antigen], which allow sensitized

T-cells to migrate into the skin, and the diminishing reactivity following the avoidance of the allergens for many years which leads to problems only after repeated fresh contact with the respective allergen [booster], e.g. in case of the repeated wear of fashion jewelry. Accordingly, the "Nickel Directive" [31], which applies to items that have a direct and prolonged contact with the skin, determines that a maximum of 0.5 µg nickel/cm²/week can be released and limits the nickel contents in piercing metals to 0.05 percent. However, such guidelines do not yet exist for implants or implant materials. In a study carried out on 242 patients, Swionkowski *et al.* [22] reported a sensitization prevalence of 0.2 percent for chromium, 1.3 percent for nickel and 1.8 percent for cobalt. Subsequent to the implantation of orthopedic implants, the sensitization rate increased to 2.7 percent for chromium, 3.8 percent for nickel and 3.8 percent for cobalt. In many cases, only minute amounts of nickel suffice to trigger allergic reactions, such as contact eczemas [32]. Therefore, titanium implants or titanium-alloy implants are often used as an alternative for patients suffering from nickel, chrome or cobalt allergies [30]. Duchna [10] conducted a study on 112 patients and did not find any allergic reactions that were associated with titanium implants. The biocompatibility of titanium materials [32] is based on the passivation of its surface. In its intact state, this surface consists of non-conductive titanium oxide, a bio-inert material that chemically corresponds to ceramics. When corrosion occurs due to an electron flow, an interaction between the body and the implant takes place. In essence, these interactions are dependent on the insulation provided by the oxide layers and thus dependent on the dielectric constant and therefore on the insulating effect of the metal oxides. The higher the dielectric constant is, the better the insulating effect and the resulting stability in vivo. Depending on the oxide type, titanium oxide has a value between $\varepsilon = 48$ and $\varepsilon = 110$, with water having a value of $\varepsilon = 78$ [32].

In contrast, the dielectric constant for cobalt oxide and nickel oxide is not measurable [32]. Therefore, an interaction with body electrolytes is likely to occur on a much larger scale than is the case for metals belonging to the refractory group [oxide formation in milliseconds], such as titanium, niobium, tantalum, vanadium and their alloys. Alternatively, "ceramic" coatings, such as titanium-niobium-oxynitride, can be used to artificially protect implant alloys against corrosion.

5. CONCLUSIONS

Our results demonstrate that titanium materials contain a small yet consistent percentage of detectable impurities, such as the elements Al, Be, Cd, Co, Cr, Cu, Fe, Hf, Mn, Mo, Ni, Pd and V. All the implant material samples thus contain a consistent yet low percentage of components to which allergies have been attributed. Under specific circumstances, even small amounts of elements, such as palladium, nickel or chromium, suffice to trigger an allergic reaction in patients suffering from the corresponding allergies. However, these allergic reactions would not be directly attributable to titanium or its alloys, but rather to the impurities contained therein. Additional research on the release of the alloy components and the reaction thresholds of the afflicted patients is urgently required. Parallel to this research, alternative production processes should be evaluated by the companies producing these metals in order to produce pure titanium and titanium alloys containing fewer impurities, for use in the human body. Titanium continues to be the implant material of choice for patients suffering from allergic reactions to cement-free implants.

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Prevalence of dysmenorrhea in female students in a Chinese university: a prospective study

Hong-Gui Zhou¹, Zheng-Wei Yang², Students Group³

¹Department of Gynecology and Obstetrics, the Affiliated Hospital of North Sichuan Medical College, Nanchong, China

²Morphometric Research Laboratory, North Sichuan Medical College, Nanchong, China; zwyang@nsmc.edu.cn

³Undergraduate students of North Sichuan Medical College, including Hong-Yu Chen, Hua-Qiu Chen, Li Chen, Ling Chen, Yu-Zi Chen, Xia Chuan, Chun-Ni Deng, Li-Tao Fan, Na Fu, Cui-Mei Gan, Yan Gou, Jing Guo, Li-Ya He, Ya-Jie Hou, Qin Hu, Xiao-Juan Huang, Lin-Ling Li, Xiao-Zuo Li, Xiao-Lan Lin, Chun-Xia Liu, Dan Liu, Xu Liu, Yin-Hua Peng, Jun Qian, Xue-Fang Ren, Wen Tang, Hai-Bo Wang, Hui-Min Wang, Qi Wang, Qian-Qian Wang, Yao Xie, Yao-Yao Yu, Ling Zeng, Huan Zhang, Qiong-Yue Zhang, Jian-Ping Zhao, Chang-Feng Zhou, Xue-Yu Zhou, Yang-Lin Zhou (in surnames' alphabetical order).

Received 28 December 2009; revised 20 January 2010; accepted 25 January 2010.

ABSTRACT

This study aimed to investigate the prevalence of dysmenorrhea in a prospective approach. Menstruation-related diary data were obtained from 2640 female college students in North Sichuan Medical College; dysmenorrhea and related factors were analyzed. Dysmenorrhea occurred in 56.4% of students; 6.5% of dysmenorrheal students suffered from “hard to bear” (unbearable) menstrual pain, and 6.5% had pre-menstrual dysmenorrhea. The more severe dysmenorrhea was, the longer dysmenorrhea lasted, and the longer the duration of menstruation and the larger the amount of menstrual blood flow appeared to be. Dysmenorrhea occurred on 37% of the menstrual dates on average and was unrelated to irregularity of menstrual cycles. The percentages of students taking medicine with mild, moderate and unbearable dysmenorrhea were 4.0%, 13.3% and 23.7%, respectively.

Keywords: College Students; Dysmenorrhea; Prospective Study

1. INTRODUCTION

Many investigations have been undertaken to determine the prevalence of dysmenorrhea (painful menstruation), the commonest (not necessarily the most serious) complaint associated with menstruation [1]. The prevalence varied widely between investigations. As published in English, for example, the prevalence (rate of occurrence) of dysmenorrhea ranged from 72.3% to 89.5% in Nigerian and Turkey university students [2,3], and from

59.7% to 85.0% in adolescent girls or young women [4-8]. In China, university/college students were the main subjects investigated and the prevalence of dysmenorrhea (reported in Chinese journals) varied from 31.5%-41.9% [9-13] to 57.1%-79.4% [14-18]. Previous investigations were, however, retrospective ones by means of questionnaires. In some investigations it was stated that the dysmenorrheal data were about the menstrual pain that occurred every month or frequently [11] or during the past 3 months [8], and in most other investigations it was unknown whether the dysmenorrhea was defined as the menstrual pain that occurred during the past 1 or several weeks, or during the last 1 or several periods. This would cloud the prevalence data. This current investigation was therefore undertaken, by adopting a prospective approach (by means of diary), to study the prevalence of dysmenorrhea in female students in a Chinese university.

2. METHODS

The students investigated were Grades 2004-2007 female undergraduate students residing on the campus of North Sichuan Medical College. 37 of them and 2 male undergraduates constituting the Students Group were recruited as volunteers to assist in the investigation.

A diary table was distributed to students on October 21, 2007. They were requested to keep record of their menstrual dates, amount of menstrual blood flow (too little, moderate or too much), dysmenorrhea (lower abdominal pain associated with menstruation) and medications taken for menstrual disorder. The completed diary tables were collected by January 18, 2008 (the end of the semester).

2876 students participated in the study. 230 of them wrote on the diary that they forgot to fill in part of the menstrual data and 6 students had no menstruation dur-

ing investigation. These students being excluded, the analysis in this study was based on data from the other 2640 students. These students were, on October 2007 at the beginning of investigation, aged 20.3 ± 1.3 (SD) full years (range, 16-26; median, 20; 5th and 95th percentiles, 18 and 22).

The first menstruation during the period of investigation was used for the analysis of dysmenorrhea (including pre-menstrual dysmenorrhea), which was divided into 3 levels: mild (not affecting life or work, without worry or concern), moderate and unbearable (the original Chinese words means “hard to bear” literally). When a student had 1 or more days of unbearable pain, she was classified into the group of unbearable dysmenorrhea. When she had no unbearable pain but had 1 or more days of moderate pain, she was grouped into moderate dysmenorrhea. Mild dysmenorrheal students had mild but no unbearable or moderate pain.

3. RESULTS

The prevalence of dysmenorrhea was 56.4%. 64.7% of the dysmenorrheal students had mild dysmenorrhea, 28.8% moderate dysmenorrhea and 6.5% unbearable dysmenorrhea (**Table 1**). 3.67% of the 2640 students had pre-menstrual dysmenorrhea and 1.10% had only pre-menstrual pain without pain during menstruation. An average of 8.0% of dysmenorrheal students took medicine; the percentages of students taking medicine with mild, moderate and unbearable dysmenorrhea were 4.0%, 13.3% and 23.7%, respectively.

The average duration of menstruation was 3.79 ± 1.22 days (median, 4 days); 261 (9.89%) students had abnormal menstrual cycles [19] > 40 days or < 18 days. Compared to non-dysmenorrheal students, dysmenorrheal students were slightly older in age, had slightly longer menstruation, and had more days on which they felt there was more menstrual blood loss (**Table 1**). The number of dates with dysmenorrhea during menstruation increased significantly with the increase of dysmenorrheal levels (**Table 1**). In dysmenorrheal students, the mean number of menstrual dates in the menstrual period was 4.84 ± 1.24 and dysmenorrhea occurred on $37.1\% \pm 22.8\%$ of the menstrual dates. [Note, for each period of menstruation, the number of menstrual dates is 1 larger than the duration (days) of menstruation.] There was no significant difference in the percentage of students with abnormal menstrual cycles between groups of students without and with dysmenorrhea (**Table 1**).

4. DISCUSSION

This is a large prospective investigation undertaken to study the prevalence of dysmenorrhea. It demonstrated

that the prevalence of dysmenorrhea in female college students in a Chinese university was 56.4%. It also demonstrated for the first time that 1) dysmenorrhea occurred on $< 40\%$ of the menstrual dates on average, and 2) the more severe dysmenorrhea was, the longer dysmenorrhea lasted: the mean number of dysmenorrheal dates during menstruation in unbearable dysmenorrheal students was $> 60\%$ larger than that in mild dysmenorrheal students (**Table 1**). It was previously reported that the severity of dysmenorrhea increased with increasing duration of menstruation or amount of menstrual flow [5-6]. As also shown in the current study, the prevalence or severity of dysmenorrhea was unrelated to irregularity of menstrual cycles (**Table 1**).

The prevalence of dysmenorrhea in college students, a relatively convenient sample to study, was reported with wide variation in previous studies (see the Introduction). A recent (data collected in June 2006) large ($n=15392$) study in 7 universities for minority nationalities reported, for example, an overall prevalence of 65.6% among the second and third year college students on campus, with no significant differences between major nationalities; however, the prevalence was 84.8% in 1 university ($n = 2097$) [18]. Strictly speaking, our result of 56.4% obtained prospectively was not comparable to previous (retrospective) results. A dysmenorrheal woman was defined in this study as suffering from dysmenorrhea in one (the first) menstrual period during investigation. So the dysmenorrheal prevalence obtained in this study reflected the prevalence among the women at certain time point. If a woman was defined as dysmenorrheal even if dysmenorrhea occurred in only one of her past 10 or 20 periods, each woman would likely be dysmenorrheal, *i.e.* the prevalence would be approximating to 100%. The prevalence (85%) of dysmenorrhea occurring during the past 3 months among high school students [8], for example, would certainly overestimate the prevalence at certain time point. Previous studies were usually retrospective, associated with recall bias, and in most previous studies it was unknown how the dysmenorrhea was defined (see the Introduction).

Severity of dysmenorrhea is a subjective feeling, which may be variable between groups of women. Severe (in comparison with mild or moderate) dysmenorrhea was reported in 10%-42% of Western dysmenorrheal women [4-6,8] while the percentage was 6.5%-6.7% in Chinese dysmenorrheal college students [9,15]. Menstruation may be likened to an internal bleeding, wound or trauma and would naturally cause some concern, painful or uncomfortable feeling, to which women would get accustomed one way or another. It is therefore those who feel “hard to bear” (unbearable) that we should pay special attention to. As estimated in the present study, this subgroup of people with unbearable

Table 1. Main results (mean \pm SD) for college students without dysmenorrhea and with mild, moderate or unbearable dysmenorrhea.

	Without (n = 1150)	Mild (n = 964)	Moderate (n = 429)	Unbearable (n = 97)
Age (full years) *	20.2 \pm 1.3 ^{abc}	20.4 \pm 1.4	20.5 \pm 1.3	20.7 \pm 1.3
Duration of menstruation (days) *	3.72 \pm 1.20	3.81 \pm 1.20	3.91 \pm 1.30	3.90 \pm 1.33
No. of dates with dysmenorrhea during menstruation *	-	1.49 \pm 0.91 ^{bc}	2.07 \pm 1.06 ^c	2.43 \pm 1.31
No. of dates with little blood flow during menstruation *	1.50 \pm 1.49	1.54 \pm 1.41	1.70 \pm 1.48	1.69 \pm 1.52
No. of dates with moderate blood flow during menstruation *	2.99 \pm 1.71	2.97 \pm 1.54	2.81 \pm 1.60	2.64 \pm 1.77
No. of dates with much blood flow during menstruation *	0.23 \pm 0.61 ^{abc}	0.29 \pm 0.60 ^{bc}	0.40 \pm 0.67	0.57 \pm 0.93
Percentage of students with abnormal menstrual cycle [#]	10.17%	9.02%	11.19%	9.28%

*Data were significantly different ($P \leq 0.05$) between the 4 groups or the 3 dysmenorrheal groups (Kruskal-Wallis one way analysis of variance on ranks); significantly different ($P \leq 0.05$) from the group of mild ^a, moderate ^b or unbearable ^c dysmenorrhea (Dunn's method for multiple comparison).

[#]Abnormal menstrual cycle: ≤ 17 or ≥ 41 days; there was no significant difference between the percentages (χ^2 test: $P > 0.50$).

dysmenorrhea accounted for 6.5% of the dysmenorrheal students, similar to the “severe” (treatment was needed and school study could not be continued) results (6.5%-6.7%) previously reported in Chinese college students [9,15]. Even with unbearable dysmenorrhea, the students just seemed to bear it without taking medicine—less than 25% of them took medicine. Menstruation-related health education programs are therefore needed on campus to promote the menstrual health of students.

The percentage of dysmenorrheal students taking medicine for dysmenorrhea was previously reported to be 50%-58% among Turkey and British undergraduate students [3,20], 52%-58% among American and Australian high school students [7,8], more than 43.8% among Chinese undergraduate students [18], and 33%-38% among young Sweden women [5,6]. These results are much higher than our result (8.0%). This may be indicative of wide variation in the attitude of medicine taking or in the tolerance to pain between groups (people) or regions (places), and/or indicative of the “cumulative effects” in retrospective studies, like the overestimation of dysmenorrheal prevalence as described above.

Dysmenorrhea in the present study is essentially primary dysmenorrhea [1] since no students in this investigation reported underlying pelvic pathology during study. In talking with some dysmenorrheal students during study, we identified a couple of students whose dysmenorrhea might be secondary, but this was not confirmed because they did not seek further consultation or examination.

5. ACKNOWLEDGMENTS

This study was supported by grants from the Nanchong Bureau of Science and Technology (N2007-SF010) and the Sichuan Department of Education (08ZA110).

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Fetal loss rates after mid-trimester amniocentesis

Ilker Arikan, Muge Harma, Aykut Barut, Mehmet Ibrahim Harma, Ulku Bayar

Department of Gynecology and Obstetrics, Faculty of Medicine, Zonguldak Karaelmas University, Zonguldak, Turkey;
driarikan@hotmail.com, ilkeristanbul@myinet.com

Received 15 December 2009; revised 10 January 2010; accepted 15 January 2010.

ABSTRACT

Objective: Amniocentesis is an invasive cytogenetic test traditionally associated with a 1/200 procedure-related pregnancy loss rate. Recent studies have questioned the validity of the traditionally stated rate. The purpose of this study was to document the results of second-trimester genetic amniocentesis performed at our perinatology clinic. **Study Design:** A retrospective review of all the amniocentesis procedures performed between 15 and 22 weeks of gestation on singleton pregnancies between May 2004 and December 2008 was performed. **Spontaneous loss** was defined as any unintentional pregnancy loss at < 24 weeks of gestation. **Setting:** Zonguldak Karaelmas University, Faculty of Medicine, Department of Obstetrics and Gynecology. **Population:** Pregnant women followed at the Obstetrics Department. **Methods:** A retrospective review of all the amniocentesis procedures performed between May 2004 and December 2008 was performed. **Main outcome measure:** Pregnancy loss due to amniocentesis. **Results:** A total of 447 amniocentesis procedures were performed during the study period. The major indication for amniocentesis was positive maternal triple screening (44%). The mean gestational age at amniocentesis was 18.80 ± 2.70 weeks. The results of cytogenetic analyses revealed an abnormal karyotype in 19 pregnancies (4.3%), nine of which were trisomy 21. The overall spontaneous loss rate was 0.89% ($n = 4$). **Conclusion:** It would be useful for each center to investigate its own pregnancy loss rate and thereby provide a firmer basis for its policy for counseling women requesting amniocentesis. If enough such investigations were reported, a true benchmark figure could also emerge.

Keywords: Genetic Amniocentesis; Mid-Trimester Amniocentesis; Pregnancy Loss; Amniocentesis-Related Fetal Loss

1. INTRODUCTION

Amniocentesis was first performed in the 1880s for decompression of polyhydramnios. In the 1950s, amniocentesis for fetal chromosome analysis was initiated as laboratory techniques for cell culture and karyotype were developed. The first reported applications were limited to fetal sex determination. The feasibility of culturing and karyotyping amniotic fluid cells was demonstrated in 1966, and the first prenatal diagnosis of a karyotype was reported in 1967 [1].

Prenatal diagnosis of fetal chromosomal abnormalities is the most common indication for invasive prenatal testing. The prevalence of chromosomal abnormalities in clinically recognized early pregnancy loss is greater than 50%. Fetuses with aneuploidy account for 6-11% of all stillbirths and neonatal deaths. Chromosomal abnormalities that are compatible with life but cause considerable morbidity occur in 0.65% of newborns [2].

There are many strategies available to screen for chromosomal abnormalities, including combined test, triple test, quad test, integrated screen, stepwise sequential screen and contingent sequential screen [3]. All of these approaches provide an adjusted risk for Down syndrome and trisomy 18, but they do not exclude the possibility of an affected fetus, because the test sensitivity is less than 100%. Therefore, amniocentesis is still the only diagnostic test in current use that is valid for diagnosis.

Amniocentesis is an invasive cytogenetic test traditionally associated with a 1/200 procedure-related pregnancy loss rate [4]. This risk was initially determined based on data from studies conducted in the 1970s [5] and limited the use of amniocentesis to clinically indicated high-risk populations rather than to all pregnancies.

Recent studies have questioned the validity of this traditionally stated fetal loss rate. For example, Eddleman *et al.* reported a 1/1600 procedure-related pregnancy loss rate after amniocentesis [6], while Odibo *et al.* documented a 1/769 amniocentesis-related fetal loss rate in a large single center cohort [7].

The purpose of this study was to document the results of the second-trimester genetic amniocenteses we performed and provide a further comparison.

2. MATERIAL AND METHODS

A retrospective review of our mid-trimester amniocentesis database for the period from May 2004 to December 2008 was carried out, with all of the amniocentesis procedures performed between 15 and 22 weeks of gestation on singleton pregnancies analyzed, and data on perinatal outcomes gathered from the hospital patient database. Spontaneous loss was defined as any unintentional pregnancy loss at < 24 weeks of gestation. Elective termination of pregnancy was not considered as pregnancy loss in this analysis.

3. RESULTS

A total of 447 amniocentesis specimens were processed during the study period. The mean (\pm SD) maternal age was 31.82 ± 6.30 years. The major indications for amniocentesis were positive maternal triple screening (44%), advanced maternal age with positive maternal triple screening (18.9%) and advanced maternal age solely (15.5%) (Table 1). The mean (\pm SD) gestational age at amniocentesis was 18.80 ± 2.70 weeks. The mean (\pm SD) gestational age at delivery was 38.06 ± 2.44 , and the mean (\pm SD) birthweight was 3261.30 ± 850.40 g.

Cytogenetic analysis revealed an abnormal karyotype in 19 pregnancies (4.3 %), nine of them being trisomy 21 (Table 2).

The overall spontaneous loss rate at less than 24 weeks of gestation was 0.89% ($n = 4$), and 41 preterm deliveries (9.2%) before 37 weeks of gestation occurred. Fifteen (3.4%) patients experienced preeclampsia. The rate of low birthweight (< 2500 g) infants was 8.6% (Table 3).

Table 1. Indications for amniocentesis.

Indication	n	%
Positive maternal serum triple screening	196	43.8
Positive maternal combined screening	42	9.4
Advanced maternal age (≥ 35 y)	69	15.4
Positive maternal serum triple screening + Positive maternal combined screening	8	1.8
Positive maternal serum triple screening + Advanced maternal age (≥ 35 y)	84	18.8
Positive maternal combined screening + Advanced maternal age (≥ 35 y)	4	0.9
Others*	44	9.8

* Previous child with chromosomal abnormality, previous fetus with malformation and unknown karyotype, thickened nuchal translucency at 11-14 wk of gestation, two soft markers for aneuploidy on genetic sonogram, parental anxiety.

Table 2. Results of cytogenetic analyses.

Results	n	%	Indication for amniocentesis			
			PM S	A M A	PMS+ AMA	Other
Culture failure	5	1.1				
Successful cell culture	442	98.9				
Normal karyotype	423	94.7				
46, XX	205	48.4				
46, XY	218	51.6				
Cytogenetic abnormality	19	4.3				
Trisomy 21	9		2	3	3	1
Trisomy 18	2		1	–	1	–
47 XXY	1		–	1	–	–
48 XXXX	1		1	–	–	–
47 XX t21(16%)	1		–	–	1	–
46 XX inv (9) (p11q13)	5		2	1	–	2

PMS, positive maternal serum screening (combined or triple);

AMA, advanced maternal age (≥ 35 y);

Other, previous child with chromosomal abnormality, previous fetus with malformation and unknown karyotype, thickened nuchal translucency at 11-14 wk of gestation, two soft markers for aneuploidy on genetic sonogram, parental anxiety.

Table 3. Pregnancy outcomes.

Outcome	n	%
Fetal loss less than 24 wk	4	0.89
Preterm delivery (< 37 wk)	41	9.2
Low Birth Weight infant (< 2500 g)	38	8.6
Preeclampsia-eclampsia	15	3.4

4. DISCUSSION

Unintended pregnancy loss has been the major concern with amniocentesis over the past four decades. The traditional estimated loss rate of 1 in 200, which was derived from the studies conducted in the 1970s, is based on recommendations by the Centers for Disease Control and Prevention and endorsed by the American College of Obstetricians and Gynecologists (ACOG) [4,8].

Recent studies have reported lower fetal loss rates and have criticized the studies conducted in 1970s because they were not randomized [6,7] (though Eddelmann [6] admitted that further randomized prospective trials could not now be performed because of ethical considerations) and were not performed under concurrent ultrasound guidance [6]. However, the figure of 1 in 1600 reported by Eddelmann *et al.* [6] in 2006 has been criticized by most investigators [9-11]. The only randomized study evaluating pregnancy loss rates, published by Tabor *et al.* in 1986, reported a procedure-related loss rate of 1%

[12], while a recent review by Seeds reported a loss rate of 0.6% [13].

In 2007, ACOG changed its 2001 recommendations, quoting the procedure-related loss rate after midtrimester amniocentesis as less than 1 in 300-500 [14]. The figure of 0.89% obtained in our study (**Table 3**) is close to the 1% obtained by Eddleman *et al.* [6] and the 0.97% of Odibo *et al.* [7]. Our rate of 9.2% preterm (< 37 weeks gestation) deliveries is less than the 11% preterm birth rate for singletons in the USA in 2005, while our 8.6% rate for low birthweight infants is similar to the 2005 US figure for singletons (7.55%) [15].

Although this study is limited by its retrospective design, its lack of a control group, and the small numbers involved, its findings agree with other recent reports. It would be useful if other centers similarly investigated and reported their own pregnancy loss rates associated with amniocentesis, and perhaps used their results to reexamine their policies for counseling women requesting amniocentesis.

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Acceleration of coxarthrosis by an exostosis causing femoroacetabular impingement

Mahmut Nedim Aytekin¹, Hakan Atalar¹, Burcu Yanik², Murat Arikan³

¹Department of Orthopedics and Traumatology, Fatih University Hospital, Ankara, Turkey; nedimaytekin@hotmail.com

²Department of Physical Therapy and Rehabilitation, Fatih University Hospital, Ankara, Turkey

³Department of Orthopedics and Traumatology, Ankara Oncology State Hospital, Ankara, Turkey

Received 18 November 2009; revised 4 January 2010; accepted 8 January 2010.

ABSTRACT

Here we describe a 28-year-old man with a history of right hip pain for the past 11 years and ankylosing spondylitis for the past 6 months. Imaging studies showed an exostosis in the femoral neck causing femoroacetabular impingement. The patient was diagnosed with coxarthrosis. This case report suggests that femoroacetabular impingement may accelerate the degenerative process in the hip joint.

Keywords: Femoroacetabular Impingement; Exostosis

1. INTRODUCTION

Femoroacetabular impingement, also known as hip impingement syndrome, refers to a condition in which the femoral head-neck junction impinges upon the acetabulum, thereby being a potential cause of hip osteoarthritis [1,2]. Two types of impingement are defined according to the anatomic structures involved. Cam impingement occurs when the contact arises from an abnormality in the femur and pincer impingement is defined as that which results when the abnormality originates from the acetabulum [3]. Both types are characterized by damage to the acetabular labrum and articular cartilage in the hip [4,5]. These changes lead to degenerative osteoarthritis in the long term [5,6]. Here, we describe a patient who has femoroacetabular impingement due to a bone mass in the femoral neck and coxarthrosis. We could not find any case which demonstrates acceleration of coxarthrosis by an exostosis causing femoroacetabular impingement in the literature.

2. CASE REPORT

A 28-year-old male presented at our hospital with a history of right hip pain for the past 11 years which had become increasingly worse in the past 3 years, up to the point at which he decided to seek treatment. He was cur-

rently working as a waiter. Six months prior to this presentation he had been diagnosed with ankylosing spondylitis and was currently taking salazoprine, methotrexate and indomethacin regularly. He was a nonsmoker and had no history of athletic activity which could explain his findings. He also reported having back pain and morning stiffness for the past 3 years.

On physical examination of the right hip, flexion, abduction, external rotation and internal rotation were limited. There was 45 degree maximum flexion, 10 degree maximum extension and 10 degree maximum abduction in the right hip joint. Internal and external rotation of the right hip was also markedly restricted. Anteflexion, extension and lateral bending of the lumbar region were limited.

Pelvic x-ray showed an exostosis in the right femoral neck. Also visible in the right hip were narrowing of the hip joint, subchondral sclerosis and osteophytes. There was also sclerosis and narrowing in the sacroiliac joint, indicating bilateral sacroileitis which is a finding of ankylosing spondylitis (**Figure 1**). CT and MRI demonstrated



Figure 1. An anteroposterior pelvic radiograph shows cam impingement due to exostosis of the femoral neck and coxarthrosis in the right hip.

femoroacetabular impingement caused by the exostosis (Figures 2,3).

Conservative treatment has been decided upon for the time being because the patient is young and can still perform his activities of daily life.

3. DISCUSSIONS

Femoroacetabular impingement has been recognized as



Figure 2. Coronal computed tomography of the right hip demonstrates femoroacetabular cam impingement due to an exostosis in the femoral neck and findings of coxarthrosis.



Figure 3. Magnetic Resonance Imaging of the right hip demonstrates femoroacetabular cam impingement due to an exostosis in the femoral neck and findings of coxarthrosis.

an underlying cause of hip pain and secondary osteoarthritis [1] and occurs in two main forms: cam-type impingement and pincer-type impingement. Cam-type impingement occurs when the anterior femoral neck abnormally impinges on the on the acetabulum and labrum, resulting in damage to the labrum [7,8]. Pincer-type impingement occurs when an osteophyte on the anterior acetabulum impinges on the anterior femoral neck during hip flexion or retroversion of the acetabulum [9,10].

We found cam-type impingement due to the exostosis on the femoral neck in our patient. For the coxarthrosis that developed, another possible etiological factor was the ankylosing spondylitis; however, the patient had no other risk factors for coxarthrosis such as smoking, alcoholism, steroid use, obesity, female gender, repetitive occupational trauma, or neuromuscular or metabolic disorders. Protrusio acetabuli may develop in as many as one third of patients and hip joint involvement typically is bilateral and symmetric. Absence of the protrusion of the both acetabulum and the unilateral nature of the coxarthrosis suggest that the impingement caused by the exostosis had accelerated the degeneration of the hip joint.

Although there are a range of treatment options for hip impingement syndrome [1], nonsurgical treatment generally does not control symptoms [11]. By preventing microtrauma, early treatment may help preserve the joint by averting the impingement that may lead to coxarthrosis [8]. Our patient presented later in the course of the disease, with hip osteoarthritis symptoms that were apparently due indirectly to the nearby tumor, which itself was not painful.

In conclusion, femoral neck exostosis may accelerate the progression of coxarthrosis by cam impingement. Early diagnosis is therefore important in preventing this degenerative process.

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Study of the mechanisms regulating human umbilical artery contractility

António José Santos-Silva¹, Elisa Cairrão^{1,2}, Ignacio Verde³

¹Centro de Investigação em Ciências da Saúde, Universidade da Beira Interior, Avenida Infante Dom Henrique, Covilhã, Portugal

²Centro Hospitalar da Cova da Beira, Quinta do Alvito, Covilhã, Portugal

³Centro de Investigação em Ciências da Saúde, Universidade da Beira Interior, Avenida Infante Dom Henrique, Covilhã, Portugal; iverde@fcsaude.ubi.pt

Received 10 November 2009; revised 27 December 2009; accepted 5 January 2010.

ABSTRACT

We studied the involvement of different types of Ca^{2+} channels, cyclic nucleotides and different kinases in the regulation of human umbilical artery (HUA) contractility. The elucidation of the precise mechanisms regulating the contractility of this artery could be very important to reveal potential therapeutic targets to treat HUA disorders such as preeclampsia. The relevancy of different types of Ca^{2+} channels on the regulation of HUA tonus was analyzed. Among the different Ca^{2+} channel inhibitors used, only the L-type calcium channels (LTCC) inhibition induced relaxation of HUA in Ca^{2+} containing medium. The inhibition of T-type calcium channels (TTCC) or TRP channels did not significantly affect HUA contractility. In presence of Ca^{2+} , the intracellular increase of a cyclic nucleotide (cAMP or cGMP) induces relaxation of HUA, which was almost complete in histamine-contracted HUA, and lower effect was observed in arteries contracted by KCl and serotonin (5-HT). Inhibition of PKA and PKG weakly reduced the relaxations induced by the increase of cAMP and cGMP respectively, suggesting that the relaxation induced by these nucleotides is not totally mediated by the activation of their respective kinases and that other mechanisms are involved. In calcium containing solution, PP2A inhibition produces relaxation of contracted HUA. In KCl contracted arteries, the OA and nifedipine relaxant effects are similar and not additive, suggesting that PP2A could activate LTCC. Besides, the increase of cyclic nucleotides significantly increased the OA effect, suggesting that the effect of PP2A inhibition is independent of the cyclic nucleotide pathways. The contractions induced by KCl, histamine and 5-HT in presence of Ca^{2+} were not significantly

affected by ROCK, ERK1/2 or p38MAPK inhibitors. In absence of extracellular Ca^{2+} , histamine and 5-HT elicited contractions of HUA characterized by two components, a rapid phasic contractile component followed by a decrease of the contraction until a tonic component. However, KCl elicited sustained contractions of HUA in absence of extracellular Ca^{2+} . As in presence of calcium, the ERK1/2 and p38MAPK inhibitors did not influence the contractions induced by KCl, histamine or 5-HT in absence of extracellular Ca^{2+} . However, in these conditions, ROCK inhibition significantly relaxed the contractions induced by KCl and reduced the phasic and tonic components of the contraction elicited either by histamine or 5-HT. Our results show that calcium-dependent contractions of HUA depend on Ca^{2+} entry by LTCC, and these channels seems to be positive regulated by PP2A. Cyclic nucleotides mediate HUA vasodilatation but their dependent kinases are not the unique responsible of this effect. HUA is able to contract independently of Ca^{2+} influx by activating the ROCK pathway and/or due to intracellular Ca^{2+} release.

Keywords: Umbilical Artery; Smooth Muscle; L-type Ca^{2+} Channels; ROCK; Cyclic AMP; Cyclic GMP

1. INTRODUCTION

The mechanisms regulating smooth muscle contractility in human umbilical artery (HUA) are very important for optimum gas and nutrient exchange between foetus and placenta. Since the umbilical blood vessels are not innervated, the control of umbilical blood flow depends of vasoactive substances either released locally or existing in the circulation [1,2]. In general, vascular smooth

muscle (VSM) contractions are initiated by receptor or ion channel activation and involve Ca^{2+} dependent and/or independent mechanisms [3]. The increase of cytosolic free Ca^{2+} can be originated by a transient and rapid increase due to the Ca^{2+} release from the sarcoplasmic reticulum and/or by a sustained extracellular Ca^{2+} influx through Ca^{2+} channels [4,5]. This Ca^{2+} increase leads to myosin light chain kinase activation and contraction due to interaction between the thick and the thin myofilaments [6]. VSM relaxation can be mediated by activation of myosin light chain phosphatase which has opposite effects than myosin light kinase activation.

Different types of Ca^{2+} channels have been involved in the control of VSM contractility, such as L-type Ca^{2+} channels (LTCC) [7,8], T-type Ca^{2+} channels (TTCC) [9], store-operated Ca^{2+} channels (SOCC) [10] or stretch-activated channels (SAC) [11,12]. Recently, the SOCC and SAC have been identified as being transient receptor potential (TRP) channels [13]. In VSM, different TRP channels were linked with Ca^{2+} store depletion, G-protein-coupled receptor activation, membrane stretch, phospholipid signals and other factors. However, the role of these channels was not clarified yet, probably due to the lack of specific pharmacological tools, such as specific activators and/or inhibitors [14]. The TTCC were mainly involved in the regulation of cell proliferation [15]. Although these channels were involved in smooth muscle contraction by some authors [16], the information linking TTCC with VSM contractility is scant and their relative importance in this process needs further analysis. On the other hand, LTCC seems to be dominant in the regulation of VSM contractility because they have been appointed as the main pathway for Ca^{2+} entry. In HUA, some authors identified different LTCC and TTCC [17] and the LTCC blockers have appeared to be the most potent relaxants of this artery [18]. On the other hand, in smooth muscle cells, LTCC could be regulated by PP2A, although the number of studies is very low and the direction of this modulation has remained somewhat controversial. Some author have shown that PP2A does not modulate LTCC in tracheal smooth muscle [19], but in intestinal smooth muscle a dual effect of phosphatase inhibitors on LTCC has been reported [20]. In human umbilical vein, Groschner *et al.* have suggested that inhibition of PP2A activates LTCC [21].

Concerning the Ca^{2+} independent mechanism, it could involve Ca^{2+} -sensitization mediated by the activation of the RhoA-kinase (ROCK) which causes inhibition of myosin light chain phosphatase, leading to an increase of myosin light chain phosphorylation [5]. In this sense, some authors have reported that rhoA/ROCK pathway can contribute to agonist-induced contractions of HUA. However, this effect seems to be limited to intracellular Ca^{2+} -induced contractions and may be more important in

sustaining contractions rather than the initial phase of force development [22]. Other authors have suggested the existence, in VSM cells, of a Ca^{2+} -dependent Rho stimulation mechanism linked to different receptor coupled to G proteins [23]. Also, it has been described that rabbit artery smooth muscle depolarization increases Ca^{2+} sensitization due to ROCK activation [24]. On the other hand, a role of some components of the MAPK cascade and its substrates (ERK1 and ERK2) in modulating the VSM contractility has been also suggested [25]. Some roles of these kinases in the regulation of cell proliferation and differentiation has been established [26] but their role in VSM contraction and relaxation is almost unknown. Some agonists inducing VSM contraction can also activate ERKs [27,28]. In cerebral arteries, it was suggested that ERKs modulate Ca^{2+} sensitivity and contractility [25]. Other authors have observed that 5-HT induces rat aorta contractions involving p38 MAPK or Erk MAPK pathways [29-31].

The cyclic nucleotides, cAMP or cGMP, are the main second messenger involved in the regulation of vasodilation [32]. Intracellular accumulation of cAMP and cGMP can be achieved by stimulation of adenylate or guanylate cyclase, respectively, or by inhibition of phosphodiesterases (PDE) [33]. Concerning cAMP, intracellular increase of this nucleotide induces relaxation of different human arteries [34-36], including HUA [37]. Among the four families of PDE expressed in this smooth muscle (PDE1, PDE3, PDE4 and PDE5), PDE4 has been shown as the key enzyme involved in the regulation of HUA relaxation associated to cAMP [37]. Concerning cGMP, the increase of the intracellular level of this nucleotide also induces artery vasodilatation [38], including HUA vasodilatation [37,39]. PDE5 has been shown as the key enzyme involved in the regulation of HUA relaxation associated to cGMP [37]. Also, distinct authors have described that in different arteries [40,41], including HUA [39], the vasodilatation induced by cGMP is mediated by activation of potassium channels. Increases in cAMP and cGMP activate cAMP-dependent protein kinase (PKA) and cGMP-dependent protein kinase (PKG), respectively [42]. In VSM cells, the inhibition of LTCC by PKG has been reported [43]. However, the cAMP pathway has been suggested to inhibit, to enhance, or to have no effect on smooth muscle LTCC [43]. Also, some authors have suggested that relaxation induced by cyclic nucleotides is not totally mediated by the activation of their respective kinases. In this sense, other mechanisms were involved, such as cross-activation between cyclic nucleotide dependent kinases [44] or the regulation of other proteins having Epac (exchange protein directly activated by cAMP) which activates the small GTPbinding protein Rap1 [45].

Despite of the great importance of HUA before and during childbirth, the mechanisms involved in the regu-

lation of the contractility of this artery have been weakly studied. Devoid of enervation, the regulation of vascular tone of the HUA depends only by the local release of humoral factors, such as 5-HT and histamine [1,2]. In this work we have evaluated the relevancy of some mechanisms involved in the contraction and relaxation of this artery, such as extracellular Ca^{2+} , cyclic nucleotides, Ca^{2+} channels and different kinase types.

2. METHODS

2.1. Tissue Preparation

Umbilical cord pieces of 3-7 cm were obtained from normal term pregnancies with the consent of the donor mothers. All procedures carried out with these samples have been approved by the Ethics Committee of "Centro Hospitalar da Cova da Beira EPE". The umbilical cord samples were collected in sterile physiological saline solution (composition, mM: NaCl 110; CaCl_2 0.15; KCl 5; MgCl_2 2; HEPES 10; NaHCO_3 10; KH_2PO_4 0.5; NaH_2PO_4 0.5; Glucose 10; EDTA 0.49). In order to avoid contamination and tissue degradation, penicillin (5 U/ml), streptomycin (5 $\mu\text{g/ml}$), amphotericin B (12.5 ng/ml) and antiproteases (leupeptine, 0.45 mg/l; benzamidine, 26 mg/l; and trypsin inhibitor, 10 mg/l) were added to the physiological saline solution. Umbilical artery rings of 3-5 mm were isolated from the surrounding connective tissue. Vascular endothelium was mechanically removed by gentle rubbing with a cotton bud introduced through the arterial lumen. These denuded HUA rings were used to perform contractility experiments.

2.2. Artery Tension Recording

2.2.1. Relaxation Studies in Ca^{2+} Containing Medium

The HUA rings were placed in organ bath chambers (LE01.004, Leticia) containing Krebs-bicarbonate solution (composition, mM: NaCl 119, KCl 5.0, NaHCO_3 25, KH_2PO_4 1.2, CaCl_2 0.5, MgSO_4 1.2, EDTA 0.03, glucose 11) at 37°C and continuously gassed with carbon dioxide. The artery rings were suspended between two parallel stainless steel wires and tension measurement was performed using isometric transducers (TRI201, Panlab SA, Spain), amplifier (ML118/D Quad Bridge, ADInstruments), interface PowerLab/4SP (ML750, ADInstruments) and a computerized system with Chart5 PowerLab software (ADInstruments). For analysis, the isometric tension measured has been expressed in milligrams (mg) of force elicited by the artery in presence of drugs. To analyze the relaxation data, we used the percentage of reduction on the maximal contraction induced by the contractile agents. During the resting periods, the organ bath solution was changed every 15 min. Initially, the rings were equili-

brated for 60 min until a resting tension of 1000 mg was achieved. After this, the rings were challenged with 5-HT (1 μM) to test their viability. Rings that induced a maximal contraction lower than 1 g when challenged with 5-HT were excluded from the study. Afterwards, the rings were contracted using KCl (60 mM), histamine (10 μM) and 5-HT (1 μM). To determine the involvement of distinct types of Ca^{2+} channels, the LTCC blocker nifedipine (10 μM), the TTCC blocker mibefradil (10 μM) and the TRP blocker 2-aminoethoxydiphenyl borate (APB; 100 μM) have been used.

To analyze the involvement of the cAMP or cGMP pathways the following drugs were used in some cases: rolipram (1 μM), a PDE4 selective inhibitor; forskolin (10 μM), an adenylate cyclase activator; KT-5720 (KTA; 1 μM), a PKA inhibitor; sodium nitroprusside (SNP; 10 μM) a guanylate cyclase stimulator; dipyridamol (3 μM), a PDE5 inhibitor; and KT-5823 (KTg; 1 μM), a PKG inhibitor. To evaluate the possible involvement of PP2A, okadaic acid (OA; 5 nM) has been used in some experiments. Control experiments with ethanol, the vehicle used to dissolve some drugs, were always performed.

2.2.2. Relaxation Studies in Ca^{2+} -free Medium

To analyze the HUA contractility in absence of Ca^{2+} , we used a Krebs solution without Ca^{2+} (composition, mM: NaCl 119, KCl 5.0, NaHCO_3 25, KH_2PO_4 1.2, MgSO_4 1.2, EDTA 0.03, EGTA 0.5, glucose 11). The rings were also contracted using KCl (60 mM), histamine (10 μM) or 5-HT (1 μM).

To analyze the modulation of contractility by some kinases in Ca^{2+} -free contractions, the following drugs were used in some experiments: Y-27632 (Y27; 10 μM), a ROCK inhibitor; PD-98059 (PD; 50 μM) an ERK1/2 inhibitor; and SB-203580 (SB; 25 μM), a p38MAPK inhibitor. In some experiments, nifedipine (10 μM) and OA were also used.

2.3. Drugs and Chemicals

All drugs and chemicals have been purchased from Sigma-Aldrich Quimica (Sintra, Portugal), except forskolin and rolipram, which were purchased from Biogen Científica (Madrid, Spain). Forskolin, rolipram and dipyridamol, were initially dissolved in ethanol and all the other drugs were initially dissolved in distilled water. Final solutions were obtained by dilution with Krebs solution. The final concentration of ethanol in the organ bath did never exceed 0.1%.

2.4. Statistical Analysis

Statistical analysis of the data has been performed using the SigmaStat Statistical Analysis System, version 1.00 (1992). Results have been expressed as mean \pm s.e.m. of

n experiments. Comparison among multiple groups was analyzed by using a one-way ANOVA followed by Tukey or Dunnet's post hoc tests to determine significant differences among the means. Comparison among two groups was analyzed by using Student-t test. Probability levels lower than 5% were considered significant ($P < 0.05$)

3. RESULTS

3.1. Effect of Ca^{2+} Channel Inhibitors in Contracted HUA

The HUA rings without endothelium were contracted by KCl (60 mM), histamine and 5-HT. The presence of KCl (60 mM), 5-HT (1 μM) and histamine (10 μM) elicited maximal contractile effects of 2041.8 ± 94.3 (n = 66), 1798.0 ± 103.9 (n = 53) and 1382.6 ± 89.4 mg (n = 44) respectively. As already exposed in a previous work [46], the contraction induced by histamine is lower than that produced by KCl or 5-HT ($P < 0.05$; one-way ANOVA with Tukey post hoc test). After contraction by these agents, the relaxant effect of blockers of different Ca^{2+} channels was analysed (Figure 1). The relaxations induced by nifedipine on KCl and histamine contracted HUA were similar, however the effect on 5-HT contracted arteries was lower ($P < 0.05$). In general, all the relaxing drugs tested in this work have lower effects on HUA contracted by 5-HT than on contractions induced by KCl or histamine. On the other hand, relaxations induced by mibefradil (TTCC blocker) and APB (TRP channels blocker) were very small, independently of the contractile agent.

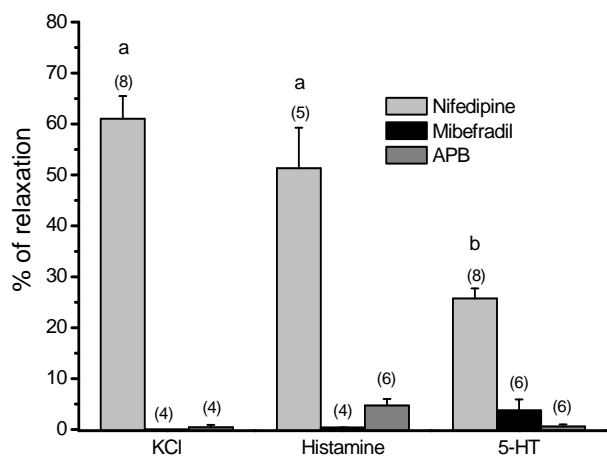


Figure 1. Effects of different Ca^{2+} channel inhibitors on HUA. Relaxation induced by nifedipine (10 μM), mibefradil (10 μM) and APB (100 μM) on contractions elicited by KCl (60 mM), histamine (10 μM) and 5-HT (1 μM). The bars represent the means and the lines the S.E.M. of the number of experiments indicated near the bars. Bars with different letters indicate significant differences in the effect of nifedipine ($P < 0.05$, one-way ANOVA with Tukey post hoc test).

Thus, TTCC or TRP inhibitors did not relax HUA and the relaxation induced by the inhibition of LTCC has been bigger when arteries were contracted by depolarisation or by histamine than by 5-HT receptor activation.

3.2. Effect of Cyclic Nucleotides in Contracted HUA

The effect of cAMP increase on HUA contracted arteries has been analysed by using forskolin (adenylate cyclase stimulator) and rolipram (PDE4 inhibitor). The conjoint application of rolipram (1 μM) and forskolin (10 μM) have relaxed the contractions induced by KCl on 41.0% (Figure 2). In contrast, the effect of these two drugs applied together on 5-HT contracted arteries was only 10% (Figure 2). These drugs almost fully relaxed the HUA contracted by histamine (10 μM). Thus, the increase of cAMP have induced almost a full relaxation on histamine contracted HUA, 41% when arteries are contracted by depolarisation and a very small effect on 5-HT contracted arteries.

The effect of PKA inhibition on these relaxations has been analysed by using KTa (1 μM). The PKA inhibition induced a significant reduction on the forskolin plus rolipram effect in KCl and histamine contracted arteries ($P < 0.05$, Figure 2), but was not efficient reducing this effect in 5-HT contracted arteries ($P > 0.05$; Figure 2). Besides, even in KCl and histamine contractions, the PKA inhibition did reduce in a tiny way the relaxation induced by the cAMP increase, suggesting that cAMP relaxation is partially independent of PKA and indicating the existence of another pathway linked to cAMP.

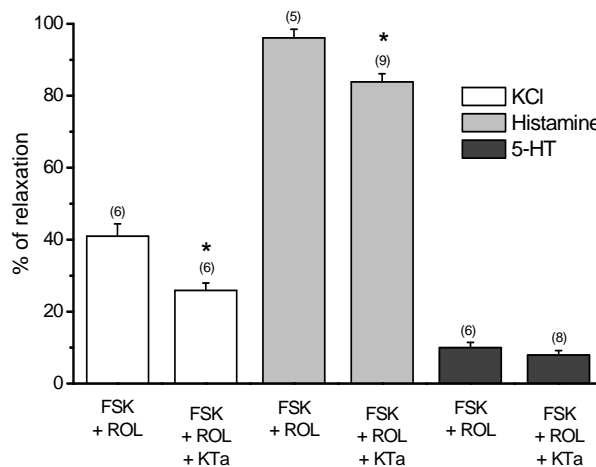


Figure 2. Relaxant effect of cAMP increase on HUA contracted arteries. Relaxation of HUA induced by the combination of forskolin (FSK; 10 μM) and rolipram (ROL; 1 μM) on contractions elicited by KCl (60 mM), histamine (10 μM) and 5-HT (1 μM). The effect of KTa (1 μM) on this relaxation is also shown. The bars represent the means and the lines the S.E.M. of the numbers of experiments indicated near the bars. Significant differences versus FSK+ROL effect are shown (* $P < 0.05$, Student-t test).

The effect of cGMP on HUA contracted arteries has been also analysed by using SNP (guanylate cyclase stimulator) and dipyridamol (PDE5 inhibitor). The bigger relaxant effect of conjoint application of dipyridamol and SNP has been obtained in HUA contracted by histamine (90.8%), being the effect on 5-HT- and KCl- contracted HUA considerably lower, 33.0% and 30.8% respectively (**Figure 3**). Thus, the increase of cGMP has induced almost a full relaxation (90.8%) on histamine contracted HUA, and around 30% of relaxation on arteries contracted by KCl or 5-HT.

The effect of PKG inhibition on these relaxations has been analysed by using KTg (1 μ M). The PKG inhibition have induced a significant reduction on the SNP plus dipyridamol effect on histamine and 5-HT contracted arteries ($P < 0.05$; **Figure 3**), but the reduction induced in KCl contracted arteries was not significant ($P > 0.05$; **Figure 3**). As for PKA, the PKG inhibition did not completely reduce the relaxation induced by the cGMP increase, also suggesting the contribution of another pathway distinct than the PKG activation.

3.3. Effect of Phosphatase 2A Inhibition on Contracted HUA

HUA rings were contracted by KCl (60 mM), histamine and 5-HT, and the effect of OA (phosphatase 2A inhibitor; 5 nM) on these contractions was analysed.

Okadaic acid (5 nM) have relaxed the contracted HUA and this effect was bigger on contractions induced by

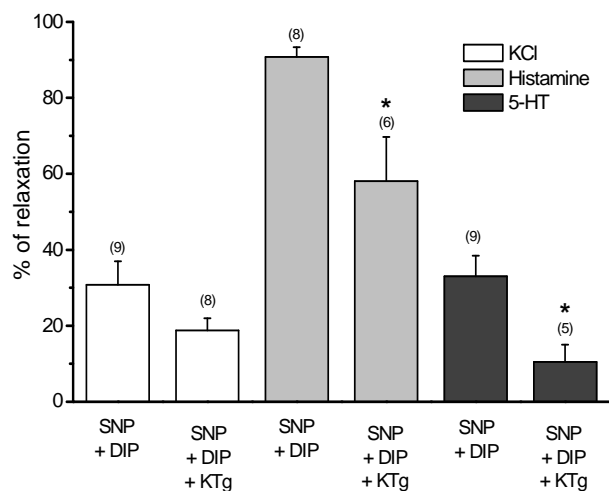


Figure 3. Relaxant effect of cGMP increase on HUA contracted arteries. Relaxation of HUA induced by the combination of SNP (10 μ M; guanylate cyclase activator) and dipyridamol (DIP; 3 μ M) on contractions elicited by KCl (60 mM), histamine (10 μ M) and 5-HT (1 μ M). The effect of KTg (1 μ M) on this relaxation is also shown. The bars represent the means and the lines the S.E.M. of the numbers of experiments indicated near the bars. Significant differences versus SNP+DIP effect are shown (* $P < 0.05$, Student-t test).

KCl than when contraction were induced by histamine or 5-HT ($P < 0.05$; **Figure 4(a)**). Thus OA relaxes better the contractions induced by depolarisation, when LTCC are activated.

In KCl contracted arteries, the OA relaxation was similar than the induced by nifedipine. When these two drugs were applied together, the relaxant effect was not bigger than the relaxation induced by nifedipine or OA applied alone ($P > 0.05$; **Figure 4(b)**). However, the presence of forskolin and rolipram, drugs which increase the cAMP levels, significantly enlarged the OA effect ($P < 0.05$; **Figure 4(b)**). Also, the presence of SNP and dipyridamol, drugs increasing the cGMP levels, significantly amplified the OA effect ($P < 0.05$; **Figure 4(b)**). Thus, the relaxant effect of LTCC inhibition and phosphatase 2A inhibition are not additive or synergic. However, the cyclic nucleotides have augmented the relaxation induced by PP2A inhibition.

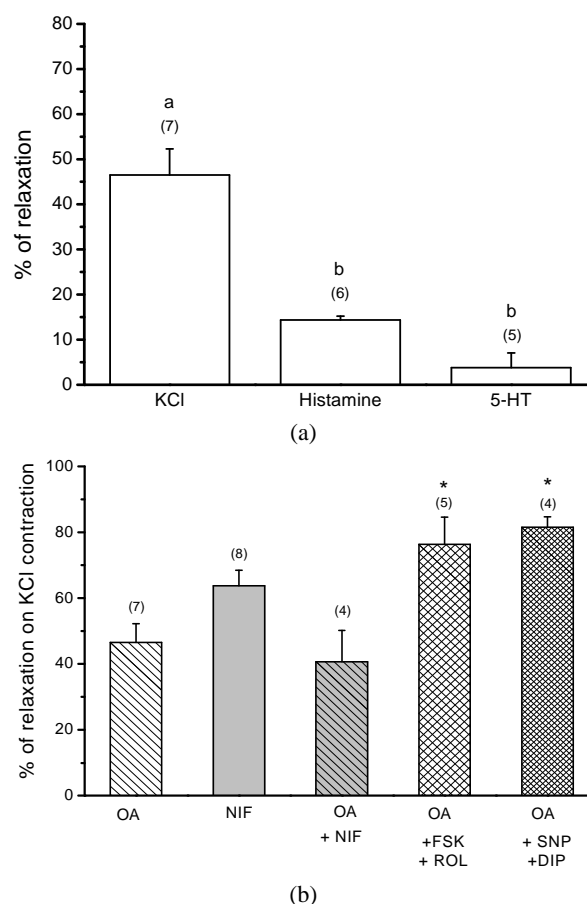


Figure 4. Relaxant effect of PP2A inhibition in HUA contracted arteries. a) Relaxation induced by OA (5 nM) on HUA contractions elicited by KCl (60 mM), histamine (10 μ M) and 5-HT (1 μ M); b) Effect of nifedipine (NIF; 10 μ M), forskolin (FSK; 10 μ M) plus rolipram (ROL; 1 μ M) and SNP (10 μ M) plus dipyridamol (DIP; 3 μ M) in the OA relaxation. Statistical significant differences versus the OA effect are indicated (* $P < 0.05$, one-way ANOVA with Dunnet's post hoc test).

3.4. Effect of Rock Inhibition in HUA Contractility

The role of different kinases in HUA contractility has been analysed by using the following inhibitors: Y27 (ROCK inhibitor; 10 μ M); PD (ERK1/2 inhibitor; 50 μ M); and SB (p38MAPK inhibitor; 25 μ M).

Firstly, the effect of these inhibitors on HUA contractility was analysed in Ca^{2+} containing medium. In these conditions, ROCK inhibition did not induce significant relaxation ($P > 0.05$) of HUA contracted by KCl ($0.3 \pm 0.2\%$, $n=8$), by histamine ($0.1 \pm 0.1\%$; $n=5$) or by 5-HT ($0.8 \pm 0.8\%$; $n=6$). Neither, in the same conditions, ERK1/2 inhibition did not induce significant relaxation ($P > 0.05$) of HUA contracted by KCl ($3.0 \pm 1.5\%$; $n=9$), by histamine ($1.7 \pm 1.7\%$; $n=5$) or by 5-HT ($1.1 \pm 1.1\%$; $n=4$). Also, in presence of extracellular Ca^{2+} , p38MAPK inhibition did not induce significant relaxation ($P > 0.05$) of HUA contracted by KCl ($1.2 \pm 1.0\%$; $n=8$), by histamine ($1.1 \pm 0.7\%$; $n=7$) or by 5-HT ($1.6 \pm 1.0\%$; $n=5$). Thus, ROCK, ERK1/2 or p38MAPK inhibition did not affect the contraction induced either by KCl, histamine or 5-HT in presence of extracellular Ca^{2+} .

The effect of these inhibitors has been also analysed in absence of extracellular Ca^{2+} (0Ca medium). In these conditions KCl has induced sustained contractions (1013.6 ± 79.2 mg; $n=18$) that were significantly lower than the induced in presence of Ca^{2+} (1859.8 ± 70.9 mg; $n=95$) ($P < 0.05$; Student-t test). The ERK1/2 or p38 MAPK inhibition did not significantly influence the contractions induced by KCl in absence of Ca^{2+} (**Figure 6(a)**). Besides, the inhibition of PP2A or LTCC did not affect the contractions induced by KCl in absence of extracellular Ca^{2+} (**Figure 6(a)**). However, ROCK inhibition by Y27 relaxed on 81.6% the contractions induced by KCl in Ca^{2+} free medium (**Figure 6(a)**). **Figure 5(a)** shows a record of an experiment in which KCl (60 mM) induces contraction of HUA in Ca^{2+} -free medium and Y27 relaxes this contraction.

On the other hand, in Ca^{2+} free medium histamine and 5-HT have elicited two step contractions characterized by a rapid phasic component, 2-3 min after the receptor stimulation, followed by a decrease of the contraction until a tonic component, 15-20 min after. The ERK1/2 or p38MAPK inhibition did not affect the phasic or tonic components of the contraction elicited by histamine or 5-HT ($P > 0.05$; **Figures 6(b)** and **6(c)**). However, ROCK inhibition significantly reduced the phasic and tonic components of the contraction elicited either by histamine or 5-HT ($P < 0.05$; **Figures 6(b)** and **6(c)**). **Figure 5(b)** shows a record of an experiment in which, in Ca^{2+} free medium, 5-HT elicited two step contractions characterized by a phasic and tonic components which were reduced after ROCK inhibition.

Thus, ROCK inhibition decreases the tension induced either by KCl, histamine or 5-HT in Ca^{2+} free medium.

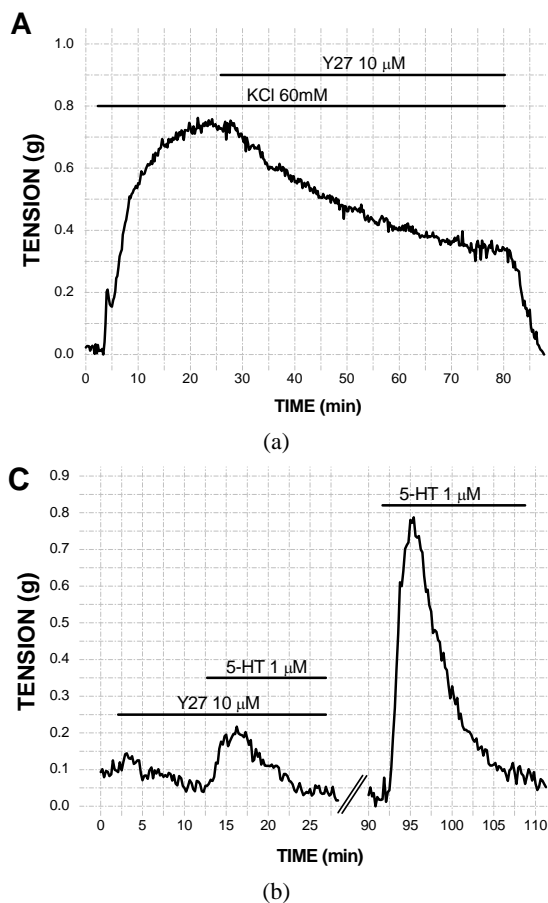


Figure 5. Original records of two tension experiments with HUA rings in Ca^{2+} free medium. a) Ca^{2+} -free medium contraction of HUA induced by KCl (60 mM) which is relaxed by Y27 (10 μ M); b) Ca^{2+} -free medium contraction of HUA induced by 5-HT (1 μ M) in presence and in absence of Y27 (10 μ M).

4. DISCUSSION

The present study investigated the involvement of some mechanism, such as different types of Ca^{2+} channels, cyclic nucleotides and different kinases, in the regulation of the HUA contractility. The elucidation of the precise mechanism regulating the contractility of this artery could be very important to detect potential therapeutic targets to treat HUA disorders such as preeclampsia.

We firstly investigated the relevancy of different types of Ca^{2+} channels on the regulation of HUA tonus. Our results show that the inhibition of LTCC relaxes contracted HUA, even if this effect does not reach 100% of relaxation. Other authors have previously reported that, among different Ca^{2+} channel blockers, nifedipine is the most potent umbilical vasodilator [18]. At the vascular level, it has been shown that nifedipine does not fully relax contractions induced by KCl. The existence of intracellular Ca^{2+} release when the arteries are contracted

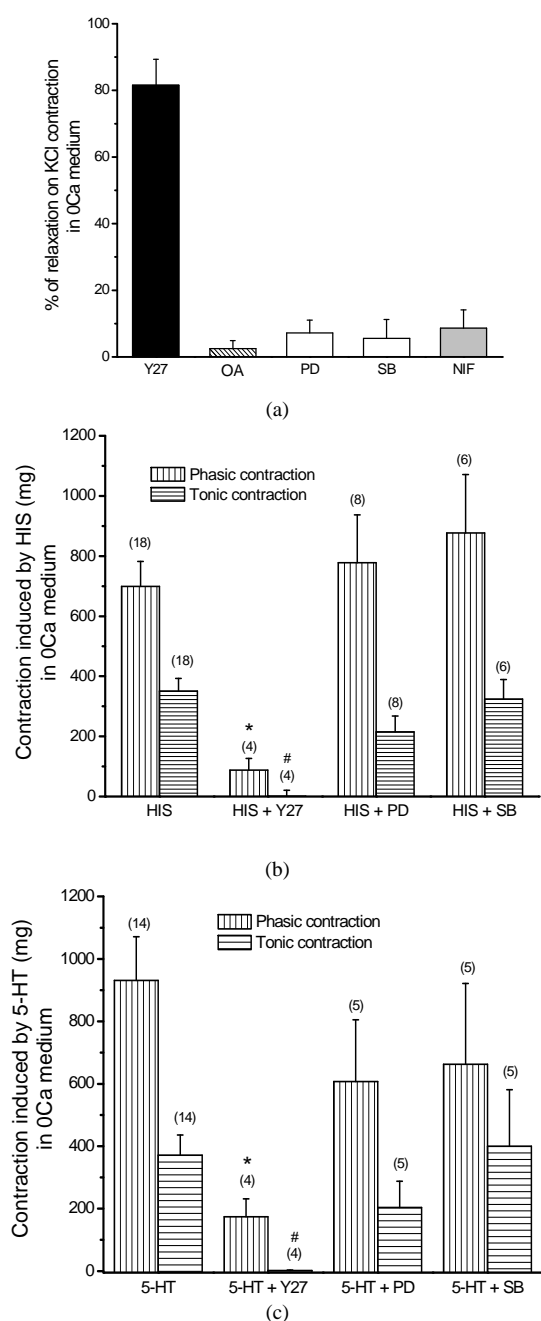


Figure 6. Relaxant effect of different inhibitors of kinases on HUA contracted in Ca^{2+} -free medium. a) Effect of Y27 (10 μM), OA (5 nM), PD (50 μM), SB (25 μM) and nifedipine (NIF; 10 μM) on KCl induced contraction in Ca^{2+} -free medium; b) Effect of Y27 (10 μM), PD (50 μM) and SB (25 μM) on the phasic and tonic contraction induced by histamine (10 μM) in Ca^{2+} -free medium; c) Effect of Y27 (10 μM), PD (50 μM) and SB (25 μM) on the phasic and tonic contraction induced by 5-HT (1 μM) in Ca^{2+} -free medium. The bars represent the means and the lines the S.E.M. of the numbers of experiments indicated near the bars. Statistical significant differences versus the effect in absence of inhibitor are indicated (* $P < 0.05$, one-way ANOVA with Dunnet's post hoc test).

by KCl has been suggested as responsible of the lack of a full relaxant effect of LTCC inhibitors [7]. In this sense, and as we will discuss later, the ability of KCl to induce HUA contractions in Ca^{2+} -free medium could be due to stimulation of intracellular Ca^{2+} release. The relaxant effect of nifedipine was stronger when HUA were contracted by depolarization or by histamine than in arteries contracted by 5-HT. Mikkelsen *et al.* also have observed that, in human pulmonary arteries, the contraction induced by 5-HT is more resistant to nifedipine than the contraction mediated by depolarization [8]. On the other hand, several studies demonstrated that TTCC and TRP channels are involved in VSM contraction in different arteries [5,9,10,47]. However, our results show the absence of effect of mibefradil and APB, suggesting that Ca^{2+} entry through TTCC and TRP channels does not contribute to HUA contraction.

The precise regulation of the intracellular levels of cAMP and cGMP plays an important role in many physiological processes, including vascular smooth muscle contractility [32]. However, the mechanisms by which increases in cAMP and cGMP concentration lead to artery relaxation are still unclear. Some authors have reported that forskolin, a direct stimulator of adenylate cyclase, induced relaxation of different human vessels such as dorsal artery [34], pulmonary artery [35], placental vessels [36] and umbilical artery [37]. On the other hand, among the four families of PDE expressed in this smooth muscle (PDE1, PDE3, PDE4 and PDE5), PDE4 has been shown as the key enzyme involved in the regulation of HUA relaxation associated to cAMP [37]. In this sense, we used forskolin (adenylate cyclase stimulator) and rolipram (PDE4 inhibitor) as drugs that can elicit the maximal increase in cAMP levels in HUA smooth muscle cells. The conjoint application of both drugs elicited different degree of relaxation depending on the contractile agent used. When applied together, these drugs almost fully relaxed histamine contracted HUA, but only 41% and 10% of relaxation was obtained on HUA contracted by KCl and 5-HT, respectively. We have previously shown that 5-HT_{2A}, 5-HT_{1B/1D} and 5-HT₇ receptors are present in HUA smooth muscle cells. The HUA contraction induced by 5-HT are mainly mediated by the activation of 5-HT_{2A}, which activation increases IP₃ levels, and 5-HT_{1B/1D} receptors, which activation inhibits adenylate cyclase [48]. Concerning the histamine receptors, H₁ receptor activation induces contraction and H₂ and H₃ receptors activation mediates HUA relaxation through the increase of cAMP intracellular levels [48]. According with these previous findings, the activation of different 5-HT receptors in HUA inhibits adenylate cyclase and the activation of different histamine receptors induces contraction, but also induces a small increase of cAMP levels due to H₂ and H₃. Thus, histamine contractions are more susceptible to activators

of adenylate cyclase or to PDE4 inhibitors than 5-HT contractions. On the other hand, apparently KCl does not affect adenylate cyclase activity and the effect of forskolin and rolipram has been lower than in histamine-contracted arteries and bigger than HUA contracted by 5-HT.

Is well known that NO activates soluble guanylate cyclase increasing cGMP levels, which induces vasodilatation [38], including HUA vasodilatation [37,39]. On the other hand, PDE5 has been shown as being the key enzyme involved in the regulation of HUA relaxation associated to cGMP [37]. In this sense, we used SNP (guanylate cyclase stimulator) and rolipram (PDE5 inhibitor) as drugs that can elicit the maximal increase in cGMP levels in HUA smooth muscle cells. As for cAMP, the conjoint application of these drugs has elicited different degree of relaxation depending on the contractile agent used. The biggest relaxant effect was observed in contraction induced by histamine, followed by contractions produced by 5-HT and by KCl depolarization. Numerous authors have described that cGMP induced vasodilatation is mediated by activation of potassium channels in different arteries [40,41], including HUA [39]. The regulation of voltage-dependent potassium channels (K_v) by KCl and 5-HT could be responsible of these differences. It has been described that vascular contraction induced by KCl is mainly due to the influx of extracellular Ca^{2+} via voltage-dependent Ca^{2+} channels [1], but K_v channel inactivation at high potassium concentrations (60 mM) was also demonstrated in some blood vessels [49]. Recently, using patch clamp techniques, some authors have demonstrated that 5-HT decreases K_v channel activity in cells of rat pulmonary [50] and mesenteric [51] arteries. Thus, as the cGMP relaxant effect seems to be mediated by potassium channels activation, this effect is lower when HUA are contracted by KCl and 5-HT because these agents inhibit these channels.

Our results have shown that the PKA inhibition induced a significant reduction on the forskolin plus rolipram effect in KCl and histamine contracted arteries, even if this effect is small. The relaxant effect of cAMP increase in 5-HT contracted arteries was very low (around 10%) and KTa have failed to significantly decrease the relaxations induced by forskolin plus rolipram. Also, the PKG inhibition induced a significant reduction on the SNP plus dipyridamol effect in histamine and 5-HT contracted arteries. The analysis of these results suggests that both PKA and PKG are involved in HUA relaxation mediated by cAMP and cGMP respectively. However, the contribution of these kinases is very small and in some cases was not significant. These results suggest that the relaxation induced by cyclic nucleotides is not totally mediated by the activation of their respective kinases and other mechanisms can be involved as described by other authors [44,45]

As we mentioned before, LTCC are critically important for regulating HUA contraction. It has been described that LTCC can be dephosphorylated by PP2A, and inhibition of PP2A was found to result in changes in functional properties of the LTCC [21]. However, there is small number of studies on this matter in smooth muscle and the direction of this modulation has remained somewhat controversial. Some authors have shown that in tracheal smooth muscle PP2A does not modulate LTCC [19]. Also, it has been shown a dual effect of phosphatase inhibitors on Ca^{2+} channels from intestinal smooth muscle cells [20]. At the vascular level, Groschner *et al.* have demonstrated that, in human umbilical vein, when PP2A is inhibited by OA there is increase of LTCC activity [21]. Our results show that PP2A inhibition produces relaxation of HUA, namely when this artery is contracted by KCl and histamine. This relaxant effect is bigger in arteries contracted by KCl. Our results also show that in KCl contracted arteries, the OA effect is similar to the nifedipine effect. When applied together, the relaxant effect is not bigger than the relaxation induced by nifedipine or OA applied alone. These results suggest that PP2A could activate LTCC in HUA. On the other hand, the increase of cAMP or cGMP induced by the conjoint application of cyclase activators and PDE inhibitors significantly increased the OA effect. These results suggest that the relaxation induced by PP2A inhibition is independent of the cyclic nucleotide pathway.

We also have analyzed the effect of some kinases on HUA contractility. Our results show that ROCK, ERK1/2 or p38MAPK inhibition does not affect the contraction induced by KCl, histamine or 5-HT in Ca^{2+} -containing extracellular solution. Other authors have obtained significant relaxation of HUA contracted by 5-HT after ROCK inhibition, but using higher concentrations of Y27 (100 μ M) which can inhibit also myosin light chain kinase directly [22]. Also, Tasaki *et al.* observed that SB significantly inhibits 5-HT-induced contractions in rat aorta [30]. Some authors indicated that 5-HT induces contractions of rat aortic smooth muscle by activating the MAPK pathway [29]. Also, activation of p38 MAPK [30] and Erk MAPK [31] by 5-HT have been shown in rat aorta.

Our results suggest that in the contractions induced by KCl, histamine and 5-HT in presence of Ca^{2+} there is not involvement of pathways concerning ROCK, ERK1/2 or p38MAPK. Sakurada *et al.* have shown the existence of a Ca^{2+} -dependent Rho stimulation mechanism in VSM from rabbit aorta which is activated by excitatory receptor agonists [23]. However, in HUA our results exclude this possibility, because in presence of extracellular Ca^{2+} ROCK does not seem to be activated by KCl, histamine or 5-HT.

On the other hand, in absence of extracellular Ca^{2+} , histamine and 5-HT elicited contractions of HUA characterized by two components, a rapid phasic contractile

component, 2-3 min after stimulation by the agonist, followed by a decrease of the contraction until a tonic component, 15-20 min after. The initial transient component has been associated with Ca^{2+} release from the sarcoplasmic reticulum, whereas the tonic component seems to be dependent on the increase on extracellular Ca^{2+} . Both contractile responses are dependent on the Ca^{2+} sensitization and two main pathways have been implicated in this phenomenon: the inhibition of myosin light chain phosphatase (MLCP) by ROCK; and the phosphorylation of the thin filament proteins by p38MAPK and ERK1/2 [5,52]. In contrast, KCl elicited sustained contractions of HUA in absence of extracellular Ca^{2+} . This effect could be induced by a progressive Ca^{2+} release from intracellular Ca^{2+} stores and/or an increase of Ca^{2+} sensitization. These data agree with the obtained by Tufan *et al.* in HUA, which have suggested that Ca^{2+} independent isozymes of protein kinase C may also be involved in the contractions produced by KCl in absence of extracellular Ca^{2+} [1]. Some authors have also suggested that depolarization by KCl induces intracellular Ca^{2+} release in human arteries [7]. Besides, it has been described that KCl depolarization increases Ca^{2+} sensitization by ROCK activation in smooth muscle cells from rabbit arteries [24]. As expected, in absence of extracellular Ca^{2+} , nifedipine did not relax the contractions elicited by KCl. Also in these conditions, OA did not relax the contractions elicited by KCl. Once more, these data suggest a functional relationship between the PP2A and LTCC in the regulation of HUA contractility.

Concerning the kinases, the ERK1/2 and p38MAPK inhibitors did not reduced or relax the contractions induced by KCl, histamine or 5-HT in absence of extracellular Ca^{2+} . Thus, these results demonstrate that KCl-induced contractions are not linked to the activation of ERK1/2 and p38MAPK. However, in Ca^{2+} -free medium, the ROCK inhibition significantly relaxed the contractions induced by KCl and reduced the phasic and tonic components of the contraction elicited either by histamine or 5-HT. Similar results were obtained by Ark *et al.* when HUA were contracted by 5-HT in Ca^{2+} free medium [22]. Our data demonstrate that ROCK is capable of mediating the contractile response after stimulation of a receptor agonist or by depolarization. Thus, in absence of Ca^{2+} , the sustained contractions elicited by KCl and the phasic and tonic components of the contraction induced by histamine or 5-HT depend on ROCK activation. Consequently, these contractions depend of Ca^{2+} sensitization by the inhibition of MLCP by ROCK. Our data show that the relevancy of the Ca^{2+} dependent or independent mechanism in HUA varies in function of the extracellular Ca^{2+} level, and further experiments are necessary to deeply study this dependence. On the other hand, our results suggest that HUA is a good sample to study the Ca^{2+} sensitization mechanism induced by ROCK.

In conclusion, our results demonstrate that the LTCC are the main way for Ca^{2+} entry and is involved in HUA contractions induced by depolarization or by agonists. A positive regulation of LTCC by PP2A seems to occur in HUA smooth muscle cells. Cyclic nucleotides are involved in HUA vasodilatation. The relaxant effect of cyclic nucleotides is partially due to the activation of their respective kinases, but other pathways are also involved. HUA is able to contract independently of Ca^{2+} influx by activating the ROCK pathway and/or due to intracellular Ca^{2+} release.

5. ACKNOWLEDGEMENTS

We thank the donor mothers and the Gynaecology-Obstetrics Department staff of "Centro Hospitalar da Cova da Beira" (Covilhã, Portugal) for their disinterested collaboration and the "Fundação para a Ciência e a Tecnologia" (Portugal) for supporting the grant SFRH/BDE/15532/2004.

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Social support and coping as moderators of perceived disability and posttraumatic stress levels among Vietnam theater veterans

Erin Martz*, Todd Bodner, Hanoch Livneh

Portland State University, Portland, USA; martzerin@gmail.com

Received 3 December 2009; revised 11 January 2010; accepted 15 January 2010.

ABSTRACT

The dual purpose of this study is to investigate whether disability predicts posttraumatic stress levels among Vietnam theater veterans, and whether coping and/or social support moderates the impact of disability on PTSD levels, after controlling for demographic, pre-military, military, and post-military factors. This research analyzed data from the U.S.'s National Vietnam Veterans Readjustment Study (NVVRS), which was a nationally representative, stratified, random sample of 3,016 Vietnam veterans. The results indicated that disability, emotional support, instrumental support, and wishful – thinking coping significantly predicted PTSD, when controlling for demographic, pre-military, military, and post-military factors. Further, interactions indicated that both emotional social support and problem-solving coping significantly decreased the impact of disability on PTSD levels. Implications of this research are briefly discussed.

Keywords: Disability; Social Support; PTSD; Vietnam Veterans

1. INTRODUCTION

An extensive amount of published research has examined psychiatric disorders, which may include posttraumatic stress disorder (PTSD), as sequelae of involvement in wars [1-4]. While some research has focused on the physical health consequences of exposure to extreme stress [5,6], limited research has been conducted on the psychological reactions occurring after the onset of an injury or permanent disability in the context of war [7,8]. Moreover, there is a paucity of research on PTSD related to disability that occurred specifically in a war-zone. There has been increasing scientific interest on coping with a disability [9]; yet, few studies have been conducted

that examined coping with a war-related disability. To date, research has not yet examined the possible moderating effect of coping or social support on disability and PTSD levels.

The need to conduct such research has been stated by Kulka and colleagues, who concluded that “Vietnam theater veterans with service-connected physical disabilities are at elevated risk for a variety of readjustment problems” [1], and more recently, by a 2006 working group on deployment-related adjustment and mental disorders [10]. The dual purpose of this study is to investigate whether disability predicts PTSD levels among Vietnam theater veterans, and whether there are possible moderators of this association, such as coping or social support, after controlling for select demographic, pre-military, military, and post-military related variables. The following sections will review the extant literature on: 1) PTSD and disability; 2) coping and PTSD; and 3) the significant findings of research that examined PTSD in analyses of the NVVRS dataset.

2. PTSD AND DISABILITY

When evidence of a traumatic event remains present in an individual's life, such as in the form of an injury or disability, it may serve as a visual or proprioceptive cue to the trauma [11,12]. Thus, injury or disability may act as a continuous reminder that can trigger anxiety for certain individuals. Research has indicated an association between PTSD and the existence of injuries, medical conditions, or disabilities [13-18], and that individuals may be susceptible to PTSD after an injury or the onset of a disability [11,14,16,17,19-23].

In research conducted among veterans, Helzer, Robins, and McEvoy [24] examined PTSD rates in a stratified sample among 2,493 individuals, 64 of whom were Vietnam veterans and 43 had experienced combat. Those who experienced combat but were not wounded had a 4% rate of PTSD, compared to 20% rate among those who were wounded. In a comparative study, Buydens-

Branchey, Noumair, and Branchey [25] found that veterans with a combat injury had significantly higher PTSD levels compared to veterans without injuries.

Martz and Cook [26] found the following rates of PTSD among 45,320 veterans: burns 13.4%, spinal cord injuries 11.6%, amputations 8.1%, major chest trauma 7.6%, heart failure/shock 7.3%, and cardiac arrest 5.1%. They found that burns, spinal cord injuries, amputations, and heart failure/shock were significant risk factors for PTSD. Delimar and Sivik [27] assessed for PTSD in three groups of soldiers ($N = 30$ per group), all of whom had at least three months of combat experience in the Croatian war of 1991-1993, and found a PTSD rate of 33.3% among soldiers with permanent disabilities (e.g., amputation). Among 312 veterans and civilians with spinal cord injuries, Martz [28] found that total PTSD levels were significantly predicted by spiritual/religious coping, pain level, severity of SCI, and number of traumatic events.

The topic of PTSD reactions after the presence of a disability among veterans is important for several reasons. Kaplan, Huguet, McFarland, and Newsom [29] found that male veterans who had activity limitations (*i.e.*, a disability) were significantly more likely to commit suicide than non-veterans without disabilities. Further, the topic of war-related physical disability as a potential trigger for PTSD reactions is imperative to study, in view of the concept of “double PTSD” [13], which in the present context can mean that the trauma of disability interacts with the trauma of war. Disability may also be a “crossover” trauma, which Terr [30] described as a one-time event with long-term, continuous consequences that may elicit a complex set of traumatic reactions.

Kulka *et al.* [1] analyzed data from the U.S.’s National Vietnam Veterans Readjustment Study (NVVRS), which was an epidemiological study of a nationally representative sample of Vietnam veterans. One part of their extensive analysis examined issues related to Vietnam veterans’ physical health problems in relation to PTSD versus no PTSD (see Exhibit VIII-2, contrast C, in [1]). The results indicated that for both males and females, individuals with PTSD reported a significantly lower level of positive perceptions of current health status and a significantly higher number of chronic health problems than those without PTSD. In a different section, Kulka *et al.* [1] reported that the group of Vietnam veterans with a service-connected physical disability (SCPD) was significantly more likely to have current PTSD (21.4%) than the group of veterans without a SCPD (14.5%) (current PTSD prevalence indicates that the individual met the PTSD criteria within 6 months of the assessment; lifetime PTSD prevalence indicates that the individual met the criteria for PTSD sometime in their life; see Reference [1]). To put the disability and PTSD research in context, the

results of the NVVRS research on war-related PTSD include the following. Kulka *et al.* [1] indicated that 15.2% of male combat veterans and 8.5% of female combat veterans from the Vietnam war-theater met the criteria for current PTSD prevalence. In contrast to the NVVRS prevalence data, the current PTSD prevalence found among Gulf War veterans were found to be 4% of men and 9% of women at first assessment, increasing to 11% of men and 21% of women at second assessment 2 years later [31]. The aforementioned current PTSD prevalence rates and the two findings related to health factors, disability, and PTSD suggest that the traumatic experience of incurring a disability is an issue that deserves more careful investigation and greater clarification of the associations, such as examining the possible predictors of PTSD levels among veterans and the possible moderators of these associations.

The present research is distinct from three studies that were published using the NVVRS database. First, in contrast to Zatzick and colleagues’ [6] research that examined PTSD and health outcomes and functioning, the present study incorporates a measure indicating an existence of a disability, in addition to also examining moderators of PTSD. Second, whereas Suvak, Vogt, Savarese, King, and King [32] researched coping as a predictor of adjustment (moderated by war-zone stress) without examining disability issues, the present study posits coping as a moderator between disability and PTSD. Third, while Martz, Bodner, and Livneh [33] examined coping as a moderator of disability and adaptation, the current study will investigate a different outcome—that of PTSD—and whether both coping and social support are moderators of disability and PTSD.

3. COPING AND PTSD

Regarding PTSD and coping research, a variety of associations have been found among various types of coping and PTSD [34-37]. Koenen, Stellman, and Stellman [38], in a follow-up study of 1,377 American legionnaires who served in Southeast Asia, concluded from their findings that perceived social support is a significant predictor of recovery from PTSD. One study examined coping and PTSD in the context of disability: Lawrence and Fauerbach [39] found that both avoidant and active coping were positively and significantly associated with PTSD at hospitalization and at 1-month follow-up among 158 individuals with burn injuries.

Using the NVVRS dataset, Suvak *et al.* [32] examined whether war-zone coping strategies predicted life adjustment, as measured by achievement, life satisfaction, and lifetime adaptation, and as moderated by combat exposure. Their study is mentioned because of its use of coping strategies, which were grouped into 3 factors called problem-focused coping (PFC), emo-

tion-focused wishful thinking (EFCWT), and emotion-focused blunting and venting (EFCBV). One finding that is relevant to the present study is that Suvak and colleagues noted that in the final step of their regression analysis, non-linear (*i.e.*, quadratic) associations were found among PFC and achievement, PFC and lifetime adaptation, and EFCWT and achievement, each varying as a function of combat exposure. This research indicated that the use of emotional coping strategies during combat predicted lowered life-adjustment after the war experience, which Suvak and colleagues interpreted the non-linear associations as suggesting that certain coping strategies are no longer useful in increasingly stressful combat situations.

The research on coping with a disability has been expanding at a rapid rate [40-44], (for a summary, see [9]). The role of coping strategies following traumatic brain injury (TBI) was investigated by Moore and Stambrook [43,44]. In their earlier study of 53 male survivors of TBI, individuals characterized by having 1) higher use of positive reappraisal and self-controlling coping strategies (as measured by the Ways of Coping-Revised Questionnaire; WOC-R), and 2) lower external locus of control, reported significantly lower mood disturbance and lower levels of depression. In the authors' latter study of 175 survivors of TBI, they reported that coping strategies that included denial, escape, and resignation were linked to poorer quality-of-life outcomes. The authors also suggested that positive reappraisal appeared to be associated with better psychosocial outcomes.

4. COPING A SOCIAL SUPPORT

Social support, which can be defined as "the perception of the value of social interactions" [45], should be distinguished from the concept of a "social network," which refer to the quantity of relationships that a person has. Lazarus and Folkman [45] further elaborated on the definition of social support as "a resource, available in the social environment, but which the person must cultivate and use" [45]. They also noted that while social support is typically deemed as a positive resource and a buffer to stress, it also may have negative effects on people (e.g., it may create problems or stress, provide misleading information, or create dependency issues).

Thoits [46] suggested that social support could be viewed as a form of coping assistance or support strategy. Livneh and Martz [9] noted that social support can be viewed as an "extra-individual" influence on coping processes, like other environmental factors. Hence, social support can be distinguished from a form of emotional coping called "seeking social support" by the former representing social support that is received and experi-

enced, versus the latter as actions taken to obtain social support.

5. PREDICTORS OF PTSD USING THE NVVRS DATA

The following NVVRS research indicated variables that should be controlled for in the present study. Fontana and Rosenheck's [47] research examined predictors of PTSD by using structural equation modeling (SEM) among a male sub-sample from the NVVRS data. One demographic predictor (ethnicity), 2 military-related variables (exposure to combat and participation in abusive violence), and 2 post-military traumas (rejection by society at homecoming, and lack of support by family and friends) directly predicted PTSD. In a later study using NVVRS data, Fontana and Rosenheck [48] focused only on war-zone stressors among male veterans. Their SEM analysis indicated that only 2 variables had direct, significant effects on PTSD: insufficiency of resources in the environment and killing of others.

King and colleagues published 3 different analyses using the NVVRS dataset. Using SEM, King, King, Gudanowski, and Vreven's [49] research, which focused only on war-zone stressors, indicated that the following war-zone stressors significantly predicted PTSD: perceived threat, malevolent environment, and atrocities/abusive violence. King, King, Foy, and Gudanowski [50] examination of pre-war factors and war-zone stressors found that for male and female theater veterans, prior trauma history, age (for men only), atrocities/abusive violence, malevolent environment, and perceived threat had significant direct effects on PTSD. King, King, Foy, Keane, and Fairbank's [51] NVVRS research indicated that the following predicted PTSD: early trauma history (women and men) and age at entry into Vietnam (men), atrocities-abusive violence and perceived threat (for both men and women) and malevolent environment (for men), additional stressful life events, hardness, and functional social support (for men and women) and structural social support (for men).

6. RESEARCH QUESTION AND HYPOTHESES

The purpose of this research is two-fold: to investigate, after controlling for demographic, pre-military, military, and post-military factors, whether 1) disability predicts PTSD levels in Vietnam theater veterans, and 2) coping and/or social support moderates the impact of disability on PTSD levels. Based on the literature about coping, it is hypothesized that emotion-focused coping is positively related to PTSD levels, while social support, social coping, and problem-solving coping are inversely related to PTSD levels.

7. METHOD

7.1. Participants

The data were obtained from the National Vietnam Veterans Readjustment Study (NVVRS), a nationally representative, stratified, random sample of 3,016 Vietnam veterans drawn from 8.2 million veterans who were on active duty during the Vietnam war and who had left U.S. military service by September, 1987 [52]. The data were collected by means of extended interviews and self-report questionnaires between 1986 and 1988 [1,2]. Several groups were intentionally over-sampled in this study: African Americans, Hispanics, women, and veterans with service-connected disabilities [49]. Refer to Kulka *et al.* [1,2] for an extensive summary of demographic characteristics of this sample. In the current study, we limited our attention to the 1618 theater veterans, that is, only those who served within the war theater.

For this study, all the NVVRS data were used in factor analyses of the variables. For the examination of the research questions, the data from the Vietnam theater veterans ($n = 1618$) were used, due the large amounts of missing data for the variables considered among other participants (e.g., non-theater veterans, civilians) in the NVVRS dataset.

7.2. Instruments

7.2.1. PTSD

In the NVVRS, a multi-method approach was used to assess PTSD with 3 primary and 7 secondary indicators of PTSD [1]. For this research, the Mississippi Scale for Combat Related Posttraumatic Stress Disorder (M-PTSD) was selected because it has been used extensively for measuring PTSD. The M-PTSD is a 35-item scale by Keane, Caddell, and Taylor [53] that measures posttraumatic stress in military situations with higher scores indicating greater likelihood of PTSD. Weiss *et al.* [52] reported on the predictive validity of the various PTSD scales used in the NVVRS; when comparing the survey data with clinicians' assessments, the M-PTSD achieved a 77.3 sensitivity rate, 82.8 specificity rate, and a Kappa of .53 for diagnosing PTSD.

7.2.2. Coping

In the NVVRS, 25 coping items were included, derived from a large number of coping items from Folkman and Lazarus's [54] Ways of Coping Checklist [see 32 for more details]. The original team of NVVRS researchers selected these items as "most appropriate to coping with the stressors of a war zone" [32]. The responses, after reverse-coding, represented how much the veteran relied on each way of coping (*i.e.*, 1 = not at all to 5 = a great deal). Prior research [33] on these items in the NVVRS dataset has identified three distinguishable coping-strategy dimensions: wishful-thinking coping (4 items;

$\alpha = .78$), social coping (2 items; $\alpha = .67$), and problem-solving coping (4 items; $\alpha = .78$). The social coping factor included items about whether the respondent "depended on others to cheer you up" and "saw someone to help feel better." Hence, social coping reflects strategies that individuals use to handle stress, which in contrast to the social support variable, refer to perceived external support that is provided to individuals.

7.2.3. Instrumental and Emotional Social Support

To measure instrumental and emotional social support, we used the 6-item Instrumental Social Support ($\alpha = .75$) and 13-item Emotional Social Support ($\alpha = .77$) scales, respectively, which King *et al.* [49-51] formulated from the NVVRS items and described in detail in their publications. Higher scale scores indicated greater levels of each type of social support.

7.2.4. Disability

To assess disability, we used participant responses to the following question (item K4b in the NVVRS database): "A military service-related wound handicapped me later." The response format was on a scale ranging from 1 "very true" to 4 "not at all true," which was reverse-scored so that higher scores indicate stronger belief that one had a service-related disability. This disability item was selected from the available NVVRS item pool because: a) it was a continuous, not categorical variable; b) the question made a direct connection between injury incurred in the service and a disabling status at a later time (*i.e.*, a permanent disability, not a temporary injury); and c) it was not a variable that represented financial compensation from the Veterans Administration. Thus, the current research team deemed it the best NVVRS item that represented disability.

7.2.5. Demographic, Pre-military, Military, and Post-military Risk Factors

Pre-military risk factors included gender (female = 1, male = 0), ethnicity (1 = white, 0 = otherwise) [these two variables, as categorical variables, were binary-coded for the multiple regression], the number of traumatic events experienced, and age at entry into Vietnam. Prior research [49-51] measured the following four facets of military-related stress in a war-zone: combat exposure (36 items), atrocities and abusive violence exposure (9 items), perceived threat (9 items), and malevolent environment (18 items). In our own analyses, we determined that many of these scales were multidimensional and that many items had large amounts of missing values. We therefore created scales for these facets based on subsets of the original itempool to improve the unidimensionality of the scales and to minimize the amount of missing data. The resulting scales had the following properties, with higher scores indicating higher levels of each military-related risk factor: combat exposure (12 items; $\alpha = .92$), atrocities and abusive violence (3 items; $\alpha = .76$), perceived threat

(8 items; $\alpha = .84$), and malevolent environment (18 items; $\alpha = .91$). One post-military factor, the level of re-adjustment problems (assessed by the NVVRS variable “number of serious readjustment problems now”), was included in this research. A list of the retained items for the present study is available from the authors on request, whereas a list of the items used by King *et al.* [49-51] can be obtained from those researchers.

7.2.6. Analysis Strategy

Because of the complex sampling methods used to generate the NVVRS data (*i.e.*, stratified random sampling with unequal sampling probabilities within strata), special procedures must be used to estimate population parameter values (e.g., means, standard deviations, correlations, and regression slopes). Therefore, we conducted all analyses using the multilevel add-on package to the M-plus 4.2 software program [55] that contains routines for analyzing data from complex survey designs.

Building the regression model to test the study hypotheses consisted of a two-step process. In the first step, a hierarchical linear regression analysis was conducted where PTSD scores were regressed on predictor variables that were entered in four successive blocks (see Results). In the second step, a predictor selection process was used to prune non-significant predictors of PTSD scores, starting with the fourth block and working backwards. For brevity, interpretations of the individual partial regression slopes are presented only for the final regression model. The statistical significance for the increase in R^2 across blocks is evaluated using χ^2 goodness-of-fit tests. The statistical significance of individual regression slopes is evaluated using Wald tests. To minimize collinearity problems encountered when including interaction terms in multiple regression models, variables involved in interactions were centered around their estimated population mean values prior to the construction of interaction terms and subsequent analysis [56].

A moderation, instead of mediation, model was used in this research, because coping was viewed as influencing the strength and direction of the associations among pre-war, military, and post-military factors, disability, and psychosocial outcomes. A mediation model would have focused on whether these factors *indirectly* influenced psychosocial outcomes through coping. Because coping strategies are more of a fluid, state-like concept [45,60,61] that is modifiable by therapeutic interventions, emphasis was placed in this study on examining coping, and how coping altered the impact of the predictor variables on PTSD outcomes. The presence of significant interactions indicates moderation by coping.

8. RESULTS

Throughout the analysis, $\alpha = .05$ was used to define statistical significance. Of the 1618 participants who served in the Vietnam combat theater, 1443 provided complete responses to all study variables. We conducted tests to investigate mean differences for the studied variables between the 1443 and 175 participants providing complete and incomplete data, respectively. The results of these tests suggest that the two participant groups did not differ significantly on most of the studied variables; even in the case of significant differences, the variance in these variables explained by respondent group was very small (*i.e.*, R^2 s = .008 and .009 for malevolent environment and perceived threat, respectively). Therefore, for modeling convenience, the following analyses were conducted only on those participants who provided complete responses.

8.1. Descriptive Statistics

Table 1 provides the estimated population means and standard deviations of the studied variables along with the estimated population correlations among these vari-

Table 1. Estimated population means and standard deviations for and correlations among study variables.

Variable	M	SD	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. PTSD Score	80.0	22.5	1.00
2. Gender	.002	.04	-.02	1.00
3. Ethnicity	.86	.35	-.15	.02	1.00
4. # of Traumas	3.47	2.89	.39	.01	.03	1.00
5. Age at Entry	22.5	5.05	-.26	.04	.01	-.18	1.00
6. AA Violence	.26	.36	.41	-.02	-.09	.26	-.15	1.00
7. Mal. Env.	2.80	.73	.53	-.01	-.18	.23	-.21	.47	1.00
8. Combat Exp.	2.11	.88	.44	-.04	-.12	.28	-.14	.67	.60	1.00
9. Perc. Threat	2.74	.76	.48	-.01	-.17	.27	-.06	.57	.70	.81	1.00
10. Readj. Probs.	1.46	.88	.56	-.01	-.13	.28	-.12	.18	.27	.23	.23	1.00
11. SR Disability	1.32	.77	.40	-.01	-.11	.21	-.06	.20	.28	.24	.28	.33	1.00
12. Emot. Support	2.07	.46	-.61	.01	.09	-.28	.19	-.24	-.38	-.27	-.29	-.41	-.23	1.00	.	.	.
13. Instr. Support	.94	.17	-.44	.01	.05	-.12	.00	-.14	-.18	-.22	-.19	-.36	-.17	.44	1.00	.	.
14. WT Coping	3.14	.95	.46	-.01	-.15	.15	-.23	.25	.56	.24	.36	.23	.20	-.26	-.11	1.00	.
15. Social Coping	3.71	.87	.27	.03	-.13	.15	-.20	.16	.34	.14	.22	.17	.11	-.15	-.05	.51	1.00
16. PS Coping	3.02	.90	.14	.02	-.07	.13	.24	.24	.25	.32	.34	.12	.09	-.14	-.17	.28	.26

Notes: $N = 1443$; AA = Atrocities/Abusive, Mal. Env.= Malevolent Environment, SR = Service Related, WT = Wishful-Thinking, PS = Problem-Solving. All correlations $> .01$ in absolute values are significant at $.05$

ables. Although the magnitude of the correlations between PTSD scores and the other study variables varied considerably, all were statistically significant.

8.2. Hierarchical Regression Analysis

In order to investigate the two research questions, a hierarchical regression analysis with interaction variables was conducted. The ordering of the regression was based on a temporal conceptualization of how the variables might theoretical occur. The regression analysis addressed the first research question of whether, after controlling for demographic, pre-military, military, and post-military factors, disability predicts PTSD levels among Vietnam theater veterans. The second research question, of whether coping and/or social support moderates the impact of disability on PTSD levels, was examined by the inclusion of interaction variables, which represents moderation, in the regression equation.

The first block of predictor variables consisted of variables prior research had identified as risk factors for PTSD (*i.e.*, gender, ethnicity, number of traumas, age at entry to Vietnam war, combat exposure, atrocities /abusive violence, malevolent environment, and perceived threat during war, readjustment problems after service). For the purpose of this investigation, these predictors served as control variables and were not pruned in the second model-selection step. These predictors account for sizable and significant variance in PTSD scores, $R^2 = .53$, $\chi^2(9) = 479.86$, $p < .001$.

The second block consisted of a single variable: service-related disability. Including this variable led to a significant increase in the variance explained in PTSD scores, $\Delta R^2 = .02$, $\chi^2(1) = 13.65$, $p < .001$, with a $R^2 = .55$ after the second step.

Variables in the third block included two indicators of social support [Emotional Support (ES), Instrumental

Support (IS)] and three coping styles [Wishful-Thinking Coping (WTC), Social Coping (SC), Problem-Solving Coping (PSC)]. These variables were added in block 3 based on the viewpoint that social support and coping may act as a buffer between various stressors (block 1) and the existence of a disability (block 2), and PTSD (the outcome). Including these variables led to a significant increase in the variance explained in PTSD scores, $\Delta R^2 = .11$, $\chi^2(5) = 268.36$, $p < .001$, resulting in a $R^2 = .66$ after the third step.

The fourth and final block of variables tested moderation of coping and social support. This block consisted of interactions between service-related disability (SRD) and the five variables in the third block (*i.e.*, SRD x ES, SRD x IS, SRD x WTC, SRD x SC, SRD x PSC). Including these variables led to a significant increase in the variance explained in PTSD scores, $\Delta R^2 = .02$, $\chi^2(5) = 12.34$, $p = .03$, resulting in a $R^2 = .68$.

8.3. Pruning and Final Regression Model

As a result of the model-pruning process, three predictor-variable interactions in block four (*i.e.*, the interactions between SRD and IS, WTC, and SC, SRD and SC) and one predictor variable in block three (*i.e.*, SC) were dropped from the model. Dropping these four variables did not result in a significant reduction in the variance of PTSD scores explained by the model, $\Delta R^2 = .01$, $\chi^2(4) = 3.49$, $p = .48$. The model $R^2 = .67$ for the final model indicated that the predictor variables explained approximately two-thirds of the variance in PTSD. **Table 2** provides the estimated regression partial regression slopes for the final regression model. For brevity, we do not interpret the slopes for the control variables (*i.e.*, variables from Block 1) except to note that the valences of these slopes are as expected based on past research.

Table 2. Final multiple regression model predicting PTSD scores.

Predictor Variable	<i>b</i>	<i>SE</i> (<i>b</i>)	
Gender	-1.62	1.05	-.00
Ethnicity	-1.57	1.40	-.02
Number of Traumas	0.84*	0.20	.11
Age at Entry Vietnam	-0.25*	0.12	-.06
Atrocities/Abusive Violence	5.85*	1.83	.09
Malevolent Environment	1.16	1.23	.04
Combat Exposure	0.56	1.16	.02
Perceived Threat	3.35*	1.48	.11
Readjustment Problems	5.81*	0.65	.23
Service-Related Disability (SRD)	2.73*	0.84	.09
Emotional Social Support (ES)	-12.88*	1.51	-.27
Instrumental Social Support	-19.71*	3.79	-.15
Wishful-Thinking Coping	4.71*	0.67	.20
Problem-Solving Coping (PSC)	-2.37*	0.65	-.10
SRD x ES Interaction	-3.20*	1.41	-.07
SRD x PSC Interaction	-1.53*	0.77	-.05

Notes: *b* represents unstandardized regression slope estimate with standard error *SE* (*b*); represents standardized regression slope estimate. Significant slopes at $\alpha = .05$ marked with an asterisk. $N = 1443$. Model $R^2 = .67$, $\chi^2(16) = 756.81$, $p < .001$.

Controlling for the other predictor variables in the model, ES, IS, and PSC (at the mean of SRD) were negatively and significantly related to PTSD scores and WTC was positively and significantly related to PTSD scores. Furthermore, controlling for the other variables in the model, SRD was positively and significantly related to PTSD scores (at the mean of the ES and PSC variables). However, the effect of SRD on PTSD scores was qualified by significant interactions with ES and PSC. In particular, the impact of SRD on PTSD scores was smaller for both those with higher levels of ES and higher levels of PSC.

9. DISCUSSIONS

The two-fold purpose of this study was to examine whether perceived disability predicted PTSD levels and whether coping and/or social support moderated the impact of perceived disability on PTSD levels, after controlling for demographic, pre-military, military, and post-military factors. The results indicated that perceived disability occurring during military service significantly predicted PTSD, when controlling for other stressors, such that more pronounced perceived disability is associated with higher PTSD levels. Further, emotional social support (ES) was found to moderate between perceived disability and PTSD, indicating that the existence of a disability had less of an influence on PTSD levels among those with higher levels of ES. Problem-solving coping (PSC) also moderated the association of perceived disability and PTSD, such that a service-related disability (SRD) had less influence on PTSD scores for individuals with higher levels of problem-solving coping.

That perceived disability was a significant predictor of PTSD scores over and above other variables already identified in the literature corresponds with what previous research has found in the general population [14-18] and among veterans [25,26]. As discussed at the beginning of this paper, Kulka and his colleagues [1,2] presented basic results regarding PTSD prevalence and disability; the present research helps to refine this association by conducting a multivariate analysis that controlled for multiple sources of stressors, and by examining coping interactions.

The direction of the significant zero-order correlation between PTSD and emotion-focused, wishful-thinking coping was in the hypothesized direction ($r = .46$, $p = .05$), indicating that higher use of wishful-thinking coping (WTC) was related to increased levels of PTSD. Yet, the direction of the zero-order correlation between PTSD and problem-solving coping was opposite (higher use of problem-solving coping was related to increased levels of PTSD) to what was hypothesized ($r = .14$, $p = .05$). However, this zero-order relationship was re-

versed ($r = -.10$, $p = .05$), when examined through regression analysis. Two possible explanations for this phenomenon include: a) the operation of a minimal amount of suppression, and/or b) the fact that after controlling for all other confounding variables, the use of problem-solving coping does act to attenuate the influence of disability on PTSD symptomatology, as originally hypothesized.

This study's finding that problem-solving coping moderates the association of perceived disability and PTSD concurs with research on the role played by problem-solving coping in decreasing the impact of a range of stressors. For example, Kennedy, Lowe, Grey, and Short [57], in a sample of people with traumatic spinal cord injuries, found a negative correlation between problem-solving coping (active coping and planning) and measures of depression, anxiety, and global psychological distress. Therapeutic interventions that integrate problem-solving components (e.g., decision making, time management, conflict resolution, money management) could become useful in countering the impact of functional impairments that are associated with disability. One example of such programs is Kennedy and Duff's program [62,63] for coping effectively with spinal cord injuries. Problem-solving is a life-skill that can be used for general problems, as well as for challenges directly related to disability-related issues. Because the existence of a disability often involves numerous challenges on multiple levels (e.g., psychological, social, vocational, environmental), therapeutic interventions to strengthen problem-solving coping can help individuals better adapt to their lives following the onset of disability.

The significant zero-order correlations between PTSD and both emotional social support ($r = -.61$, $p = .05$) and instrumental social support ($r = -.44$, $p = .05$) were in the hypothesized directions, indicating that the use of social support is inversely related to the presence of PTSD symptoms, as hypothesized. Further, this study's finding of moderation by emotional social support—indicating that the effect of disability on PTSD scores is smaller for those with higher levels of emotional social support—is also noteworthy. This corroborates with the earlier reported findings [see 58] that the availability of social support for people with disabilities bolsters one's ability to cope adaptively with life's crises. The finding that emotional social support statistically decreases the impact of disability on PTSD levels suggests that therapeutic interventions that include interpersonal components, such as family and group counseling, can help facilitate the individual with a disability's functioning and adaptation to the onset of a chronic medical condition. There is a range of empirically-based therapies, from cognitive behavior approaches to exposure therapies, which directly target the PTSD symptomatology [for an overview

of the range of treatments, see 64-67]. Yet, more comprehensive strategies are needed, in order to address the complex traumatic reactions (e.g., “double PTSD” [13]) that may be experienced when a disability occurs in a war-zone.

9.1. Limitations

The findings of this study must be interpreted with caution. First, the generalizability of the findings obtained in this study is limited, because the data were drawn only from U.S. Vietnam veterans. King and King [59] published a detailed article critiquing possible validity issues related to research among Vietnam veterans. More recently, debates about calculating the PTSD rates from the NVVRS dataset have been published in several volumes of the *Journal of Traumatic Stress*. The conditions and uniqueness of the Vietnam war itself may have created differences in PTSD rates among veterans of various wars [68,69].

In addition, because this research was retrospective and used cross-sectional data, no causal patterns can be established. Further, the disability variable was based on a single, self-reported item that did not encompass the wide range of possible disability definitions; yet, in this secondary data analysis, the researchers determined it as the best representation of a war injury with permanent consequences, *i.e.* disability. Finally, while the percentage of variance explained in PTSD was notable (67%), unexplained variance still exists, which means that variables not included in this study also are influencing PTSD levels.

10. CONCLUSIONS

The results of this study provide unique information contributing to the knowledge about PTSD, which has been generated by the decades of research using data from the NVVRS and other sources. The present research examined whether disability predicted PTSD (while controlling for specific variables), in addition whether social support and coping were modifiers of that association; these two issues had not yet been studied in previous research using the NVVRS data.

As expected, medical conditions or disabilities may be one source of the veterans' PTSD. The findings clarified that emotional social support and problem-solving coping both decrease the impact of perceived disability on PTSD levels. In view of such knowledge, psychosocial, therapeutic interventions may help to facilitate the individual with a disability's functioning and adaptation to the onset of a permanent medical condition. Future research should test whether the findings of this study, which used a nationally representative data from the Vietnam era, can be replicated among veterans from the Iraq and Afghanistan wars.

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Efficacy of the structured life review and the short-term life review on the spiritual well-being of terminally ill cancer patients

Michiyo Ando¹, Tatsuya Morita²

¹ Faculty of Nursing, St. Mary's College, Kurume, Japan; andou@st-mary.ac.jp

² Department of Palliative and Supportive Care, Palliative Care Team, and Seirei Hospice, Seirei Mikatahara General Hospital, Shizuoka, Japan

Received 27 November 2009; revised 4 January 2010; accepted 20 January 2010.

ABSTRACT

GOALS: The aims of this study were to evaluate the treatment efficacy of the structured life review interviews and the Short-Term Life Review on the spiritual well-being of terminally ill cancer patients, in order to improve patients' quality of life. **SUBJECTS and METHODS:** Participants were patients in palliative care units in Japan. In the study 1, the structured life review interviews were conducted with 12 patients. They completed the QOL scale of the SELT-M (Skalen zur Erfassung von Lebens qualitat bei Tumorkranken-Modified Version) questionnaire before and after the interviews. In the study 2, the Short-Term Life Review was conducted with 30 patients. They reviewed their lives in the first session and they confirmed the contents in the album based on the life review in the second session. Duration of the treatment was one week. Measurement instruments included Functional Assessment Chronic Illness Therapy-Spiritual (FACIT-Sp). **RESULTS:** After the structured life review, the mean overall QOL score and Spirituality subscale score of the SELT-M significantly increased, from 2.57 ± 0.61 to 3.58 ± 1.0 ($p=0.013$) and 2.57 ± 0.61 to 3.14 ± 2.25 ($P=0.023$), respectively. After the Short-Term Life Review, the mean FACIT-Sp scores significantly increased from 16 ± 8.2 to 24 ± 7.1 . **CONCLUSION:** Both the structured life review and the Short-Term Life Review may be effective in improving the spiritual well-being of terminally ill cancer patients, being higher feasibility for the Short-Term Life Review. We need to use these therapies understanding characteristics of each therapy.

Keywords: Psychotherapy; Life Review; Terminally Patients; Spiritual Well-Being

1. INTRODUCTION

Meaning of life or meaning of existence is an important for human, and it is called spirituality. World Health Organization demonstrates that spirituality is an important factor for human's quality of life. From the study of Japanese terminally ill cancer patients, Murata & Morita [1] defined spiritual well-being as meaning of life and peaceful of mind, and psycho-existential sufferings as loss of meaning of life or meaning of existence. Although terminally ill cancer patients often experience psycho-existential sufferings, there have been few interventions for these problems. The present study describes an intervention to elevate spiritual well-being in terminally ill cancer patients.

Butler [2] reported that the life review process is a mean of reintegration and can give new significance and meaning to an individual's life. It is defined as "the progressive return to consciousness of prior experience, which can be re-evaluated with the intention of resolving and integrating past conflict, thereby giving new significance to one's life." A life review is a type of reminiscence therapy [2]. Reminiscence itself is an activity of life review or reminiscence therapy. Reminiscence is an interpersonal or communicative psychosocial process that can be carried out individually or in groups [3]. Previous studies have shown the effects of life review on depression [4-6], self-esteem [4], and life satisfaction [7].

There are relatively few studies concerning life review interviews for cancer patients, particularly in the form of empirical studies, though there are some studies. To use life review effectively in the clinical situation, we had to know the efficacy of life review empirically. We are reviewing two empirical studies [8,9].

2. STUDY 1 STRUCTURED LIFE REVIEW

2.1. Purpose

In the Study 1, we evaluate the treatment efficacy of the

structured life review interviews on the spiritual well-being of terminally ill cancer patients.

2.2. Methods

2.2.1. Participants

Participants were patients with incurable cancer receiving specialized care in the palliative care unit of a general hospital in Japan. The inclusion criteria for this study were 1) the patient had incurable cancer, 2) the patient was 20 years of age or older, and 3) the primary physicians were in agreement that the patient would benefit from the psychological interventions. Exclusion criteria were, 1) the patients had strong physical symptoms, 2) the patients had cognitive impairment. During 6 month study periods, a total of 21 patients were recruited from the primary physicians; however 9 patients were later excluded from this study for various reasons. Thus, 12 patients (2 males, 10 females) finally participated in the study. The patients' ages ranged from 54 to 82, with a mean of 63. The primary tumor sites were breast ($n = 3$), liver ($n = 2$), colon ($n = 2$), lung ($n = 2$), thyroid ($n = 1$), stomach ($n = 1$), and gallbladder ($n = 1$).

2.2.2. Interventions and Outcome Measurements

The interviewer was a clinical psychologist. The interview procedure entailed a structured life review interview [7] in which patients reviewed their own childhood, adolescence, adult life, and current situation along Erikson's development stages [10]. Some of the questions asked were 1) Please tell me about your childhood, 2) What do you remember to be the most impressive events in your childhood?, and 3) How do you feel now when you review those impressive events (Table 1). Four sessions were planned for each patient, addition to one session for making relationships between a patient and the interviewer. Interviews were conducted in the dayroom or at the bedside. The patient reviews were recorded in the form of notes taken during or immediately after the session.

To evaluate the spiritual well-being, we used the

Table 1. The question items in the structured life review.

Times	Examples of question items
1	Making relationships and listening to patients' feeling now Theme: feeling life in the hospitals, problems, etc.
2	Child period: Theme: parents, home town, family members, playing, etc.
3	Young Adult period: Theme: study, work, human-relation in social life, etc.
4	Adult period: Theme: marriage, born in children, role in family or social life, etc.
5	From adult to now: Theme: illness, medical treatment, human-relationships, etc.

SELT-M (Skalen zur Erfassung von Lebens qualitat bei Tumorkranken-Modified Version) [11]. The patients completed the Japanese version of the SELT-M [12] before and after the intervention. The reliability and validity of the Japanese version of the SELT-M has been reported. The SELT-M consisted of 6 subscales, namely, Physical well-being (3 items), Mood (6 items), Support (3 items), Orientation (3 items, e.g., "Today, I see many things in a more positive light"), Spirituality (3 items, e.g., "It is difficult for me to see positive meaning in my illness"), and Overall QOL (1 item). We used the overall QOL score and Spirituality subscale score in the post-interview results. Each subscale ranged from 1 to 5, with a higher score indicating a higher level of patient-perceived QOL.

The institutional review board of this hospital approved this study from both ethical and scientific aspects, and a patient gave written consent.

2.2.3. Statistical Analysis

In order to evaluate the treatment efficacy of life review interviews on the patient's spiritual well-being, a Wilcoxon sign rank test was conducted on the sections of the SELT-M before and after the life review interviews.

2.3. Results

As shown in Table 2, overall QOL and Spirituality subscale scores, in addition to Mood and Orientation subscale scores, significantly increased after the intervention.

3. STUDY 2 SHORT-TERM LIFE REVIEW

Although structured life review may be effective in improving the spiritual well-being of terminally ill cancer patients, the long sessions decrease the feasibility, thus we need a shorter version of life review therapy. We propose a new psychotherapy-the Short-Term Life Review with short sessions for terminally ill cancer patients. A Short-Term Life Review involves only two sessions. Patients review or narrate their lives about questions which promote the integration of lives referring other previous studies. In the first session, patients review their lives, and the review is then recorded and edited. The therapist makes an album after the first session. In the second session, the patient and therapist view the album, and confirm the contents with appreciation.

3.1. Purpose

The primary aim of this study was to assess the efficacy of the Short-Term Life Review on the spiritual well-being, as well as anxiety, depression, sufferings, and happiness, or terminally ill cancer patients.

Table 2. Mean scores and P value by the Wilcoxon sign rank test on scores of SELT-M.

	SELT-M					
	Physical well-being	Mood	Support	Orientation	Spirituality	Overall QOL
Before	2.33 (SD = 0.79)	3.16 (SD = 0.65)	3.28 (SD = 0.65)	2.93 (SD = 0.75)	2.57 (SD = 0.61)	2.57 (SD = 0.61)
After	2.80 (SD = 1.16)	3.79 (SD = 0.74)	3.61 (SD = 0.83)	3.65 (SD = 1.03)	3.14 (SD = 2.25)	3.58 (SD = 1.0)
P value	Z = -1.02, P = 0.307	Z = -2.67, P = 0.008	Z = -1.18, P = 0.237	Z = -2.05, P = 0.041	Z = -2.23, P = 0.023	Z = -2.49, P = 0.013

Table 3. Patient backgrounds.

Primary tumor site	Gender
Lung	n = 8 Male (n = 8)
Stomach	n = 5 Female (n = 22)
Pancreas	n = 2 Age
Gallbladder	n = 2 Mean age; 74 (Total SD = 9.1)
Uterine	n = 2 Marital states
Breast	n = 1 Married (n = 29); Widow (n = 11), Widower (n = 3)
Kidney	n = 1 Non-married (1)
Leukemia	n = 1 Religion
Rectal	n = 1 Christian (n = 4)
Tongue	n = 1 Buddhism (n = 3) None (n = 22)
Colon	n = 1 ECOG-PS
Mesothelioma	n = 1 1 (n = 1), 2 (n = 3)
Myeloma	n = 1 3 (n = 13), (n = 13)
Lymphoma	n = 1 Duration from the interview to patients' death Mean; 67 days

3.2. Methods

3.2.1. Participants

The participants were cancer patients from the palliative care unit of two general hospitals and one home-care clinic. The inclusion criteria for this study were 1) patients with incurable cancer; 2) patients without cognitive impairment; 3) patients 20 years of age or older; and 4) patients for whom the primary physicians agreed would benefit from the psychological interventions. During the 11-month study period, 35 patients were recruited through primary physicians. **Table 3** shows the patients' background.

3.2.2. Intervention

Ethical aspect of this study was validated by both the board and the ethical committee of St. Mary's Hospital and St. Mary's College.

The Short-Term Life Review has two parts. In the first part, patients review their lives, and in the second, they re-evaluate, re-construct, and appreciate their life. The interviewer was a clinical psychologist (therapist). The interview procedure was based on a structured life review interview that was conducted individually, and the patient was asked to re-evaluate both good and bad memories. Question items were mainly based on the structured life review, and some items from Chochinov *et al.* [13] were added. The following questions were asked in the reviewing session (**Table 4**):

- 1) What is the most important thing in your life and why?
- 2) What are the most impressive memories in your life ? 3)

In your life, what was the event that or the person who affected you the most? 4) What is the most important role in your life? 5) Which is the proudest moment of your life? 6) Is there anything about you that your family would need to know, are there things you would want them to tell you, and, if possible, are there things you would want them to remember? 7) What advice or word of guidance would you wish to pass on to the important people in your life or to the younger generation?

The patient's narratives were recorded, and the therapist tried to listen to each word uttered by the patient. After the first session, the interview was first transcribed verbatim and the therapist made the album. To make the album, 1) key words in the answer to each question were selected. Words or phrases used by the patient were written in the album as often as possible; both good and bad things were included and feelings or re-framed thoughts-how he feels now-were written in the album. 2) The therapist pasted photos or drawings from various books or magazines that were related to the patients' words or phrases, to make the album more beautiful and more memory-provoking. It took a week for the treatment.

3.2.3. Outcome Measurements

To measure the effects of the Short-Term Life Review, we used the Japanese version of FACIT-Sp [14]. The validity and reliability of the FACIT-Sp Japanese version is well-established [15].

3.2.4. Statistical Analysis

To evaluate the efficacy of the Short-Term Life Review in improving the patients' spiritual well-being, the Wil-

Table 4. The question items in the short-term life review.

Question Items
1) What is the most important thing in your life and why?
2) What are the most impressive memories in your life ?
3) In your life, what was the event that or the person who affected you the most?
4) What is the most important role in your life?
5) Which is the proudest moment of your life?
6) Is there anything about you that your family would need to know, are there things you would want them to tell you, and, if possible, are there things you would want them to remember?
7) What advice or word of guidance would you wish to pass on to the important people in your life or to the younger generation?

coxon signed rank test was conducted on all scores of each scale before and after the Short-Term Life Review.

3.3. Results

Five of the patients were excluded from this study because of unexpected deterioration in health. Thus, a total of 30 patients completed all sessions. After the Short-Term Life Review, the mean FACIT-Sp scores significantly increased from 16 ± 8.2 to 24 ± 7.1 ($Z = -4.2$, $P = 0.001$).

4. DISCUSSION

4.1. Effects of the Structured Life Review and the Short Term-Life Review

After the structured life review, the significant increase in the SELT-M scores, as well as the Spirituality, Orientation, and Mood subscales shows the efficacy of this therapy on spiritual well-being of terminally ill cancer patients. As the Spirituality and Orientation subscale quantify the levels of the patients' meaningfulness and positive outlook, the patients may have found a meaning to life and had more positive thoughts. The significant increase in the Mood subscale for terminally ill cancer patients is accord with previous studies for elders [3-5].

After the Short-Term Life Review, the FACIT-Sp scores significantly increased. It shows the effect of this therapy on spiritual well-being of cancer patients. We confirm the efficacy of this therapy even when we compare it with the control group [16]. Adapting these process with the previous theoretical model [17,18], we can explain the effects of Short-Term Life Review. A patient has a purpose or a goal for his life when he is healthy. However, when he falls into serious illness, it is often difficult for him to attain his purpose or a goal because of unexpected serious illness, and he feels much distress. In this situation, when he receives the Short-Term Life Review and he can re-think and modifies his original purpose or goals into attainable ones, he feels positive mood. Short-Term Life Review may contribute for a patient to reconstruct his life being congruent with cancer in his life.

4.2. Feasibility

It would be remarkable for Short-Term Life Review to have high feasibility. The percentage of patients deteriorating with this therapy was only 17%, although, it was 30% for patients using the structured life review. We describe some factors related to feasibility. 1) The Short-Term Life Review is completed in a week, and this short-term intervention enables terminally ill cancer patients to complete an intervention. 2) Patients with very low functionality in ADL can participate because the patients review their lives while lying on bed. If patients' ADL is high, the Problem-solving therapy for cancer patients [19] and cognitive behavior therapy for cancer patients [20], or meaning-centered group psychotherapy [21] are also effective.

4.3. Characteristics of Each Life Review

There are some characteristics of each therapy. The structured life review is suitable for patients who have much time for interviews and require little cognitive load. Oppositely, The Short-Term Life review is suitable for patients who do not have much time for interviews and require a little more cognitive load. In the Short-Term Life review, a patient needs to think of his life profoundly, resulting in requiring much cognitive load. We use these therapies understanding patients' physical and mental states.

5. CONCLUSIONS

Both the structured life review and the Short-Term Life Review have efficacy on spiritual well-being of terminally ill cancer patients, being higher feasibility for the Short-Term Life Review. We need to use these therapies understanding characteristics of each therapy.

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Inhibition of salivary peroxidase by cigarette smoke

Reyhaneh Sariri¹, Aabdolali Varasteh¹, Ali Erfani², Afsaneh Rezaei³, Zahra Heidari⁴

¹Department of Biochemistry, University of Guilan, Rasht, Iran; Sariri@guilan.ac.ir

²Department of Internal Medicine, Iran University of Medical Sciences, Tehran, Iran

³Department of Periodontology, University of East, Manila, Philippines

⁴Department of Biology, Islamic Azad University, Zanzjan, Iran

Received 9 October 2009; revised 9 November 2009; accepted 15 November 2009.

ABSTRACT

Saliva is the first biological fluid to encounter many dangerous chemicals present in cigarette smoke. The oral peroxidase system is responsible for the antioxidant capacity of saliva. In this research, the interaction of cigarette smoke and oral peroxidase in smokers and non-smokers was compared. It was found that after smoking one cigarette, oral peroxidase activity was decreased in both groups: 42.5% in smokers and 58.5% in non-smokers ($p < 0.05$). After 30 min, the level of activity returned to 90-100% of the pre-smoking level, mainly due to the secretion of new saliva.

Keywords: Cigarette Smoke; Oxidative Stress; Peroxidase, Saliva; Free Radicals.

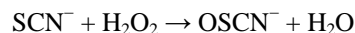
1. INTRODUCTION

In addition to its local toxic effect in the oral cavity, tobacco smoke is involved in the pathogenesis of several diseases of other body systems, including cardiovascular and respiratory. The noxious effects of smoke compounds explain the reason for high incidence of periodontal diseases, caries, and neoplastic diseases of oral tissues in smokers. Cigarette smoke is seriously injurious to the oral cavity and is associated with several periodontal diseases and cancer. More than 4000 different chemicals have been detected in cigarette smoke, 1/10th of which are known carcinogens. Tobacco smoke also contains potent oxidants such as oxygen free radicals and volatile aldehydes [1,2]. The oxidizing agents can seriously damage biomolecules such as proteins and enzymes leading to various physiological problems.

Saliva is the first biological fluid to encounter the cigarette smoke. Saliva is well known for its highly protective functions against deleterious agents such as microorganisms, toxins and various oxidants [3]. It has been known that the antioxidant capacity and reducing

power of saliva may be diminished by various factors [4]. It has been shown that *in vitro* exposure to cigarette smoke could significantly decrease some enzymatic activities, both in plasma and in saliva [5]. The most widely known toxic components of tobacco smoke include unsaturated and saturated aldehydes. These compounds can interact with thiol rich compounds, leading to structural and functional modification. The peroxidase system of oral cavity is predominately involved in the high antioxidant capacity of saliva [6]. This system is composed of two peroxidase enzymes, salivary peroxidase and myeloperoxidase. The salivary peroxidase secreted from the major salivary glands, mainly the parotid gland [7], contributes 80% of oral peroxidase activity, while myeloperoxidase, produced by leukocytes in inflammatory regions of the oral cavity [8], contributes only 20% of oral peroxidase activity. The term oral peroxidase is used here to denote the total activity of both isoforms, since the 2-nitrobenzoic acid-thiocyanate (NBS-SCN) assay used is capable of measuring the activity of both enzymes.

In addition to the capacity of oral peroxidase to reduce the level of hydrogen peroxide (H_2O_2) excreted into the oral cavity from the salivary glands by bacteria and by leukocytes, it can also increase specific antibacterial activity by inhibiting the metabolism and proliferation of various bacteria in the oral cavity [9]. Reduction of H_2O_2 by thiocyanate ion by is the common reaction catalysed by oral peroxidase.



SCN^- is the detoxification product of cyanide secreted mainly from the parotid gland. In the above reaction, SCN^- acts as the electron-donating component, similar to glutathione (GSH) in other biological systems [8,10]. Two potent antibacterial oxidizing products evolve from this reaction: hypothiocyanous acid (HOSCN) and its conjugated anion, hypothiocyanite ($OSCN^-$) both of which can oxidize intracellular reduced GSH [11].

Although antibacterial characteristics of oral peroxidase have widely been investigated, its possible anticarcinogenic role against the most prevalent and lethal can-

cer of the oral cavity, squamous cell carcinoma (SCC), has rarely been reported in scientific literature and case reports. Oral SCC is the most common malignancy of the head and neck, with a worldwide incidence of over 300,000 new cases annually [12]. The disease is characterized by a high rate of morbidity and mortality (about 50%) and in this respect is similar to malignant melanoma [12-15]. The major inducer of oral SCC is exposure to tobacco, which is considered to be responsible for 50-90% of cases worldwide [16]. The incidence of oral SCC in cigarette smokers is four to seven times higher than in non-smokers [17]. In heavy smokers, there is a constant and direct attack of various smoke reagents on the oral epithelial cells, which gradually accumulate and cause a step-wise malignant transformation. Free radicals, reactive oxygen species (ROS), and reactive nitrogen species (RNS) in the inhaled smoke can induce this process [16]. Despite the importance of cigarette smoke effect on salivary antioxidants in the pathogenesis of oral cancer, the subject has received little attention by researchers. The aim of the current research was to analyze the effect of smoke on oral peroxidase activity.

2. MATERIALS AND METHODS

2.1. Saliva Collection

58 healthy university students aged 25-30 (28 smokers, over 20 cigarettes daily for at least 3 years) and 30 non-smokers volunteered to enter the study. About 2 ml of their un-stimulated whole saliva was collected in sterile, dry tubes. Saliva samples were immediately centrifuged at $800 \times g$ for 10 min at 4°C to remove squamous cells and cell debris. The resulting supernatant was used for determination of peroxidase activity.

2.2. Cigarettes

The cigarettes used in this study were popular commercial cigarettes containing 14 mg of tar and 0.9 mg of nicotine.

2.3. Peroxidase Activity

The biological activity of peroxidase on 4-amino antipyrine was measured spectrophotometrically. In a typical experiment, oxidation of 4-amino antipyrine was measured at 25°C in 3 ml of 0.3 M phosphate buffer, pH 7.4, containing 0.0010 M hydrogen peroxide, 0.002 M 4-amino antipyrine and 0.15 M phenol. 40ml of enzyme solution (6×10^{-4} mg/ml in 0.3 M phosphate buffer pH 7.4) was then added and the change in absorption at 510 nm (DA/min) was recorded. The change in absorption at 510 nm is due to the formation of a chromogen product with a λ_{max} at 510 nm. One unit of activity was defined as the amount of enzyme that caused an absorbance change of 0.001 per min under standard conditions.

2.4. Thiocyanate Determination

The level of SCN^- in smokers and non-smokers was determined using the method of Densen *et al.* [18]. Saliva was de-proteinized with 10% trichloroacetic acid, the supernatant was reacted with ferric nitrate and nitric acid, and the color reaction was measured at 460 nm using a standard solution of potassium thiocyanate.

2.5. Statistical Analysis

The results for statistical analysis were compared between the smoking and non-smoking groups. Data on the activities of each group were observed and calculated, and statistical significance was set at $p < 0.05$.

3. RESULTS

3.1. Peroxidase Activity

Table 1 shows peroxidase activity at time 0 in both the smoking and non-smoking groups. The effect of smoking one cigarette on peroxidase activity in the saliva of the smoking group as compared with the non-smoking group is shown in Figure 1. A sharp decrease in peroxidase activity was observed in saliva of both groups collected shortly after smoking a single cigarette. Drop

Table 1. Oral peroxidase activity (mU/ml) at time 0 in smokers and non-smokers.

Measurement	Smokers	Non-smokers
n	20	18
Range	182-1721	190-1020
Mean	696.2	569.3
SD	418.2	253.1

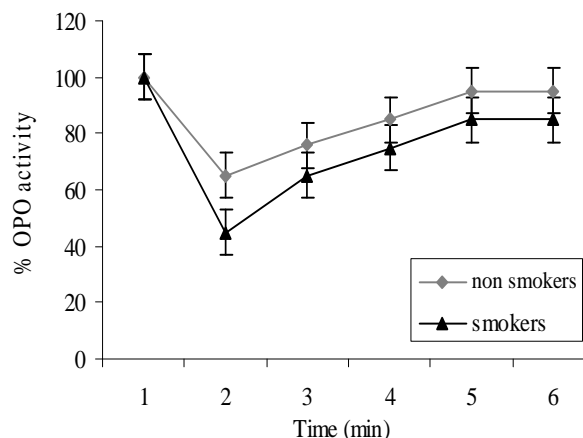


Figure 1. Effect of smoking one cigarette on oral peroxidase activity. The oral peroxidase activity of 20 smokers and 18 non-smokers was measured prior to and after smoking one cigarette ($p < 0.05$).

of peroxidase activity was much higher in non-smokers (61.8%) than in smokers (43.2%) ($p < 0.05$). The volunteers were told not to smoke, and their peroxidase activity was measured at 10, 30, 45 and 60 min after the onset of the experiment. It was noted that in most cases, the level of peroxidase activity returned to 90-100% of the initial pre-smoking level after 30 minutes.

It is clear from **Figure 1** that oral peroxidase activity is higher and more resistant to inhibition by one cigarette smoke in smokers as compared with non-smokers. In order to explain this finding, the effect of the exogenous addition of SCN^- was measured in non-smokers *in-vitro*. It is known that the saliva of non-smokers contains between 0.3 and 1.5 mM of SCN^- , while the level of SCN^- in the saliva of heavy smokers is 1.4-4.0 mM [19]. Using the ferric nitrate-nitric acid method, the ranges and means of SCN^- were: a) in the range of 0.33-1.42 mM in non-smokers (0.725 ± 2.44 mM, mean \pm SD, $n = 10$), and b) 1.35-2.76 mM in smokers (1.82 ± 0.50 mM, mean \pm SD, $n = 9$). It is, therefore, suggested that higher quantities of SCN^- in heavy smokers might provide some protection against damage of smoke to oral peroxidase. **Figure 2** shows the effect of adding KSCN (range, 0.4-4.5 mM) to the saliva of three non-smoking volunteers, after overnight dialysis to remove endogenous SCN^- and measuring oral peroxidase activity before and after smoking one cigarette. It can be seen that the addition of SCN^- increased oral peroxidase activity considerably after smoking one cigarette and, indeed, provided some protection of enzyme activity as compared with the controls.

As mentioned above, it is known that peroxidase system in oral cavity is composed of two peroxidase enzymes, salivary peroxidase and myeloperoxidase. Therefore, the final part of research was designed with the aim to elucidate which of the two components of oral

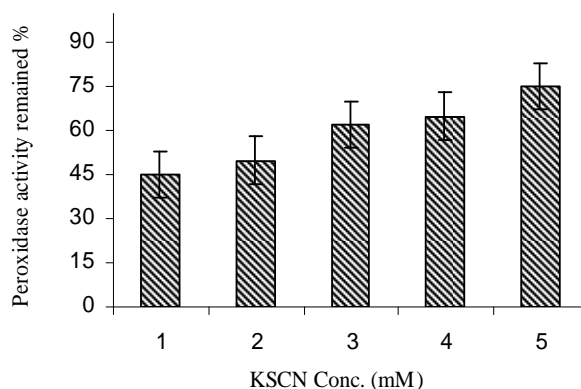


Figure 2. Oral peroxidase residual activity after *in-vitro* exposure of the saliva of three non-smokers to one cigarette in control (line1, no added SCN^-) and increasing concentrations of KSCN, lines 2-5 (0.4, 1.2, 3.6 and 4.5 mM respectively). Each value represents an average of at least three different volunteers \pm SD.

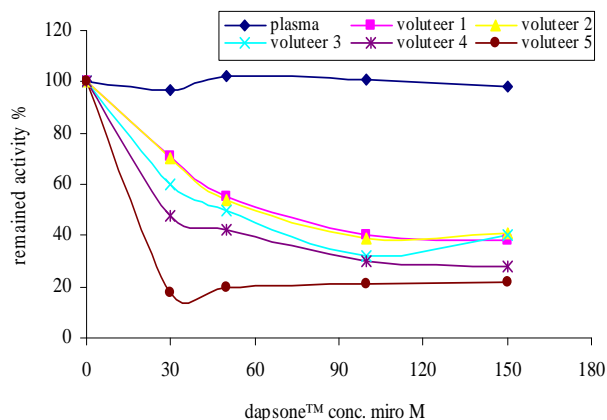


Figure 3. Effect of incubation of various concentrations of dapsone™ on oral peroxidase activity in saliva of five subjects compared to plasma.

peroxidase are affected by cigarette smoke. It has been demonstrated that 4, 4'-diaminodiphenylsulfone (commercially named as dapsone™) can specifically inhibit lactoperoxidase and not myeloperoxidase at acidic pH [19].

In this part of research, oral peroxidase activity of five volunteers was examined in the presence of increasing concentrations of (dapsone™, 50-150 μM). **Figure 3** shows that oral peroxidase activity was reduced to 22-62% its original value in the presence of 150 μM dapsone™, while under the same conditions, plasma peroxidase activity (myeloperoxidase) was not affected at all. It was observed that cigarette smoke caused a loss of peroxidase activity at the same rate, whether or not it contained dapsone (150 μM). Therefore, cigarette smoke seemed to be decreasing salivary myeloperoxidase activity to the same degree that it decreased salivary total peroxidase activity.

4. DISCUSSIONS

The enzymatic system plays a prime part in oral defense mechanism, especially against the attack of free radicals related to smoke and the evolution of oral cancer. The salivary antioxidant system, in which the peroxidase is the most important, has received more attention in recent years [7,19]. It has been shown that oral peroxidase inhibits the initiation and progression of oral cancer [20]. Moreover, it is demonstrated that patients with oral lichen planus (a premalignant lesion) have a lower salivary antioxidant capability [21].

Cigarette smoke contains hydrogen cyanide, which is metabolized by the liver to SCN^- . The SCN^- is specifically sequestered from the plasma by the parotid gland and is secreted by this gland into the oral cavity. Its concentration in normal saliva is in the range of 0.3-1.5 mM. In heavy smokers this range is increased to approximately 1.4-4.0 mM, depending on the number of ciga-

rettes smoked per day, with a prolonged half-life of 9.5 h [19]. Following SCN^- secretion via the saliva, it reacts with the H_2O_2 in the oral cavity, leading to elimination of the H_2O_2 . This reaction is catalyzed by the peroxidase. In the case of peroxidase inhibition, e.g. when saliva is exposed to cigarette smoke, H_2O_2 is not removed and reacts with redox-active metal ions that are secreted via the parotid saliva.

Redox-active metals such as iron and copper in the presence of H_2O_2 and other free radicals found in smoke, participate in Haber-Weiss and Fenton reactions, leading to the production of free radicals. The free radicals are highly reactive and immediately react with neighboring cellular macromolecules including DNA. This may result in malignant transformation and could be the indirect reason of cigarette smoke-induced role of free radicals in oral SCC alongside other direct effects of carcinogens contained in cigarette smoke. Accordingly, the importance of the current demonstration of a cigarette smoke-induced 60% reduction in oral peroxidase activity (in the *in vitro* study) stems from the fact that, in this condition, H_2O_2 is not removed from the oral cavity and, hence, is available to participate in the DNA-injurious reactions previously mentioned.

It can be emphasized that cigarette smoke – induced inactivation of peroxidase activity is reversible. This can be explained by the continuous secretion of saliva into the oral cavity (0.1-1.0 ml/min secretion rate) containing fresh peroxidase. Therefore, the affected enzyme is almost continuously removed by fresh saliva. On the other hand, *in-vitro* studies using smoking flasks showed that the loss of activity was irreversible, as no new saliva could replace the damaged enzyme (the results are not included here). However, it is shown in **Figure 1** that the recovery process is time-consuming, taking approximately 30 min to fully regain baseline values. Heavy smokers, who smoke over 20 cigarettes per day, have significantly reduced salivary peroxidase activity as recovery does not occur before their next smoking. Their saliva remains almost un-protected by salivary peroxidase against cigarette smoke and other free radicals that penetrate their oral cavity via food, drink, and/or inhaled ingredients.

Another interesting observation in this study was the relative stability of the peroxidase in smokers compared to non-smokers after exposure to one cigarette, which may have resulted from partial protection rendered by the high concentrations of SCN^- found in the saliva of smokers. Such a protective role was demonstrated in the experiment in which we added exogenous SCN^- to the saliva of non-smokers, rendering its peroxidase less sensitive to cigarette smoke. However, the continuous protective capacity of SCN^- , which may be explained by the extended biological half-life of the molecule (9.5 h), was only partial and could not prevent the immediate injurious effect of smoke on peroxidase that did not recover

during the first 30 min after smoking.

From a clinical point of view, our findings are important because the specific peroxidase sensitivity to CS is unique as compared with other salivary antioxidants, such as uric acid or GSH molecules. These antioxidants are not affected by smoking a single cigarette [19,22]. In other words, it is not the general reduction in salivary antioxidant capacity that may account for the much higher prevalence of oral pathologies associated with the ROS attack in smokers, but the inhibition of peroxidase as a crucial phenomenon. This specific peroxidase activity loss leaves the oral epithelial cells almost completely unprotected against the deleterious effects of both SCN^- ions and hydroxyl free radicals produced by un-removed H_2O_2 in the presence of salivary redox-active metal ions. It has been shown that SCN^- ions and free radicals may adversely react with DNA [23,24], thus paving the way for CS induction and saliva-mediated initiation and progression of oral cancer [25].

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Antiuro lithiatic activity of aqueous extract of bark of *moringa oleifera* (lam.) in rats

Jameel Fahad¹, Vijayalakshmi¹, M. C. Satish Kumar¹, Sanjeeva¹, G. Prabhath Kodancha¹, Benegal Adarsh¹, A. L. Udupa^{2*}, U. P. Rathnakar³

¹Department of pharmacology, Kasturba Medical College, Manipal, India

²Department of Pharmacology, Faculty of Medical Sciences, University of the West Indies, Cave Hill, Barbados;
aludupa2002@yahoo.com

³Department of pharmacology, Kasturba Medical College, Mangalore, Karnataka, India

Received 8 January 2010; revised 20 February 2010; accepted 22 February 2010.

ABSTRACT

In the present study, aqueous extract of bark of *Moringa oleifera* administered orally, was evaluated for its antiuro lithiatic potential in albino rats of Wistar strains. The stones were produced in this study by zinc disc foreign body insertion in the bladder supplemented with 1% ethylene glycol in drinking water. The reduction in weight of the stones was used as criteria for assessing the preventive or curative antiuro lithiatic effect of the bark of this plant. Two doses of extract for prophylactic and curative groups were used. In both groups the oral administration of the extract of bark of *Moringa oleifera* has resulted in significant reduction in the weight of bladder stones compared to the control group.

Keywords: *Moringa oleifera* bark; Antiuro lithiatic activity; Introduction

1. INTRODUCTION

Moringa oleifera is an important medicinal herb, belonging to the family Moringaceae. It is considered as *miracle tree* as all the parts of the plant are useful for human health. It is also known as drum stick tree, horseradish tree, clarifier tree and mother's best friend in different parts of the world, based on their appearance and unique uses. It is mostly cultivated all over the plains of India, in the old world tropics and it is used medicinally in Guinea, Madagascar, and Burma [1-3].

The various parts of the plant used include flower, pod, leaves, bark and roots. The *flowers*, after cooking, are eaten either mixed with other foods or fried in butter and have been shown to be rich in potassium and calcium [4]. The *leaves* are eaten as greens, in salads,

in vegetable curries, as pickles and for seasoning. The *Bark* is regarded as an antiscorbutic, and it exudes a reddish gum sometimes used for the treatment of diarrhoea. The *roots* are bitter, act as a tonic to the body and lungs, and have an expectorant activity. They are a mildly diuretic and are used as a stimulant in paralytic affections, epilepsy and hysteria. The pods are the most valued and widely used of all the tree parts. The pods are extremely nutritious, containing all the essential amino acids along with many vitamins and other nutrients. The immature pod can be eaten raw or prepared like green peas or green beans, while the mature pods are usually fried and possess a peanut-like flavour. The pods also yield 38-40% of non-drying, edible oil known as Ben Oil which is clear, sweet and odourless, and never becomes rancid. Overall, its nutritional value most closely resembles olive oil [4].

In recent decades, many scientific studies using the extracts of leaves, pods and roots of *Moringa oleifera* are being carried out to confirm many potential uses including wound healing [5,6], anti-tumour [7], antihepatotoxic [8], antifertility [9], hypotensive [10], antiuro lithiatic [11] acute anti-inflammatory [6,12,13], and analgesic activity [14].

Even though the root extracts of *Moringa oleifera* have been studied for diuretic activity [11], there is no scientific study showing antiuro lithiatic activity of *Moringa oleifera* bark extract. Therefore this study was conducted in order to evaluate its antiuro lithiatic activity in rats.

2. MATERIALS AND METHODS

2.1. Approval for the Project

Approval for the experiment was obtained from the Institutional animal ethics committee (IAEC), Kasturba Medical College, Manipal, vide letter No. IAEC/KMC 07/2008-2009.

2.2. Plant Material

The bark of *Moringa oleifera* Lam. available locally were collected between February and May in and around Udupi. The botanical identity has been confirmed by department of botany, Sri Poornapagna College, Udupi. The voucher specimen is preserved in our laboratory for future reference.

2.3. Method of Extraction

The fresh bark collected were cut into small pieces, partially crushed and soaked in water overnight which was later subjected to boiling for 6 hours. The resultant extract was then drained and concentrated in a water bath to get the concentrated extract. The yield of the extract was 10.5%. The extract was stored in desiccators and used for further experiments after dissolving it in distilled water.

2.4. Animals

Inbred albino rats of Wistar strain, of either sex, aged around 2 to 3 months and weighing 150-200 g were used. They were housed in standard conditions of temperature ($25 \pm 2^\circ\text{C}$), relative humidity of 45-55%, and maintained on 12-hour light: 12-hour dark cycle in animal house of Kasturba Medical College, Manipal. They were fed standard pellet diet (Hindustan Lever rat pellets) and water *ad libitum*.

3. ACUTE TOXICITY STUDY

The aqueous extract of *Moringa oleifera* bark was administered orally in the escalating dosages, up to 8 g/kg to different groups of rats ($n = 6$, in each). The animals were observed for behavioural and physiological variations initially continuously for 4 hours, followed by 4th hourly for 12 hours and there after once daily for 14 days.

3.1. Antiuro lithiatic Study: Method of Induction of Urolithiasis by Insertion of Zinc Disc [15-19]

Rats were anaesthetized with intraperitoneal ketamine (50 mg/kg). A suprapubic incision was made and the abdomen was opened. The urinary bladder was then carefully exposed and the urine in the bladder was aspirated with a sterile syringe. A small nick was made at the apex end of urinary bladder and the sterile zinc disc (previously weighed) was carefully inserted into the bladder. Then the bladder was closed in a single stitch using chromic catgut (4-0). The abdomen was then closed in layers with chromic catgut and skin was closed with silk thread. The rats were allowed to recover from anaesthesia. Food and 1% ethylene glycol

in water was given *ad libitum*. The stone was allowed to form and grow inside the bladder during the study period. After the study period the rats were sacrificed and zinc disc implants/stones were removed from the bladder and dried. Stones taken out were weighed. The difference between initial and final weights indicated the amount of stone formed.

3.2. Grouping of Animals for Different Treatments and Procedure of the Study

Adult albino rats of Wistar strain, weighing between 150-200 g, were selected for the study. In this study using aqueous extract of bark of *Moringa oleifera*, the rats were divided into 6 groups with 6 animals in each group receiving different treatments. Group I–Prophylactic control (1% ethylene glycol for 4 weeks), Group II–Prophylactic treatment (1% ethylene glycol+aqueous extract of bark of *Moringa oleifera* 400 mg/kg orally for 4 weeks), Group III–Prophylactic treatment (1% ethylene glycol+aqueous extract of bark of *Moringa oleifera* 800 mg/kg orally for 4 weeks), Group IV–Curative control (1% ethylene glycol for 4 weeks followed by water for 4 weeks), Group V–Curative treatment (1% ethylene glycol for 4 weeks followed by aqueous extract of bark of *Moringa oleifera* 400 mg/kg for 4 weeks), Group VI–Curative treatment (1% ethylene glycol for 4 weeks followed by aqueous extract bark of *Moringa oleifera* 800 mg/kg for 4 weeks).

Prophylactic activity against urolithiasis was tested using Groups I to III in this study and after 4 weeks, animals were sacrificed and vesical calculi were collected, weighed and statistically evaluated.

Curative property was tested in using Groups IV to VI in the study and, at the end of eight weeks, animals were sacrificed and vesical calculi were collected, weighed and statistically evaluated.

3.3. Weight of Stones

The difference between the weight of the implanted zinc discs at the time of implantation and final weight of the dried calculi taken out from the bladder at the end of the 4th and 8th week period indicated the weight of deposited stone.

4. STATISTICAL ANALYSIS

The data obtained from the study was statistically evaluated using a parametric test ANOVA (Analysis of Variance) and Tukey as post hoc test. This was done with SPSS (Statistical package for social science) software.

5. RESULTS

The results of different groups studied using aqueous

Table 1. Weight of stone material deposit on zinc discs in control and after using aqueous extract of bark of *Moringa oleifera* 400 mg/kg & 800 mg/kg treated rats.

Groups	No. of rats	Weight of stone (mg) (Mean \pm SEM)
Group I–prophylactic control	6	278.16 \pm 7.35
Group II–prophylactic treatment (400 mg/kg)	6	139.5 \pm 8.89*
Group III–prophylactic treatment (800 mg/kg)	6	105.6 \pm 5.61*
Group IV–curative control	6	269 \pm 10.45
Group V–curative treatment (400 mg/kg)	6	170.6 \pm 12.98*
Group VI–curative treatment (800 mg/kg)	6	114.6 \pm 4.43*

* $p < 0.001$, $df = 2$, SEM = Standard error of mean, $n = 6$

extract of bark of *Moringa oleifera* 400 mg/kg & 800 mg/kg are shown in **Table 1**. In this study, the extracts from bark of the plant showed significant decrease in the weight of stones compared to control after the study period.

6. DISCUSSIONS

In the present study, aqueous extract of bark of *Moringa oleifera* 400 mg/kg & 800 mg/ were evaluated for the antiurolithiatic potential in albino rats of Wistar strains. The method used for induction of stones in this study was zinc disc foreign body insertion technique supplemented with 1% ethylene glycol, which is commonly used in these type of studies.

In this study, the weight of the stones was used as criteria for assessing the preventive or curative effect of the bark of *Moringa oleifera*. Two doses of extract for prophylactic and curative groups were used. In both groups the administration of the extract have resulted in significant ($p < 0.01$) reduction in the weight of stones compared to the control group.

The decrease in the bladder stone formation was inconsistent with the increase in the dose of the extract which could be due to variability in the response due to physiological variation. The stone formation in the control group itself was variable to a certain extent ranging from 240 mg to 300 mg in prophylactic control group and 248 mg to 310 mg in curative control group, which is indicative of normal physiological variation. Among the different strains of rats used for preclinical studies, Wistar rats are much less susceptible to persistent bladder infection and struvite stone formation in comparison to other strains. In a study where female Fischer 344 (F344), Lewis (LEW), Sprague-Dawley (SD), and Wistar (WIS) rats were inoculated with a host-adapted strain of *Ureaplasma parvum*, and necropsied at 2 weeks post

inoculation; 100% of F344, 42% of SD, 10% of LEW, and 10% of WIS rats remained infected. Severe bladder lesions and struvite calculi were seen in 64% of F344 rats; in other rat strains, bladder lesions were mild or absent [20].

7. CONCLUSIONS

In conclusion we can confidently confirm the possibility of antiurolithiatic activity of bark of *Moringa oleifera* as there was reduction in size of the stones. Further studies are needed to prove the stone dissolving property of aqueous extract of bark of *Moringa oleifera* (400 mg/kg & 800 mg/kg) in other animal models

8. ACKNOWLEDGEMENT

We the authors are thankful to the Dean of Kasturba Medical College, Manipal and to the K.M.C. Trust Manipal for the financial support rendered for this study.

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Health of children less than 5 years old in an upper middle income country: parents' views

Paul Andrew Bourne

Department of Community Health and Psychiatry, Faculty of Medical Sciences, Kingston, Jamaica; paulbourne1@yahoo.com

Received 25 November 2009; revised 10 December 2009; accepted 16 December 2009.

ABSTRACT

Health literature in the Caribbean, and in particular Jamaica, has continued to use objective indices such as mortality and morbidity to examine children's health. The current study uses subjective indices such as parent-reported health conditions and health status to evaluate the health of children instead of traditional objective indices. The study seeks 1) to examine the health and health care-seeking behaviour of the sample from the parents' viewpoints; and 2) to compute the mean age of the sample with a particular illness and describe whether there is an epidemiological shift in these conditions. Two nationally representative cross-sectional surveys were used for this study (2002 and 2007). The sample for the current study is 3,062 respondents aged less than 5 years. For 2002, the study extracted a sample of 2,448 under 5 year olds from the national survey of 25,018 respondents, and 614 under 5 year olds were extracted from the 2007 survey of 6,728 respondents. Parents-reported information was used to measure issues on children under 5 years old. In 2007, 43.4% of the sample had very good health status; 46.7% good health status; 2.5% poor health and 0.3% very poor health status. Almost 15% of children had illnesses in 2002, and 6% more had illnesses in 2007 over 2002. In 2002, the percentage of the sample with particular chronic illnesses was: diabetes mellitus (0.6%); hypertension (0.3%) and arthritis (0.3%). However, none was recorded in 2007. The mean age of children less than 5 years old with acute health conditions (*i.e.* diarrhoea, respiratory diseases and influenza) increased over 2002. In 2007, 43.4% of children less than 5 years old had very good health status; 46.7% good health status; 7.1% fair health status; 2.5% poor and 0.3% very poor health status. The association between health status and parent-

reported illness was $-x^2$ ($df = 4$) = 57.494, $P < 0.001$ -with the relationship being a weak one, correlation coefficient=0.297. A cross-tabulation between health status and parent-reported diagnosed illness found that a significant statistical correlation existed between the two variables $-x^2$ ($df = 16$) = 26.621, $P < 0.05$, $cc = 0.422$, – with the association being a moderate one, correlation coefficient = 0.422. A cross tabulation between health status and health care-seeking behaviour found a significant statistical association between the two variables $-x^2$ ($df = 4$) = 10.513, $P < 0.033$ -with the correlation being a weak one-correlation coefficient = 0.281. Rural children had the least health status. The health disparity that existed between rural and urban less than 5 year olds showed that this will not be removed simply because of the abolition of health care utilization fees.

Keywords: Health Conditions; Acute Condition; Chronic Conditions; Health Status; Child Health; Jamaica

1. INTRODUCTION

In many contemporary nations, objective indices such as life expectancy, mortality and diagnosed morbidity are still being widely used to measure the health of people, a society and/or a nation [1-6]. The World Health Organisation (WHO) in the Preamble to its Constitution in the 1940s wrote that health is more important than disease, as it expands to the social, psychological and physical wellbeing of an individual [7]; and lately that during the 21st century the emphasis must be on healthy life expectancy [8,9]. In keeping with its opined emphasis, the WHO formulated a mathematical approach that diminished life expectancy by the length and severity of time spent in illness as the new thrust in measuring and examining health. Although healthy life expectancy removes time spent

in illness and severity of dysfunctions, it fundamentally rests on mortality. The WHO therefore, instead of moving forward, has given some scholars, who are inclined to use objective indices in measuring health, a guilty feeling about continuing this practice.

The Caribbean, and in particular Jamaica, continues to use mortality and morbidity to measure the health of children or infants [1-6]. The use of mortality, morbidity and life expectancy is the practice of Caribbean scholars, and is widely used in Jamaica by the: Ministry of Health (MOHJ) [10]; Statistical Institute of Jamaica (STATIN) [11]; Planning Institute of Jamaica (PIOJ) [12]; PIOJ and STATIN [13] as well as the Pan American Health Organization (PAHO) [14] in measuring health. In spite of the conceptual definition opined by the WHO in the Preamble to its Constitution in 1946, the health of children who are less than 5 years old in Jamaica is still measured primarily by using mortality and morbidity statistics. Recently a book entitled 'Health Issues in the Caribbean' [15] had a section on *Child Health*; however the articles were on 1) nutrition and child health development [16] and 2) school achievement and behaviour in Jamaican children [17], indicating the void in health literature regarding health conditions.

An extensive review of health literature in the Caribbean region found no study that has used national survey data to examine the health status of children less than 5 years of age. The current study fills this gap in the literature by examining the health status of children less than 5 years of age using cross-sectional survey data which are based on the views of patients. The objectives of this study are 1) to examine the health and health care-seeking behaviour of the sample; and 2) to evaluate the mean age of the sample with a particular illness and to describe whether there is an epidemiological shift in these conditions.

2. MATERIALS AND METHODS

2.1. Sample

The current study used two secondary nationally representative cross-sectional surveys (for 2002 and 2007) to carry out this work. The sub-samples are children less than 5 years old, and the only criterion for selection was being less than 5 years old. The sample in the current study is 3,062 respondents of ages less than 5 years. For 2002, a sub-sample of 2,448 less than-5 year olds was extracted from the national survey of 25,018 respondents in 2002, and information on 614 less than-5 year olds was extracted from the 2007 survey. The survey (Jamaica Survey of Living Conditions) began in 1989 to collect data from Jamaicans in order to assess government policies. Since 1989, the JSLC has added a new module each year in order to examine that phenomenon, which is critical within the nation [18,19]. In 2002, the focus was on 1) social safety nets, and 2) crime and vic-

timization, while for 2007, there was no focus.

2.2. Methods

Stratified random sampling technique was used to draw the sample for the JSLC. This design was a two-stage stratified random sampling design where there was a Primary Sampling Unit (PSU) and a selection of dwellings from the primary units. The PSU is an Enumeration District (ED), which comprises a minimum of 100 residences in rural areas and 150 in urban areas. An ED is an independent geographical unit that shares a common boundary. This means that the country was grouped into strata of equal size based on dwellings (EDs). Based on the PSUs, a listing of all the dwellings was made, and this became the sampling frame from which a Master Sample of dwellings was compiled, which in turn provided the sampling frame for the labour force. One third of the Labour Force Survey (*i.e.* LFS) was selected for the JSLC [18,19]. The sample was weighted to reflect the population of the nation [18-20].

The JSLC 2007 was conducted in May and August of that year; while the JSLC 2002 was administered between July and October of that year. The researchers chose this survey based on the fact that it is the latest survey on the national population, and that it has data on the self-reported health status of Jamaicans. An administered questionnaire was used to collect the data from parents on children less than 5 years old, and the data were stored, retrieved and analyzed using SPSS for Windows 16.0 (SPSS Inc; Chicago, IL, USA). The questionnaire was modelled on the World Bank's Living Standards Measurement Study (LSMS) household survey. There are some modifications to the LSMS, as the JSLC is more focused on policy impacts. The questionnaire covered areas of socio-demographic variables such as education; daily expenses (for the past 7 days); food and other consumption expenditures; inventory of durable goods; health variables; crime and victimization; social safety net and anthropometry. The non-response rates for the 2002 and 2007 surveys were 26.2% and 27.7% respectively. The non-response includes refusals and cases rejected in data cleaning.

2.3. Measures

Social class: This variable was measured based on the income quintiles: The upper classes were those in the wealthy quintiles (quintiles 4 and 5); the middle class was quintile 3 and the poor were the lower quintiles (quintiles 1 and 2).

Age is a continuous variable in years.

Health conditions (*i.e.* parent-reported illness or parent-reported dysfunction): The question was asked: "Is this a diagnosed recurring illness?" The answering options are: Yes, Cold; Yes, Diarrhoea; Yes, Asthma; Yes, Diabetes; Yes, Hypertension; Yes, Arthritis; Yes, Other; and No.

Self-rated health status: "How is your health in general?" And the options were: Very Good; Good; Fair; Poor and Very Poor.

Medical care-seeking behaviour was taken from the question 'Has a health care practitioner, healer or pharmacist been visited in the last 4 weeks?' with there being two options: Yes or No.

Parent-reported illness status. The question is 'Have you had any illness other than due to injury (for example a cold, diarrhoea, asthma, hypertension, diabetes or any other illness) in the past four weeks? Here the options were Yes or No.

2.4. Statistical Analysis

Descriptive statistics, such as mean, standard deviation (SD), frequency and percentage were used to analyze the socio-demographic characteristics of the sample. Chi-square was used to examine the association between non-metric variables, and Analysis of Variance (ANOVA) was used to test the relationships between metric and non-dichotomous categorical variables, whereas an independent sample t-test was used to examine the statistical correlation between a metric variable and a dichotomous categorical variable. The level of significance used in this research was 5% (*i.e.* 95% confidence interval).

3. RESULTS

3.1. Demographic Characteristic of Sample

In 2002, the sex ratio was 98.8 males (less than 5 years old) to 100 females (less than 5 years old), which shifted to 116.2 less than-5 year old males to 100 less than-5 year old females. The sample over the 6 year period (2002 to 2007) revealed internal migrations to urban zones (**Table 1**): In 2002, 59.6% of respondents resided with their parents and/or guardians in rural areas, which declined to 5.07%. The percentage of children less than 5 years of age whose parents were in the poorest 20% fell to 25.4% in 2007 over 29.6% in 2002. In 2007 over 2002, 1.7 times less children less than 5 years of age were taken to public hospitals, compared to 1.2 times less taken to private hospitals (**Table 1**). Approximately 6% more children less than 5 years were ill in 2007 over 2002. Based on **Table 1**, less than-5 year olds with particular chronic illnesses had: diabetes mellitus (0.6%); hypertension (0.3%) and arthritis (0.3%). However, none was recorded in 2007.

There were some occasions on which the response rates were less than 50%: In 2002, health care-seeking behaviour was 14.3%; parent-reported diagnosed health conditions, 14.2%; and visits to health care institutions, 8.9% (**Table 1**). For 2007, the response rate for health care-seeking behaviour was 20.2%; parent-reported diagnosed health conditions, 20.2%, and less than 11% for

cost of medical care.

3.2. Health Conditions

Based on **Table 1**, the percentage of less than-5 year olds with particular acute conditions saw a decline in colds and asthmatic cases, as well as chronic conditions. **Figure 1** revealed that in 2007 the mean age of children less than 5 years old with acute health conditions (*i.e.* diarrhoea, respiratory diseases and influenza) increased over 2002. On the other hand, the mean age of those with unspecified illnesses declined from 1.76 years (SD = 1.36 years) to 1.64 years (SD = 1.36 years). Concomitantly, the greatest mean age of the sample was 2.71 years (SD = 1.21 years) for asthmatics in 2007 and 2.59 years (1.24 years) in 2002. It should be noted here that the mean age of a child less than 5 years of age in 2002 with diabetes mellitus was 1.50 years (2.12 years).

3.3. Health Status

In 2002, the JSLC did not collect data on the general health status of Jamaicans, although this was done in 2007. Therefore, no figures were available for health status for 2002. In 2007, 43.4% of children less than 5 years old had very good health status; 46.7% good health status; 7.1% fair health status; 2.5% poor and 0.3% very poor health status. The response rate for the health status question was 96.9%.

Ninety-seven percent of the sample was used to examine the association between health status and parent-reported illness $-x^2$ (df = 4) = 57.494, $P < 0.001$ -with the relationship being a weak one, correlation coefficient = 0.297. **Table 2** revealed that 24.2% of children less than 5 years of age who reported an illness had very good health status, compared to 2 times more of those who did not report an illness. One percent of parents indicated that their children (of less than 5 years) who had no illness had poor health status, compared to 5.6 times more of those with illness who had poor health status.

3.4. Health Conditions, Health Status and Medical Care-Seeking Behaviour

A cross-tabulation between health status and parent-reported diagnosed illness found that a significant statistical correlation existed between the two variables $-x^2$ (df = 16) = 26.621, $P < 0.05$, cc = 0.422, -with the association being a moderate one, correlation coefficient = 0.422 (**Table 3**). Based on **Table 3**, children less than 5 years old with asthma were less likely to report very good health status (5.9%), compared to those with colds (30.5%); diarrhoea (22.2%); and unspecified health conditions (22.7%).

When health status by parent-reported illness (in %) was examined by gender, a significant statistical relationship was found, $P < 0.001$: males $-x^2$ (df = 4)

Table 1. Socio-demographic characteristic of sample, 2002 and 2007.

Variable	2002		2007	
	n	%	n	%
Sex				
Male	1216	49.7	330	53.7
Female	1231	50.3	284	46.7
Income quintile				
Poorest 20%	725	29.6	156	25.4
Poor	554	22.6	140	22.8
Middle	474	19.4	126	20.5
Wealthy	402	16.4	117	19.1
Wealthiest 20%	293	12.0	75	12.2
Self-reported illness				
Yes	345	14.9	125	20.8
No	1969	85.0	475	79.2
Visits to health care facilities (hospitals)				
Private, yes	17	7.8	5	6.7
Public, yes	100	46.3	20	26.7
Area of residence				
Rural	1460	59.6	311	50.7
Semi-urban	682	27.9	125	20.4
Urban	306	12.5	178	29.0
Health (or, medical) care-seeking behaviour				
Yes	221	63.3	76	61.3
No	128	36.7	48	38.7
Health insurance coverage				
Yes, private	211	9.0	66	11.1
Yes, public	*	*	33	5.5
No	2123	91.0	496	83.4
Self-reported diagnosed health conditions				
<i>Acute</i>				
Cold	185	53.3	60	48.4
Diarrhoea	20	5.8	9	7.3
Asthma	46	13.3	17	13.7
<i>Chronic</i>				
Diabetes mellitus	2	0.6	0	0
Hypertension	1	0.3	0	0
Arthritis	1	0.3	0	0
<i>Other (unspecified)</i>	54	15.6	22	17.7
<i>Not diagnosed</i>	38	11.0	16	12.9
Number of visits to health care institutions	1.53 (SD = 0.927)		1.43 (SD = 0.989)	
Duration of illness Mean (SD)	8.51 days (6.952 days)		8.07 days (7.058 days)	
Cost of medical care				
<i>Public facilities Median (Range) in USD</i>	2.36 (157.26) ¹		0.00 (64.62) ²	
<i>Private facilities Median (Range) in USD</i>	13.76 (117.95) ¹		10.56 (49.71) ²	

¹USD1.00 = Ja. \$50.87; ²USD1.00 = Ja. \$80.47

*In 2002, all health insurance coverage was private and this was change in 2005 to include some public option.

=25.932, $P < 0.05$, $cc = 0.320$, and females $-x^2$ (df = 4) = 39.675, $P < 0.05$, $cc = 0.356$. The health statuses of males less than 5 years old in the very good and good categories were greater than those of females (**Figure 2**). However, the females had greater health statuses in fair and poor health status than males, with more males reporting very poor health status than females.

Based on **Figure 3**, even after controlling health status and parent-reported illness (in %) by area of residence, a significant statistical association was found: urban $-x^2$ (df = 3) = 10.358, $P < 0.05$, $cc = 0.238$; semi-urban $-x^2$ (df = 3) = 9.887, $P = 0.021$, $cc = 0.273$, and rural $-x^2$ (df = 3) = 45.978, $P < 0.001$, $cc = 0.365$. Concomitantly,

Table 2. Health status by self-reported illness.

Health status	Self-reported illness	
	Yes n (%)	No n (%)
Very good	30 (24.2)	227 (48.3)
Good	61 (49.2)	217 (46.2)
Fair	23 (18.5)	19 (4.0)
Poor	9 (7.3)	6 (1.3)
Very poor	1 (0.1)	1 (0.2)
Total	124	470

 x^2 (df = 4) = 57.494, $P < 0.001$, $cc = 0.297$, $n = 594$

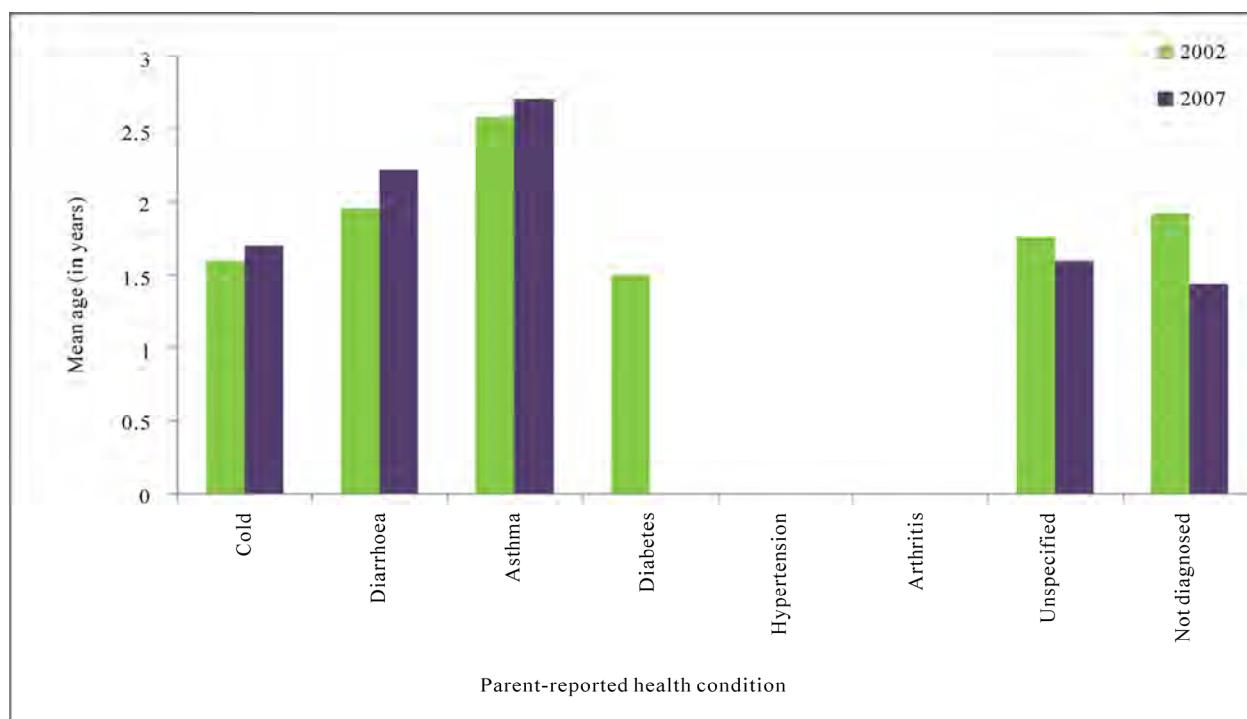


Figure 1. Mean age of health conditions of children less than 5 years old, 2002 and 2007.

Table 3. Health status by self-reported diagnosed illness.

Health status	Self-reported diagnosed illness				
	Cold	Diarrhoea	Asthma	Unspecified	No
Very good	18 (30.5)	2 (22.2)	1 (5.9)	5 (22.7)	5 (31.3)
Good	31 (52.5)	5 (55.6)	4 (23.5)	11 (50.0)	8 (50.0)
Fair	7 (11.9)	2 (22.2)	8 (47.1)	3 (13.6)	3 (18.8)
Poor	2 (3.4)	0 (0.0)	4 (23.5)	3 (13.6)	0 (0.0)
Very good	1 (1.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	59	9	17	22	16

children less than 5 years of age were the least likely to have very good health status (19.4%) compared to rural (25.8%) and semi-urban children (25.9%). Furthermore, the respondents who resided in urban areas were 2.1 times more likely to have parent-reported very poor health status, compared to rural respondents.

In examining health status and reported illness (in %) by social classes, significant statistical relationships were found, $P < 0.05$: poor-to-poorest classes $-x^2$ (df = 4) = 52.374, $P = 0.021$, cc = 0.393; middle class $-x^2$ (df = 3) = 8.821, $P = 0.032$, cc = 0.259, and wealthy class $-x^2$ (df = 3) = 10.691, $P = 0.02$, cc = 0.234. Based on **Figure 4**, middle class children who are less than 5 years old had the greatest very good health status (37%) compared to the wealthy class (26.8%) and the poor-to-poorest

classes (16.1%). Fourteen percent of poor-to-poorest class children who are less than 5 years old had at most poor health status compared to 0% of the middle class and 4.9% of the wealthy class, while 1.8% of poor-to-poorest classes less than 5 years of age had very poor health status.

When health status and parent-reported illness was examined by age, sex, social class, and area of residence, the correlation was a weak one-correlation coefficient = 0.295, $P < 0.001$, $n=583$. A cross tabulation between health status and health care-seeking behaviour found a significant statistical association between the two variables $-x^2$ (df=4) = 10.513, $P < 0.033$ -with the correlation being a weak one-correlation coefficient = 0.281. A child less than 5 years old was 2.44 times more likely to be taken for medical care if he/she had at most poor health status. On the other hand, a child who had very good health status was 1.97 times more likely not to be taken to health care practitioners (**Figure 5**).

In 2007, an examination of the health care-seeking behaviour and parent-reported illness of the sample revealed no statistical correlation $-x^2$ (df = 1) = 0.430, $P = 0.618$. Sixty-two percent of the sample, who was ill, was taken to health care practitioners, while 38.5% were not. On the other hand, more were taken for medical care than in 2007 in the 4-week period of the survey. No statistical correlation was noted for the aforementioned variables in 2002 $-x^2$ (df = 1) = 1.188, $P = 0.276$. Of those who reported ill, 63.7% were taken to health care practitioners.

4. DISCUSSIONS

Infant mortality has been declining since the 1970s, and this has further decreased since 2004 [14]; this, as the literature shows, is not a good measure of health. The current study found that, using general health status,

children less than 5 years of age in Jamaica had good health. The findings revealed that 90 out of every 100 less than-5 year olds had at least good health status, with 44 out of every 100 having very good health status. In spite of the good health status of less than-5 year olds in Jamaica in 2007, 20.8% of them had an illness in the

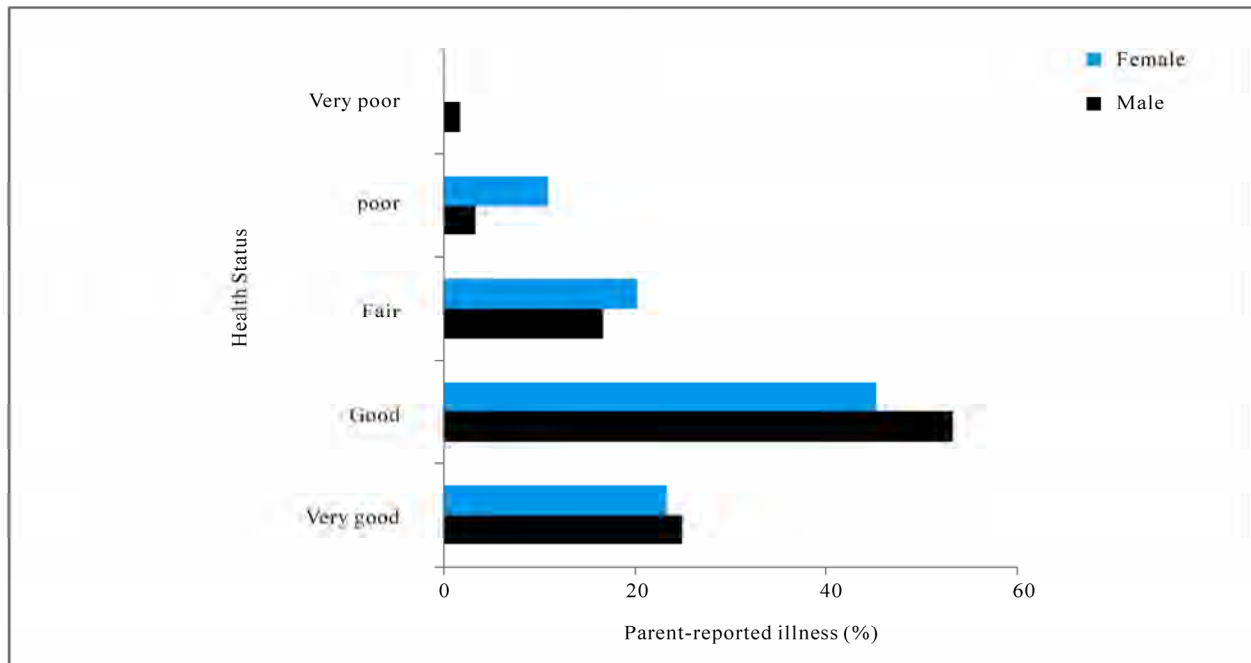


Figure 2. Health status by Parent-reported illness (in %) examined by gender.

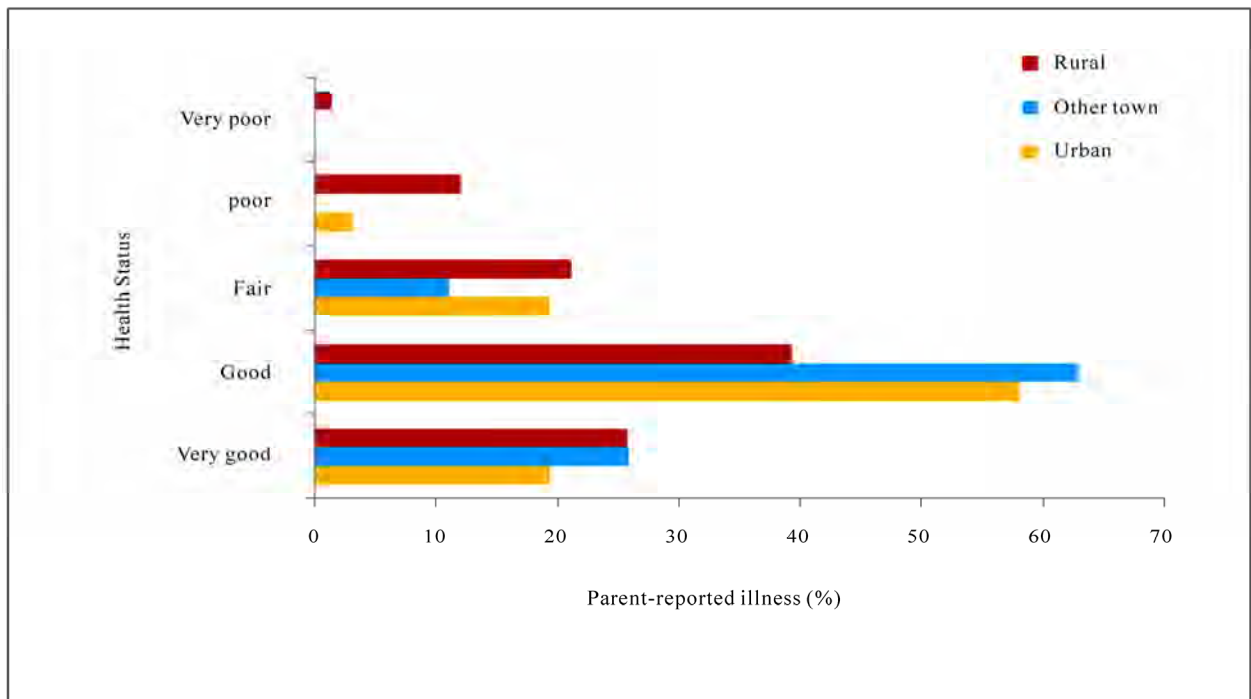


Figure 3. Health status by parent-reported illness (in %) examined by area of residence.

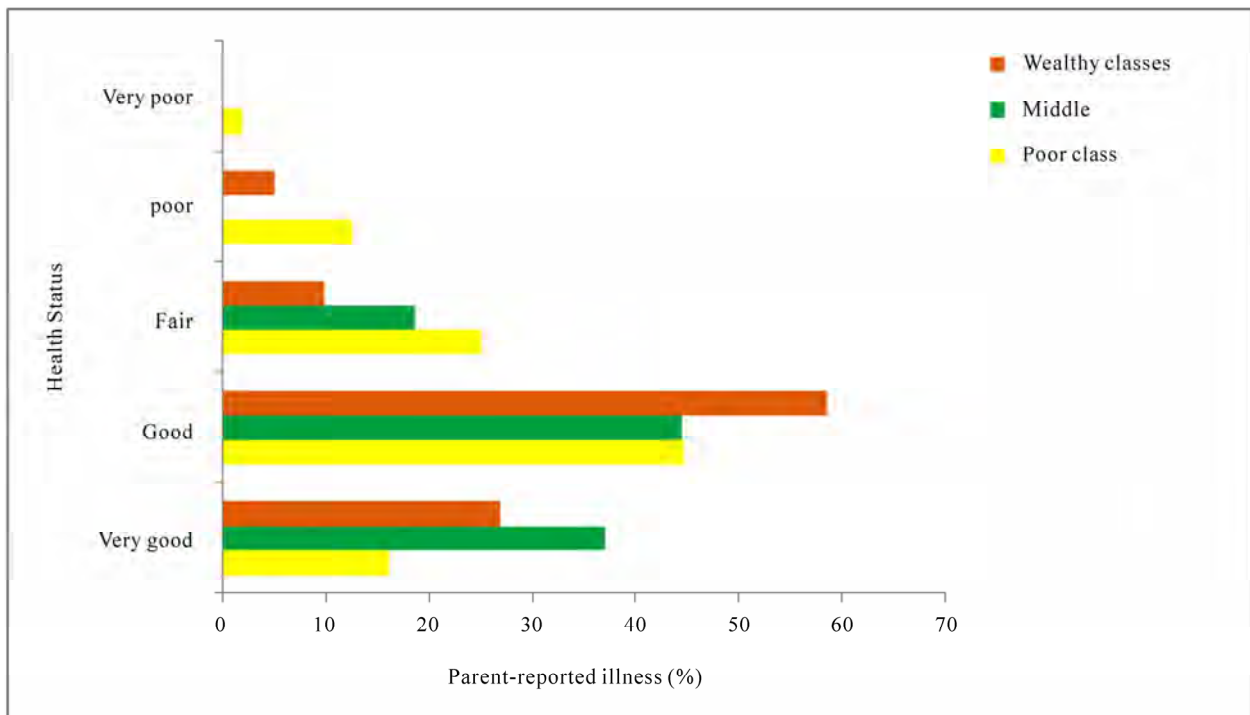


Figure 4. Health status by parent-reported illness (in %) examined by social classes.

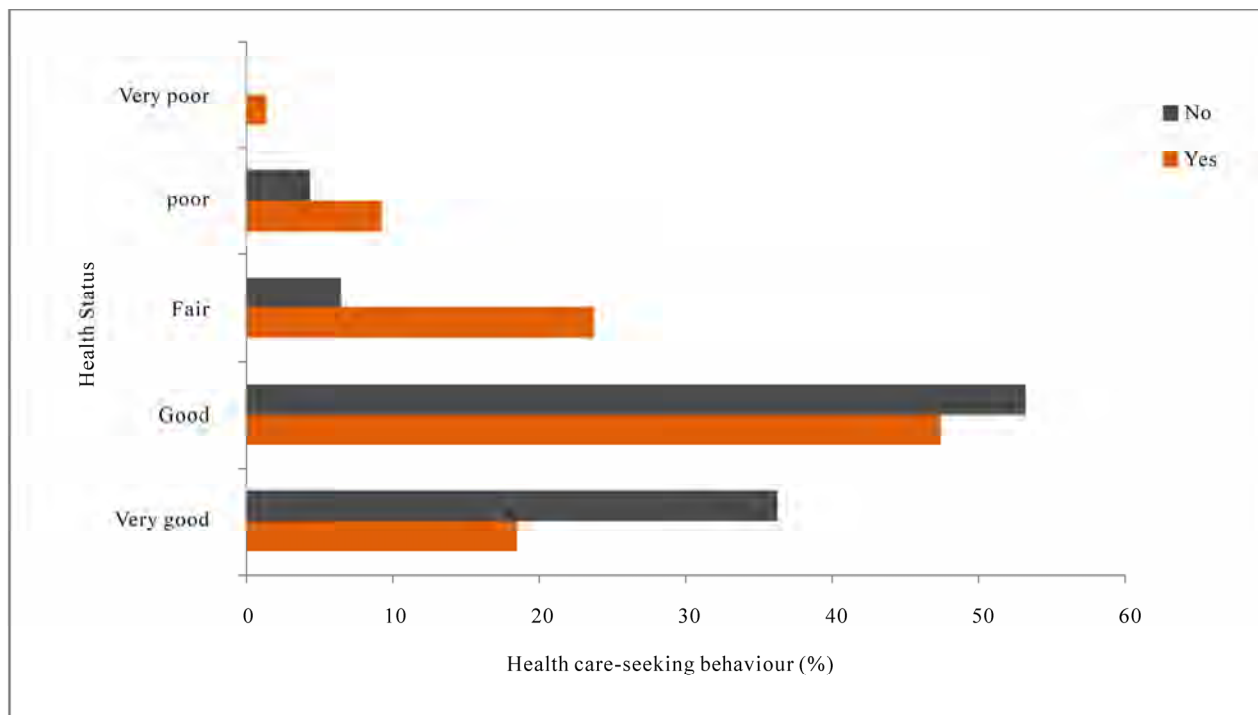


Figure 5. Health status by health care-seeking behaviour.

4-week period of the survey, which is a 5.9% increase over 2002. It is interesting to note the shift in this study away from specific chronic illnesses. In 2002, 30 out of every 1,000 less than-5 year olds in Jamaica were diag-

nosed with hypertension and arthritis (*i.e.* parent-reported), with 60 out of 1,000 having been parent-reported with diabetes mellitus. None such cases were found in 2007, suggesting that in the case of the children who had

those particular chronic illnesses, their parents had either migrated with them or they had died. Concomitantly, the country is seeing a reduction in children less than 5 years old with colds; however, marginal increases were seen in diarrhoea, asthma and unspecified health conditions over the last 6 years. Although there were increased reported cases of illness over the studied period, in 2007, 62 out of every 100 ill children were taken to medical practitioners, and this fell from 64 in every 100 in 2002. One of the arguments put forward by some people is that what retards or abates health care-seeking behaviour is medical cost. With the abolition of health care user fees for children since 2007, the culture must be playing a role in parents and/or guardians not taking children who are ill to medical care facilities for treatment.

Medical cost cannot be divorced from the expenditure that must be incurred in taking the child to the health care facility. In 2007, 25 out of every 100 children less than 5 years of age had parents and/or guardians who were less than the poverty line. Although this has declined by 4.2% since 2002, it nevertheless means that there are children whose parents are incapacitated by other factors. Marmot [21] opined that the financial inability of the poor is what accounts for their lowered health status, compared to other social classes. The current study concurs with the findings of Marmot, as it was revealed that children less than 5 years of age from poor households had the least health status. This means that poverty is not merely eroding the health status of poor Jamaicans, but that equally it is decreasing the health status of poor children.

Rural poverty in Jamaica is at least twice as great as urban poverty, and approximately 4 times more than semi-urban [13], which provides another explanation for the poor health status of children less than 5 years of age. The current study found that 3.2% of those children dwelling in urban zones recorded at most poor health status, compared to 13.6% of rural children, suggesting that the health status of the latter group is 4.3 times worse than the former. This means that poverty in rural zones is exponential, eroding the quality of life of children who are less than 5 years old. Poverty in semi-urban areas was 4% which is 2.5 times less than that for the nation; and those less than 5 years of age recorded the greatest health status, supporting Marmot's perspective that poverty erodes the health status of a people. Hence, the decline in health care-seeking behaviour for this sample is embedded in the financial constraints of parents and/or guardians as well as their geographical challenges. The terrain in rural zones in Jamaica is such that medical care facilities are not easily accessible to residents compared to urban dwellers. With this terrain constraint comes the additional financial burden of attending medical care facilities at a location which is not in close proximity to the home of rural residents, and this accounts for the vast health disparity between rural

and urban children. As a result of the above, the removal of health care utilization fees for children less than 18 years of age does not correspond to an increased utilization of medical care services, or lowered numbers of unhealthy children less than 5 years of age. If rural parents are plagued with financial and location challenges, their children will not have been immunized or properly fed, and their nutritional deficiency would explain the health disparity that exists between them and urban children who have easier access to health care facilities.

The removal of health care utilization fees is not synonymous with an increased utilization of medical care for children less than 5 years old, as 46.5% of the sample attended public hospitals for treatment in 2002, and after the abolition of user fees in April 2007 utilization fell by 1.7 times compared to 2002. In order to understand why there is a switch from health care utilization to mere survival, we can examine the inflation rate. In 2007, the inflation rate was 16.8% which is a 133% increase over 2002 (*i.e.* 7.2%), which translates into a 24.7% increase in the prices of food and non-alcoholic beverages, and a 3.4% increase in health care costs [22]. Here the choice is between basic necessities and health care utilization, which further erodes health care utilization in spite of the removal of user fees for children.

Health status uses the individual self-rating of a person's overall health status [23], which ranges from excellent to poor. Health status therefore captures more of people's health than diagnosed illness, life expectancy, or mortality. However, how good a measure is it? Empirical studies show that self-reported health is an indicator of general health. Schwarz & Strack [24] cited that a person's judgments are prone to systematic and non-systematic biases, suggesting that it may not be a good measure of health. Diener, [25] however, argued that the subjective index seemed to contain substantial amounts of valid variance, indicating that subjective measures provide some validity in assessing health, a position with which Smith concurred [26]. Smith [26] argued that subjective indices do have good construct validity and that they are a respectably powerful predictor of mortality risks [27], disability and morbidity [27], though these properties vary somewhat with national or cultural contexts. Studies have examined self-reported health and mortality, and have found a significant correlation between a subjective and an objective measure [27-29]: life expectancy [30]; and disability [28]. Bourne [30] found that the correlation between life expectancy and self-reported health status was a strong one (correlation coefficient, $R=0.731$); and that self-rated health accounted for 53% of the variance in life expectancy. Hence, the issue of the validity of subjective and objective indices is good, with Smith [26] opining that the construct validity between the two is a good one.

The current research found that parent-reported illness and the health status of children less than 5 years of age

are significantly correlated. However, the statistical association was a weak one (correlation coefficient=0.297), suggesting that only 8% of the variance in health status can be explained by parent-reported children's illnesses. This is a critical finding which reinforces the position that self-reported illnesses (or health conditions) only constitute a small proportion of people's health. Therefore, using illness to measure the health status of children who are less than 5 years of age is not a good measure of their health, as illness only accounts for 8% of health status. However, based on Bourne's work [30], health status is equally as good a measure of health as life expectancy. One of the positives for the using of health status instead of life expectancy is its coverage in assessing more of people's general health status by using mortality or even morbidity data.

5. CONCLUSIONS

In summary, the general health status of children who are less than 5 years old is good; however, social and public health programmes are needed to improve the health status of the rural population, which will translate into increased health status for their children. The health disparity that existed between rural and urban children less than 5 years of age showed that this will not be removed simply because of the abolition of health care utilization fees. In keeping with this reality, public health specialists need to take health care to residents in order to further improve the health status of children who are less than 5 years old.

5.1. Conflict of Interest

The author has no conflict of interest to report.

5.2. Disclaimer

The researcher would like to note that while this study used secondary data from the Jamaica Survey of Living Conditions, 2007, none of the errors that are within this paper should be ascribed to the Planning Institute of Jamaica or the Statistical Institute of Jamaica as they are not there, but owing to the researcher.

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Cutting and other forms of derma-abuse in adolescents

Hari D. Maharajh¹, Rainah Seepersad²

¹Department of Clinical Medicine, University of the West Indies, Trinidad, West Indies; drharim@carib-link.net

²Psychologist, Department of Clinical Medicine, University of the West Indies, Trinidad, West Indies; rainahs@hotmail.com

Received 22 November 2009; revised 25 December 2009; accepted 28 December 2009.

ABSTRACT

Cutting or self inflicted epidermal damage (derma-abuse) describes a number of blood-letting behaviours among adolescents. Unlike suicidal behaviour, it is associated with low lethality and the absence of suicidal attempts. The purpose of this study is two-fold: Firstly, to present and discuss vignettes of four young adolescents and secondly, to study the dynamics and characteristics of six derma-abusers who have attended Dual Group Therapy (DGT) concurrently with their parents for a six month period. Our findings suggest that patients involved in derma-abuse are generally non-suicidal but engage in comfort cutting for the psychological release of pain, tension reduction and anger management. There is a preponderance of females (80%) with an over-representation of mixed origin and borderline cultural states. In this small group, males amounted to 20% and were more bizarre, gruesome and brutal in their self-abuse. Of the total sample, 10% were of African origin, 60% were of Indian descent and 30% were of mixed ancestry. Psychodynamic factors explored in Dual Group Therapy (DGT) are the emphasis on non-suicidal intent, association with tension reduction, reclaiming power and mastery over self and others, life and death instincts, the significance of bloodletting in a socio-cultural context, trans-generational conflicts, dysfunctional family dynamics frequently with parental separation and sexual abuse and early sexual induction.

Keywords: Derma-Abuse; Cutting, Self-Harm; Adolescents; Non-Suicidal Intent

1. INTRODUCTION

Self-inflicted epidermal damage, referred to as derma-abrasion and derma-contusion are common practices among young adolescents. There is much confusion in

the classification of suicidal behaviours with the general view that self-inflicted human blood release is equated to suicidal behaviour. The literature is replete with descriptive terminologies: Suicide and parasuicide [1] suicide and deliberate self poisoning/injury [2], and Non-fatal deliberate self-harm [3]. Other synonyms are “self injury” (SI), “self-harm” (SH) “self-mutilation,” “deliberate self-harm”, (DSH) “self injurious behaviour” (SIB), and “self inflicted violence” (SIV) which are used interchangeably to explain common patterns of behaviour where demonstrable injury is self inflicted [4-9].

Self-mutilation has its origin in many cultures around the world. In ancient Mayan civilizations, Sadhus or Hindu ascetics and early Catholic and Jewish Canaanite rituals, all involved some form of bloodletting or self flagellation that are associated with great religious and spiritual sacrifice or rites of passage [10]. In the 1880's, this form of behaviour was the norm among cultures and was not distinguished from other behavioural problems. In 1935 and 1938, an important distinction was made with a modification of the term self-mutilation that was initially introduced by L. E Emerson [10]. This differentiation considered the view that suicidal behaviour and self-mutilation were two separate entities. As Menninger stated in his book “self-mutilation was a non-fatal expression of an attenuated death wish” [10].

Internationally, the most common form of clinically determined self-harm is skin cutting. This occurs in 70% of the individuals that harm themselves, followed by the act of banging or hitting oneself (21% to 44%) and lastly 15% to 35% of persons who engage in acts of burning themselves [11,12]. In non-clinical populations such as college samples, the most common form of self-injurious behaviour was severe forms of scratching and pinching which results in bleeding and scarring (51.6%). This was followed by acts of hitting objects to the point of blood release (37.6%), then cutting (33.7%) followed by acts of punching and banging with blood release (24.5%). Body surface areas targeted by self-harmers are the areas that are of easiest access such as arms, hands, wrists, thighs and abdomen [13-15].

However, there seems to be no agreement on findings of deliberate self-harm since similar rates have been re-

ported in both institutional and community populations. In non-clinical populations, 4% of the adult population had engaged in deliberate self-harm with a similar finding of 4% in military recruits, (33). College populations as a rule have reported higher rates of DSH, ranging from 14%-38% [4,12,16,17]. In Europe, for persons over the age of fifteen (15), there is an average rate of 0.14% for males and 0.19% for females [18].

In the local setting, self-harm has been on a steady rise over the past decade. A conservative estimate of the incidence rate of students referred to a psychiatric clinic is about 0.5% percent of secondary school students in Trinidad. Approximately four cases per month are reported at the Eric Williams Medical Sciences Complex at Mt. Hope with an emphasis on derma-abuse or skin cutting. In comparison to larger countries such as in England 6.9% of students' ages 15 and 16 in a cross sectional study of 41 schools reported acts of deliberate self-harm, [19].

In 2007, a newspaper report on 'cutting' among girls in Trinidad sparked the issue of a mental health crisis [20]. Further, two recent surveys conducted on non-clinical populations have revealed high rates of self-harmers. In a sample of 215 students at the University of the West Indies, the overall prevalence of self-harmers was found to be 24.2 percent with 9.3 percent notated as recent self-harmers and 14.9 percent engaged in self-harming behaviour over the past year [21]. Among the students reporting recent (within the past twelve months) self-harm, the most frequently utilized methods were cutting (70%), sticking oneself with sharp objects (50%), and scratching oneself (45%). Students invariably utilized multiple forms of derma-abuse.

Analysis of the self-harmers over a one year period revealed that those who reported recent self-harm behaviour had an average of seven times as many ($M = 35.6$ $s.d = 54$) incidents than those with a past history of self-harm behaviour. In another study of 174 students, [22] there was an overall prevalence rate of 31.6 percent with a history of self-harm. In terms of recent self-harm 11.5 percent indicated this in comparison to 20.1 percent who engaged in self-harm behaviour more than a year ago. Within this sample 8.6 percent reported cutting, 8.6 percent indicated severe scratching and 6.9 percent, needle sticking. Of interest, 9.2 percent admitted to consuming pills, consumption of excessive amounts of alcohol, hair pulling (trichotillomania) and food refusal, [22]. An interesting finding is that although these studies were conducted at the same University in Trinidad during the same year, differential rates of 31.6% [22] and 24.2% [21] were recorded for self-harm behaviours.

It is evident that many researchers have described a medley of behaviours that have been categorized as life threatening and equated with suicidal intent. While some authorities [19,23,24] have commented on the low lethality of derma-abusers, the boundaries appear to be blurred.

The purpose of this study therefore is two-fold: Firstly, to present and discuss vignettes of four young adolescents and secondly to study the dynamics and characteristics of six derma-abusers who have attended group psychotherapy for a six-month period with emphasis on their suicidality and treatment.

1.1. Theories of Self Harm

There are many explanatory models of self-harm that encompass various theories in psychology. Self-harm has been described through Behavioral and Systems theories, Psychodynamic and Psychoanalytical models as well as Interpersonal and Object relations approaches [25,26].

Behavioral and Environmental models theorized that self-mutilation creates internal or environmental responses that are reinforcing to the individual. The Drive models purport a psychoanalytical understanding of the self-harm behavior, specifically with the Anti-suicide and Sexual model. The Anti-Suicide model claims that self-mutilation is a suicide replacement, an attempt to avoid suicide, a compromise between life and death drives, and a sort of 'microsuicide.' The Sexual model states that self-mutilation stems from conflicts over sexuality, sexual development, masturbation, menarche and menstruation [25,26].

The Affect Regulation Models offer a psychodynamic explanation through the affect regulation model and the dissociation model. The Affect Regulation model claims self-mutilation stems from the need to express or control anger, anxiety, or pain that cannot be expressed verbally or through other means whereas the dissociation model states that self-mutilation is a way to end or cope with the effects of dissociation that results from the intensity of affect. Many self-harmers report that they want to feel alive again and acts such as skin cutting removes their feelings of numbness [25,26].

The Boundaries Model which builds its explanatory power on interpersonal and object relations theories state that self-mutilation is an attempt to create a distinction between self and others. It creates boundaries or an identity to protect against feelings of being engulfed, on the other hand a fear of loss of identity. It reinforces self-mutilation as evidence of familial or environmental dysfunction [25,26].

1.2. Objective

In this clinical study, prefaced with a comprehensive review of the local and international literature, four vignettes and six patients and their families in Dual Group Therapy (DGT) are studied over a six month period. The purpose is to define socio-demographics characteristics and to understand the dynamics of derma-abusers in the context of interpersonal, trans-generational and environmental factors. An appropriate management strategy is devised.

2. RESULTS

2.1. Vignette 1

I. S. is a 13 year old female student, of Caucasian descent, who resides in Singapore. She was born in Florida, of mixed origin and of the Roman Catholic faith. She came to Trinidad for treatment since she could not be contained in Singapore. She has Trinidadian roots as most of her family is originally from the island. The patient has been reportedly skin-cutting since 2007 when she was eleven years old. She reported that she had accidentally cut herself with a broken tea cup during one of the many arguments of her parents. She further stated that her 'accidental injury' had alleviated her emotional confusion and made her feel relaxed. In 2008, she was hospitalized for a two (2) week period after a cutting incident while in Singapore. On her release, it was discovered that she smuggled a piece of glass into the hospital by concealing it in her clothing and had continued cutting herself on her thighs and was further warded at the facility. In July, in Trinidad, she became so distraught and tense, she begged her Aunt who was visiting from Florida to allow her 'to make just a little nick on her wrist to alleviate her confusion'. Her most recent episode was in September 2009. She presented for cutting her left wrist at the Health Facility and subsequently taken to the University Hospital and warded at the Paediatric Ward. The patient with a history of skin cutting and burning indicated her most recent cutting was not a suicidal attempt but was used to eliminate stressors in her life, inclusive of a strained relationship with her cousins. In a review of her developmental, personal and family history she has had somewhat of a tumultuous past from an early age. As a toddler, she exhibited temper tantrums at age 3, her parents divorced when she was age five (5), and she reported that her mother has been in abusive relationships, not only with her father, resulting in her having to move between Malaysia and Singapore.

Her developmental milestones were normal but early visits to her Paediatrician had shown evidence of precocious development. At a routine pediatric checkup at age 6, the patient was noted to have a unilateral breast bud and pubic hair. The pediatrician referred her to an Endocrinologist where FSH, LH and other hormonal levels testing were done. They were all within normal range. A bone age scan was also done which showed that the age of her bones were consistent with her chronological age. Subsequently, a unilateral ovarian cyst was discovered via ultrasound. This was monitored for six months and at the second ultrasound no cysts were found. The endocrinologist has since disconfirmed precocious development despite her advanced sexual development. In Trinidad, brain scans CT and

MRI were found to be normal as well as Electroencephalographic (EEG) studies.

In her personal history, she has shied away from her usual extracurricular activities such as netball, football and swimming when she started cutting. Her attempts to conceal the scars have resulted in her lack of interest in other activities but she still continued in the school choir. I.S. described herself as always being below average, and always had difficulty concentrating since very young expressing that she has always been taken long periods to complete assignments. She has never got into any physical fights at school or otherwise. Her history of friendships has been mixed with some "bad" friendships, but presently she has trustworthy friends. She lives with her mother, her mother's fiancée and his children and will be returning to Singapore soon. Her maternal aunts and mother are being treated for depression.

2.2. Vignette 2

M. A is a 16 year old female student of East Indian descent who resides in Trinidad. She is the youngest of three children. She presented on this occasion with ingestion of six (6) Painol tablets and two (2) painkillers. The incident occurred in August 2009 and was precipitated by an argument with her current boyfriend who is eight years older and a friend of her brother. She described herself as feeling depressed, hopeless and frustrated, with loss of interest in activities. She normally enjoyed listening to music and watching television, but did not have any intent to die. After this incident, she reported feelings of sadness for long periods over the next two days. M. A has a prior history of skin cutting which had started two years ago.

Her first incidence of skin cutting was in early Form 3 (age 13). She described feeling angry but cannot remember the details of the incident. She also stated that she engaged in banging her fists against the walls in her bedroom when she felt upset and frustrated due to arguments with her parents surrounding incidents with her boyfriend.

Both her parents' family has a history of depression. Her father's two cousins have depression and one of her mother's brothers has been committed to a mental institution following a nervous breakdown. One has also committed suicide. The patient herself has also been treated for depression on her initial visits to the psychiatrist.

Her developmental history was insignificant as developmental milestones were reported in congruence with her age. She began puberty at around age twelve (12). She has had no major accidents or illness to require hospitalizations. She has visited a psychologist for a few sessions after she broke school on the first occasion. Her relationship with her parents and brother has been somewhat average since the incidents occurred but

presently the family ties are improving.

In terms of her personal history, M. A has reported to having two previous relationships from the age of 12 which lasted two (2) months, and then at age 13 with a twenty one (21) year old man, which lasted one (1) year and eight (8) months. M. A's current boyfriend is twenty five (25) and this relationship developed as she enjoyed conversing with him as well as being a family friend. She expressed that she enjoys school very much and gets along with everyone including her friends. She has no history of aggressive behavior. Her performance in school is fair, but her grades have been falling due to her involvement with her boyfriend.

Both her parents family have a history of depression. Her father's two cousins have depression and her mother's family has depression. One of her mother's brother has been committed to a mental institution following a nervous breakdown and one committed suicide. The patient herself has also been treated for depression on her initial visits to the psychiatrist.

2.3. Vignette 3

S. M is a fourteen year old Secondary School student who was referred to the Psychiatric Services for self-harm, following a self inflicted tattoo which he carved on his left arm with a symbol of his initial S. He did this without the permission of his parents because "he wanted to feel pain". He mutilated his forearm with a razor blade and covered it with ink in order to make a tattoo.

In addition, the school guard found letters in his possession written in blood and ink which were messages of hate. He stuck a fountain pen into the vein of his forearm thereby withdrawing blood and wrote a letter to his alleged girlfriend.

In his past history, at the age of five years on a school excursion he was separated from the class and claimed that people stamped on his chest. He was found by two strangers who carried him back to school. At the age of nine years, he received electric shocks from open wires with no serious injuries. It is not known whether these were accidental.

Both his parents are alive and he will drink with them on special occasions and will even smoke cigarettes. He has no sexual relationship but claims that he has many girlfriends, defining a girlfriend as "someone to be with when feeling down." At the age of fourteen, he suffered a fracture of the radius due to a fight at school. He was close to his grandfather who recently died.

On interview, he was properly groomed adequately clothed with a relaxed behavior. His affect was appropriate and speech fluent. He said he did not believe in God. He gave no reasons for his behavior and appeared to be smug about it.

2.4. Vignette 4

A. P is a thirteen (13) year old male Form 3 secondary

school student of Indo-Guyanese descent. He was referred by the school guidance officer with a two month history of carving a tattoo on his left forearm with the inscription 'Sasha' his girlfriend. He painted it with ink creating a self-made tattoo. His mother reported that he is aggressive at home, stealing jewelry and money allegedly giving it to his girlfriend. He spends a considerable amount of time at night speaking to the girl on the phone which his mother attributes to his poor performance at school.

He was born in Guyana and was kept at the hospital for an extra week due to an infection. His developmental milestones were normal but his mother noted that he is extremely short tempered and responds with rage at the slightest provocation. He is the last of three (3) siblings with an older sister and brother. He does not get along with his brother and recently pulled a knife at him.

He came to Trinidad six (6) years ago with his mother, who has been separated from his father for nine (9) years. Presently he lives with his grandmother, grandfather and brother aged seventeen (17). His mother is now in a second relationship with a new husband for the past eight (8) years. A. P does not get along with his stepfather and accuses him of stealing the lost money and jewels. All members of his family except his stepfather are of Guyanese origin. He denies the use of tobacco, alcohol and drugs. He was diagnosed as having an impulse control disorder in his first contact with the psychiatric services on the island.

With respect to his derma-abuse, he feels no pain on carving and is supported by his girlfriend who is extremely thrilled at his show of love. His mother has contacted her on this issue and she has denied receiving money and stolen rings from him but is adamant that no one can stop him from seeing her.

3. ANALYSIS OF STUDIED GROUP

3.1. General Classification of Suicidal Behavior with and without Intent

In the figure below, a classification based on a small sample of ten (10) derma-abusers is presented. Patients involved in derma-abuse are generally non suicidal but engage in comfort cutting for the psychological release of pain, tension reduction and anger management. In this small group, males amounted to 20% and were more bizarre, gruesome and brutal in their self-abuse. Females accounted for the majority of the sample (80%) and among these; approximately 38% were of mixed origin. Of the total sample 10% were of African origin, 60% were of Indian descent and 30% were of mixed ancestry. The high percentage of abusers of mixed origin was unexpected and a plausible explanation is that these adolescents find themselves in a borderline cultural state.

These are individuals who are unable to conceptualize which culture they belong to and consequently develop identity issues in their attempts to please both parents. The value assigned to each parent is often based on stereotyped racial pecking order and the environmental influences of parental dominance and autonomy.

In this small sample of ten derma-abusers, patients were categorized into three groups: those without suicidal intent, those with suicide in mind and a third category of delayed onset, secondary suicidal thoughts. It is noteworthy that in more than 80% of the sample, suicide

or thoughts of death was not the initial intent and apparently developed following intervention, after the patient's discovery of its importance as a powerful manipulative tool. (**Table 1**)

In **Table 2** below, a number of characteristics of derma-abusers are outlined. These are observations taken from group psychotherapy and concurrence with the group therapist following the sessions.

In **Table 3** above the socio-demographic characteristics of adolescents in group psychotherapy were tabularized to highlight commonalities among derma-abusers.

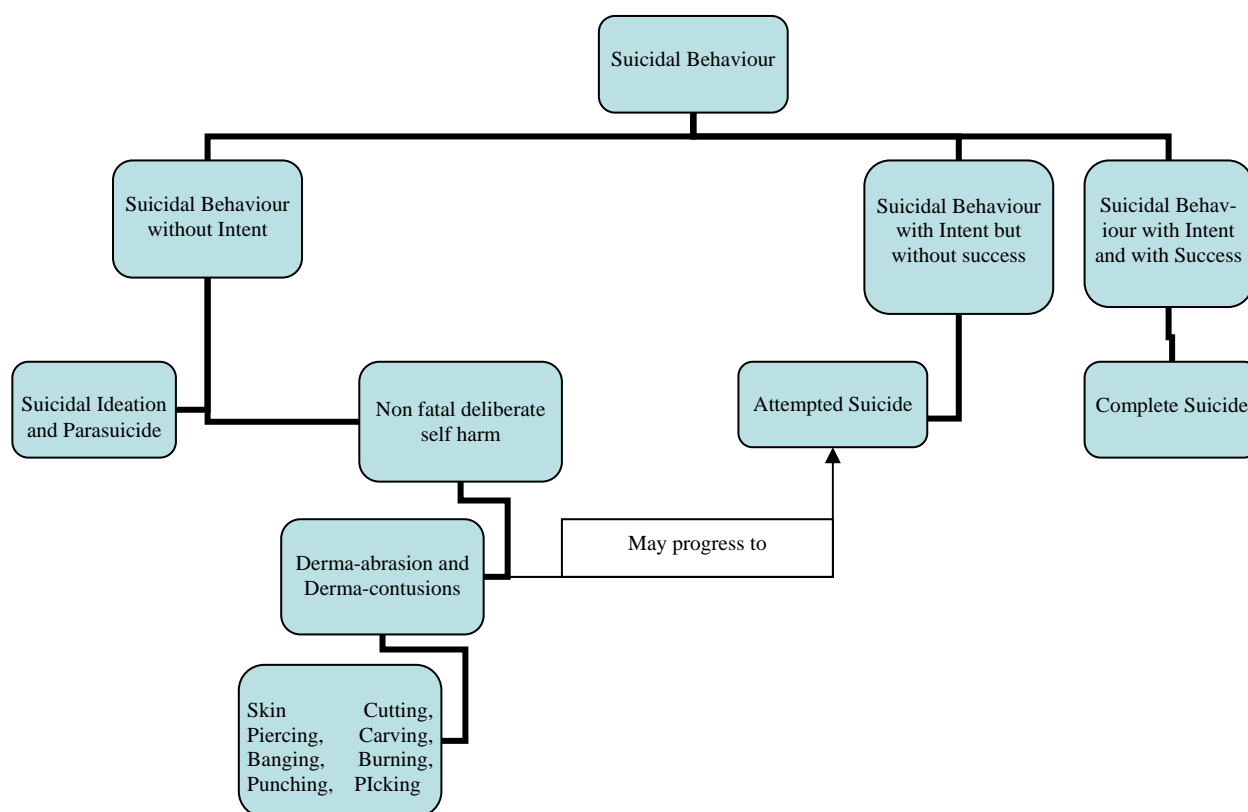


Figure 1. General classification of derma abusers.

Table 1. Categorization of derma abusers in trinidad.

Derma-Abusers		
Without Suicidal Intent	With Suicidal Intent	Mixed Group with later suicidal onset
<ul style="list-style-type: none"> • Chronic Harmers • High Predictability <ul style="list-style-type: none"> • Low lethality • Intense family Pathology • Low impulse control 	<ul style="list-style-type: none"> • Harmer usually fits the criteria below: <ul style="list-style-type: none"> Plan-distinct Lethality-High Intent-High Method Timing • Intense personal Pathology 	<ul style="list-style-type: none"> • Chronic attempters • Any available method • Family pathology • Personal Pathology

Table 2. Characteristics of derma-abusers as recorded in group psychotherapy.

Psychodynamics of adolescent Derma-abusers in Trinidad	
1.	Emphasis on non-suicidal intent
2.	Associated with tension reduction
3.	Spontaneous overflow of emotion with low impulse control
4.	Rejuvenation of loss of emotional resonance
5.	Life and Death instincts- Eros and Thanatos considered
6.	Reclaiming power and mastery over self and others
7.	Significance of bloodletting in a socio-cultural context
8.	Transgenerational dysfunctional family dynamics frequently with parental separation and sexual abuse
9.	Physical and developmental disorders in early childhood
10.	Morbid relationship with creativity with respect to body carving and architectural designs
11.	Contemporaneous influences of youth culture
12.	Reinforcement by family and help-seeking services

Table 3. Socio-demographic characteristics of the four (4) Patients presented in the vignettes and six (6) in group psychotherapy.

Demographics	Vignettes* and Group Psychotherapy Cases									
	I. S*	M. A*	S. M*	A. P*	R. A	K. E	A. M	O. M	A. T	T. S
Sex	F	F	M	M	F	F	F	F	F	F
Ethnicity	Mixed	East Indian	East Indian	East Indian	Mixed	East Indian	Mixed	East Indian	African	East Indian
Religion	Roman Catholic	Roman Catholic	Hindu	Hindu	Roman Catholic	Hindu	Roman Catholic	Roman Catholic	Christian (Pentecostal)	Pentecostal
Educational Level	Secondary (Junior)	Secondary (Senior)	Secondary (Junior)	Secondary (Junior)	Tertiary (University)	Tertiary (University)	Secondary (Senior)	Tertiary (University)	Secondary (Senior)	Secondary (Junior)
Family Structure	Single Parent	Nuclear	Nuclear	Single Parent	Nuclear	Single Parent	Single Parent (father died)	Adopted blended	Single Parent	Nuclear dysfunctional
School Performance	Borderline	Fair	Fair	Poor	Borderline	Good	Poor	Poor	Poor	Poor
Intermarriage by race/religion/nationality	Caucasian white & Indian	Hindu & Muslim	Presbyterian & Hindu	Guyanese & Trinidadian	Indian & African	Guyanese & Trinidadian	Indian & African	Muslim & Roman Catholic	No	No
Method	Skin Cutting	Banging & Cutting	Carving	Carving	Skin Cutting	Skin Cutting	Skin Cutting	Skin Cutting	Wrist/Skin Cutting	Skin cutting
Onset of self harm	11	13	13	15	20	16	13	18	16	13
Co morbidity	Depression	Depression	Non	Family Dysfunction	Depression	Depression	Childhood Epilepsy & Conduct Disorder	Borderline Personality Disorder	Conduct Disorder & Hypersexuality	Conduct Disorder; early sexual induction & Somatic complaints
Family history of Psychopathology	Bipolar disorder	Depression	Non	Non	Depression & Alcohol Dependence	Alcohol Dependence	Non	Personality Disorder (mother) Psychopath (brother)	Non	Nervous disorder (mother)

3.2. Profile of Adolescent Derma-Abusers

From the sample investigated, a general profile was deduced to represent the description of a typical derma-abusing Trinidadian adolescent. Eighty percent (80%) of the cases in Trinidad appear to be adolescent girls, rang-

ing from ages 11 to 16 years with onset of self harm in early teenage years. They appear to be of East Indian or mixed descent of both the Roman Catholic or Hindu faith and attending Secondary School. The derma-abusing adolescent seems to have an equal chance of coming from either a nuclear or single parent family (absence of

father figure), though, within the nuclear family there is usually a dominant parent, the mother. Their family background seems to be of mixed origin, either by religion, race or nationality and there is a history of family dysfunction and instability. Their performance at school spans the poor to borderline ranges and the individual is usually diagnosed with a depressive disorder due to relational conflicts. In some cases there is a history of Conduct Disorder in childhood.

Associated psychosocial factors are low self-esteem, body image and identity disturbances and early courtship and sexual induction. This is on a background of Trinidad and Tobago having the second highest rate of suicide in the Caribbean region and may be a precursor for such behaviour in adulthood. Different forms of derma-abrasion and derma-contusion observed among the sample were skin cutting, banging, scraping, carving, burning and branding, picking at the skin and removal of blood with instruments (pen and needles). Skin cutting was more prevalent amongst the female sample and carving with tattooing was present in all the male derma-contusion cases. It seems the intensity of the latter paints a more bravado picture of sacrifice to a loved object when compared to skin cutting by the females. Sexual drives are a major operative factor in both male and female derma-abusers.

4. DISCUSSIONS

An examination of the four (4) vignettes and six (6) cases treated in Dual Group Therapy (DGT) highlights significant commonalities in the life histories and presenting concerns of all patients. The vignettes and group psychotherapy cases presented are of adolescent individuals who began self-harm between the ages of 11 to 20, with a mean of 14.8 years and 80% between the 11-16 age group. According to the literature, studies have reinforced that individuals aged 11-25 have been known to self-injure [27].

Eighty percent (80%) of the cases discussed here were children of intermarriages by race, religion or nationality. It raises the issue of identity confusion and misunderstanding of culture and practice as it starts at the family level. The author is of the opinion that these individuals suffer from a borderline cultural state which results in their poor conceptualization of which culture they belong to. This cultural confusion in ethnicity, religion and nationality is often stratified by the environment in which one lives and can result in identity splitting and confusion. In psychodynamic terms, blood-letting can be viewed as an individual attempt to remove the bad blood or bile of their mixtures in a purging process. Durkheim's theories of anomie, egoistical and altruistic behaviors and Erikson's stages of development are applicable here.

On closer inspection, the precipitating cause of self-harm is strongly associated with the establishment of

early relationships with sexual induction as evidenced by 70% of the cases. This holds commonalities as those purported by the Sexual Model of self-mutilation. Among the teenage population, sexual experimentation and risk taking behavior is a common aspect of this age group. With numerous coping strategies to aid in the tension reduction needed, caused by volatile partnerships, derma-contusions seemed to be prevalent. Within the present sample of self-harmers skin cutting was observed in all of the females and carving, being the derma-contusion of choice among the male cases. It seems the intensity of the latter paints a more bravado picture matching the male image, in comparison to 'skin cutting' portraying a slightly less gruesome, 'romantic' sacrifice. Though two different forms, both make the assumption of the ultimate sacrifice, bloodshed.

The cases presented underlie the occurrence of trans-generational dysfunctional family dynamics as shown in **Table 3**. Approximately 60% of cases report family separation, divorce, transcultural differences and family psychopathology. In the nuclear family there was invariably the presence of a dominant parent which served as a major stressor in the individuals' life. In addition, there were high rates of psychiatric disturbances (80%) and psychosocial difficulties (100%), especially the prominence of mood disorders (40%) in individuals who self-harm within the present sample, as reported by previous studies [28]. It is likely that a substantive proportion of these patients will progress to Bipolar disease. Aggressive tendencies, emotional disorders, temper tantrums, conduct disorders and teenage angst were prevalent. The aggregate of emotions that are expected of this age group coupled by intense family psychopathology as expressed by 60% of the sample and personal psychopathology as indicated by 90% of the cases seem to be antecedents of self-harming behavior. Most of the individuals in the vignettes and group therapy cases have stated that they use these behaviors as a way of expressing anger and frustration when emotions are at a high and the overflow is unbearable, whereas some individuals self-harm to prevent suicide, or escape unwanted feelings, as indicated by the Anti Suicide Model [25,26].

As expressed by Vignette 2, she was unable to explain the situations surrounding her first skin cutting episode but was certain of the fact that she was extremely overwhelmed by anger. Since banging her fists on the wall ceased to work anymore, she upped the ante to a more punitive method that she felt helped at stressful times. In a recent interview with M.A she stated that she was faced with a situation concerning an assignment, in which she had to redo a portion that she assumed was finished. She reported that for a brief moment she thought of cutting but reconsidered her actions. M.A's behavior seemed to hold commonalities to the Affect Regulation Model of self-mutilation as she was overwhelmed by emotion. The extent of the behaviors and

meaning of their acts are unknown to them and are often given interpretative credence in treatment. Also, their behavior is significantly different from suicidal behavior with intent as the individuals in these cases have made their scars public. As suggested by Hawton [29] self harm behavior is distinctly suicidal if the act is "planned for, carried out and followed through in such a way as to keep it from the notice of others." Even though derma-abusers may try to hide their wounds the target areas are easily noticeable despite concealment with hand bands or clothing.

The new found control that has been indicated by some of the above cases and vignettes has been the main function of the deliberate derma-contusions that are self-inflicted. It may be apparent that the manifestation of family psychopathology and family strife is showing itself in adolescence as creative forms of 'bloodletting' as it parallels Hippocrates early assumptions of 'purging of bad humors.' [30]. Seventy five percent of the cases report their bloodletting as an addiction that they desperately need to engage in with the likes of alcohol and drugs. It seems that a derma-contusion returns it users to an equilibrium state that is required for their existence. This supports the underlying commonality among the cases with the lack of suicidal intent. They perceive their self-harming behavior as a form of rejuvenation by letting the bad blood out rather than as a destruction of body tissue.

An important observation is the predictability and chronicity of the self-harming behavior without suicidal intent. In most of the vignettes derma-abusers repeated these behaviors as certain events presented in their lives, indicative of maladaptive coping mechanisms as well as a need to remedy the situation at the moment, suggestive of perhaps a hopeful future. An emphasis here can be placed on a compromise being made between life and death instincts of psychoanalytical theory, specifically the Anti Suicide Model. They derive pleasure not from stereotypical pleasurable behaviors but rather from aggressive, self punitive behaviors that widens the power differential between themselves and others. In an effort to heal, they set themselves apart from normal methods of remedy, lending resemblance to the Boundaries model of self-mutilation. In Vignette 4, A. P felt that he was showing his commitment to his cause when he carved the name of his girlfriend along his forearm. He seemed to be giving of himself wholly in a way he probably could not express in words as proffered by the Affect Regulation Model of self-mutilation [25,26].

The excessive compulsion and obsessive psychological dependence of derma-abusers seems to be cognizant of its chronicity among the individuals inflicted with the addiction. Fifty percent of them seem to experiment with different methods of self-harm before the addiction of the tool of choice develops. It seems though, when in desperation the tool of choice may best be substituted

with an available option. As illustrated by Vignette 1, I.S went to great lengths to conceal a piece of glass within her clothing, and thought nothing of it as she continued to deliberately harm herself at the hospital, even though she found comfort using razorblades. Prior evidence of this kind of dependent behaviour can also be seen where she begged a relative to allow her to cut her wrist in an attempt to equilibrate herself again.

In this study, derma-abusers were not generally involved in acts with suicidal intent (**Figure 1, Table 1**). The ten patients were categorized into three groups, those without suicidal intent, those with suicide in mind and a third category of delayed onset, secondary suicidal thoughts. It is noteworthy that in more than 80% of the sample, suicide or thoughts of death was not the initial intent. These thoughts apparently developed following intervention, on discovery that their behaviors were enmeshed with the feelings of power and mastery over self and others, effects on tension reduction and as a form of revenge and hostility directed against family members. The act itself became the most powerful manipulative tool reinforced in a Caribbean setting with a history of aggression, violence and more recently high murder rates in Trinidad (**Table 2**).

The treatment of derma-abusers is difficult and presents a major challenge. After failure of individual therapy designed along the lines of Linehan's Dialectical Behaviour Therapy model (DBT) [31] another method was employed. DBT was unsuccessful for the following reasons: Patients were too young and disturbed to acquire what Linehan calls wisemind. With respect to the 'what' and 'how' skills, they could not focus on mindfulness. They could not develop interpersonal effectiveness to say no, or resist their urges of cutting and had little distress tolerance and emotional regulation.

A novel system of Dual Group Therapy (DGT) was devised. This is the simultaneous occurrence of two group sessions of one and a half hours held concurrently in two adjoining sound proof rooms of the same building once per week. The following screening process was instituted. First the initial interview with the patient and the attendants in the presentation of the problem (30 minutes). In the local setting, it is customary for the entire extended family, caretakers and friends to accompany the patient. In the second stage, the patient was interviewed individually (45 minutes) being allowed to tell her story and assessing significant others in her life. In the third stage, two significant others, determined by the patient and therapist were asked to be seen together with the patient. In a client centered approach, the dynamics of interaction of family members and patients were observed. The therapist had to be cautious in not ascribing blame to anyone person, attempting to avoid confrontation and acting-out behavior commonly found in our setting. Information on ethno-historiography, that is, the characteristics of origin, race, culture, religion and

lifestyle were sought. This socio-cultural academic exercise presented little threat to those involved. In the final stage, an agreement (non-signed contract) was reached by the parents (caretakers) and the patient to attend two concurrently run groups namely 'The Adolescent Group' and 'The Parent Support Group'. They were asked to attend for a twenty four sessions (six months) period. The third and fourth stages lasted approximately one hour with a total assessment time of two hours. Parents were given the charge of bringing the patients to the groups. Two trained psychologists conducted the groups and met together with two co-therapists who also were in the group and the psychiatrist for weekly reviews following the meetings. With shared information and emotions from both groups, a tailored management approach was devised for each patient introduced within the dynamics of group therapy.

This study investigates derma-abuse in adolescents. However, two other studies done on adults in Trinidad indicate that this practice continues into adult life. In the University sample [21,22], students in the psychology field were more prone to self-harm than those in natural sciences and engineering fields of study. It seemed the trend of cutting and derma abuse has continued into the tertiary level institution and it may be useful to investigate whether these students were derma abusers during their secondary school life, or has the behavioural pattern emerged during their University life. If the former is suggested, a reduction in numbers may be expected if early detection plans by appropriate service providers and personnel are put into place at the secondary school level.

The cases and vignettes presented are individuals who have used maladaptive coping in an attempt to remove dysfunctional events in their life. The adolescents have challenged the status quo of 'normal behavior' in the hope of normalizing their own lives. Their commonalities are striking and lend itself to distinct characterization of the phenomena of derma-abusing in Trinidad.

5. CONCLUSIONS

This is a preliminary study that highlights a growing problem among secondary school children in Trinidad. The author works closely with the School Supervision Unit of the Ministry of Education in Trinidad where a number of students are referred by the Guidance Officers who are not equipped to deal with the intensity of problems encountered. The behavior of these students are devastating to both fellow students and staff members alike and can undermine the spiritual and moral values of the schools' discipline. The fact that there is an element of contagion or copy cat behavior has led many school authorities, especially those of the denominational Christian schools to perceive the problem to be one of demonical possession and it is not unusual to have these students ostracized and referred for exorcism and spiritual healing.

In the presentation of this small sample of ten patients, the intention is to demonstrate the similarities of behavior, personal and family psychopathology and dynamics. The psychopathology of the individuals and their families must be emphasized as these may be major precursors to their condition. While there is room in any institution for pastoral care and counseling, the presentation of four (4) vignettes and study of the dynamics of six (6) students in group therapy provide a better understanding of these patients and provide a psychological framework for treatment. While this paper does not address their outcome in treatment, it is necessary to recognize that in group therapy, the inclusion of non-derma abusers in groups can lead to the recruitment of deviants. Family involvement is mandatory since disturbed kids invariably come from disturbed families.

Notwithstanding the limitations of it being a small, descriptive observational study, it is however the first clinical study of this nature coming out of the Caribbean region. As highlighted in a daily newspaper two years ago as 'a mental health crisis' [20], it is understandable that this will be a major public health issue of adolescents in the future, especially in Trinidad and Tobago now on the threshold of first world status.

The increased attention to derma-abusing cases may be an indication of additional adolescents engaging in the behavior. So too, it can be attributed to the changing attitudes of adolescents in today's culture as they freely engage in risky behavior. As previously stated [32], service providers may now have a more keen ability to recognize and report derma-abusing behavior with a better understanding of the dynamics involved.

6. ACKNOWLEDGEMENT

I wish to thank Dayna Mohammed of the Psychology Department of the University of the West Indies and Katija Khan, Psychologist of University of Hull in England for their useful comments and contribution to this paper.

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Cardio-Toxicity during chemotherapy: feasibility of new diagnostic approaches

Fable Zustovich¹, Mario Trivellato², Renato Ceravolo¹, Davide Pastorelli¹, Rosanna Canton², Lorena Buratin², Monica Mion³, Martina Zaninotto³, Martina Mattiazzi¹, Mario Plebani³, Giuseppe Cartei¹

¹Oncologia Medica 1° piano, IOV – IRCCS, Padova, Italy; fable.zustovich@ioveneto.it

²Servizio di Cardiologia ULSS16 c/o IRA, Padova, Italy

³Dipartimento di Medicina di Laboratorio, Università – Azienda Ospedaliera, Padova, Italy

Received 25 October 2009; revised 28 November 2009; accepted 5 December 2009.

ABSTRACT

Chemotherapy induced cardio-toxicity is a well known side effect of anticancer treatments, moreover the 70% of all tumors involve patients over 65 years-old, frequently with cardiac comorbidity. We evaluated the feasibility of the application during chemotherapy administration of some of the most recent diagnostic techniques as 12-diagnostic leads telemetry, 7 days EKG monitoring device (R-Test Evolution 3^R), blood level dosage of Brain Natriuretic Peptide (BNP) and Ischemia Modified Albumin (IMA). Some sub-clinical changes in the investigated parameters were found in patients undergoing chemotherapy, mostly containing fluorouracil, as shown in the following paper. Far from suggesting a widespread use of these methods during chemotherapy administration, we think that some more tools are needed to prevent cardiac toxicity in high-risk patients and some of what we studied may deserve further valuation in chemotherapy clinical trials.

Keywords: Cardio-Toxicity Diagnostic

1. INTRODUCTION

The rising percentage of older subjects among cancer patients has led oncologists to manage chemotherapy administration often dealing with severe concomitant diseases, mostly cardiovascular.

Chemotherapy induced cardio-toxicity is a well known side effect of anticancer treatment. Among the most cardio-toxic agents there are anthracyclines, 5-fluorouracil, taxanes and Trastuzumab. These agents may cause left ventricular dysfunction as well as arrhythmias and/or ST-T wave changes [1].

The evaluation of the cardiac safety during cardio toxic chemotherapy is usually based on standard EKG, EKG Holter and mostly using echo-color doppler cardiography. All these methods reveal cardiac damage when macroscopic morphological alterations have been reached, thus when damage is already present and often irreversible.

New methods of investigation are needed in attempt to detect a subclinical damage and to prevent the symptomatic cardiac failure. We evaluated the feasibility of some new techniques planning prospective preliminary studies monitoring patients undergoing potentially cardio toxic chemotherapy with 12-diagnostic leads EKG telemetry (TDLT), 7 days EKG monitoring device (R-Test Evolution 3^R), blood level dosages of Brain Natriuretic Peptide (BNP) and Ischemia Modified Albumin (IMA).

2. TWELVE-DIAGNOSTIC LEADS TELEMETRY (TDLT)

The TDLT monitors continuously 12 diagnostic leads electrocardiogram and gives a real time analysis with events comparing with basal reference.

The telemetry monitor (Mortara Rangoni Europe) had the following characteristics: acquisition data amplifier with analogue digital converter (ADC) = 20 bit; sampling sample channel (SSC) = 10000 Hz; low significant bit (LSB) = 1.2 microVolt; band with 0.05-100 Hz; telemetry transmission = 2.4 GHz. The system has high accuracy for arrhythmias and ischemia. Comparison among different time events and ST movements histograms with automatic continuous bit by bit data storage were also available. Patients were allowed to walk during TDLT.

We firstly studied prospectively some aspects of cardiac toxicity in patients during gemcitabine chemotherapy infusion for non-small cell lung cancer (NSCLC) [2]. Seventeen patients were evaluated, 12 male and 5 female,

median age was 63 years (range 37-84) and ECOG (Eastern Cooperative Oncology Group) performance status was 0 in 12 patients and 1 in 5. Gemcitabine was administered in a 30 minute iv. infusion in normal saline solution at the median dose of 1000 mg/m² (range 500 - 1200 according to age) on day 1, 8 and 15 every 28 days. Patients were monitored during the chemotherapy administration.

At basal condition a single patient had ventricular ectopic beats (VEB) arrhythmias and another one had abnormal ST and T waves. During the Gemcitabine administration 13 patients had EKG changes as supra-ventricular ectopic beats (SVEB), aberrance, VEB, fusion beats, multiform VEB and couplet as 4A grading. Two patients had minimal changes of ST as a sign of ischemia. Four patients did not develop arrhythmias and 1 of them was already in therapy with propafenone.

These preliminary data offered the chance of a much skillfull control on asymptomatic cardiovascular toxicity during chemotherapy and induced us to further investigation. With the same technique, we studied patients with colo-rectal cancer during the infusion of folate modulated 5-fluorouracil [3]. Cardiac toxicity due to 5-fluorouracil infusion is well known [4] and standard EKG is most used evaluation method of cardiac ischemia and/or arrhythmia. We enrolled 6 patients with age respectively of 61, 61, 66, 66, 71 and 75 years old, 3 were males and 3 females. All patients had ECOG (Eastern Cooperative Oncology Group) performance status of 0. All patients were receiving post-operative adjuvant chemotherapy treatment (5 patients receiving 5-fluorouracil 365 mg/m² iv and 50 mg/m² iv days 1 to 5 every 28 days; 1 patient receiving weekly 5-fluorouracil 600 mg/m² iv and folinic acid 100 mg/m² iv). Four patients had normal basal EKG and 2 had right bundle block. Patients were monitored during and after the entire chemotherapy treatment. The following EKG changes were found: QT lengthening in 2 patients, VEB in 2 patients (1 also with a triplet), SVEB in 2 patients. One patient had no EKG changes. Then 5 out of 6 healthy patients had asymptomatic EKG changes sometimes relevant during adjuvant fluoro-folate based chemotherapy after colorectal cancer operation.

3. SEVEN DAYS EKG MONITORING DEVICE (R-TEST EVOLUTION 3^R)

Patients undergoing chemotherapy with 5-fluorouracil in protracted infusion were studied over a period of 7 days using a 7-days monitoring by R-Test Evolution 3^R (Novacor), able of a real time EKG acquisition and processing [5]. This device stored only significant events with an acquisition system favoring also the CM5 lead for excellent results in arrhythmia detection and ST shifts measurements.

We enrolled 12 patients. Median age was 65 (range: 51-81), 8 patients were males and 4 were females. Nine patients had gastric cancer and 3 had colorectal cancer. Three patients were chemo-treated in the adjuvant setting and 9 for advanced disease. Median ECOG performance status was 1 (range 0-2). Patients received 5-fluorouracil in protracted infusion at the daily dose of 200 mg/m². Soon after the 5-fluorouracil in protracted infusion beginning we started a 7 days monitoring by R-Test Evolution 3^R.

The following EKG changes were found: supra-ventricular tachyarrhythmia ranging among 110 and 187 bpm in 7 patients; SVEB in 1; sinus tachycardia in 2 patients; VEB in 4 patients (1 monomorphyc, 1 polymorphyc, 1 couplets and 1 polymorphyc couplets); ST changes among 1 and 3 mm depression in 4 patients and T waves changes in 2 patients. No patient complained cardiac symptoms except one, having an angina-like pain with sinus tachycardia (110 bpm) without ST/T wave changes.

These data shows that the majority of these patients treated with 5-fluorouracil in protracted infusion had asymptomatic EKG changes, sometimes relevant.

4. BRAIN NATRIURETIC PEPTIDE (BNP)

BNP is widely known as a serum marker of clinical and sub-clinical heart failure [6]. To evaluate a potential chemotherapy cardio-toxicity we focused on BNP values obtained before and after chemotherapy infusion [7,8]. BNP, Troponin I (TI), Myoglobin (MY) dosage and EKG were performed before and 5 hours after the end of the chemotherapy infusion. BNP was evaluated using the ADVIA-Centaur BNP method (Bayer Diagnostics, Tarrytown, NY).

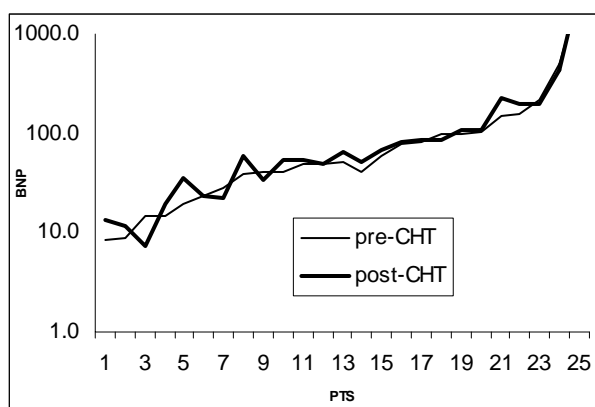
Twenty-five consecutive patients with different solid tumors undergoing different chemotherapy treatments (mostly 5-Fluorouracil and gemcitabine) were enrolled. Nineteen were males and 6 females, mean age was 62 years (range: 48-75). All patients had a baseline cardiac echo-color Doppler and cardiologic evaluation. Mean ejection fraction was 57% (range 52-72%). No prior pathological cardiac conditions were recognized.

See results in **Table 1** and **Figure 1**. One patient with BNP over 2000 ng/L had a chronic renal failure in dialytic treatment. A statistically significant difference (one-tail Student t-test $p = 0.02$) was found between the pre and post chemotherapy BNP mean values of patients with a normal basal BNP (< 100 ng/L). No changes were seen in the pre and post chemotherapy means of TI and MY.

These data suggest BNP as a possible early biomarker of sub-clinical cardiac damage. Future studies will evaluate a possible correlation between the observed BNP increases and the possible subsequent clinically

Table 1. Results of BNP valuation before and after chemotherapy administration.

	All PTS (n = 25)		PTS with BNP < 100 ng/L (n = 19)		PTS with BNP < 90 ng/L (n=18)	
	Pre- CHT	Post- CHT	Pre- CHT	Post- CHT	Pre- CHT	Post- CHT
BNP mean	180.8	203.2	40.4	44.6	38.1	42.6
BNP range	8.6- 2556.0	13.2- 2992.0	8.6- 99.0	7.4- 86.0	8.6- 82.0	7.4- 84.0
t-test	P = 0.112		P = 0.02		P = 0.006	

**Figure 1.** Graphic representation of BNP results before and after chemotherapy administration.**Table 2.** Results of IMA, BNP, Troponine I (cTnI), Cromogranine A (CRA), Myoglobine (MYO) evaluation before and after chemotherapy administration.

	IMA (< 85 U/mL)		cTnI (< 0.15 ug/L)		MYO (< 70 ug/L)		CRA (2-18 U/L)		BNP (< 100 ng/L)	
PT	t0	t1	t0	t1	t0	t1	t0	t1	t0	t1
1	110	117	0.20	0.18	77	85	> 562	> 562	2556.21	2992.01
2	94	107	0.06	0.03	88	68	12	9.8	8.57	13.20
3	84	83	0.02	0.04	35	29	7.4	4.4	58.24	68.78
4	102	91	0.00	0.00	28	21	4.8	6.1	41	55
5	83	86	0.00	0.01	56	64	8.6	11.7	40	34
6	95	125	0.00	0.00	25	20	16.9	12.7	23	23
Mean	94.667	101.500	0.047	0.043	51.500	47.833	9.940	8.940	454.503	530.998

evident cardio-toxicity in patients undergoing chemotherapy.

5. ISCHEMIA MODIFIED ALBUMIN (IMA)

IMA is an emerging biomarker of cardiac ischemia [9]. Moreover, cromogranine A (CRA) and Brain Natriuretic Peptide (BNP) are reported to be possible ischemia markers too [10,11]. We have already put on evidence as a protracted 5-fluorouracil infusion may induce asymptomatic relevant EKG changes in cancer patients, while 5-fluorouracil's cardiotoxicity, ischemic in particular, is well known.

We analysed IMA, troponin I (cTnI), Myoglobine (MYO), CRA and BNP in 6 colorectal advanced cancer patients [8]. Five patients were males and 1 female; age was respectively of 64, 71, 71, 62, 67 and 63 years old. Biomarkers valuation was performed before and 5 hours after 5-fluorouracil 400 mg/m² iv. Bolus infusion and a concomitant monitoring by TDLT (see previous paragraph).

See **Table 2** for results. Patient n. 1 had a chronic renal failure in dialytic treatment and his basal EKG showed sinus rhythm with left ventricular hypertrophy and strain. In the overall analysis IMA and BNP means showed a slight increase while cTnI, MYO and CRA means did not show a significant trend. In particular, we noted that IMA increased in 3 PTS out of 6. At the EKG monitoring all the patients showed only few VEB only during the 5-fluorouracil bolus infusion without any other significant changes.

Thus, chemotherapy administration could induce asymptomatic cardiac ischemia potentially detectable by the new biomarker IMA.

6. DISCUSSIONS

Preliminary data obtained with TDLT in gemcitabine treated patients showed something of usually missed about asymptomatic cardiac effects of chemotherapy. Further evaluation in patients treated with bolus of 5-fluorouracil protracted infusion for gastrointestinal diseases, confirmed and enforced this observation showing sometimes relevant asymptomatic EKG changes. On the other hand R-Test Evolution 3 R allowed us to monitor patients undergoing treatment with 5-Fluorouracil in continuous infusion as adjuvant chemotherapy after surgery colorectal cancer. The high incidence of sub clinical cardiac effects were confirmed as 5 out of 6 healthy patients had relevant EKG changes as QT lengthening, VEB and SVEB.

This data are might be of great impact if we consider that 5-fluorouracil is a milestone in the treatment of gastrointestinal tumors. In our clinical practice, the availability of these techniques, especially TDLT, might allow us to administer 5-fluorouracil based chemotherapy also to cardiac high-risk patients, otherwise excluded from a potentially curative treatment. This issue might be of a great impact if we consider the high prevalence of colorectal cancers (147500 new cases in the USA in the 2003 [12]) and that the cancer-related overall survival benefit obtained by 5-fluorouracil based adjuvant chemotherapy is 74% of patients alive at 5 years vs. 63% treated with surgery alone [13].

Evaluation of BNP and IMA before and after chemotherapy administration revealed their potential role as early bio-marker of sub-clinical cardiac damage. In particular BNP reveals a myocardic contractile failure [6]. Drugs as anthracyclines are well known as cardiotoxic, especially for the contractile function of the myocardium. Anthracyclines are largely used in the oncological practice as the milestone in the adjuvant treatment of operated breast cancer and in the curative treatment of lymphomas. Broeyer *et al.* [14] showed as patients receiving anthracyclines based chemotherapy had a significant BNP elevation in comparison to a control population and suggested that what observed was the expression of the toxic effect on the myocardium. Late cardiac toxicity of these drugs is a very important issues in this long term cancers survivors. BNP, other biomarkers or modern EKG techniques, could be predictive of late cardiac toxicity and could help to define a high risk subset of patients eligible for a prophylactic administration of cardio protectors as dexrazoxane or to the administration of alternative antitlastic drugs as liposomal doxorubicin.

Indeed, we hope that future studies will evaluate a

possible correlation between the observed BNP increases and the subsequent late evidence of cardio-toxicity in patients undergoing cardio toxic chemotherapy. This approach will define the possible predictive role of these bio-marker.

7. CONCLUSIONS

The potential availability of new methods able to discover the cardiac damage in an early stage is meaningful. We think that some new tools are needed in cardiac toxicity prevention and some of what we tested deserve further evaluation in chemotherapy clinical trials.

Moreover, basing on modern monitoring instruments, cardiac high-risk patients maybe safely candidate to a chemotherapy treatment otherwise unmanageable.

8. ACKNOWLEDGEMENTS

To Dr. Claudio Bano for his great help in valuating telemetry EKG data.

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Influence of production processes in quality of fermented milk “Laban” in Lebanon

Zeineddine Mayssoun¹, Nassif Nadine²

¹National Beverage Company, Quality Department, Choueifat, Lebanon; misso_zd@hotmail.com

²Lebanese University, Faculty of Agricultural Sciences, Environmental Department, Dekwaneh, Lebanon; nadinenassif3@hotmail.com

Received 6 December 2009; revised 20 January 2010; accepted 25 January 2010.

ABSTRACT

Yoghurt (Laban) is one of the most consumed food products in Lebanon. Thus its quality has given a concern. In this study, the sensory, chemical and rheological properties of commercial and traditional samples were investigated in order to characterize this fermented milk. Hence, Laban samples were collected from 14 areas in Lebanon; especially from mountainous regions and from the capital Beirut. Forty-two samples were provided by processing industry whether at small, medium, or large scale. A statistical analysis was carried out, and thus sensory and physicochemical properties were subjected to two approaches of variance analysis. Pearson correlation coefficients between attributes were also calculated. Both, the analyses of variance and correlations were conducted using SPSS 3. The physicochemical analysis and the microbiological analysis exhibit a significant effect of the date, and the manufacturing process. Also, the instrumental data showed no significant correlation between physicochemical and microbiological parameters, which indicates that they are completely independent. Moreover, the general appreciation of descriptive sensory analysis of products display that this appreciation is not dependant on the production process. It is also noticed that some sensory characteristics can be dread by instrumental measures. This research endorses the essential role of quality control for the manufacturing of yoghurt in Lebanon.

Keywords: Laban; Rheology; Manufacturing Process; Sensory Analysis; Lebanon

1. INTRODUCTION

Yoghurt (Laban) is one of the most consumed prod-

ucts in Lebanon. It is obtained through lactic acid fermentation of heat-treated milk by *Streptococcus thermophilus* and *Lactobacillus bulgaricus* at 40-45°C [1]. The annual consumption of Laban is approximately 20 Kg per capita in Lebanon [2]. Laban may be prepared from full fat milk or low fat milk, but it must conform to the different standards set by the government regarding its fat and total solids content [3].

The possibility of using some artisanal strains as starter for the development of fermented milks with characteristics similar to traditional products, at an industrial scale, is very promising. However their potential use depends on further assessment of their aptitude to be reproduced by fermentation and preserved by freezing and/or by freeze drying. Moreover, these strains should first be tested in mixed cultures, in order to obtain fermented milks with rheological and flavor characteristics similar to those of artisanal products.

In Lebanon, Laban is manufactured by both industrial and small traditional producers. Lately in Lebanon, a number of yoghurt factories have been developed. There is an obvious competition between these factories considering mainly, in addition to production rate, the quality control. Quality and reproducibility of fermented milks and processes are ensured by using industrial starters. Nevertheless, consumers prefer traditional fermented milks since artisanal starters give these products more typical flavors [4], where small producers inoculate the milk with an artisanal starter obtained from the previous day's preparation.

The production of yoghurt is a very classical procedure that does not necessitate variations. However, post fermentation handling is a very critical to induce desirable properties to the product. Indeed, the Laban, being a highly consumed product in Lebanon, its need to have produced under hygienic conditions to be more widely available to the consumer, prior to becoming too acidic and to avoid any texture modification.

Milk testing and quality control is an essential component of any milk processing industry whether small, medium or large scale. Milk being made up of 87% water is prone to adulteration by unscrupulous middlemen

and unfaithful farm workers. Moreover, its high nutritive value makes it an ideal medium for the rapid multiplication of bacteria, particularly under unhygienic production and storage at ambient temperatures. We know that, in order for any processor to make good dairy products, good quality raw materials are essential “*Quality, it starts at the farm*”. Different technological and microbiological parameters have been reported to influence yoghurt quality: composition, heat treatment of milk, conditions of incubation (temperature and pH) and storage (temperature and duration) [5-7].

Yoghurt is a very prominent media for yeast. Even though pasteurization eliminates them from raw milk, they appear again when they are subjected to handling and manufacture. Heat treatment of yoghurt was efficient in improving its shelf life and its microbial count. In September 1971, the milk committee had agreed that the future standard on yoghurt should not be considered as “yoghurt” even with a qualifying term. The excuse for this that the appellation “yoghurt” must denote a product with specific bacteria in viable form and in abundance this did not conform to the heated yoghurt [8]. In this way the Lebanese standard precise that these bacteria should be stay alive in finished product.

The addition of stabilizers was shown to be necessary. Regarding preservation alone, different yeasts had different reactions to sodium benzoate and potassium sorbate. For instance *Trichosporon cutaneum* was most sensitive to potassium sorbate. Indeed 100 mg/Kg was enough to ensure less than 10^5 cfu g⁻¹ for a 14 days storage period, whereas 200 mg/Kg limited the count to less than 10^5 cfu g⁻¹ for a 21 days storage period [9]. Potassium sorbate was shown to be more effective than sodium benzoate. Thus, as we see, preservation of yoghurt requires more than 100 mg/Kg of preservatives. The latter could have an identical effect than heat treatment at 65°C. Indeed, the yeast and mold count was limited to less than 10 cfu g⁻¹ after 12 months of storage at 20°C. This showed the efficiency of the preservatives and their very delicate usage. Indeed if extra amounts were used some off-flavors, health problems and extra costs might occur [9]. According to Lebanese standard [10], the maximal content in sorbic acid and its salts must be never exceed 50 mg/Kg and the use of benzoic acid are forbidden.

The efficient production of milk under good hygienic conditions is the key to successful dairying. The principal constraints in Lebanese smallholder systems are inadequate feeding, low genetic potential in animals and the major farms don't have a milking room. If milking doesn't take place manually, mostly, it takes places with movable milking machine for larger herds with the possibility of transmission of many germs leading the product to spoilage before reaching the market. The transportation of milk from the producer to the processor and

finally to the consumer take place by the producer itself or through a conveyor. Generally, milk was collected in plastic or aluminum containers and transported to the processor via refrigerating car or no according to the distance covered [11].

Therefore, The objective of the current study is to compare the quality of Laban sample collected from 2 representative regions (Mount Lebanon and Beirut) in Lebanon with the International Standards in order to assess the suitability of this nutritious material for health.

2. MATERIALS AND METHOD

2.1. Laban samples

Laban samples were collected from 14 areas distributed throughout the Lebanese territory especially from Mount Lebanon and Beirut regions (see **Table 1**). Forty-two samples were provided by processing industry whether small, medium or large scale. Samples were collected during 3 periods for 1 month. A replication of the descriptive sensory evaluation, coupled with instrumental measurements, was done for each sample.

2.2. Physicochemical Analysis

Titrate acidity was determined by titrating Laban samples with 0,1N NaOH using phenolphthalein as an indicator as outlined by AFNOR [12]. Results were expressed in g of lactic acid per Kg of sample. The fat content was determined by Gerber method [13]. The dry solids content by drying method as outlined by the Association of Official Analytical Chemists (AOAC) [14]. All analyses were performed in duplicate. Apparent viscosity (η), expressed in Pa.s, was determined by using a Brookfield viscosimeter mod DV-II (Brookfield Eng. Lab., Middleborro, MA, U.S.A.), operating at 20°C. A LV4 spindle was used at different shear rates: 2, 5, 10, 20, 50 and 100 rpm.e.

2.3. Microbiological Analyses

The Total and Faecal coliform bacteria were isolated by plating the diluted samples (dilution of 1 g of sample in sterile distilled water with 0,8% NaCl) on Maconkey Agar incubated for 24 h at 37°C for Total coliform [15], and at 44°C for Faecal coliform [16]. *Salmonella* spp. was isolated after enrichment in selenit broth at 37°C for 24 h, than plating on SS Agar incubated at 37°C for 24-48 h [17]. *Staphylococcus aureus* were isolated by plating the diluted samples on Chapman Agar at 37°C for 24 h [18]. Yeasts and molds are also isolated using “Chloramphenical yeast Agar” at 30°C for 5 days [19]. Bacteria counts were expressed as colony-forming units (cfu) per 1 g of sample. All tests were duplicated for each isolate.

Table 1. Distribution of Laban samples throughout the Lebanese territory.

N°	Code	Date of fondation	origin	Scale
1	LMO	1961	ELGHOBEIRI (Mount Lebanon)	Small
2	ER	2000	ELJAHLEH (Mount Lebanon)	Small
3	LKA	2005	ELCHOUWEIFAT (Mount Lebanon)	Small
4	LW	2002	KABER CHMOUN (Mount Lebanon)	Small
5	LR	1992	ALLAY (Mount Lebanon)	Small
6	LD	1992	BORJ EL BARAJNI (Beirut)	Medium
7	GD	1994	ELAMROUSAYEH (Mount Lebanon)	Medium
8	LG	1960	HARET HREIK (Mount Lebanon)	Medium
9	LK	1990	JISER EL BACHA (Beirut)	Medium
10	ES	1976	DMIT (Mount Lebanon)	Medium
11	LM	2005	DEKWANEH (Mount Lebanon)	Medium
12	PR	1991	SIN EL FIL (Beirut)	Medium
13	DD	1857	KFARCHIMA (Mount Lebanon)	Large
14	Dk	1978	AIN EL SINDIYANA (Mount Lebanon)	Large

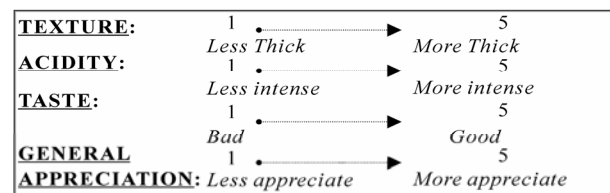
2.4. Descriptive Sensory Analysis

Sensory analysis was carried out in Lebanon with the collaboration of a panel of 16 volunteer members. Two hours were conducted to train the panelists in the good use of the intensity scale and to compare their perception. The 4 characteristics tested are given in **Figure 1**. Samples were classified for each descriptor according to a 5-category intensity scale (scale 1 denoting an absence of the considered perception and scale 5 denoting a very intense perception).

Each product was coded randomly by 1 letter and 2 digits. Samples, stored at 4°C for 4 days, were presented in a simultaneous way. Extrinsic factors such as temperature, quantity presented, and container were homogeneous for all the products. Spring water was provided for mouth rinsing between samples. In order to control the sort out of samples, the distribution of samples to panelists is realized according to “latin square plane of williams”.

3. STATISTICAL ANALYSIS

Sensory and physicochemical properties were subjected to two-way analysis of variance. It allowed studying the variability between samples, by taking into account the variability between subjects. Pearson correlation coefficients between attributes were also calculated. Analyses of variance and correlations were conducted using Software Statgraphics Plus version 3,0 (Stastical Graphics Corp., Rockville, MD, U.S.A.).

**Figure 1.** Attributes used in the sensory evaluation of Laban.

4. RESULTS AND DISCUSSIONS

4.1. Physicochemical Analyses

Results of physicochemical analyses are shown in **Figure 2**. Significant differences ($\alpha < 0,05$) in titratable acidity (see **Table 2**), total solid content, and fat content were found between samples collected from different origins. In plus, a significant effect of date was observed. A typical yoghurt sample was described by the Lebanese Institut of Normalisation (LIBNOR) [10], should contain 3% milk fat and, not less than 8,5% milk solids non-fat with a titratable acidity of no high than 1,5%. The total solid content of Laban samples varied between 6,78 and 13,25%.

Therefore, almost 31% of samples were conformed to Lebanese Standard. However Toufeili and others [20], showed that the composition varied according of feeding, milk production period, and the race. Concerning fat content, the mean value obtained deviate widely with minimum of 2,3% and maximum of 8,4%. Results showed that 86% are conformed to Lebanese standard. Two industries are under standard (GD “medium scale”

and DD “large scale”). These lower values can result from 2 causes: milk in its original composition, is poor in fat content or due to fraud practice on behalf of industrialist be possible during the moment of reception of milk or during obtaining finished product.

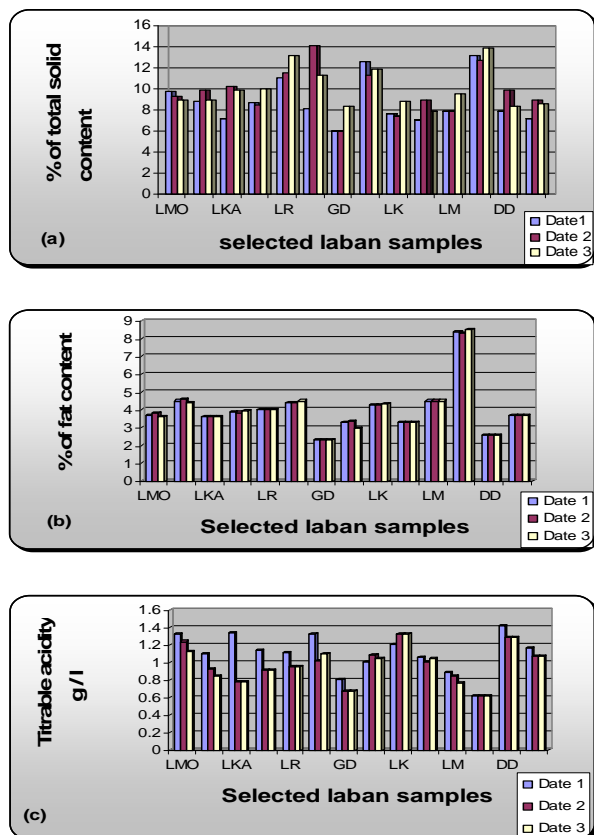


Figure 2. Physicochemical characteristics of selected Laban samples. (a) percentage of total solid content during 3 dates in the 2 regions, (b) percentage of fat content during 3 dates in the 2 regions, and titratable acidity (g/l) (c).

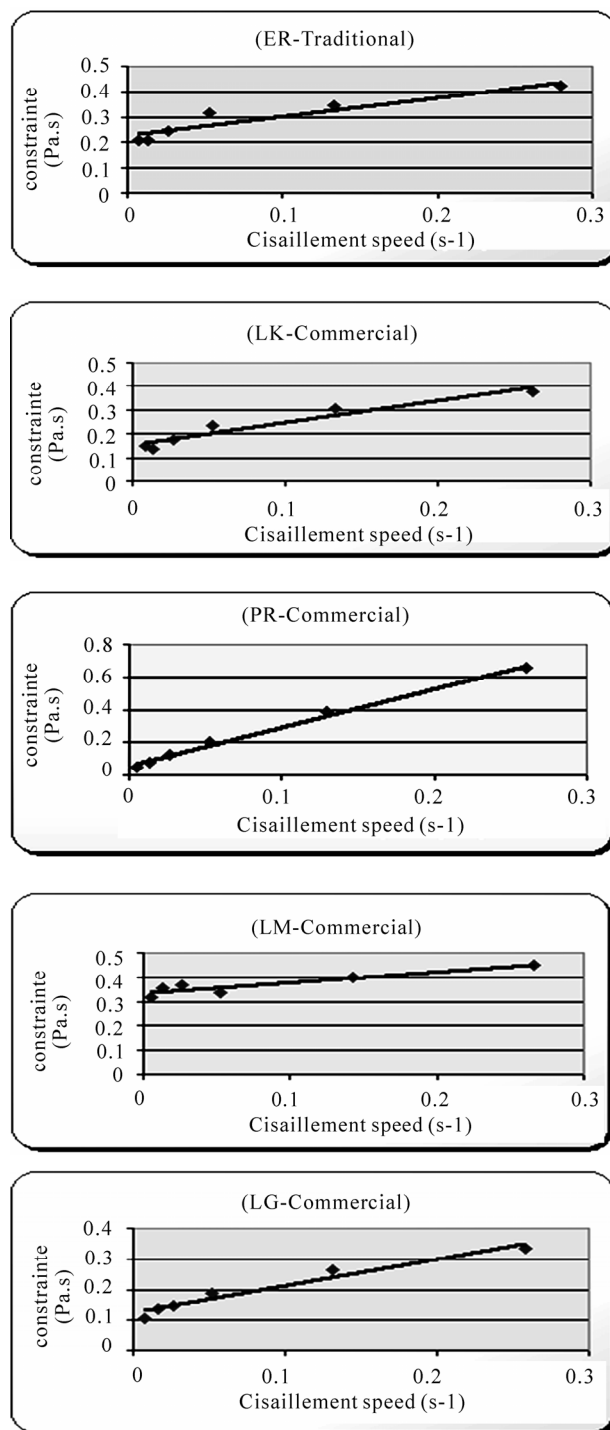
Table 2. Two way analysis of variance for titratable acidity.

Test of Between-Subjects Effects					
Dependent Variable: RDT					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.758 ^a	15	.117	11.915	.000
Intercept	43.513	1	43.513	4423.361	.000
DATE	.166	2	8.295E-02	8.432	.002
USINE	1.592	13	.122	12.451	.000
Error	.256	26	9.837E-03		
Total	45.527	42			
Corrected Total	2.014	41			

^aR Squared= .873 (Adjusted R Squared = .800)

Finally, lactic acid content varied between 0,63% for PR industry (medium scale) and 1,37% for DD industry (large scale). Consequently, the values of lactic acid content obtained for the Laban samples were in agreement with those described by Lebanese Standard. In previous researches, fresh yoghurts had lower titratable acidity.

The apparent viscosities of traditional samples (see **Figure 3**) were lower than that of the commercial product



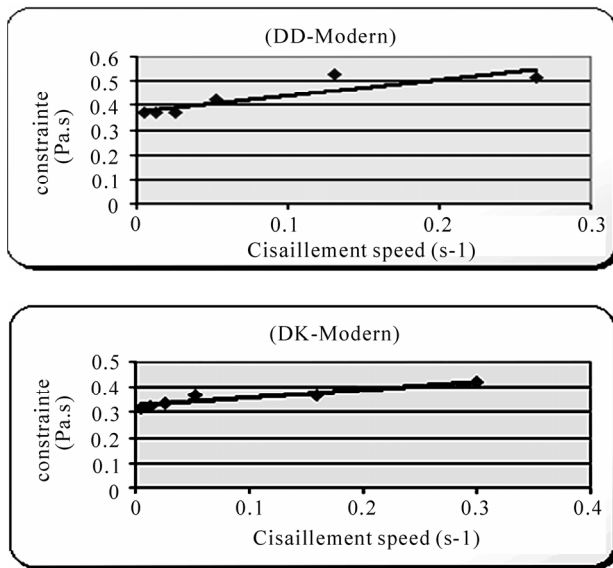


Figure 3. Evolution of cisaillement speed according to the applied constraint during the second date in the 2 regions.

(DD and DK). These observations are in agreement with the results of Guizani and others [21], who found that commercial fermented milk samples had higher viscosities than home made products. Owing to additional solids in the formulation of the commercial products. In lack for any reference that described the normal viscosity of yoghurt, these higher values probably explained by homogenization of milk with milk powder or casein without proportional addition of water content.

4.2. Microbiological Analyses

Considering the microbiological part of the study, Analysis of variance showed significant differences ($\alpha < 0.05$) between industries for those of 6 parameters (see **Table 3**). As described by the Lebanese and French Standards, the Total and Faecal coliform count must be lower than 100 cfu per 1 g of Yoghurt [15,16, 22] the absence of salmonella in 25 g of sample and not higher than 10 cfu g⁻¹ of Saphylococcus aureus [23]. Indeed, the yeast and mold count must be lower than 10³ cfu g⁻¹ [19-23].

For years, Total and Faecal coliforms (FC) were widely thought to be meaningful indicators. FC, also called Thermotolerant coliforms, have been used throughout this study as fecal indicators to evaluate the levels and sources of microbiological contamination of yoghurt produced in Mount Lebanon and Beirut regions. **Table 4** shows that Total coliforms concentrations exceeded the Lebanese and French standards in GD and LG industry (medium scale) and FC concentrations in GD and LK (medium scale). Therefore 14% are none conforms. These Higher values can be results from the non respect of good hygienic practices (GHPs) in farms, insufficient heat treatment of milk during

manufacturing, and keeping out the milk without refrigeration.

As can be seen in **Table 4**, they are 2 traditional factory (ER and LKA), who that exceed the standard of Staphylococcus aureus count. The importance of Staphylococcus aureus in yoghurt may be result from bad hygiene or high contamination of dairy product [24]. Taken the hygienic quality of containers into account [25,26], and the absence of refrigeration, Staphylococcus aureus is responsible for alimentary intoxication.

Concerning salmonella count, the mean values varied between 40 and 95 cfu g⁻¹ for these 4 industry: LKA, GD, LG and LK (See **Figure 4**). But these suspected germs are present only in 1 date among the 3 dates. It's prove that it is necessary to be a casual contamination and may be result from poor hygiene of farms, personnel, utensils, the starter used, and insufficient heat treatment. Further tests should be done to confirm the presence of salmonella species other than plating.

There is also possible examination of pathogenic organisms, but what is more significant is the examination of yeasts and molds as these are capable of spoiling yoghurt well within an anticipated sell-by-date [1]. They reproduce by "budding". Indeed no growth of yeast was observed in commercial fermented milk samples (DD and DK). However, a higher count was observed in the others Laban samples were manufacturing according to traditional methods (inoculation with an artisanal starter culture obtained from the previous day's preparation). Therefore 71% are none conforms (see **Table 4**). This high count can be influenced moreover by the age of product than the containers used and the method of transformations [27]. These lower values can be explained by usage of preservatives such sorbic acid who have a anti-fungic effect. It has been suggested that a limitation in the count to less than 100 viable yeast cell/ml should be put. Indeed a count of 1000 cells/ml could imply a serious risk of deterioration [1]. Low fat yoghurts appear to be less suitable as a growth medium than full fat but the reason is not known yet. Oxidative yeasts are also important spoilage organisms. They could be limited by the availability of oxygen. They may develop moist flat colonies. They usually grow on the walls of the container whereas the fermentative yeasts dominate in the middle.

4.3. Correlation between Technological Parameters

As can be seen in **Table 5**, the high correlation was observed between Total coliforms and Faecal coliforms ($r=0.995$). Fat content was correlated to total solids content ($r=0.635$). Significant but poor correlations were observed between yeasts and the presence of salmonella

Table 3. Two way analysis for Faecal coliforms.

Test of Between-Subjects Effects						
Dependent Variable: RDT						
Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	3221458.333	1	3221458.333	12026.778	.006
	Error	267.857	1	267.857 ^a		
REP	Hypothesis	267.857	1	267.857	.001	.976
	Error	19868660.7	67	296547.175 ^b		
USINE	Hypothesis	35425625.0	13	2725048.077	9.189	.000
	Error	19868660.7	67	296547.175 ^b		
DATE	Hypothesis	1046488.095	2	523244.048	1.764	.179
	Error	19868660.7	67	296547.175 ^b		

a. MS(REP)

b. MS(Error)

Table 4. Microbiological characteristics of selected Laban samples.

N	Code ^a	Mean of 6 repetitions (cfu g ⁻¹)					
		Total coliforms	Faecal coliforms	<i>Staphylococcus aureus</i>	<i>Salmonella Shigella</i>	Yeasts	Molds
1	LM	0	0	0	0	1100	48
2	ER	0	0	50	0	467	12
3	LKA	50	0	33	40	1147	0
4	LW	0	0	0	0	350	0
5	LR	0	0	0	0	46	0
6	LD	0	0	0	0	275	2
7	GD	1733	2533	0	42	1008	18
8	LG	150	75	0	67	1400	5
9	LK	0	133	0	95	7833	0
10	ES	0	0	0	0	6667	148
11	LM	0	0	0	0	1267	2
12	PR	83	0	0	0	78	2
13	DD	67	0	0	0	75	0
14	DK	18	0	0	0	33	2

($r=0,547$). This result are in according to the study carry out in Bekaa region [28]. It's proving that there are no correlations between physicochemical parameters and microbiological parameters and that they are completely independent.

4.4. Descriptive Sensory Analysis

ANOVA univariates results, taking into account the effects of both laban samples and subjects, showed no significant difference ($\alpha < 0,05$) between the 14 laban samples tested

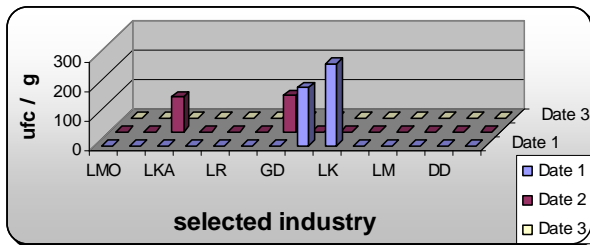


Figure 4. Salmonella count during the 3 dates in the 2 regions.

for the 4 characteristics: texture, taste, acidity and, general appreciation. As an illustration, **Figure 5** shows the score obtained for 4 selected attributes.

A general appreciation of descriptive sensory analysis of products display that this appreciation does not depend on the production process. In **Table 6**, general appreciation of products were correlated with taste ($r = 1$), and acidity ($r = 0,901$). Correlation results revealed that there were 2 groups of attributes for the flavor of Laban.

No correlation was observed between general appreciation and texture in our study. Pearson correlation coefficients were highly significant between acidity and taste ($r = 0,904$), it's indicate that these criteria are very important in the evaluation of Laban.

4.5. Relationships between Sensory Data and Physicochemical Measurements

The statistical relationship between sensory and instrumental data was examined using Pearson's correlation procedure. Some sensory characteristics can be read by instrumental measures (see **Table 6**). The sensory values for viscosity were correlated with the viscosity measured with a viscosimeter type Brookfield ($r = 0,789$).

These correlations were also observed by Marshall and Rawson [29], indicated that texture measurement were correlated with sensory evaluation of viscosity. In the same way, fat content was negatively correlated with the acidity ($r = -0,855$).

Table 5. Correlation analysis between instrumental parameters of Laban samples for 3 dates.

	TC	FC	Staph.	Salmo.	Yeast	Molds	T.S.C.	Fat C.	Acidity
TC	1	0,995^b	-0,118	0,258	-0,012	-0,084	-0,377	-0,34	-0,447
FC	0,995	1	-0,12	0,277	0,001	-0,026	-0,428	-0,355	-0,418
Staph.	-0,118	-0,12	1	-0,018	-0,103	-0,136	-0,075	0,03	0,041
Salmo.	0,258	0,277	-0,018	1	-0,188	0,547	-0,152	-0,198	0,072
Yeast	-0,012	0,001	-0,0103	-0,188	1	0,55	-0,28	-0,199	0,002
Molds	-0,084	-0,026	-0,136	0,547	0,55	1	-0,387	-0,118	0,159
T.S.C.	-0,377	-0,428	-0,075	-0,152	-0,28	-0,387	1	0,635	-0,221
Fat C.	-0,34	-0,355	0,03	-0,198	-0,199	-0,118	0,635	1	-0,508
Acidity	-0,447	-0,418	0,041	0,072	0,002	0,159	-0,221	-0,508	1

^bIn bold, significant values at the level of significance $\alpha = 0,05$ (two-tailed test). Abbreviations: Total coliform (TC), Faecal coliform (FC), Staphylococcus aureus (Staph.), Salmonella (Salmo.), Total solid Content (T.S.C.), and Fat content (Fat C.).

Table 6. Relationships between sensory data and physicochemical measurements.

	T.S.C	Fat C.	Acidity	Visco.	S.Acidity	Taste	Texture	G.A.
T.S.C	1	0.628	-0.695	0.515	-0.522	-0.394	0.361	-0.392
Fat C.	0.628	1	-0.855 c	0.728	-0.514	-0.456	0.4	-0.45
Acidity	-0.695	-0.855	1	-0.535	0.74	0.533	-0.17	0.525
Visco.	0.515	0.728	-0.535	1	-0.057	-0.041	0.789	-0.046
S.Acidity	-0.522	-0.514	0.74	-0.057	1	0.904	0.445	0.901
Taste	-0.394	-0.456	0.533	-0.041	0.904	1	0.525	1
Texture	0.361	0.4	-0.17	0.789	0.445	0.525	1	0.522
G.A.	-0.392	-0.45	0.525	-0.046	0.901	1	0.522	1

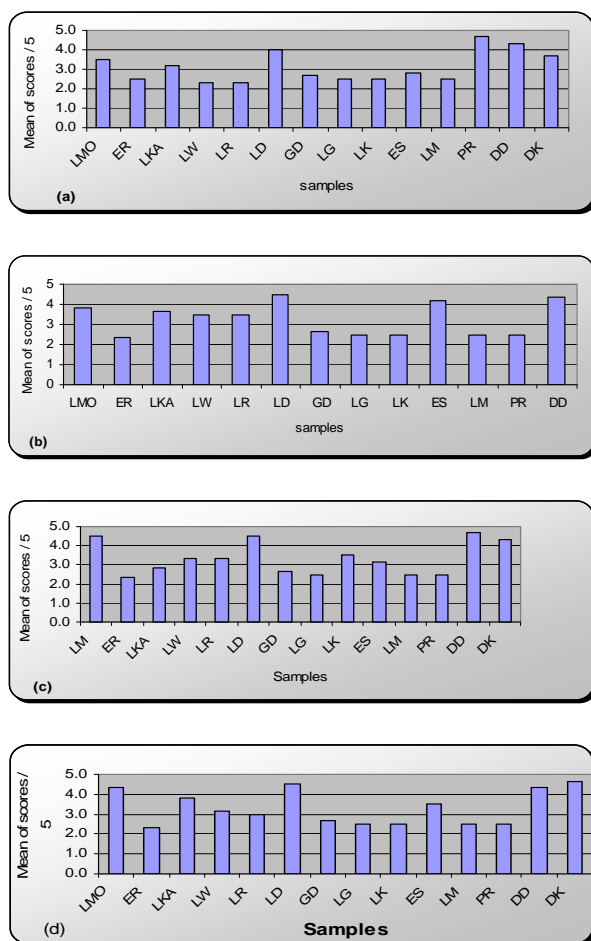


Figure 5. (a) Determination of viscosity, (b) Determination of taste, (c) Determination of acidity, and (d) General appreciation of products during the second date.

5. CONCLUSIONS

Both instrumental and sensory data distinguished large differences between laban samples collected from Mount Lebanon and Beirut. Faecal coliforms have been used throughout this study as fecal indicators to evaluate the levels and sources of microbiological contamination of yoghurt produced in Mount Lebanon and Beirut regions. Significant differences in titratable acidity, total solid content, and fat content were found between samples collected from different origins. In plus, a significant effect of date was observed. The instrumental data showed no significant correlation between physico-chemical and microbiological parameters. It's proving that they are completely independent.

A general appreciation of descriptive sensory analysis of products display that this appreciation does not depend on the production process. Pearson correlation coefficients were highly significant between acidity and taste, it's indicate that these criteria are very important in

the evaluation of Laban. Some sensory characteristics can be dread by instrumental measures. The texture measurement was correlated with sensory evaluation of viscosity.

Nevertheless, results were based on a limited number of industries, and further testing with larger numbers may be necessary to confirm current results. However, over the coming years other stabilizers must be tried in a quest for having an even more desirable body and texture as well as taste. The possibility of using some artisanal strains as starter for the development of fermented milks with characteristics similar to traditional products, at an industrial scale, is very promising. A future identification of the different yeast species contaminating yoghurt over the time from different commercial sources could be done. This is to help in incrimination of the final products once contaminated by a tolerant yeast organism.

6. ACKNOWLEDGEMENT

The authors gratefully acknowledge the financial support of the Lebanese University, Faculty of agricultural sciences. We wish to express our appreciation for the panelists for their participation in the sensory assessments.

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