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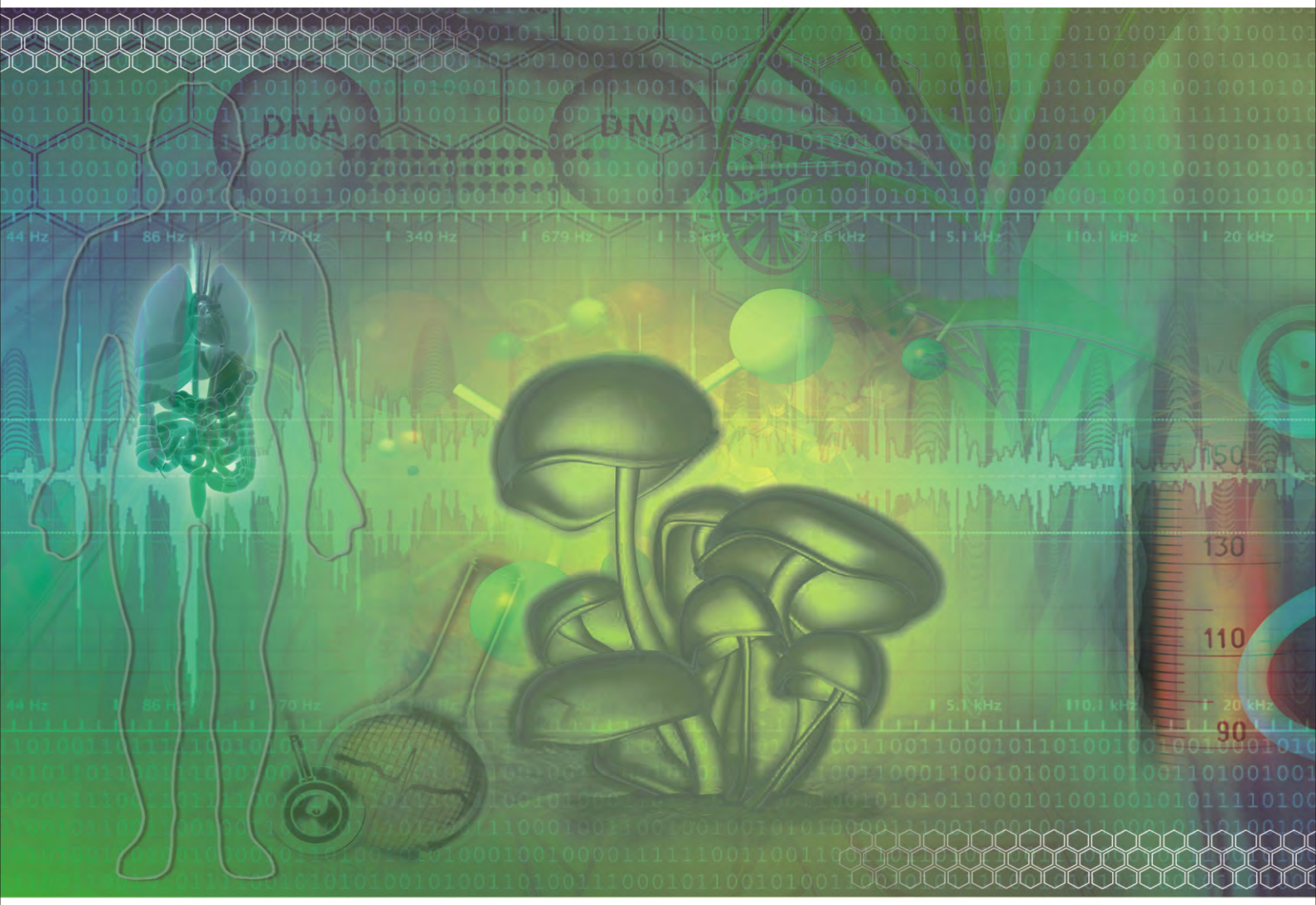

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Escin may exert a synergistic anti-inflammatory effect with glucocorticoids

Lei-Ming Zhang, Tian Wang, Hua-Ying Fan, Xin Yu, Bing Han, Mei Zhu, Feng-Hua Fu

Department of Pharmacology, School of Pharmacy, Yantai University, Yantai, China; fufenghua@sohu.com

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ABSTRACT

Escin is a natural mixture of triterpenoid asponin isolated from the seed of the horse chestnut and demonstrates anti-oedematous and anti-inflammatory effects. As yet, the precise mechanisms by which escin exerts its anti-inflammatory effects remain unclear. The data from current studies indicate that the anti-inflammatory properties of escin were attributed to its ability to reduce the adhesiveness of neutrophils and the associated release of inflammatory mediators; its ability to decrease histaminic and serotoninergic activities; its ability to inhibit phospholipase A_2; its ability to decrease nuclear factor-κ B activation and down-regulate the expression of tumor necrosis factor-α. All these effects are similar to glucocorticoids. Moreover, escin depends on adrenal glands to exert its anti-inflammatory effects. Also, our recent research showed that the serum corticosterone level in mice did not increase after a 7-day intravenous injection of escin. The results support the hypothesis that escin may exert a synergistic anti-inflammatory effect with glucocorticoids. Confirming this hypothesis will play a role in elucidating the anti-inflammatory mechanisms of escin.

Keywords: Escin; Glucocorticoids; Inflammation; Synergism

1. INTRODUCTION

Escin, the major active principle from Aesculus hippocastanum (Hippocastanaceae), the horse chestnut tree, possesses diverse biochemical and pharmacological actions. It has been reported that escin has anti-oedematous, anti-inflammatory, and venotonic effects [1], and which currently has wide clinical use. Accumulated experimental evidence also suggests that escin exerts anti-oedematous and anti-inflammatory effects. Escin has been shown to be effective in preventing the formation of oedema in models of inflammation that reproduce the initial exudative phase, such as oedema induced in the paw by a series of irritative agents [2]. Additionally, escin shows a significant inhibition not only of the increase of capillary permeability induced by acetic acid, but also of the adhesion formation in animal model [3]. However, the anti-inflammatory mechanisms of escin are still unclear.

2. PROOFS FOR THE HYPOTHESIS

The studies showed that escin could attenuate brain injury, down-regulate the protein expressions of intercellular adhesion molecule (ICAM)-1 and E-selectin, and reduce the adhesiveness and migration of neutrophils [2, 4]. According to Matsuda [5], the anti-inflammatory effects of escin are mainly dependent on their anti-histaminic and antiserotoninergic activities. Another research [6] reported that escin dose-dependently prevented the hypoxia-induced activation of human endothelial cells, as evidenced by the inhibition of hypoxia-increased phospholipase A2, an enzyme responsible for the release of precursors of inflammatory mediators. In addition, escin can well alleviate the formation of inflammatory edema by blocking the increase in permeability through enhancing generation of prostaglandin F2α [7]. Escin could significantly inhibit nuclear factor-κ B (NF-κ B) activation and down-regulate the expression of tumor necrosis factor-α (TNF-α), alleviating brain edema in traumatic brain injured rats [8].

The experiments listed above characterized that escin has potent anti-inflammatory effects and its anti-inflammatory mechanisms are similar to glucocorticoids (GCs) [9-11]. Additionally, an intriguing finding was that the anti-inflammatory effects of escin disappeared following adrenalectomy [12]. It suggests that the anti-inflammatory effects of escin depend on GCs. However, our recent research showed the serum corticosterone level in mice did not increase after a 7-day intravenous

injection of escin [13]. In another study, we further found escin to be a safe and potent anti-inflammatory drug with long effective anti-inflammation and without immunosuppression [14].

It is well known that GCs possess both anti-inflammatory and immunosuppressive effects. The anti-inflammatory and immunosuppressive effects of GCs rely on several molecular mechanisms, including direct effects on gene expression by the binding of glucocorticoid receptors (GR) to GC-responsive elements (i.e., the induction of annexin I and MAPK phosphatase 1), indirect effects on gene expression through the interactions of GR with other transcription factors (i.e., NF-κB and activator protein 1), and GR–mediated effects on second-messenger cascades (i.e., the PI3K–Akt–eNOS pathway) [15]. Unfortunately, because some of these mechanisms are also involved in physiologic signaling rather than inflammatory signaling, the therapeutic effects of GCs in inflammation are often accompanied by clinically significant side effects.

3. THE HYPOTHESIS

Based on the aforementioned data, we hypothesize that escin may exert a synergistic anti-inflammatory effect with GCs, which could explain the relationship described between escin and its anti-inflammatory mechanism, and the molecular mechanisms of the synergistic anti-inflammatory effect between escin and GCs may be derived from amplification of endogenous GC action through affecting GR or other elements in the signaling pathways. In fact, this hypothesis is not difficult to test. We can design experiments to confirm whether the combination of escin with GCs, which alone had no anti-inflammatory action in rodent animals by adrenalectomy, can greatly inhibit inflammation after the administration of physiological dose corticosterone. However, the difficulty in conducting these studies is how to precisely discover the molecular mechanisms of synergistic anti-inflammatory effects between escin and GCs, that is, to determine escin how to affect the GC signaling pathway, from GR to other elements [15].

4. CONSEQUENCES OF THE HPOTHESIS

GCs are widely used to treat inflammatory diseases. However, GCs has multiple effects to inhibit the immune system and it is also associated with an increased susceptibility to infection and a risk for reactivation of latent tuberculosis. If our hypothesis could be proved correct, escin has its own virtue compared with GCs, as escin is not only a safe and potent anti-inflammatory drug, but also an anti-gastric ulcer agent [16]. Furthermore, it is easy to obtain and can be taken orally and venously with less side effects and complications. In conclusion, escin is a promising anti-inflammatory drug, promising wide clinical use within the population.

5. ACKNOWLEDGEMENTS

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6. CONFLICT OF INTEREST STATEMENT

All authors declare that there are no conflicts of interest.

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Health

Pseudomonas aeruginosa ventilator associated pneumonia: improved outcomes with earlier follow-up

Elpis Giantsou[1,2], Nikolaos Liratzopoulos[1], Eleni Efraimidou[1], Konstantinos I. Manolas[1], J. Duncan Young[2]

[1]Intensive Care Unit, Demokritus University of Thrace, Greece
[2]Adult intensive Care Unit, John Radcliffe Hospital, Oxford, United Kingdom; elpisgiantsou@yahoo.com

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ABSTRACT

It is not clear what is the appropriate timing to follow-up patients with ventilator-associated pneumonia (VAP) and Clinical Pulmonary Infection Score >6 between days 3-5 of an appropriate antibiotic treatment. We studied 122 patients with Pseudomonas aeruginosa VAP. A follow-up respiratory sample was collected on days three or five ("day-three" and "day-five" group) and treatment was modified 48h later. Molecular typing identified super-infections or persistence. For serial data another respiratory sample was collected, on day three from the "day-five" group and on day five from the "day-three" group. Sixty patients, in the "day-three" group compared to 62 in the "day-five" group, had reduced fourteen-day mortality (18.3% and 38.7%; p=0.01) and fewer days in intensive care unit (17.2 ± 4.3 compared to 27.3 ± 4.7, p<0.05). Eighteen patients of the "day-five" group were diagnosed with super-infection and 22 with persistence on day five, of whom 14 and 19 had been having these patterns since day three. For patients with Pseudomonas aeruginosa VAP and Clinical Pulmonary Infection Score >6, improved fourteen-day mortality and shorter duration of stay in health-care facilities were observed with earlier follow-up.

Keywords: Ventilator-Associated Pneumonia; Clinical Pulmonary Infection Score; Pseudomonas Aeruginosa

1. INTRODUCTION

In addition to its value in diagnosing ventilator-associated pneumonia (VAP), the Clinical Pulmonary Infection Score (CPIS) has other uses [1]. Initial values of CPIS may guide the duration of antibiotic therapy for patients with VAP [2], while serial measurements may identify patients as potentially un-responsive to antibiotic therapy, when the value of the score is above six from day three to day five of antibiotic treatment [3]. Further investtigation of patients identified as potentially un-responsive should ensure that the administered antimicrobials are appropriate and that extra-pulmonary infections and non-infectious conditions are not involved [4,5]. When the lung remains the suspected focus of infection, the next step should involve a follow-up respiratory sample to investigate treatment failure [6]. However, it is not clear from the data available [3,7] what is the appropriate timing to collect a follow-up respiratory sample from patients with VAP, in whom CPIS remains >6, between days three to five of antibiotic treatment. Should the follow-up respiratory sample be collected on day three or a little later?

The objective was to evaluate an earlier compared with a later timing to retrieve respiratory pathogens and determine treatment failure, for patients with initially appropriately treated *Pseudomonas aeruginosa* (*P. Aeruginosa*) VAP, in whom the simplified CPIS [3] remained >6 between days 3-5 of antibiotic therapy.

2. MATERIALS AND METHODS

The study was conducted at the University Hospital of Thrace, during a 48-month period. Patients were entered into the study if they met all the following: clinical suspicion for VAP [1]; two identical positive solely for P. aeruginosa quantitative cultures, one of tracheal aspirate and one of bronchoalveolar lavage (BAL) (thresholds of $>10^6$ and $>10^4$ colony–forming units/ml, respectively); and simplified CPIS>6 [3] between days 3-5 of treatment. Patients were excluded if they had received solid organ or bone marrow transplant or had evidence of rapid deterioration within 72hr of treatment [8]. The diagnostic and therapeutic approach is presented in **Figure 1**. Initial antibiotic treatment for P. aeruginosa with daily infusion of Amikacin (20mg/kg per day) combined with 6h bolus administration of Piperacillin- Tazobactam (4.5gm) was instigated within 6h of bronchoscopy.

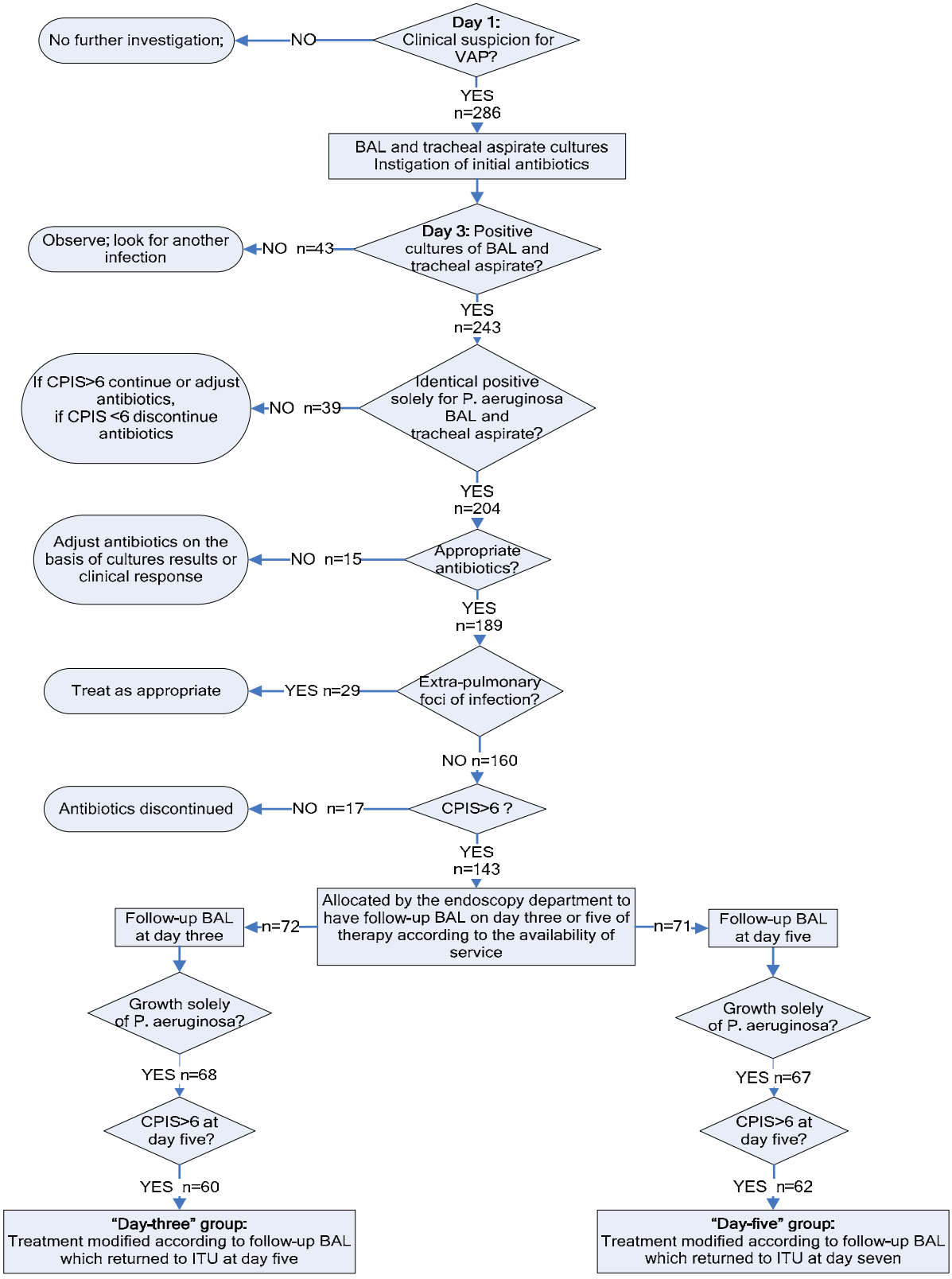

Figure 1. Schematic presentation of the diagnostic and therapeutic approach. VAP, ventilator-associated pneumonia; BAL, bronchoalveolar-lavage; P.aeruginosa, Pseudomonas aeruginosa; CPIS, Clinical Pulmonary Infection Score.

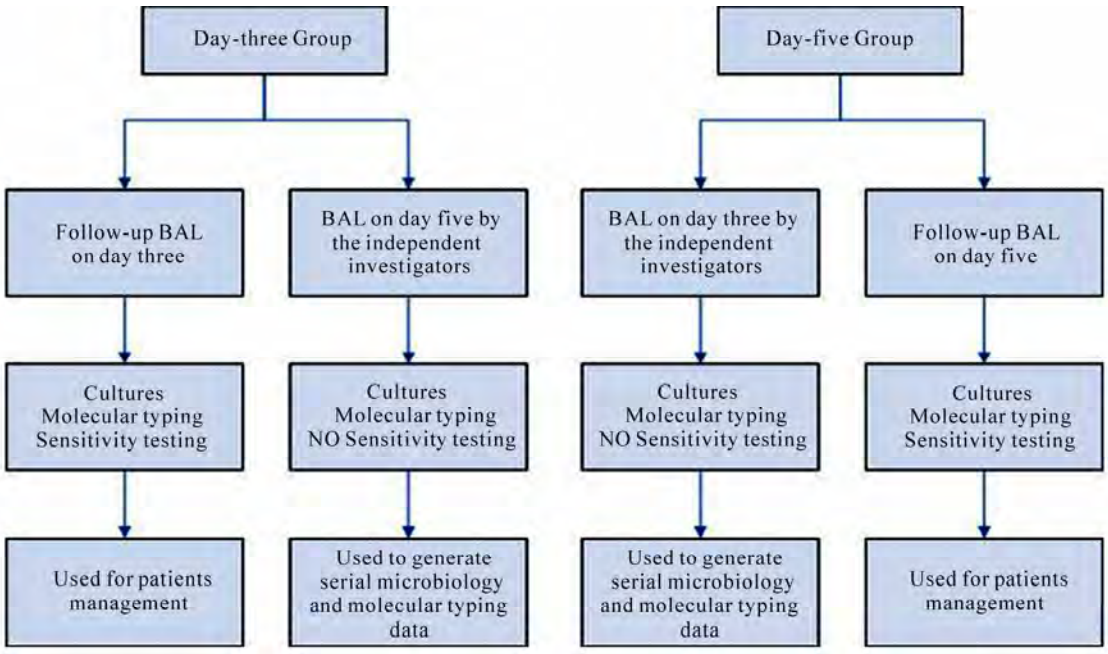

Figure 2. Schematic representation of the approach used for serial microbiology and molecular typing.

The follow-up BAL was collected by the endoscopy group on days three or five as determined by the availability of service and patients shall be referred to as the "day-three" and the "day-five" group respectively. The follow-up BAL cultures and sensitivity tests results returned to the Intensive Care Unit (ICU) 48hr later and were used to modify the antimicrobials. The duration of treatment was 14 days [9].

Pulsed gel electrophoresis was applied to the BAL samples at study entry and follow-up, to identify whether P. aeruginosa isolated at follow-up was super-infection or persistence of the initial isolate. The DNA fragment patterns were interpreted as genetically indistinguishable, closely related or unrelated [10,11]. Isolates genetically unrelated to those grown at study entry were considered super-infections, whereas isolates genetically indistinguishable or closely related, were considered persistence.

For serial microbiologic and molecular typing data two independent investigators performed BAL on day three at the "day-five" group and on day five at the "day-three" group (**Figure 2**). For cost reasons, no sensitivity testing was undertaken for these samples, when P. aeruginosa was isolated, because it was previously tested from the same source. If growth, other than P. aeruginosa, was isolated, then patients were excluded and sensitivity was tested. The outcomes evaluated were mortality, SAPS II [12] and SOFA [13] on day 14, mortality on day 28, mortality in ICU and hospital, duration of mechanical ventilation and duration of stay in ICU and hospital after VAP. CPIS and organ failures [13] were assessed on day 14.

3. STATISTICS

Data were expressed as mean ± standard deviation (SD) or as percentages of total. Continuous data were compared using Student's t-test. The chi-square test with Yates correction for proportions was used for categorical variables. All tests were two sided. Significance was accepted for $p<.05$. Data were analyzed using SPSS 11 (SPSS, Chicago, IL).

4. RESULTS

The admission and study entry characteristics of 122 patients with P.aeruginosa VAP appear in **Table 1**. The CPIS score for the "day-three" and the "day-five" group respectively it was 7.33 (± 0.47) vs 7.32 (± 0.59), p=0.9 on day three and 7.08 (± 0.27) vs 7.1 (0.42) , p=0.46 on day five .

Super-infection and persistence rates of P. aeruginosa as revealed by the follow-up BAL are presented in **Table 2** and schematically in **Figure 3**. For both study groups, the strains of P. aeruginosa identified at follow-up as super-infection or persistence at significant counts were resistant to Piperacillin-Tazobactam and sensitive to Meropenem and Amikacin which replaced the initial combination of Piperacillin-Tazobactam with Amikacin. *P. aeruginosa* strains persistent at insignificant counts at follow-up remained sensitive to the initial antibiotics which remained unchanged.

Significantly lower mortality, SAPS II and SOFA were noted on day 14 for the "day-three" group (**Table 3**). Schematic presentation of mortality and length of

Table 1. Admission and study entry characteristics.

Characteristics	"day-three" group n=60	"day-five" group n=62	P value
On admission			
Age, mean (SD)	55.4 (11.9)	55.6 (13)	0.2
Men, n (%)	39 (65)	40 (64)	0.9
SAPS II, mean, SD	43.3 (5.8)	45.7 (6)	0.7
SOFA, mean, SD	6.7 (2.2)	6.5 (2.1)	0.8
Admission, n (%)			
Medical	33 (55)	31 (50)	0.8
Emergency surgery	14 (23)	16 (26)	
Elective surgery	13 (22)	15 (24)	
Reason for MV, n (%)			
Status asthmaticus	7 (12)	8 (13)	0.9
COPD	11 (18)	10 (16)	
CAP	9 (15)	7 (11)	
Drug overdose	9 (15)	8 (13)	
Abdominal Surgery	4 (7)	5 (8)	
Other surgery	5 (8)	6 (10)	
CHF	5 (8)	6 (10)	
Neurological emergency	10 (17)	12 (19)	
On study entry			
MV before VAP, mean (SD), d	6.9 (1.2)	7 (1.2)	0.9
Antibiotics before VAP, n(%)	47 (78)	46 (74)	0.8
SAPS II, mean (SD)	45.3 (5.8)	46 (6.3)	0.1
SOFA, mean (SD)	6.8 (2.1)	6.7 (2.3)	0.7
CPIS, mean (SD)	7.68 (0.85)	7.6 (0.7)	0.8

Abbreviations: SAPS, Simplified Acute Physiology Score; SOFA, Sequential Organ Failure Assessment; MV, Mechanical Ventilation; COPD, Chronic Obstructive Pulmonary Disease; CAP, Community Acquired Pneumonia; CHF, Congestive Heart Failure; VAP, Ventilator-associated pneumonia.

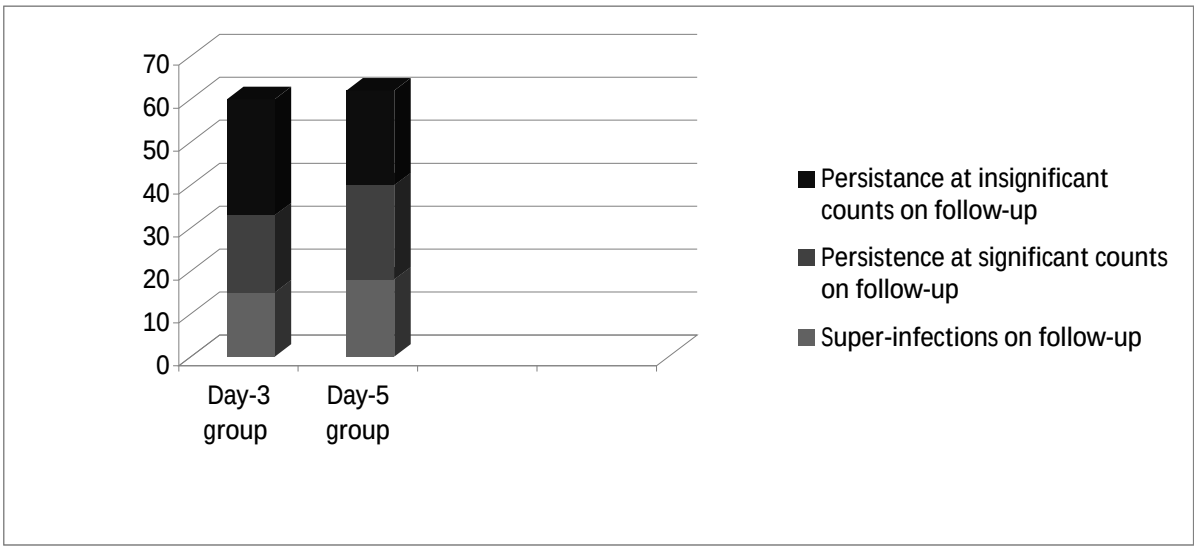

Figure 3. Schematic presentation of P.aeruginosa patterns on follow-up.

Table 2. *P.aeruginosa* patterns on follow-up.

"Day- three" group on follow-up[a], n=60	Patients n (%)	Final antibiotics
Super-infection[b]	15 (25)	M / A
Persistence[c] at significant counts	18 (30)	M / A
Persistence[c] at insignificant counts	27 (45)	PT / A
"Day- five" group on follow-up[a], n=62		
Super-infection[b]	18 (29)	M / A
Persistence[c] at significant counts	22 (35.5)	M / A
Persistence[c] at insignificant counts	22 (35.5)	PT / A

Abbreviations: M, Meropenem; A, Amikacin; PT,Piperacillin-Tazobactam.
[a]The follow-up BAL was performed on day three of therapy for the "day-three" group and on day five of therapy for the "day-five" group.
[b]These *Pseudomonas aeruginosa* strains were identified as super-infections, because they were genetically unrelated to the clone isolated at study entry.
[c]These *Pseudomonas aeruginosa* strains were identified as persistence, because they were closely related or indistinguishable to the clone isolated at study entry.

Table 3. Study outcomes.

End Point	"day-three" group (n=60)	"day-five" group (n=62)	p value
Mortality on day 14, n (%)	11 (18.3)	24 (38.7)	
SAPS[b] on day 14	42.1 (5.8)	49.5 (9.7)	0.01
SOFA[b] on day 14	5.9 (1.6)	8.1 (2)	<0.05
Mortality on day 28, n (%)	17 (28.3)	39 (62.9)	<0.05
Mortality in ICU, n (%)	16 (26.6)	40 (64.5)	<0.05
Mortality in hospital, (n %)	18 (30)	42 (67.7)	<0.05
MV after VAP, d	14.3 (2)	22.7 (2.6)	<0.05
ICU stay after VAP, d	17.2 (4.3)	27.3 (4.7)	<0.05
Hospital stay after VAP, d	23.1 (3.7)	35.5 (4.5)	<0.05
CPIS on day-14	4.2 (1.7)	4.4 (1.5)	<0.05
			<0.05
Organ failure on day- 14 [b], n (%)	19 (38.7)	24 (63.1)	0.59
Cardiovascular	17 (34.6)	23 (60.5)	
Renal	10 (20.4)	14 (36.8)	
Central nervous	2 (4)	4 (10.5)	
Hepatic	2 (4)	4 (10.5)	
Coagulation			

Abbreviations: SAPS, Simplified Acute Physiology Score; SOFA, Sequential Organ Failure Assessment Score; MV, mechanical ventilation; CPIS, Clinical Pulmonary Infection Score [b]For patients alive on day 14. Values are expressed as mean (SD) unless otherwise indicated. Organ failures may not sum up to hundred as some patients may have >1.

stay in health care facilities appear in **Figure 4**.

Eighteen patients of the "day-five" group were diagnosed with super-infection with a new strain of P. aeruginosa at follow-up on day five. Of them 14 had been having super-infection and required treatment change, since day three, as it was diagnosed by the independent investigators on day three (**Table 4**). Five of the 14 (35.7%) P. aeruginosa strains that were responsible for super-infections in the" day-five" group, were present at insignificant concentrations in the BAL that was performed initially to diagnose VAP. Similarly, for the "day-three" group six of the 15 (40%) superinfections on day 3 were due to overgrowth of P. aeruginosa, that was present in insignificant concentrations in the BAL performed initially to diagnose VAP. The evolution over time of superinfection is presented schematically in **Figure 5** for both groups.

Twenty two patients of the "day-five" group were diagnosed at follow-up on day five with persistence at significant counts, of the initially isolated P. aeruginosa

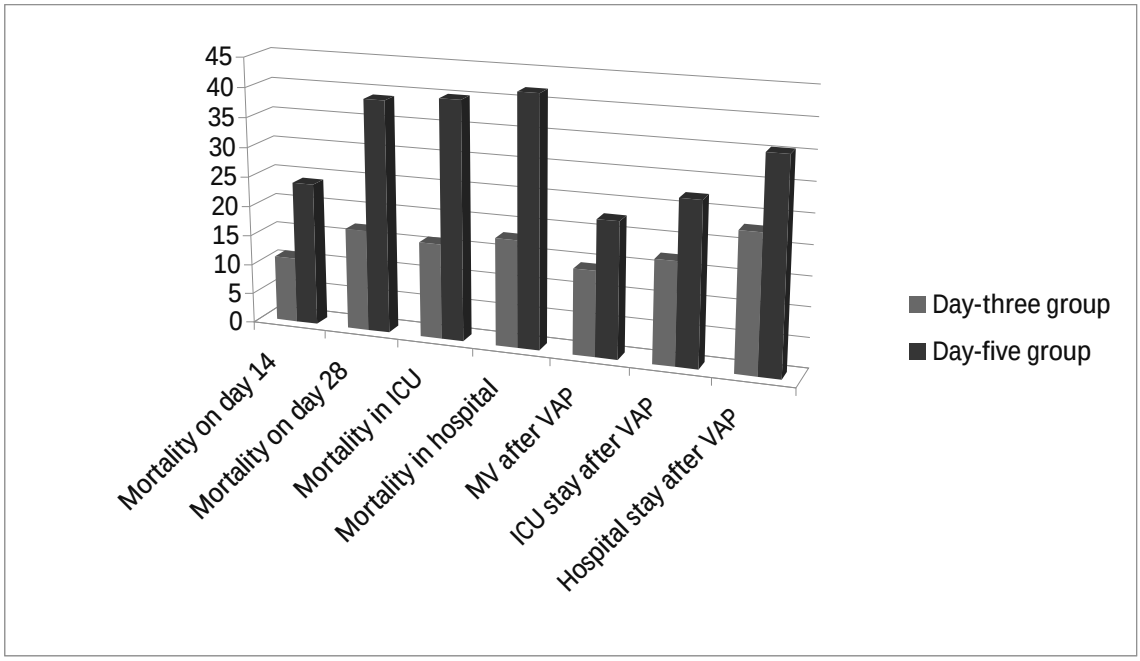

Figure 4. Schematic presentation of mortality and length of stay in health care facilities

Table 4. Super-infection and persistence over time.

Patients, n (%)	"day-three" group[a] (n=60)	"day-five" group[b] (n=62)	p
Super-infections on			
Day three of therapy	15 (25)	14 (22.5)	0.7
Day five of therapy	20 (33.3)	18 (29)	0.8
Persistence at significant counts on			
Day three of therapy	18 (30)	19 (30.6)	0.9
Day five of therapy	22 (36.6)	22 (35.4)	0.8

[a]For the "day-three" group super-infections and persistence at significant counts on day three of therapy were derived from the follow-up BAL and on day five of therapy from the BAL collected by the independent investigators.
[b]For the "day-five" group super-infections and persistence at significant counts on day three of therapy were derived from the BAL collected by the independent investigators and on day five of therapy from the follow-up BAL.

(**Table 4**).Of them 19 had been having this pattern and required treatment adjustment, since day three. Fourteen-day mortality for patients of the "day-five" group, who had super-infection or persistence at significant counts since day three and in whom treatment was adjusted on day seven, was 8 of 14 (57.1%) and 11 of 19 (58%) respectively. By contrast, fourteen-day mortality for patients of the "day-three" group, who had super-infection or persistence at significant counts on day three and in whom treatment was adjusted on day five was 3 of 15 (20%) and 4 of 18 (22%) respectively. The evolution over time of persistence at significant counts is presented schematically in Figure 5 for both groups.

5. DISCUSSION

In this study, improved fourteen-day mortality, severity scores and duration of stay in ICU and hospital were observed with earlier follow-up and re-institution of an appropriate antibiotic regimen for patients with P. aeruginosa VAP, who were initially appropriately treated and in whom CPIS remained >6 six between days 3-5 of treatment.

Few data are available, on clinical outcomes with an earlier or a later recognition of treatment failure for patients with initially appropriately treated VAP. Our study showed mortality of 18.3% for patients re-evaluated on day three and 38.7% for patients re-evaluated on day five. Montravers et al. reported 35% mortality, for patients, who on clinical suspicion for VAP were treated appropriately and on day three were microbiologically re-evaluated, to identify and treat super-infections and persistence [7].

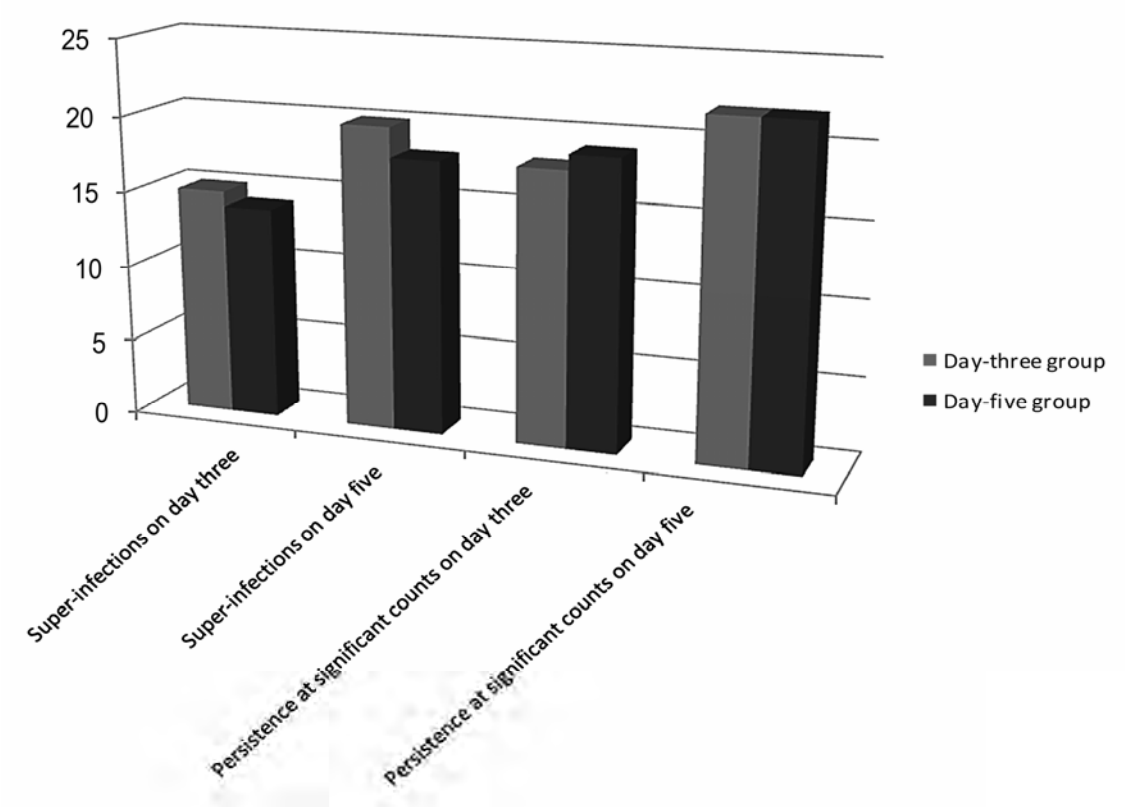

Figure 5. Schematic presentation of super-infection and persistence at significant counts over time

The American Thoracic Society guidelines suggest that the earliest time point to re-assess the antibiotic regimen is day three of treatment [9]. Although, BAL data can optimize antibiotic therapy in VAP, this is translated to improved clinical outcomes when the antibiotics administered on clinical suspicion for VAP are adequate and BAL data become available in a timely manner [14-17]. In our data, both groups had received an appropriate antibiotic therapy on clinical suspicion for VAP, which by day-three became inappropriate due to super-infections and persistence at significant counts. We observed improved outcomes with early recognition and treatment of non-response to therapy. Appropriate antibiotic therapy administered in a timely manner is suggested to be one of the primary determinants of hospital outcome [18] and our data reinforce this view.

Our study has limitations. First, we used the availability of service to determine assignment and not randomization because we could not ensure endoscopy support on the assigned day. This would introduce bias if the availability correlated with aspects of clinical care. Second, the study was necessarily un-blinded, but bias should not have occurred from this as the primary outcome was mortality. Third, it was conducted within a single ICU and a relatively large number of patients were excluded. Therefore the results cannot necessarily be extended to other populations. Fourth, patients in the "day-five" group had CPIS >6 from day three of therapy and treatment were modified on day seven. However, the CPIS provided no evidence for deterioration because it was continuously dropping. Finally, our study was not specifically designed to test whether an earlier follow-up is superior to a later one. To answer this question we need a double blind randomized trial.

Although clearly important, an accurate diagnostic technique and an appropriate initial empirical therapy may not be sufficient to reduce mortality in patients with P.aeruginosa VAP [19,20]. In our data, improved mortality and shorter duration of stay in health–care facilities were observed with earlier follow-up for patients with P.aeruginosa VAP. This finding suggest that clinicians should have low threshold to re-sample early and if necessary to revise therapy for patients with P. aeruginosa VAP, who failed to reduce CPIS to values below six between days three to five of antibiotic treatment.

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Subgroups of chronic fatigue syndrome based on psychiatric disorder onset and current psychiatric status

Molly M. Brown, Carly Kaplan, Leonard A. Jason, Christopher B. Keys

Center for Community Research, DePaul University, Chicago, USA; mbrown59@depaul.edu

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ABSTRACT

Few studies have examined the effects of psychiatric disorders occurring over a long duration among patients with chronic fatigue syndrome (CFS). The role of premorbid and current psychiatric disorders in impairment was explored with a sample of 113 participants with CFS. Subgroups were created based on past and current psychiatric status including those whose psychiatric history was premorbid and current, postmorbid and current, past but not current, and those with no lifetime diagnosis. Results from a one-way MANOVA revealed that patients with a premorbid and current psychiatric disorder reported significantly higher pain severity, more somatic symptoms, poorer sleep quality, and poorer quality of life than those with no psychiatric history. Levels of fatigue and physical functioning among patients with CFS were unrelated to the four subgroups in this study. Although those with a premorbid and current psychiatric disorder were differentiated from those with no psychiatric history on some markers of impairment, the sample as a whole had severe fatigue-related impairment, which is the cardinal symptom of CFS. Implications for research are discussed.

Keywords: Chronic Fatigue Syndrome; Psychiatric Comorbidity; Subgroups; Impairment

1. INTRODUCTION

Chronic fatigue syndrome (CFS) is a chronic, debilitating illness that remains poorly understood. Some patients with CFS experience psychiatric symptoms, but the role of these symptoms in the development, maintenance, and severity of the illness is unclear. Several studies have found high rates of psychiatric comorbidity among patients with CFS in the range of 45% to 50%, [1, 2] and up to 82% for a lifetime psychiatric diagnosis, [3] exceeding rates in the general population.

A number of studies have examined the role of comorbid psychiatric issues in the course of CFS. While one study found that patients with CFS who had a comorbid psychiatric diagnosis had significantly more severe fatigue than those without a psychiatric disorder, [4] most research has not found that psychiatric comorbidity increases impairment in CFS. Studies comparing patients with and without psychiatric comorbidity have found no differences in impairment in sleep, [5] neurocognitive functioning, [6,8] physical functioning, [9,11] fatigue severity, [5] and widespread pain. [11] These results suggest that psychiatric comorbidity is not related to higher illness severity in this population.

Due to the unexpected lack of evidence for a relationship between psychiatric comorbidity and illness severity in CFS, it is perhaps more important to explore long-term psychiatric status in CFS, as opposed to present psychiatric comorbidity only. Individuals with psychiatric illness early in life may be more likely to develop CFS later in life, [12] indicating that a subgroup of patients with CFS may have a long-term history of psychiatric disorder beginning prior to the onset of CFS. It has been hypothesized that an onset of psychiatric disorder predating CFS may be indicative of a long history of poor coping skills, leading to increased impairment. [13].

Tiersky, Matheis, DeLuca, Lange, and Natelson [13] developed psychiatric subgroups based upon whether the onset of the psychiatric disorder was before (premorbid) or after (postmorbid) CFS onset. They found that patients with CFS with a premorbid and current psychiatric diagnosis performed significantly worse on neuropsychological tests than patients with no history of psychiatric disorder and healthy controls. However, they did not find increased physical impairment in this subgroup. Tiersky *et al*. developed more specific psychiatric subgroups of patients with CFS compared to previous studies. Nonetheless, their study did not include patients who had a psychiatric disorder in the past but did not cur-

rently meet criteria for the disorder. In other words, it may also be important to examine CFS severity for people who have recovered from a past psychiatric disorder, as remission from a psychiatric illness is associated with increased self-efficacy [14] and may be indicative of adaptive coping leading to better functioning. Further, the authors did not explore some key features of CFS severity such as sleep quality, pain severity, and diversity of symptoms.

The present study examined four groups of patients with CFS: those with a premorbid and current psychiatric diagnosis, those with a postmorbid and current psychiatric diagnosis, those with a past (either premorbid or postmorbid) but no current psychiatric diagnosis, and those with no history of psychiatric diagnosis. We hypothesized that patients with a premorbid and current psychiatric disorder would have increased physical and psychiatric impairment compared to patients with a postmorbid and current psychiatric diagnosis, those with a past but not current psychiatric disorder, and those with no history of psychiatric disorder.

2. METHOD

2.1. Participants

The present investigation used baseline data derived from a larger longitudinal study of non-pharmacological treatment interventions for CFS. [15] Participants were recruited from physician referrals, media advertisements, and CFS support groups.

Participants were at least 18 years of age, not pregnant, able to read and speak English, and considered to be physically capable of attending scheduled appointments. Participants were included if they met the Fukuda *et al*. [16] criteria for CFS (i.e., six or more months of persistent fatigue accompanied by four of the following eight symptoms: tender lymph nodes, sore throat, new or different headaches, muscle pain, joint pain, post-exertional malaise, unrefreshing sleep, and memory or concentration difficulties). Medical and psychiatric examinations were provided to rule out exclusionary medical or psychiatric diagnoses according to the Fukuda *et al*. criteria. In addition, participants in the current study must have provided self-reported date of onset for CFS and psychiatric diagnoses. A total of 113 participants were included in the present investigation. The DePaul University Institutional Review Board approved all procedures. All participants provided written informed consent.

2.2. Materials

2.2.1. CFS Questionnaire

The CFS Questionnaire was used to collect date of CFS onset, demographic, health status, medication usage, and symptom data. This screening scale has demonstrated adequate validity and inter-rater and test-retest reliability. [17,18]

Jason, Corradi, and Torres-Harding [19] explored 22 theoretically derived symptoms of CFS from the CFS Questionnaire for diagnostic importance. The authors found that these 22 symptoms are common among patients with CFS and are indicators of symptom clusters, including: neurocognitive (e.g., slowness of thought), vascular (e.g., dizzy after standing), inflammatory (e.g., allergies), muscle/joint (e.g., muscle pain), infectious (e.g., flu-like symptoms), and sleep/post-exertional (e.g., unrefreshing sleep) symptoms. This study utilized these 22 symptoms to measure total somatic complaints among participants. The total number of currently present symptoms endorsed by participants was used to determine the breadth of current symptoms experienced by participants.

2.2.2. Structured Clinical Interview for DSM-IV (SCID)

The SCID [20] was administered in order to establish Axis I psychiatric diagnoses. While a coding scheme is included in the instrument, the SCID allowed for clinical judgment in the assignment of symptoms to psychiatric or medical categories, a crucial distinction in the assessment of symptoms that overlap between CFS and psychiatric disorders, e.g., fatigue, concentration difficulty, and sleep disturbance. [21] A psychodiagnostic study [22] validated the use of the SCID in a sample of CFS patients. Through questioning about psychiatric symptom onset, an approximate date of psychiatric disorder onset is determined on the SCID.

2.2.3. Medical Outcomes Study-Short Form-36 (SF-36)

The SF-36, was used to measure self-reported functional status related to health. [23] The full measure included 36 self-report items that identified eight health concepts, or scales. This study used the Physical Functioning scale to measure overall disability. Higher scores indicated better health**.** Test construction studies for the SF-36 [24,25] found adequate internal consistency and discriminant validity across the eight scales of this measure.

2.2.4. Fatigue Severity Scale (FSS)

The FSS [26] was used to measure fatigue. This scale included 9 items rated on 7-point scales, and higher scores indicated more severe behavioral consequences of fatigue. Previous findings have demonstrated the utility of the FSS to discriminate between individuals with CFS, multiple sclerosis, and primary depression. [27] Within a CFS-like group, the FSS was found to be closely associated with severity ratings for the eight Fukuda *et al.* [16] CFS symptoms as well as with functional outcomes related to fatigue. [28]

2.2.5. Brief Pain Inventory (BPI)

The BPI [29] was administered to measure the intensity of pain (pain severity) and the interference of pain in the patient's life (pain interference). This measure consisted of 14 questions with scores on each scale ranging from 0 to 10. Higher scores indicated more pain. This measure exhibited adequate levels of reliability to assess pain in non-cancer samples, with alpha coefficients of .70 and above. [29] It also evidenced good concurrent validity with other generic pain measures. [30]

2.2.6. Pittsburgh Sleep Quality Index (PSQI)

The PSQI was a self-report measure developed to assess sleep quality in psychiatric research. [31] This index measured sleep disruptions and sleep quality. There were 19 questions (on 0-3 scale) which generated seven "component" scores: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction. The sum of scores for these seven components yielded one global score, which ranged from 0 to 21. Higher scores indicated worse sleep quality. The global scale demonstrated adequate internal consistency, test-retest reliability, and discriminant and concurrent validity. [31]

2.2.7. Quality of Life Scale (QLS)

The QLS measured satisfaction with different life activities for individuals with various chronic illnesses. [32] The scale consisted of 16 items answered on a Likert-type 1 to 7 scale asking how satisfied individuals are with each item. Higher scores indicated more overall life satisfaction. This scale demonstrated high test-retest reliability, convergent, discriminant, and construct validity among individuals with various stable chronic illnesses. [32]

2.3. Procedure

Once study eligibility criteria were met and no exclusionary illnesses were found based on the medical examination or SCID findings, participants completed self-report measures. The 113 participants were then divided into four psychiatric subgroups based on onset of psychiatric disorder in relation to CFS onset and current psychiatric status. In order to determine whether participants had a psychiatric disorder that predated the onset of CFS, the date of psychiatric disorder onset derived from the SCID was compared with self-reported date of CFS onset from the CFS Questionnaire. Specifically, participants with a current psychiatric disorder who reported having a psychiatric disorder at any point in their life prior to developing CFS were considered to have a premorbid and current psychiatric disorder (n = 32; 28.3%). Those with a current psychiatric diagnosis and a history of psychiatric disorder that never predated CFS onset were considered to have a postmorbid and current disorder (n = 12; 10.6%). Those with a history of psychiatric disorder who do not currently meet criteria for a psychiatric disorder comprised a third subgroup referred to past but not current psychiatric disorder (n = 28; 24.8%). Finally, patients with no lifetime psychiatric diagnosis were placed into a fourth subgroup (n = 41; 36.3%).

2.4. Statistical Analyses

Pearson chi-square analyses and one-way analysis of variance (ANOVA) were used to examine differences among the four psychiatric groups with regard to demographic variables. Fisher's Exact Tests were computed using SAS Proc Freq to compare the three groups with a history of psychiatric disorders on DSM-IV diagnostic categories. A one-way ANOVA was used to compare total number of lifetime psychiatric diagnoses between the three groups.

One-way multivariate analysis of variance (MANOVA) was used to test the primary hypotheses of this study with psychiatric group (premorbid and current, postmorbid and current, past but not current, and no lifetime psychiatric disorder) as the independent variable. The use of MANOVA reduced the likelihood of Type I error when making comparisons of multiple related dependent variables. [33] Upon examination of the correlation matrix of the outcome variables used in this study, all outcome variables were moderately correlated ($r \geq .3$) with at least two other outcomes, indicating that MANOVA would be appropriate. Wilks' Lambda F approximation was used for interpretation of the multivariate test, and Bonferroni tests were used for post hoc comparisons.

3. RESULTS

3.1. Demographic Characteristics

In regards to demographic characteristics, 83.2% of participants were female. The average age was 43.8 years. Regarding ethnicity, 87.6% were White, 4.4% were African American, 4.4% were Latino, and 3.5% were Asian American. As for marital status, 48.7% were married or living with a partner, 32.7% were single, and 17.7% were divorced or separated. In terms of work status, 40.7% were working or full time students and 59.3% were part-time students, retired, unemployed or on disability. With regards to education, 46.9% had earned a standard college degree, 22.1% had a graduate or professional degree, 21.2% had partial college, and 9.7% had a high school/GED degree or less. No significant

Table 1. Lifetime Psychiatric Diagnoses for Psychiatric Subgroups.

	Premorbid and current (n = 32)	Postmorbid and current (n = 12)	Past but not current (n = 28)	Sig.
Any Mood Disorder	59.4%	58.3%	50.0%	
Major Depressive Disorder	50.0%	58.3%	46.4%	
Dysthymic Disorder	18.8%	0.0%	0.0%	*
Other Mood Disorder	0.0%	0.0%	3.6%	
Any Anxiety Disorder	56.3%	33.3%	46.4%	
Generalized Anxiety Disorder	9.4%	8.3%	3.6%	
Panic Disorder (with or without Agoraphobia)	25.0%	16.7%	17.9%	
Posttraumatic Stress Disorder	37.5%	0.0%	10.7%	**
Other Anxiety Disorder	6.3%	8.3%	10.7%	
Adjustment Disorder	21.9%	33.3%	7.1%	
Other	25.0%	0.0%	14.3%	
Total Lifetime Psychiatric Disorders (Mean)	1.97 [a, b]	1.25 [a]	1.25 [b]	***

Notes: *p < .05, **p < .01, ***p < .001; Similar letters across rows indicate significant difference

differences were found among the four groups in terms of demographic variables.

3.2. Diagnostic Outcomes

Total lifetime psychiatric diagnoses and rates of different psychiatric diagnostic categories were compared for the three groups with a history of psychiatric disorder (**Table 1**). Fisher's Exact Tests revealed that the three groups did not differ in terms of lifetime presence of anxiety or mood disorders. When comparing differences among the three psychiatric groups for specific psychiatric diagnoses, a significant difference was revealed for lifetime dysthymic disorder (p = .02), and the premorbid and current psychiatric group had the highest rate (18.8%) compared to the other two groups with rates of 0%. A significant difference between groups was also found for lifetime posttraumatic stress disorder (p = .01). The premorbid and current group had the highest rate of posttraumatic stress disorder (37.5%), the past but not current group had the second highest rate (10.7%), and no participants from the postmorbid and current group met criteria during their lifetime.

A one-way ANOVA revealed that the groups significantly differed on total number of lifetime psychiatric diagnoses, $F(2, 69) = 9.19$, $p < .001$. Bonferroni post hoc analyses indicated that the premorbid and current psychiatric group had significantly more lifetime psychiatric diagnoses than both the postmorbid and current group (p = .01), and the past but not current group (p = .003).

3.3. Main Outcomes

Sixteen participants had missing data for one or more of the self-report outcome measures and were therefore excluded, which left a total of 97 participants for analysis of main outcomes (See **Table 2**). Results from the one-way MANOVA revealed a significant overall multivariate effect for psychiatric group on the combined DVs, Wilks' Lambda = .68, p = .03.

Descriptive statistics for outcomes are reported in **Table 2**. Upon examination of univariate effects, significant differences were found for pain severity [$F(3, 93) = 3.08$, $p = .03$], pain interference [$F(3, 93) = 3.27$, $p = .03$], total somatic symptoms [$F(3, 93) = 3.86$, $p = .01$], sleep quality [$F(3, 93) = 4.59$, $p = .01$], and quality of life [$F(3, 93) = 3.31$, $p = .02$]. No significant univariate effects were found for fatigue severity or physical functioning. Bonferroni post hoc tests revealed the premorbid and current group scored significantly worse than the no lifetime diagnosis group for pain severity (p = .02), pain interference (p = .02), total somatic symptoms (p = .03), sleep quality (p = .003), and quality of life (p = .02).

4. DISCUSSIONS

Patients with CFS with a premorbid and current psychiatric disorder reported significantly higher pain severity and interference, more somatic symptoms, poorer sleep quality, and poorer quality of life than those who have never been diagnosed with a psychiatric disorder. No significant differences in impairment were found for

Table 2. Means (and Standard Deviations) for Psychiatric Subgroups on Outcome Measures.

	Premorbid and current (n = 29)	Postmorbid and current (n = 11)	Past but not current (n = 22)	No lifetime diagnosis (n = 35)	Sig.
Physical Functioning[1]	38.72 (24.00)	40.91 (28.88)	48.64 (18.01)	46.57 (22.68)	
Fatigue Severity[2]	6.19 (1.01)	6.29 (0.64)	6.16 (0.71)	5.98 (0.69)	
Pain Severity[2]	4.94 (2.19) [a]	3.98 (2.39)	4.30 (1.58)	3.33 (2.31) [a]	*
Pain Interference[2]	5.33 (3.15) [a]	4.81 (3.25)	4.40 (2.01)	3.24 (2.56) [a]	*
Somatic Symptoms[2]	16.21 (3.56) [a]	17.00 (3.23)	15.36 (4.67)	13.37 (3.99) [a]	**
Sleep Quality[2]	9.55 (1.77) [a]	8.36 (3.29)	8.05 (2.28)	7.49 (2.23) [a]	**
Quality of Life[1]	59.48 (15.87) [a]	69.91 (18.11)	65.68 (12.42)	71.03 (15.16) [a]	*

Notes: Similar letters across rows indicate significant difference; [1]Higher numbers are better; [2]Lower numbers are better; *p < .05, **p < .01

participants in the other two psychiatric history groups compared to the no lifetime diagnosis group. These results in combination with two of Tiersky *et al.*'s [13] neuropsychological findings suggest that patients with CFS who have a premorbid and current psychiatric disorder have significantly more impairment than those with no psychiatric history, while patients with other categorizations of psychiatric history are not differentiated from patients without a psychiatric history in terms of impairment. However, the postmorbid and current psychiatric group did, in fact, demonstrate the highest level of fatigue severity and the most somatic symptoms compared to the other three groups, but significant differences were not revealed due to the small sample size for this group. Although patients with a premorbid and current psychiatric history were found to represent a subset of the CFS population that experiences particularly severe illness symptomatology, patients with a postmorbid and current diagnosis may also have increased illness severity.

Despite findings of increased impairment on some outcomes for those who had premorbid and current psychiatric diagnoses, no differences were found between groups for physical functioning or fatigue severity. Moreover, it is evident from this study that patients with and without psychiatric disorders exhibit notably high levels of fatigue and disability. These findings are consistent with previous research demonstrating that psychiatric comorbidity does not differentiate patients in terms of physical functioning [9] or fatigue severity. [5] Of note is that fatigue is the cardinal symptom of CFS, and findings from this and previous studies suggest that this symptom is present at a severe level regardless of psychiatric status.

In terms of severity of psychiatric functioning among patients with CFS, our prediction that the premorbid and current psychiatric group would evidence the most psychiatric dysfunction as defined by more lifetime psychiatric diagnoses was confirmed and was consistent with prior research. [13] This finding suggests that patients who have had ongoing mental health issues beginning prior to CFS onset tend to have more pervasive emotional problems. High levels of psychiatric impairment over time, in turn, may be related to increased illness-related impairment compared to those with a shorter-term history of psychiatric disorder.

The premorbid and current group had, on average, a total of 16.21 somatic symptoms compared to the no history group which had an average of 13.37 symptoms. This greater number of somatic symptoms suggests that the clinical presentation of patients with longstanding mental health issues is more complex than those without a psychiatric history. Exclusion of premorbid psychiatric disorder in CFS samples has been used to reduce sample heterogeneity in some studies. [34] Results from this study provide support for sample selection strategies that take into consideration premorbid psychiatric functioning, as long-term psychiatric status adds complexity to the illness symptomatology.

Several limitations can be noted for the present investigation. The use of self-reported onsets of psychiatric disorder and CFS to determine psychiatric subgroups may be problematic, as both are vulnerable to recall bias. [35,36] Further, research has shown that those with a gradual CFS onset were more likely to report long-term depressive symptomatology gradually leading to the onset of the illness. [37] Thus, the self-reported onset of CFS in the present study may not have been accurate for all participants, particularly those who also had a gradual illness onset. Finally, differences between groups may not have been revealed in the analysis due to small sample sizes and low power. Future research with a larger sample size is needed to more fully explore functioning among patients with a postmorbid and current psychiatric diagnosis.

This study added to previous research demonstrating that neuropsychological functioning is impacted based on premorbid and current psychiatric status. [13] The

findings indicated that long term psychiatric dysfunction increased impairment on several key indicators of CFS severity: sleep difficulties, pain, and wide ranging somatic symptoms. However, the sample as a whole had high levels of fatigue and physical impairment which were unrelated to the four subtypes, suggesting that even patients without a psychiatric history suffer from severe disability. Since the premorbid and current psychiatric history group also evidenced the most psychiatric dysfunction, treatment targeting mental health issues may help reduce symptom severity for this particular subgroup of patients. Past research suggests that examining current psychiatric status may not differentiate patient in terms of symptomatology. Based on the findings from this study, future research exploring the role of psychiatric functioning in CFS should examine psychiatric subgroups based on the onset and current status of the psychiatric disorder.

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Comparative study of haemagglutination inhibition, Agar gel precipitation test, Serum neutralization and Enzyme linked immunosorbent assay for detection to avian influenza viruses

Shahid Faraz, Muhammad Abubakar[1], Mohammad Farooque[2], Sarfaraz Ali Fazlani, Ghluam Hussain Jaffar

Faculty of Veterinary and Animal Sciences, Lasbela University of Agriculture, Water and Marine Sciences, Uthal.
[1]National Veterinary Laboratory, Park Road, Islamabad.
[2]Quarantine Officer, Animal Quarantine Department, Islamabad.

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ABSTRACT

The sensitivity, specificity and reproducibility of the serological tests for detection of avian influenza viruses were carried-out by using Hamagglutination inhibition (HI), Agar gel precipitation test (AGPT), and Enzyme linked immunosorbent assay (ELISA) and Serum neutralization test. The geometric mean titre (GMT) of haemagglutination inhibition antibodies recor- ded as log_2 indicated that the post vaccination titres in the field were on higher side i.e., 7.9 for H7 and 5.9 for H9. The correlation between HI titre and AGPT affirmed that for the AGPT test need high antibody titre for positive reaction. The pooled sera were also used to correlate the serum neutralization test and enzyme linked immuno-sorbent assay. The serial two fold diluteons were tested for the serum neutralization activity and concluded that the HI titre log_2 4 provided 100% protection, than 52% and 45% protection in 1:2 and 1:4 dilution was recorded, respectively. Similarly, the ELISA test showed positive results up to 1:16 HI titre, i.e. log_2 4 and confirmed the linear relation between these two serological tests. In HI test, the concentration of antigen can influence the result. It also needs careful preparation of concentration of erythrocyte suspension. Agar Gel immuno-diffusion is basically a qualitative test as it can not determine the quantity of antigen or antibody with the help of this test. It lacks the level of sensitivity as offered by other test. If serum neutralization test is performed on a pooled serum samples, then it could lead to a false conclusion on antibodies status. ELISA is most sensitive, specific and accurate as compare to all other serological tests.

Keywords: Serology; HI; ELISA and Avian Influenza Virus.

1. INTRODUCTION

Infectious viral diseases are a major threat to poultry. Avian influenza is one of most important among them which inflicts heavy economical losses. It is caused by a virus that belongs to family Orthomixoviridae, genus influenza A virus [1].

Avian influenza virus is classified into subtypes on the basis of antigenic differences in their surface glycoprotein hemagglutinin (HA; H) and neuraminidase, (NA; N). To date 16H subtypes (H1-H16) and 9N subtypes (N1-N9) have been recognized [2]. It rapidly infects the poultry when heavy outbreak occurs. Many species of birds are found to be susceptible to avian influenza virus.

Avian influenza viruses are circulating periodically among the domestic poultry over 100 years [3]. Wild waterfowl and shorebirds are considered to be reservoir of influenza A virus because the species harbor all 16 HA subtypes [4]. Although chicken and turkeys are not natural host species for avian influenza but these viruses routinely cross over from wild bird's reservoir to infect poultry birds [5].

During 2003 and 2004, there were quite a few outbreaks of avian influenza throughout the Asia, including South Korea, Japan, Indonesia, Vietnam, Thailand and China. The outbreak resulted not only tremendous economic loses in the poultry industry but also claimed death in human in Vietnam and Thailand in 2004.

Table 1. Calculation of the 50% endpoint of a neutralization test by the method of Reed and Muench.

Serum Dilution			Response		Accumulated Values		Ratio Infected / Total	% Infected Embryos
Numerical Value	Log	Infection Ratio	Infected embryos	Non Infected embryos	Infected embryos	Non Infected Embryos		
Undiluted	-	0/5	-	-	-	-	-	-
1:2	$10^{-0.3}$	0/5	5	0	18	0	18/18	100%
1:4	$10^{-0.6}$	1/5	4	1	13	12	13/25	52%
1:8	$10^{-0.9}$	2/5	3	2	9	11	9/20	45%
1:16	$10^{-1.2}$	3/5	3	2	6	9	6/15	40%
1:32	$10^{-1.5}$	3/5	2	3	3	7	3/10	30%
1:64	$10^{-1.8}$	4/5	1	4	1	4	1/5	20%
Negative Control Sera	-	05	-	-	-	-	-	-
Positive Control Sera	-	00	-	-	-	-	-	-

Table 2. Correlation among HI (log_2) AGP and ELISA.

H7			H9		
HI	AGPT	ELISA	HI	AGPT	ELISA
00	00	-	2	-	0.0
00	00	-	4	-	0.1
00	00	-	8	-	0.2
16	-	0.4	16	-	0.5
32	-	0.8	32	-	1.01
64	-	0.9	64	-	1.32
128	+	1.02	128	-	1.28
256	+	1.43	256	+	1.42
512	++	1.72	512	++	1.59
1024	++	1.78	1024	++	1.62
2048	++	1.82	2048	++	1.80

Shows positive and negative Pattern reactivity of AGPT
- Negative
+ Positive
++ Strong Positive

Avian Influenza viruses have been reported to cause high mortality in Pakistan. The first outbreak of avian influenza virus in Pakistan was recorded in 1995 when highly pathogenic influenza of H7 subtype was identified Naeem and Hussain [6]. In 1998 Avian Influenza subtype H9N2 was isolated from breeder flock showed a reduction in egg production along with respiratory infection Naeem *at el.* [7]. The H7 and H9 strains were still being isolated in Karachi in 2004.

New diagnostic test for emerging avian diseases are developed using molecular biology techniques. These mainly rely on detection of nucleic acid (either RNA or DNA) unique to that pathogen. Analysis is being used to identify the species, subspecies, type and subtype and even in some cases individual strains. These tests are based on molecular techniques such as Restriction fragment length polymorphism (RFLP), Polymerase chain reaction (PCR) and nucleic acid sequencing. The serological detection of antibodies for avian Influenza virus in the poultry birds is of the great importance in preventing and controlling the avian influenza. This study revealed standard sero-diagnostic techniques used to evaluate sensitivity, specificity and accuracy of the techniques in identification of avian influenza in birds.

2. MATERIALS AND METHODS

Day-old broiler chicks were purchased and used for the experiment. The broiler chickens were divided into three groups A, B and C. Group A and B were vaccinated with H7N3 and H9N2, respectively, while group C was kept as control. Before vaccination, the blood samples were collected randomly from 5% birds prior to vaccination at 3rd week of age to determine the status of maternal antibodies in the sera of chicks.

2.1. Serum Samples

To assess the susceptibility of chickens the serum samples were checked prior to vaccinate and found zero titre against avian influenza. Blood samples were allowed to coagulate than serum was collected, marked, centrifuged and stored at 20 ^{o}C prior to use.

2.2. Source Virus

A local virus isolate chicken/Pakistan/23/99 (H9N2) virus was used with ELD 50 of $10^{9.26}$/0.1ml. Another virus isolate H7N3 was also received from Sindh Poultry Vaccine Centre (SPVC) with ELD 50 of $10^{7.56}$/0.1ml. Both serotypes were confirmed from southeast poultry research institute Georgia.

2.3. Haemagglutination Inhibition Test

The (HI) test was performed by adopting the technique described in (OIE) manual of diagnostic tests using 4HA units of H7 and H9 viruses with 1% suspension of washed chicken RBCs. Serum was diluted while antigen was constant.

2.4. Agar Gel Precipitation (AGP) Test

This test was performed to adopt the method described by Beard in 1998. Agar was prepared in borate buffer, than allowed to solidify. Cylinder of gel were cut with a gel cutter, than antigen was placed in the central wells while the sera were in the peripheral wells than the Petri plates were kept in moist chamber to avoid drying of gel.

2.5. Virus Neutralization Test

The virus neutralization test was performed to titrate the antibodies against avian influenza virus; both strains were checked and confirmed for sterility. Test was done by using the embryo inoculation, ß-method (constant virus diluted serum) and followed the technique described by Beard [8], while endpoint titre was calculated as $\log_2$ exponent that was 50% neutralization endpoint or PD50 by the formula of Reed and Munch.

2.6. ELISA Test

The ELISA test was run in accordance as described by Terry and Tony in 1991 [9] and as described in the monograph of Australian Centre for International Agricultural Research (ACIAR 1995), in which plates were read at 405nm absorbancy in a microplate ELISA reader.

3. RESULTS

The geometric mean titer (GMT) in $\log_2$ of serotype H7 was recorded as 7.9±0.23 (66) and for H9 was calculated as 5.7±0.29 (66). The mean H7 titre was significantly ($p<0.05$) higher than H9.

3.1. Agar Gel Precipitation Test

The pattern of reactivity of both strains is similar and no remarkable difference has been observed. However, the precipitation line can be seen in those samples which have HI titres more than 7 $\log_2$ in H9 subtype. Moreover, there is a linear relation between precipitates of antibodies and antigen. Results also showed that H7 subtype was not positive till the HI titre was 9 $\log_2$.

3.2. Virus Neutralization Test

Before the using of strains for serum Neutralization Test, the ELD 50 was determined and recorded as $10^{11.26}$/ml for H9 and $10^{8.31}$/ml for H7subtype. The results in (Table 1) indicated that undiluted and 1:2 dilution of serum showed 100% protection and than gradually decreased i-e 52% and 45% protection in 1:4 and 1:8 dilution respectively. However, negative control sera showed no protection while positive showed 100%.

3.3. ELISA Test

The average optical density value (OD405nm) of positive sera showed 0.4 OD up to 1:16 dilution, i.e, 4 on log 2. Both serotypes showed positive correlation between HI titre and ELISA at GMT $\text{Log}_2$4. However there is a linear relation between HI titre, ELISA and AGPT shown in **Table 2.**

4. DISCUSSION

Present study revealed that ELISA is better than HI in identifying sera with low antibody titer. HI titer within range of 1:2 to 1:4 are considered as suspected whereas 1:8 may be considered as positive and according to the manufacturer of ELISA kit, 0.2 value considered as a suspected positive value if value was recorded more than 0.6 considered as positive Ewing *et al.*[10].

Meilinjin *et al.* [11] conducted the comparative study on newly developed ELISA technique, in which they used nucleoprotein as antigen for detecting the antibodies to Avian Influenza Virus. They compared this technique with (HI) and ELISA test. Comparative study indicated that these two tests had a high agreement ratio and

no statistically significant difference.

Haem-agglutination inhibition test and titration of antibodies by geometric mean titer (GMT) is the convenient and best technique to measure the level of protection in vaccinated chickens as well as to check the efficacy of vaccine, such types of results were also reported by Meulemans, *et al.* [12] who carried out a study to run HI, AGP and ELISA for measuring the antibodies against avian influenza virus infection. His studies showed a linear relation among these three techniques, when the chickens were exposed with Avian Influenza Virus the antibody status was measured 157 day post infection. It was suggested that AGP is a type-specific; the HI detects only haemagglutinin subtypes, however only ELISA is most sensitive test to detect the antibodies.

The findings of present study showed a degree of correlation between HI, AGP, ELISA and virus Neutralization test, however the degree of accuracy depends on the standardization of reagents, buffers and all other parameters related to the standard procedures of the test. This statement correlates with the availability of equipments and expertise in different laboratories as they perform the same test. That's why the repeatability and reproducibility are two key features that a test must have to be accepted for different laboratories.

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Socio-demographic determinants of health status of elderly with self-reported diagnosed chronic medical conditions in Jamaica

Paul. A. Bourne[1]*, Donovan. A. McGrowder[2]

[1]Departments of Community Health and Psychiatry, The University of the West Indies, Kingston, Jamaica; paulbourne1@yahoo.com
[2]Pathology, Faculty of Medical Sciences, the University of the West Indies, Kingston, Jamaica

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ABSTRACT

Objectives: The aim of the current study is to examine the health status of elderly in rural, peri-urban and urban areas of residence in Jamaica, and to propose a model to predict the social determinants of poor health status of elderly Jamaicans with at least one chronic disease. Methods: A sub-sample of 287 respondents 60 years and older was extracted from a larger nationally cross-sectional survey of 6783 respondents. The stratified multistage probability sampling technique was used to draw the survey respondents. A self-administered questionnaire was used to collect the data from the sample. Descriptive statistics were used to examine the demographic characteristics of the sample; chi-square was used to investigate non-metric variables, and logistic regression was the multivariate technique chosen to determine predictors of poor health status. Results: Almost thirty six percent of the samples had poor health status. Majority (43.2%) of the sample reported hypertension, 25.4% diabetes mellitus and 13.2% rheumatoid arthritis. Only 35.4% of those who indicated that they had at least one chronic illness reported poor health status and there was a statistical relation between health status and area of residence [χ^2 (df = 4) = 11.569, *P* = 0.021, n = 287]. Rural residents reported the highest poor health status (44.2%) compared to other town (27.3%) and urban area residents (23.7%). Conclusions: Majority of the respondents in the sample had good health, and those with poor health status were more likely to report having hypertension followed by diabetes mellitus and rheumatoid arthritis. Poor health status was more prevalent among those of lower economic status in rural areas who reported greater medical health care expenditure. The prevalence of chronic diseases and levels of disability in older people can be reduced with appropriate health promotion and strategies to prevent non-communicable diseases.

Keywords: Older; Chronic Illness; Social Determinants; Jamaica

1. INTRODUCTION

The Caribbean has been identified as the most rapidly ageing region of the world. Between 1960 and 1995, there was a 76.8% increase in the elderly population [1]. Among its regional island states, the average growth rate in the elderly population was approximately 5.3% for the 1995-2000 periods. The elderly as a percentage of total population was 4.3% in 1950 and is estimated to reach about 15% by 2020 [1]. In Jamaica, a similar pattern has been observed with a clear and rapidly rising trend in the elderly as a proportion of the population [2]. By 2025 as much as 1 in 7 persons will be elderly. Moreover, characterizing this pattern of increasing elderly is the differential growth rates within the various sub-age groups over age 60, with the 75 years and above age group expected to double moving from 2.8% currently to 4.0 % in 2025 [3]. Eldemire [4] noted that the elderly in Jamaica represents 10% of the population, and that they were for the most part mentally competent and physically independent. With a calculated life expectancy of 75.5 years [5], the burden on the healthcare system can be expected to increase.

The epidemiologic transition in the Caribbean over the last 40 years has produced an epidemic of lifestyle-related chronic non-communicable diseases [6]. Among these are obesity, diabetes mellitus, and hypertension, along with such complications as stroke, heart disease, and amputations [6]. Cardiovascular disease is by far the leading cause of death at older ages in developing countries, although the impact of communicable

diseases remains considerable [7]. One comprehensive analysis attributes nearly 46 percent of all deaths among women aged 60 and over in developing countries in the early 1990s to cardiovascular disease; the corresponding figure for older men was 42 percent [7]. Older people with diabetes mellitus are at particularly high risk for heart disease, stroke, eye damage, kidney disease, limb amputation and depression. In the Survey on Health and Well-Being of Elders (SABE), among those reporting diabetes, at least 60% reported visual problems with or without eye glasses. Among those reporting at least two chronic diseases, 25% had symptoms of depression [8]. Furthermore, SABE indicates that an average of 70% of women aged 60 years and older have at least one potentially disabling condition, such as low vision, rheumatoid arthritis, or urinary incontinence [8].

In developed countries, the health and social status of the elderly has received a fair amount of attention [9]. Within the Caribbean, some progress has been made in terms of research on the elderly. Braithwaite [10] noted that data on the Caribbean elderly were extremely limited. With the continuing aging of the population in the Caribbean, gerontological research has devoted increasing attention to those at very advanced ages [11] and in recent years, there has been increasing interest in issues relating to health of the elderly in the Caribbean. Patterns of mortality at the most advanced ages are of interest in their own right, indicating variation in health status and well-being among this group. Moreover, differences in mortality and trends in them may give clues about the likelihood of a further extension of life expectancy [12].

Rural populations in Caribbean countries generally experience excessive deficiencies in health care access, social services, and other goods and services needed for healthy living. Rural residence has significantly influenced health care access and health status. Urban residents consistently reported better health status than rural residents and greater satisfaction with their health care [13]. Rural residents are more often uninsured [14], have greater distance to travel for their health care needs [13], and are more often plagued by resource inaccessibility [15]. Using poverty to proxy resource inadequacies which increased inaccessibility, in 2007; rural poverty was 2.5 times more than urban poverty (i.e. 6.2%) and 3.8 times more than urban poverty (i.e. 4.0%) [16,17]. Rural residents in Jamaica are poor and a greater proportion of them reported having chronic illnesses, with an even smaller population having insurance of any kind (7.6% in rural areas versus 25.0% in urban areas) [16].

While national averages provide insights into the inequalities in the nation, the current study on a sub-population provides health policy practitioners with a comprehensive understanding of the issues experienced by elderly Jamaicans particularly public health problems that presently exist in this population. Among these is the fact that rural and peri-urban residents spent 11 and 14 times more days experiencing illnesses than urban residents. Another public health problem is the percentage of elderly population with chronic illness compared to the general population. Statistics revealed that 12% of Jamaicans had diabetes mellitus; 22.4% had hypertension and 8.8% had rheumatoid arthritis [17]. However, the prevalence of diabetes mellitus in the elderly was 2.1 times more than the general population. Similarly, the prevalence of hypertension and rheumatoid arthritis in elderly Jamaicans were 1.9 and 2.1 times respectively more than in the general population. The public health problem also includes reasons why some elderly are unable to seek care despite health care being free for this group (since 2006). Of those who did not seek medical care, 18% indicated that they could not afford it and 38% reported that they were not ill enough (i.e. after self-assessment of health and image of health care). Hence, the aims of the study were to 1) examine the health status of elderly Jamaicans in rural, peri-urban and urban areas of residence; 2) establish a model to predict the social determinants of poor health status of elderly Jamaicans who have reported at least one chronic disease, and 3) provide information that could assist health care professionals to specifically and adequately address the health needs of the elderly in Jamaica.

2. MATERIALS AND METHODS

The current study used cross-sectional survey data collected by the Planning Institute of Jamaica (PIOJ) and the Statistical Institute of Jamaica (STATIN) [17] between May and August 2007. The sample for this study was 287 individuals who indicated having being diagnosed with a chronic illness and who are older than 60 years. The study was extracted from a larger nationally representative cross-sectional survey of 6,783 Jamaicans. The survey was drawn using stratified random sampling. 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 constitutes of a minimum of 100 dwellings in rural areas and 150 in urban areas. An ED is an independent geographic 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 dwelling was compiled, which in turn provided the sampling frame for the labour force. One third of the 2007 Labour Force Survey (LFS) was selected for the Jamaican Survey of Living Conditions (JSLC, 2007) [17]. The sample was weighted to reflect the population of the nation.

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 health status of Jamaicans. A self-administered questionnaire was used to collect the data, which were stored and analyzed using SPSS for Windows 16.0 (SPSS Inc; Chicago, IL, USA). The questionnaire was modeled from the World Bank's Living Standards Measurement Study (LSMS) household survey. There are some modifications to the LSMS, as JSLC is more focused on policy impacts. The questionnaire covered areas such as socio-demographic, economic and health variables. The non-response rate for the survey was 26.2%.

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 an Analysis of Variance (ANOVA) was used to test the relationships between metric and non-dichotomous categorical variables. Logistic regression examined the relationship between the dependent variable and some predisposed independent (explanatory) variables, because the dependent variable was a binary one (health status: 1 if reported poor health status and 0 if otherwise).

The results were presented using unstandardized B-coefficients, Wald statistics, Odds ratio and confidence interval (95% CI). The predictive power of the model was tested using the Omnibus Test of Model and Hosmer and Lemeshow [18] was used to examine goodness of fit of the model. The correlation matrix was examined in order to ascertain whether autocorrelation (or multicollinearity) existed between variables. Based on Cohen and Holliday [19] correlation can be low (weak)—from 0 to 0.39, moderate—0.4-0.69, and strong —0.7-1.0. This was used to exclude (or allow) a variable in the model. Wald statistics were used to determine the magnitude (or contribution) of each statistically significant variable in comparison with the others, and the Odds Ratio (OR) for the interpreting of each significant variable.

Multivariate regression framework was utilized to assess the relative importance of various demographic, socio-economic characteristics, physical environment and psychological characteristics, in determining the health status of Jamaicans; and this has also been employed outside of Jamaica. This approach allowed for the analysis of a number of variables simultaneously. Secondly, the dependent variable is a binary dichotomous one and this statistic technique has been utilized in the past to do similar studies. Having identified the determinants of health status from previous studies, using logistic regression techniques, final models were built for Jamaicans as well as for each of the geographical sub-regions (rural, peri-urban and urban areas) and sex of respondents using only those predictors that independently predict the outcome. A p-value of 0.05 was used to for all tests of significance.

2.1. Model

The use of multivariate analysis in the study of health and subjective wellbeing (i.e. self-reported health or happiness) is well established [20,21] and this is equally the case in Jamaica and Barbados [22,23]. The current study will employ multivariate analyses in the study of health status of elderly Jamaicans with diagnosed chronic medical conditions. The use of this approach is better than bivariate analyses as many variables can be tested simultaneously for their impact (if any) on a dependent variable.

The current study seeks to examine the social determinants of poor health status of old Jamaicans who reported having at least one chronic medical condition (**Eq.1**):

$$H_t = f(A_i, G_i, AR_i, FC_i, NFC_i, MR_i, S_i, HI_i, CR_i, MC_t, SA_i, \varepsilon_i) \quad (1)$$

where H_t (self-rated current health status in time t) is a function of age of respondents, A_i ; sex of individual i, G_i; area of residence, AR_i; food consumption per person per household member, FC_i; non-food consumption per person per household member, NFC_i; marital status of person i, MR_i; social class of person i, S_i; health insurance coverage of person i, HI_i; crowding of individual i, CR_i; medical expenditure of individual i in time period t, MC_t; social assistance of individual i, SA_i and an error term (ie. residual error).

2.2. Measure

Age is a continuous variable which is the number of years alive since birth (using last birthday). Age group is a non-binary measure: young-old (ages 60 to 74 years); old-old (ages 75 to 84 years) and oldest-old (ages 85 years and older).

Elderly denotes the chronological age of 60 years and beyond. Self-reported illness (or self-reported dysfunction): The question was asked: "Is this a diagnosed recurring illness?" The answering options were: Yes, cold; Yes, diarrhoea; Yes, asthma; Yes, diabetes mellitus; Yes, hypertension; Yes, arthritis; Yes, Other; and No. A binary vari- able was later created from this construct (1 = yes, 0 = otherwise) in order to use in the logistic regression.

Health status: "How is your health in general?" And the options were very good; good; fair; poor and very poor. For this study the construct was categorized into 3 groups with (i) good; (ii) fair, and (iii) poor. A binary variable was later created from this variable (1 = good and fair 0 = otherwise).

Social class: This variable was measured based on income quintile: The upper classes were those in the weal-

Table 1. Socio-demographic characteristics of sample.

Variable	Frequency	Percent
Sex		
Men	110	38.3
Women	177	61.7
Diagnosed chronic medical condition		
Diabetes mellitus	73	25.4
Hypertension	124	43.2
Arthritis	38	13.2
Other (unspecified)	52	18.2
Health care-seeking behavior		
Sought care	201	70.8
Did not seek care	83	29.2
Why didn't you seek care		
Could not afford it	14	17.7
Was not ill enough	29	36.7
Preferred home remedies	11	13.9
Didn't have time to go	6	7.6
Unspecified	19	24.1
Purchased medication		
Prescribed medicine	198	72.0
Partial prescription	8	2.9
Prescribed/over the counter	6	2.2
Over counter	6	2.2
Prescribed, but did not buy	9	3.3
No	48	17.4
Health insurance coverage		
Private	23	8.0
Public	72	25.2
No	191	66.8
Health status		
Good	49	17.1
Fair	136	47.4
Poor	102	35.5
Area of residence		
Urban	76	26.5
Other town	55	19.1
Rural	156	54.4
Social class		
Poor	114	39.7
Middle	62	21.6
Wealthy	111	38.7
Household head		
No	85	29.6
Yes	202	70.2

thy quintiles (quintiles 4 and 5); middle class was quintile 3 and poor those in lower quintiles (quintiles 1 and 2).

3. RESULTS

3.1. Socio-Demographic Characteristics

The sample was 287 elderly respondents (38.3% of men and 61.7% of women), with 57.1% young-old; 33.1% old-old and 9.8% oldest-old. Seventy percent of the sample was head of household; 35.5% had poor health status; 70.8% sought health care; 72.0% purchased the prescribed medication; 33.2% had public health insurance coverage; 39.7% were poor; 26.5% lived in urban areas, 19.2% in other towns and 54.4% in rural areas (**Table 1**). Majority (43.2%) of the sample reported hypertension; 25.4% diabetes mellitus; 13.2% rheumatoid arthritis and 18.2% unspecified the type of chronic illness that they were diagnosed with (**Table 1**). Approximately eighteen percent of those who indicated that they did not seek care indicated that they could not afford it; 36.7% indicated that they were not ill enough; 13.9% reported that they use home remedy.

3.2. Bivariate Analyses

There was no statistical correlation between health status and self-reported dysfunction (χ^2 = 1.810, P = 0.404, n= 286) (**Table 2**). Based on **Table 2**, only 35.4% of those who indicated that they had at least one chronic medical condition reported poor health status. **Table 3** revealed a statistical relation between health status and area of

Table 2. Health status by self-reported dysfunction.

Health status	Self-reported Dysfunction		Total n (%)
	No n (%)	Yes n (%)	
Good	0 (0.0)	49 (17.2)	49 (17.1)
Fair	0 (0.0)	135 (47.4)	135 (47.2)
Poor	1 (100.0)	101 (35.4)	102 (35.7)
Total	1	285	286

χ^2 (df = 2) = 1.810, P = 0.404, n = 286

Health status	Area of residence			Total
	Urban	Other town	Rural	
Good	16 (21.1)	11 (20.0)	22 (14.1)	49 (17.1)
Fair	42 (55.3)	29 (52.7)	65 (41.7)	136 (47.4)
Poor	18 (23.7)	15 (27.3)	69 (44.2)	102 (35.5)
Total	76	55	156	287

χ^2 (df = 4) = 11.569, P = 0.021, n=287

Table 3. Health status by area of residence.

Table 4. Diagnosed chronic medical condition by area of residence.

Diagnosed chronic medical condition	Area of residence			Total
	Urban	Other town	Rural	
Diabetes mellitus	25 (32.9)	17 (30.9)	31 (19.9)	73 (25.4)
Hypertension	25 (32.9)	22 (40.0)	77 (49.4)	124 (43.2)
Rheumatoid arthritis	9 (11.8)	5 (9.1)	24 (15.4)	38 (13.2)
Other (unspecified)	17 (22.4)	11 (20.0)	24 (15.4)	52 (18.1)
Total	76	55	156	287

χ^2 (df = 6) = 10.455, P = 0.107, n=287

Table 5. Self-reported chronic medical condition by social class.

Self-reported chronic medical condition	Social Class			Total
	Poor	Middle class	Upper class	
Diabetes mellitus	21 (18.4)	11(17.7)	41 (36.9)	73 (25.4)
Hypertension	55 (48.2)	32 (51.6)	37 (33.3)	124(43.2)
Rheumatoid arthritis	19 (16.7)	8 (12.9)	11 (9.9)	38 (13.2)
Other (unspecified)	19 (16.7)	11 (17.7)	22 (19.8)	52 (18.1)
Total	114	62	111	287

χ^2 (df = 6) = 15.870, P = *0.014*, n=287

residence [χ^2 (df = 4) = 11.569, P = 0.021, n = 287]. Rural residents reported the highest poor health status (44.2%) compared to other town (27.3%) and urban area residents (23.7%). On the other hand, greatest good health status was reported by urban residents (21.1%), compared with other town (20.0%) and rural area residents (14.1%) (**Table 3**). No statistical association was found between diagnosed chronic medical condition and area of residence [χ^2 (df = 6) = 10.455, P = 0.107, n = 287] (**Table 4**).

A statistical correlation was found between self-reported chronic medical condition and social class [χ^2 (df = 6) = 15.870, P = 0.014, n = 287]. The wealthy was most likely to have diabetes mellitus (36.9%) while the poor (48.2%) and the middle class (51.6%) were mostly likely to indicated hypertension. Approximately ten percent of the wealthy had arthritis compared to 12.9% of middle class and 16.7% of poor (**Table 5**).

The mean number of day reported to have illness was 71.6 days (SD = 185.1, 95% CI = 49.1 – 94.2 days). Urban dwellers reported the least number of days in illness (mean = 7.5 days, SD=10.96, 95% CI = 4.7 – 10.2 days) compared to other town residents (mean = 98 days, SD = 216.4, 95% CI = 38.3 – 157.6 days) and rural residents

Table 6. Annual consumption expenditure, length of illness, total medical expenditure, public medical expenditure, private medical expenditure by area of residence.

Variable	Area of residence	n	Mean	Std. Deviation	95% Confidence Interval
†Annual consumption expenditure*	Urban	76	8711.95	6761.20	716695 - 10256.95
	Other Town	55	7388.90	5271.25	5963.88 - 8813.91
	Rural	156	5445.09	4470.72	4738.01 - 6152.17
	Total	287	6682.69	5485.63	6045.34 - 7320.03
††Length of illness	Urban	64	7.45	10.96	4.72 - 10.19
(days)	Other Town	53	97.98	216.44	38.32 - 157.64
	Rural	143	90.55	206.90	56.35 - 124.76
	Total	260	71.61	185.10	49.01 - 94.22
†††Number of visit to health care practitioner	Urban	55	1.65	1.58	1.23 - 2.08
	Other town	39	1.21	.61	1.01 - 1.40
	Rural	101	1.42	.85	1.25 - 1.58
	Total	195	1.44	1.08	1.29 - 1.59
††††Medical expenditure*	Urban	57	1481.58	1988.75	953.89 - 2009.27
	Other town	39	1817.95	2377.57	1047.23 - 2588.67
	Rural	103	1805.34	5154.02	798.04 - 2812.64
	Total	199	1715.07	3988.73	1157.48 - 2272.67

† F statistic [2,284] = 10.248, $P < 0.001$; †† F statistic [2,257] = 5.031, $P = 0.006$; ††† F statistic [2,192] = 2.057, $P = 0.131$; †††† F statistic [2,196] = 0.136, $P = 0.001$

Table 7. Logistic regression: Predictors of poor health status of those diagnosed with chronic medical condition.

Variable	Coefficient	Std. Error	Wald statistic	Odds ratio	95.0% C.I.
Middle class	0.647	0.527	1.507	1.909	0.680 - 5.360
Upper class	0.427	0.639	0.446	1.533	0.438 - 5.366
†Poor Man	0.765	0.386	3.937*	1.000 2.150	1.009 - 4.578
Urban areas	-0.314	0.439	0.512	0.730	0.309 - 1.727
Other towns	-0.449	0.466	0.931	0.638	0.256 - 1.589
†rural areas Social assistance (1=yes)	-0.112	0.461	0.059	1.000 0.894	0.362 - 2.207
Crowding	0.173	0.119	2.124	1.189	0.942 - 1.499
Age	0.033	0.022	2.182	1.033	0.989 - 1.079
Married	0.257	0.403	0.406	1.293	0.587 - 2.847
Divorced, separated or widowed	0.629	0.461	1.858	1.875	0.759 - 4.628
†Never married Non-food consumption	0.000	0.000	0.017	1.000 1.000	1.000 - 1.000
Food consumption	0.000	0.000	4.088*	1.000	1.000 - 1.000
Health insurance (1=yes)	0.390	0.382	1.039	1.476	0.698 - 3.123

χ^2 (df = 13) = 20.249, $P < 0.001$; n = 285
-2 Log likelihood = 238.17
Nagelkerke R^2 =0.115
Hosmer and Lemeshow goodness of fit χ^2=7.565, $P = 0.477$
Overall correct classification = 83.5%
Correct classification of cases of self-rated poor health status = 99.2%
Correct classification of cases of self-rated good health status = 6.3%
†Reference group
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

(mean = 90.6 days, SD = 206.9, 95% CI = 56.4 – 124.8 days) - F statistic [2,257] = 5.031, p = 0.006. This was similar for medical health care expenditure - F statistic [2,196] = 0.136, P = 0.001. The mean amount spent on medical care for urban residents was US $21.85 compared to US $26.12 for other town residents and US $26.81 for rural respondents. On the other hand, there was a statistical difference between annual consumption expenditure and area of residence - F statistic [2,284] = 10.248, $P < 0.001$. The mean annual amount spent by urban dwellers was US $8, 711.95 than other town dwellers US $7, 388.90 and rural residents US $5, 445.09 (**Table 6**).

3.3. Multivariate Analyses

The socio-demographic determinants of poor health status of those who indicated being diagnosed with chronic illness were sex of respondents (OR = 2.15, 95% CI = 1.009 – 4.578) and food consumption (OR = 1.00, 95% CI = 1.00 – 1.00) (**Table 7**). Elderly men who revealed that they were diagnosed with chronic illness were 2.15 times more likely to indicated poor health than elderly women (**Table 7**).

4. DISCUSSION

The current revealed that 43 out of every 100 elderly Jamaican who reported chronic illness had hypertension, 25 in every 100 had diabetes mellitus and 13 in every 100 had rheumatoid arthritis. Thirty-five in every 100 indicated poor self-reported health status; 70 out of every 100 were household heads; 29 out of every 100 did not seek care and of those who did not seek care 37% indicated that they were not ill enough to visit a medical practitioner or health facility. Rural residents had greatest percentage with hypertension (49.4%) and rheumatoid arthritis (15.4%) compared to other area of residents. However, urban residents had the greatest percent of diabetes mellitus (32.9%) compared to peri-urban (30.9%) and rural residents (19.9%). Upper class people recorded the most diabetes mellitus cases (37%) compared to the poor (18%) and the middle class (18%). Middle class however recorded the most hypertensive cases (52%) compared to the poor (48%) and the wealthy (33%). Concurrently, the poor recorded the most rheumatoid arthritis cases (17%) compared to the middle class (13%) and the wealthy (10%). Only sex and food consumption were found to be correlated with self-reported health status. Older men self-reported health status was almost 2.2 times more than that for older women, and those who consumed more food recorded better health status. Furthermore, the duration of illness (in days) for rural residents was 12 times more than that for urban residents and their medical expenditure was 1.2 times more than that of those in urban areas. Concurrently, periurban residents spent 13 times more days in illness than urban residents and spent 1.2 times more on medical expenditure.

Self-reported health status has been widely used in censuses, surveys, and observational studies and there is evidence suggesting that self-reported health is an indicator of general health with good construct validity [24] and is a respectably powerful predictor of mortality risks [25], disability [26] and morbidity [27]. The results of this study showed that the majority of those sampled reported themselves to be experiencing good or fair health, while approximately one-third indicated poor health. These results concur with those by other researchers from Dominica [28] and Trinidad [29]. In a recent island wide survey of persons aged 65 years and older conducted in Trinidad in 2002, 44% reported their health as fairly good or good. In reviews of the literature, Benyamini & Idler [30] and Idler & Benyamini [25], showed that in most studies conducted since the 1980s, the elderly people who self-rated their health as bad presented greater incidence of death than did those who considered it to be excellent. Among elderly people, self-rated health may present greater sensitivity for men than for women. Since women live longer than men and experience more years with diseases and incapacities, they tend to rate their health more negatively than do men, but do not necessarily die because of this, over the short term. Thus, negative self-rated health expressed by women may be more associated with quality of life. On the other hand, when men rate their health negatively, they present a greater risk of succumbing to a fatal event [31].

There has been a general epidemiological shift from infectious to chronic diseases and the elderly are one of the main at risk groups. In this study, just over one-third of the respondents who reported poor health indicated that they had at least one chronic disease. This is less than the 80% reported in a study in Trinidad [29]. The main chronic illnesses reported by the respondents in this study were hypertension, diabetes mellitus and rheumatoid arthritis. This is in keeping with the study by Rawlins *et al.* [29] and other Caribbean studies on this age group [32,33]. Furthermore, a study conducted on elderly Jamaicans showed that this age cohort was mainly affected by chronic non-communicable diseases [34]. The most common chronic diseases identified among the elderly in Jamaica are hypertension, arthritis, diabetes mellitus, cardiovascular arrest, stroke and cancer. Patients in the 60 and over age groups accounted for 37.2% and 41.1%, respectively, of new hypertensive and diabetic cases [35]. Some gender differences have been reported in respect of chronic illnesses with women at greater risk for hypertension and men cardiovascular

diseases [36]. Furthermore, in 1991, cardiovascular diseases followed by diabetes mellitus and neoplasms were the diseases for which Jamaicans 65 years older were most often hospitalized [37].

Data for the Caribbean showed that hypertension and rheumatoid arthritis are morbidities that significantly affect both men and women [38]. The current study revealed that hypertension was the leading cause of illness among older and oldest elderly in Jamaica, followed by diabetes mellitus, and rheumatoid arthritis, which concurs somewhat with a past study [39] that had hypertension as the leading cause of morbidity of the elderly, followed by rheumatoid arthritis and diabetes mellitus. In another reported study, the most common chronic diseases identified among the elderly were hypertension, rheumatoid arthritis, diabetes mellitus, cardiovascular arrest, stroke and cancer [35]. Some gender differences have been reported in respect of chronic illnesses with women at greater risk for hypertension and men cardiovascular diseases [36]. In a recent study by Bourne, 1.4 times more women had diabetes mellitus than men and this was the same for hypertensive older and oldest elderly Jamaicans [39]. On the other hand, there were 1.6 times more old and oldest elderly Jamaican men with self-reported rheumatoid arthritis than women [39]. These chronic non-communicable diseases continue to interface within the functional lives of the elderly, which means that they are indeed living longer but are faced with lower levels of good health than young adults (ages 15 to 29 years) and middle-aged adults (ages 30 to 59 years). According to the JSLC there has been significant increase in illness/injury among older persons since 1997 [40]. Data from the 2002 survey indicate that 34.6 percent of the elderly population surveyed, reported an illness or injury during the four-week reference period [41].

Hypertension is one of the most important treatable causes of morbidity and mortality and accounts for a large proportion of cardiovascular diseases in elderly in Jamaica [42]. It is known to be a major risk factor for the development of diabetic renal disease, and hyperglycaemia also has a role in the development of diabetic nephropathy [43]. Studies from developed countries have reported prevalence of raised blood pressure among elderly to vary from 60% to 80% [44]. Furthermore, diabetes mellitus is one of the leading causes of morbidity and mortality among persons aged 65 and older [45]. About 20% of persons in this age group are estimated to have diabetes mellitus, with another 25% in pre-diabetic stages [46]. Moreover, because diabetes can be asymptomatic for many years, about 50% of older individuals with diabetes are thought to be undiagnosed [47]. In Jamaica, diabetes-related deaths in 1994 had increased 147% over the 1980 level and represented the third leading cause of loss of years of potential life among women and tenth among men [48]. There is evidence that this is due to the low rates of awareness, treatment and control among patients with hypertension and diabetes [49,50].

One of the silent illnesses which emerged from the current study is unspecified health conditions. Eight out of every 100 elderly Jamaicans who reported a chronic illness stipulated unspecified conditions. Based on causes of mortality and morbidity statistics in Jamaica, the other includes heart diseases; malignant neoplasm of the prostate; malignant neoplasm of the breast; and malignant neoplasm of the trachea, bronchus [51,52]. Statistics revealed that other heart diseases, malignant neoplasm of the breast, malignant neoplasm of the prostate and malignant neoplasm of the trachea and bronchus are among the 10 leading causes of mortality for males and/or females [51]. The prevalence of diseases in this category (i.e. unspecified condition) is greater than those with rheumatoid arthritis, and statistics have showed malignant neoplasm of the prostate is the 5th leading cause of mortality of male 50 years and older [52]. Heart diseases and malignant neoplasm of the breast were the 6th and 7th leading cause of death respectively among females 50 years and older [52]. The unspecified health conditions are therefore silent killer among the elderly with chronic diseases.

Seventy-two percent of poverty lies in rural are compared to 20% in urban and 9% in peri-urban area [17], indicating that poverty is accounting for illnesses experienced by rural residents as well as the length of time they spent in illness. The current study showed that length of time spent in illnesses by rural residents was 12 times more than that for those in urban area, and so justifying why they spend 1.2 times more on medical care compared to urban dwellers. The typology of illness that is experienced by rural residents (i.e. hypertension) is such that they require frequent visits to health care providers (i.e. doctors, nurses, pharmacists). Seemingly the afore-mentioned should be the case, but we found that there was no significant statistical difference between the number of visits made to health care providers and area of residents. Like those who were unable to attend health care during the time of illness, they were either unable to afford it (18%) or diagnosed themselves as being not ill enough (37%). Inspite of this fact, 48% of the poor elderly with chronic illnesses had hypertension and 18% had diabetes mellitus, which are illness which require treatment and cannot be left to prayer, faith or abstinence from medical care.

Rural populations generally experience excessive deficiencies in healthcare access, social services and other goods and services needed for healthy living. Furthermore, 23% of people from rural Jamaica who reported having a chronic medical condition were not actively engaged in seeking health care because of affordability issues, compared with 9.4% from urban areas. Urban residents consistently reported better health status than rural residents, and greater satisfaction with their health

care [53]. There was a statistical correlation between good health status and area of residence, or self-reported (chronic) recurring illness and age cohort. Furthermore, the data showed that elderly Jamaicans who dwelled in rural area had the lowest self-reported good health compared to those who resided in other towns and urban areas. Continuing, those who resided in urban residence reported the greatest good health status. In 1997, statistics from PIOJ and STATIN [54] revealed that 54.3 percent of elderly (ages 60 years and over) lived in rural areas. A study by Bourne [39] showed that approximately 7 out of every 10 old and oldest elderly in Jamaica lived in rural areas, compared to 6 out of 10 for those 60 years and older of the population. In addition, 20 out of every 100 Jamaicans were below the poverty line, compared to 25 out of every 100 in rural Jamaica. Given that the elderly substantially lived in rural areas and that poverty for this group was 10.2 percent [55], it is not surprising that the elderly in this area of residence had a lower level of good health status than the urban elderly in Jamaica.

The wealthiest in the society are expected to experience better health due to their knowledge of health risks and their access to the resources necessary to avoid such risks and treat emerging health conditions [56]. But with increasing wealth and development these has been an increase in chronic disease as lifestyle changes have had a negative impact. The studies found that there were large gaps between the mean amounts of money spend by urban residents compared with their rural counterparts. Furthermore, the elderly who are wealthy were more likely to have diabetes mellitus while the poor and the middle class were more likely to report hypertension. This suggests the consumption patterns of the wealthy contribute to ill-health. Thus whereas the poor become ill due to their inability to access their basic human rights, the rich become ill as a result of their harmful consumption patterns. According to Sobal and Stunkard [57], in developing societies there is a higher likelihood of obesity among men in higher socioeconomic strata. These men are at increased risk of developing type 2 diabetes mellitus [58] which is increasing in the adult population. Among the demographic correlates of health is the cost of medical care. It is established that medical care [20] and cost of medical care [21] are among the social determinants of health.

5. CONCLUSIONS

The general epidemiological shift from infectious to chronic non-communicable diseases in Jamaica puts the elderly at risk. Majority of the respondents in the sample had good or fair health, and those with poor health status were more likely to report having hypertension followed by diabetes mellitus and rheumatoid arthritis. Poor health status was more prevalent among those of lower economic status in rural areas who reported the greatest number of sick days of illness and medical health care expenditure. The prevalence of chronic diseases and levels of disability in older people can be reduced with appropriate health promotion and strategies to prevent non-communicable diseases. This research provides valuable information on health status and the non-communicable diseases which affect the elderly in Jamaica, and particular socioeconomic group respond being diagnosed with particular chronic illnesses. These findings can assist health care professionals to specifically and adequately address the health needs of the elderly in Jamaica.

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Comparative effects of idazoxan, efaroxan, and BU 224 on insulin secretion in the rabbit: Not only interaction with pancreatic imidazoline I_2 binding sites

María José García-Barrado[1], María Francisca Pastor[1], María Carmen Iglesias-Osma[1], Christian Carpéné[2], and Julio Moratinos[1]

[1]Departamento de Fisiología y Farmacología, Facultad de Medicina, Universidad de Salamanca, Salamanca, Spain; barrado@usal.es
[2]INSERM, U586 (Institut National de la Santé et de la Recherche Médicale), Unité de Recherches sur les Obésités, Université Paul Sabatier, Institut Louis Bugnard IFR31, Toulouse, France

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ABSTRACT

The nature of the binding site(s) involved in the insulin secretory activity of imidazoline compounds remains unclear. An imidazoline I_2 binding site (I_2BS) has been neglected since the classic I_2 ligand, idazoxan, does not release insulin. Using the rabbit as an appropriate model for the study of this type of binding sites, we have tried to re-evaluate the effects of idazoxan, the selective I_2 compound BU 224, and efaroxan on insulin secretion. Mimicking efaroxan, idazoxan and BU 224 potentiated insulin release from perifused islets in the presence of 8 mM glucose. In static incubation, insulin secretion induced by idazoxan and BU 224 exhibited both dose and glucose dependencies. ATP-sensitive K^+ (K_{ATP}) channel blockade, though at a different site from the SUR1 receptor, with subsequent Ca^{2+} entry, mediates the insulin releasing effect of the three ligands. However, additional MAO independent intracellular steps in stimulus-secretion coupling linked to PKA and PKC activation are only involved in the effect of BU 224. Therefore, both an I_2 related binding site at the channel level shared by the three ligands and a putative I_3-intracellularly located binding site stimulated by BU 224 would be mediating insulin release by these compounds. In vivo experiments reassess the abilities of idazoxan and BU 224 to enhance glucose-induced insulin secretion and to elicit a modest blood glucose lowering response.

Keywords: BU 224; Efaroxan; Idazoxan; Imidazoline Ligands; Insulin Secretion; IVGTT (Intravenous Glucose Tolerance Test); K_{ATP} Channel; PK Activity; Rabbit Pancreatic Islets

1. INTRODUCTION

A number of imidazoline containing compounds have been previously shown to induce insulin release from the perifused pancreas or isolated islets [1, 2] and to improve glucose tolerance in rats [3-5] and mice [6].

In accordance with the mechanisms of their insulinotropic effect, two groups of imidazoline compounds can be considered: classical imidazolines, i.e., imidazoline derivatives possessing both ATP-sensitive K^+(K_{ATP}) channel activity and a direct effect on exocytosis, like RX871024 [7], and a new generation of compounds without effect on K_{ATP} channels though possessing a pure glucose-dependent insulinotropic effect like BL11282 [8]. The K_{ATP} channel consists of two subunits: a sulphonylurea receptor (SUR1) and a Kir6.2 subunit. Classical imidazoline drugs bind to the transmembrane protein Kir6.2 [9] considered to be the pore-forming subunit of the channel whereas sulphonylureas bind to the SUR1 receptor. It is also established that the binding site for imidazolines and the sulphonylurea receptor are not identical since the first drugs do not displace binding from the SUR sites [10]. Additional sites located at more distal stages of the stimulus-secretion coupling pathway, mediating activation of protein kinase A (PKA) and protein kinase C (PKC) have also been reported [8,11,12].

However, when trying to analyze the nature of the binding sites or receptors involved in their insulin secretory response a number of difficulties have emerged. An I_2 imidazoline binding site (I_2-site) has been discarded since the classic I_2 ligand idazoxan exhibited a mild concentration independent increase in insulin release [13], failed to evoke any effect [14] or even blocked the response induced by efaroxan (an α_2-adrenoceptor antagonist) with an imidazoline structure [15]. Similarly, the monoamine oxidase A (MAO-A) inhibitor clorgyline did

not modify the insulin secretory response induced by the imidazoline compound RX871024 [16]. Radioligand binding studies performed in membranes from RINm5F and MIN6 cells, in the presence of [3H]-RX821002 (methoxy-idazoxan an imidazoline α_2-adrenoceptor antagonist) showed a low affinity non-adrenergic binding site which could be displaced by efaroxan but not by idazoxan [18,19]. Therefore, evidence for a novel putative I_3 binding site involved in the insulin secretory response is being accepted. Efaroxan is tentatively considered an I_3 ligand and 2-(2-ethyl-2,3-dihydro-benzofuran-2-yl)–imidazole (KU 14R), a close structural efaroxan analogue able to block its effect, an I_3 antagonist [18,20].

However, some recent data have yielded intriguing results: even high doses of efaroxan did not increase circulating insulin in mouse [21] and the selective I_2 ligand: 2-(2-benzofuranyl)-2-imidazoline (2-BFI) releases insulin from isolated rat islets [10]. Considering the heterogeneity of imidazoline binding sites [22] and that the putative I_3 binding site encompasses a nebulous group of loci, we have tried to re-evaluate the effect of imidazoline ligands on insulin release in rabbits. The rabbit was chosen as a suitable model in view of the paucity of this type of data for an animal species otherwise very rich in I_2-binding sites [17,23,24]. 2-(4,5-dihydroimidazol-2-yl)-quinoline (BU 224), considered a selective I_2 ligand [25-27], idazoxan (a typical α_2-adrenoceptor antagonist and I_2 ligand), methoxy-idazoxan (an α_2-adrenoceptor antagonist) and efaroxan (I_3 putative ligand) were employed in both in vitro and in vivo experiments to delineate insulin secretion and glycaemic control.

2. MATERIALS AND METHODS

2.1. Chemicals and Solutions

Forskolin, diazoxide, tolbutamide, methoxy-idazoxan, nimodipine, yohimbine, 3-isobutyl-1-methylxanthine (IBMX), chelerythrine and pargyline were provided by Sigma-Aldrich (Spain); idazoxan was obtained from Rekilt-Colman Pharmaceutical Company (Germany); brimonidine (UK 14,304) came from Pfizer (UK); 2-(4,5-dihydroimidazol-2-yl)-quinoline (BU 224 hydrochloride), efaroxan hydrochloride, and 2-(2-ethyl-2,3-dihydro-benzofuran-2-yl) -imidazole (KU 14R) were obtained from Tocris (Bristol, UK), calphostin and Rp-Adenosine-3′,5′-cyclic monophosphothioate triethylamine (Rp-cAMPS) were from Bionova (Spain). Forskolin and chelerythrine were prepared in DMSO, and final concentrations of DMSO were 0.1% or less in each case.

2.2. Animals

The experiments were performed using male New Zealand white rabbits aged 7-12 months (body weight between 2.5-3.5 kg). The animals were maintained in a 12 h light-dark cycle and were provided with free access to food and water. The study was conducted in accordance with the European Communities Council Directives for experimental animal care.

2.3. In Vitro Experiments

2.3.1. Islet Isolation and Incubation

The rabbits were sacrificed after the induction of general anaesthesia with 30 mg kg^{-1} i.v. of sodium pentobarbital (Abbott, Spain). The pancreas was removed and distended with bicarbonate-buffered physiological salt solution. The islets were isolated by collagenase (Inmunogenetic, Spain) and hand-picked using a glass loop pipette under a stereo microscope. They were free of visible exocrine contamination. The medium used for islet isolation was a bicarbonate-buffered solution containing 120 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 5 mM HEPES and 24 mM $NaHCO_3$. It was gassed with O_2-CO_2 (94: 6) to maintain a pH of 7.4 and was supplemented with 1 mg ml^{-1} BSA and 10 mM glucose. When the concentration of KCl was increased to 30 mM, that of NaCl was decreased accordingly. The concentration of glucose was adjusted and test substances were added as required.

2.3.2. Measurements of Insulin Secretion

After isolation, in the first type of experiments, the islets were pre-incubated for 60 min in a medium containing 15 mM glucose before being distributed into batches of three. Each batch of islets was then incubated for 60 min in 1 ml at 37º C of medium containing 8 mM glucose and test substances, Pargyline was added to the preincubation medium 40 min before incubation. A portion of the medium was withdrawn at the end of the incubation and its insulin content was measured by a double antibody-RIA (insulin CT, Schering, Spain).

In the other type of experiment, the isolated islets were divided in equal batches of 45-50 and placed in a parallel perifusion chamber at 37º C and perifused for 30 min before the start of the experiment at a flow rate of 1.1 ml min^{-1}. After a 30 min stabilisation period they were perifused with 8 mM glucose and the appropriate compounds as indicated in the figure legends. Effluent fractions collected at 2 min intervals were chilled until their insulin content was measured by RIA.

2.2.3. In Vivo Experiments

The experimental design carried out on conscious 24 h fasted animals has been fully described in other publications [28, 29]. Arterial blood was sampled by means of an indwelling cannula placed in the central artery of one ear. Two control samples, separated by an interval of 30 min, were taken before drug infusion started. Drug solutions were infused at a constant rate (0.15 ml min^{-1}) for 30 min through an indwelling cannula, which was kept functional by a slow constant infusion of physiological saline (0.07 ml min^{-1}). Plasma glucose was estimated by means of the glucose oxidase procedure using a kit from Atom (Madrid, Spain). Insulin was determined by using a radioimmunoassay kit (Schering, Spain), with human insulin as standard.

2.2.4. Statistics

Statistical significance was determined using the Student's *t* test for unpaired data or analysis of variance in conjunction with the Newman-Keuls test for unpaired data. A *P* value ≤0.05 was taken as significant. Values presented in the Figures and Results represent means ± s.e.m. of at least 6 observations.

3. RESULTS

3.1. Effects of Imidazoline Ligands on Insulin Release in Perifused Islets

The time course of the effects of imidazoline ligands on insulin release was studied in perifused islets. Idazoxan, BU 224 and efaroxan, each at the equivalent dose of 100 µM, potentiated the insulin secretory response induced by 8 mM glucose (**Figure 1**).

3.2. Effects of Imidazoline Ligands and α_2-Adrenoceptor Antagonists on Insulin Release from Isolated Islets. Glucose Dependency

When islets were incubated in 8 mM glucose, methoxy-idazoxan in the range 10 µM to 1 mM was unable to evoke insulin release. However, idazoxan at the same concentration range induced a clear dose dependent increase in insulin secretion (**Figure 2A**). Similar results were found when islets were incubated in the presence of efaroxan and BU 224 (1-100 µM, **Figure 2B**). As a marked significant increase in insulin release was observed at 100 µM of either ligand, this particular imidazoline drug concentration was used for further studies.

Interestingly, the inhibitory effect on glucose induced insulin release mediated by 1 µM of the selective α_2-adrenoceptor agonist brimonidine (BRM, 55% reduction) was

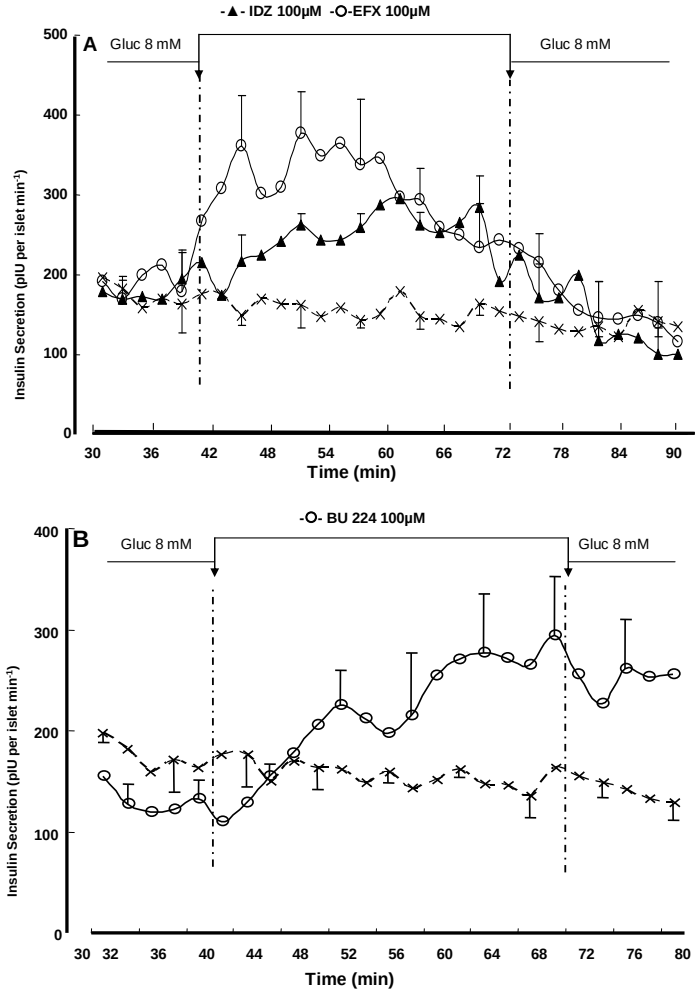

Figure 1. Effects of imidazolines on insulin release from rabbit perifused islets. Groups of 40 islets were perifused throughout the experiment with a medium containing 8 mM glucose (-X-). Test substances were introduced between 40 and 70min: (A) 100 µM idazoxan (-▲-) or 100 µM efaroxan (-O-); (B) 100 µM BU224 (-O-). Values are mean±s.e.m. for four to six experiments.

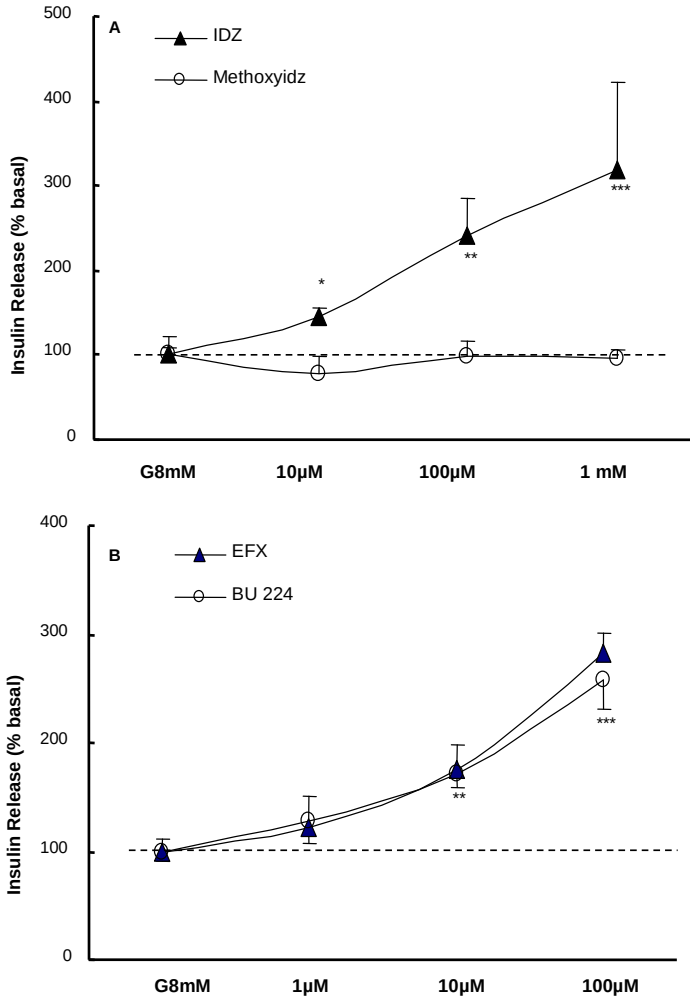

Figure 2. Dose-dependent effects of idazoxan (IDZ), methoxy-idazoxan (Methoxyidz) in (A); efaroxan (EFX) and BU 224 in (B) on insulin release from rabbit islets incubated in the presence of 8 mM glucose in static condition. Each value represents the mean±s.e.m. from at least 10 observations. **P*<0.05, ***P*<0.01, and ****P*<0.001 indicate statistically significant percentage increase relative to 8 mM glucose. Basal insulin release at this particular nutrient concen tration was: 9.65±1.1 µIU ml^{-1} $islet^{-1}$ h^{-1}.

completely reversed by 1 µM idazoxan, thus demonstrating the dual nature (I_2 ligand and α_2-adrenoceptor antagonist) of this compound. However, the α_2-adrenoceptor agonist clearly blunted the response to BU 224 (**Figure 3**). As expected, neither yohimbine nor methoxy-idazoxan affected the response to glucose, though the latter antagonist blocked the inhibitory response to brimonidine.

It is known that efaroxan is able to potentiate glucose induced insulin release over the range of 4-10 mM glucose [30]. Therefore, the effects of idazoxan and BU 224 (100 µM) were also investigated at different glucose concentrations. Both ligands, mimicking efaroxan, enhanced insulin secretion from 3-15 mM glucose (**Figure 4**). In the absence of nutrient these imidazolines failed to release insulin and they did not modify the maximal secretory response to 30 mM glucose.

3.3. Interaction with K_{ATP} Channels

The effects of the three ligands on glucose induced insulin secretion were tested in the presence of 250 µM diazoxide. As expected, the K_{ATP} channel opener inhibited the response to glucose (a 42.5% reduction) and completely suppressed the effect of idazoxan. However, both efaroxan and BU 224 significantly reversed the inhibitory effect mediated by the channel agonist (**Figure 5A**).

Since imidazoline compounds and sulphonylureas block the K_{ATP} channel, though interacting with different and specific binding sites, the effect of the simultaneous addition of BU 224 plus tolbutamide on glucose mediated insulin secretion was also studied. When added alone BU 224 or 200 µM of the sulphonylurea compound both drug induced significant increases in insulin release (80% and

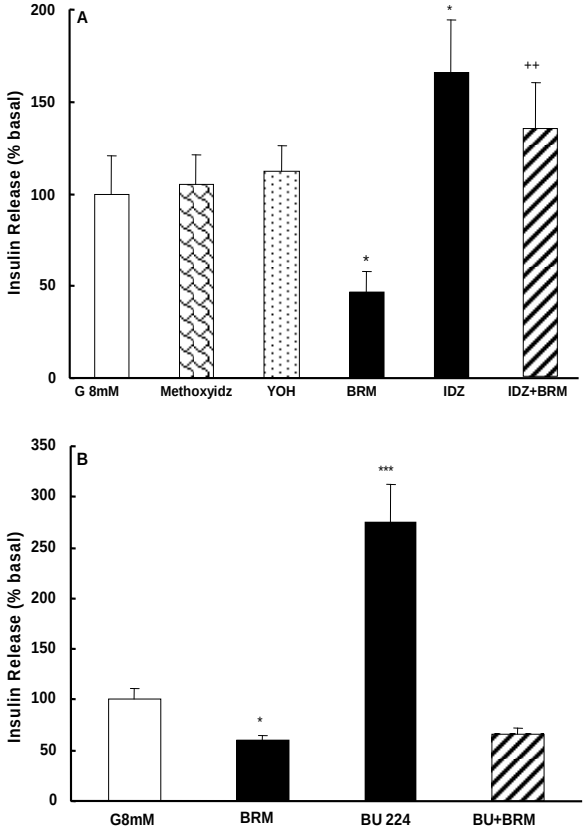

Figure 3. Inhibition of glucose induced insulin release by the α_2-adrenoceptor agonist brimonidine (BRM, 1 µM) when added alone (■) or in the presence of 100 µM of either idazoxan (▨, A) or BU 224 (▨, B). The effects of both imidazoline ligands (100 µM), the α_2-adrenoceptor antagonist yohimbine (YOH, 5 µM ▨), and methoxy-idazoxan (Methoxyidz, 5 µM) by themselves are also shown. **P*<0.05 and ****P*<0.001 represent significant percentage decrease or increase relative to 8 mM glucose. ++*P*<0.01 when comparing to BRM alone. Basal insulin release from rabbit islets in static incubation was 12.32±1.4 µIU ml^{-1} islet^{-1} h^{-1}. Data are from at least 12 experiments.

and 51%, respectively). When added together, the insulin secretory response was clearly enhanced (Δ=190%), thus the effect found with this drug association was significantly higher than the response induced by either drug alone (data not shown).

Interestingly in fresh isolated rabbit islets, idazoxan did not block the response to efaroxan. However neither synergism nor antagonism was found when two imidazoline ligands (either efaroxan-idazoxan, efaroxan-BU 224) were added together (**Figure 5B**).

3.4. Role of Ca^{2+} on Insulin Release Induced by Imidazoline Ligands

The calcium channel blocker nimodipine (5 µM) attenuated the insulin secretory response to 8 mM glucose (by 36.5%, *P*<0.05) and significantly reduced the

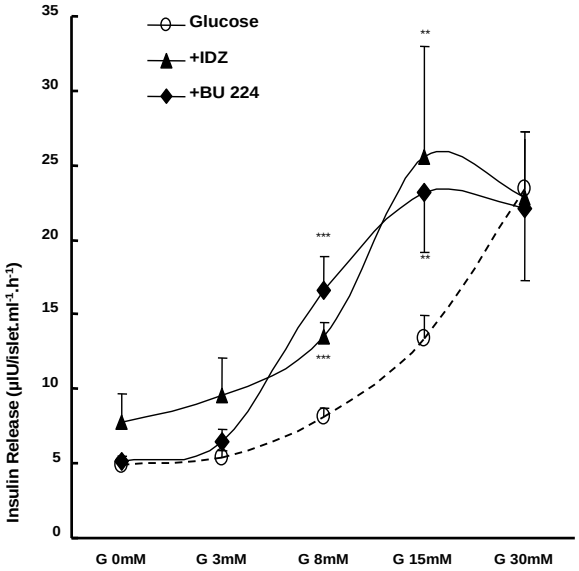

Figure 4. The effect of idazoxan or BU 224 (100 µM, ◆ each) on insulin release in isolated rabbit pancreatic islets in the presence of different glucose (G) concentrations. Results are mean±s.e.m. from at least 14 experiments. ***P*<0.01 and ****P*<0.001, values significantly different relative to their corresponding glucose concentration.

stimulatory effect of BU 224. Interestingly, nimodipine abolished the response to idazoxan (inhibitory degree: 67.6%, **Figure 6A**).

The effects of both ligands were also explored in a low calcium medium (1 mM Ca^{2+}), but in the presence of a higher glucose concentration (15 mM). Ca^{2+} reduction significantly attenuated the responses to glucose and BU 224 (from 11.90±2.10 to 5.35±0.35 µIU ml^{-1} and from 23.25±4.30 to 12.45±0.65 µIU ml^{-1}, respectively, *P*<0.01), though, interestingly, the per-centage increase in insulin secretion mediated by the ligand was of similar magnitude in both media (**Figure 6A**). Idazoxan elicited a tiny effect.

3.5. Role of the K_{ATP}-Independent Pathway on Insulin Release Mediated by Imidazolines

The experimental approach followed was as has been described [31]. The response to 8 mM glucose was already enhanced when islets were incubated in a medium containing 30 mM potassium and 250 µM diazoxide (64.7% increase). BU 224, but neither idazoxan or efaroxan, still induced a significant insulin secretory effect (Δ=80%, P<0.05, **Figure 6B**).

3.6. Intracellular Sites Involved in the Insulin Secretory Activity of Imidazolines

Since true I_2 ligands are reported to be linked to MAO enzymes, it was necessary to test the effect of a non-selective MAO inhibitor on insulin release in the presence of the three ligands. Pargyline (10 µM) did not modify the

response to either glucose or any of the ligands under investigation (**Figure 9B**).

When either BU 224 or efaroxan was assayed in the presence of forskolin (10 µM), drug interaction led to an enhanced insulin secretory response (percentage increases with BU 224, forskolin and both together were: 135.5, 149.7 and 438.2, respectively; similarly, in the case of efaroxan, the results were: 100.9, 149.7, and 407.9). In the same way, the stimulatory response to BU 224 was potentiated by 100 µM of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX, 312%). Again, the increase derived from drug combination (association with either forskolin or IBMX) was significantly higher than the effect found with any drug alone (**Figure 7**, Δ to IBMX alone=177.3±24.5 %).

Neither glucose nor efaroxan and idazoxan induced insulin release were affected by the presence of Rp-

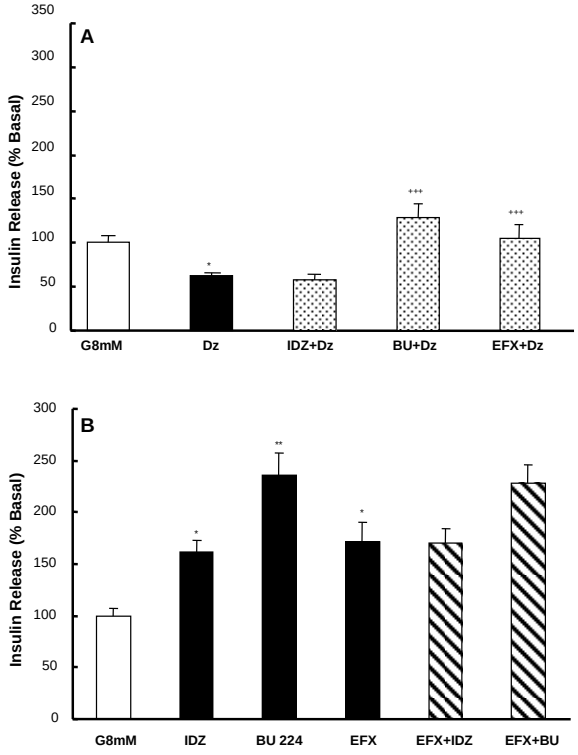

Figure 5. (A): Reversal of diazoxide induced inhibition of secretion by imidazoline ligands. 250 µM of the channel opener were added to islets incubated in 8 mM glucose in the absence (■) or presence (⚃) of 100µM of the three different ligands: idazoxan, BU 224 and efaroxan. **P*<0.05 statistically significant percentage reduction relative to glucose; +++*P*<0.001 when comparing to diazoxide. Basal insulin release was 6.7±0.8 µIU ml^{-1} islet^{-1} h^{-1}. Each value represents the mean±s.e.m. from at least 12 experiments. (B): Insulin release from isolated islets in the presence of the different imidazoline ligands added either separately (■) or together (⚅) as shown. 100 µM of each ligand was always applied. Basal insulin release in the presence of 8 mM glucose was: 7.45±0.55 µIU ml^{-1} islet^{-1} h^{-1}. Data represent the mean from at least 17 observations.

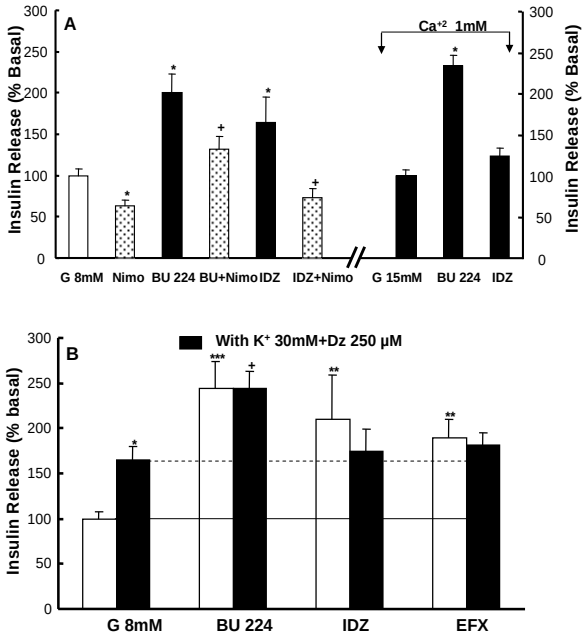

Figure 6. (A): Effects of BU 224 and idazoxan on insulin release in the absence (■) and presence (⚃) of 5 µM nimodipine **P*<0.05 represents statistically significant percentage inhibition relative to glucose; +*P*<0.05, percentage when comparing to either ligand. Basal insulin release was: 7.6±0.6 µIU ml^{-1} islet^{-1} h^{-1}. *On the right*: rabbit islets were incubated in a low calcium medium (1 mM) but with a higher glucose concentration (15 mM). In these experimental conditions insulin release was 5.35±0.3 µIU ml^{-1} islet^{-1} h^{-1}. The effects of BU 224 and IDZ are shown. (B): The insulin secretory responses induced by BU 224, idazoxan and efaroxan in the presence of 30 mM KCl and 250 µM diazoxide (■). The effect found in normal medium (□) is also presented for comparison. **P*<0.05, significant percentage increase relative to normal medium; +*P*<0.05, percentage increase when compare to insulin release from the medium enriched whit KCl and diazoxide. Basal insulin secretion in normal medium: 9.1±0.9 µIU ml^{-1} islet^{-1} h^{-1}. Data from these different experimental designs come from at least 14 observations.

Adenosine-3′,5′-cyclic monophosphothioate triethylamine (Rp-cAMPS, 200 µM). This PKA inhibitor did attenuate the effect of BU 224, though a residual significant increase of 34% above basal levels was still observed with this ligand (**Figure 8A**). The PKC inhibitor chelerytrine 1µM [32] did not alter glucose induced insulin release though significantly blocked the response to BU 224 **Figure 8B**). However insulin secretion in the presence of idazoxan or efaroxan was not modified by chelerytrine. Similarly, calphostin (1 µM), another Protein-Kinase C inhibitor, completely suppressed the effect of (BU 224 (from 21.3±2.9 to 11.9±1.7 µIU ml^{-1} islet^{-1} h^{-1}, insulin secretion in the presence of 8 mM glucose being= 11.6±1.4 µIU ml^{-1} islet $^{-1}$ h^{-1}), the response to efaroxan (22.2±4.7 and 22.0±4.4 µIU ml^{-1} islet^{-1} h^{-1} remaining unaltered in the absence and presence of the inhibitor. Data are from at least 12 experiments).

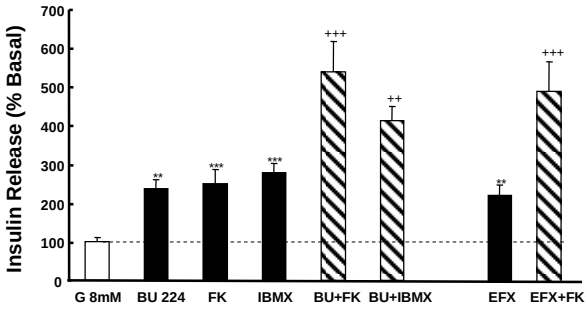

Figure 7. Stimulation of insulin release by BU 224 and efaroxan when added alone (■) or in the presence of either 10 µM forskolin or 100 µM 3-isobutyl-1-methylxanthine (IBMX, ▨). Rabbit islets were incubated in 8 mM glucose throughout the experiment. ***P*<0.01 and ****P*<0.001 represent significant percentage increase relative to glucose; ++*P*<0.01 and +++*P*<0.001, synergistic percentage increase. Basal insulin release: 7.1±1.0 µIU ml^{-1} islet^{-1} h^{-1}. Data are from at least 14 observations.

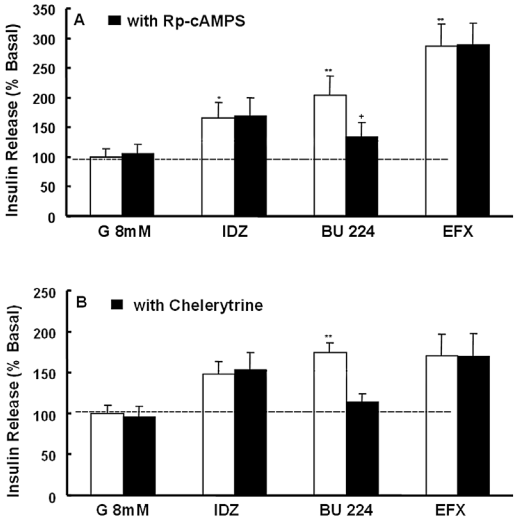

Figure 8. (A): Stimulatory effect on insulin secretion induced by idazoxan, BU 224 and efaroxan when added alone (□) (100 µM, of either ligand) or in the presence of 200 µM of the selective PKA inhibitor Rp-cAMPS (■). (B): The insulin secretory response of the three imidazoline ligands, alone (□) or in the presence of 1 µM of the PKC inhibitor Chelerythrine (■). The effects of both inhibitors on glucose induced insulin release are also shown. **P*<0.05 and ***P*<0.01, significant percentage increase relative to glucose; +*P*<0.05 and ++*P*<0.01 when comparing to ligand alone. Basal insulin release: (A) 12.9 ±1.3 and (B) 11.9 ±1.1 µIU ml^{-1} islet^{-1} h^{-1}. Data are from at least 13 observations.

The compound 2-(2-ethyl-2,3-dihydro-benzofuran-2-yl)-imidazole (KU 14R, 100 µM) did not alter glucose induced insulin release, but selectively reduced the effect of BU 224 (from a 124.5% to a 30.5% increase), therefore, responses to idazoxan and efaroxan remained unchanged. This reported antagonist also lowered forskolin induced insulin secretion (from a 329% to a 158% increase, *P*<0.05), (**Figure 9A**).

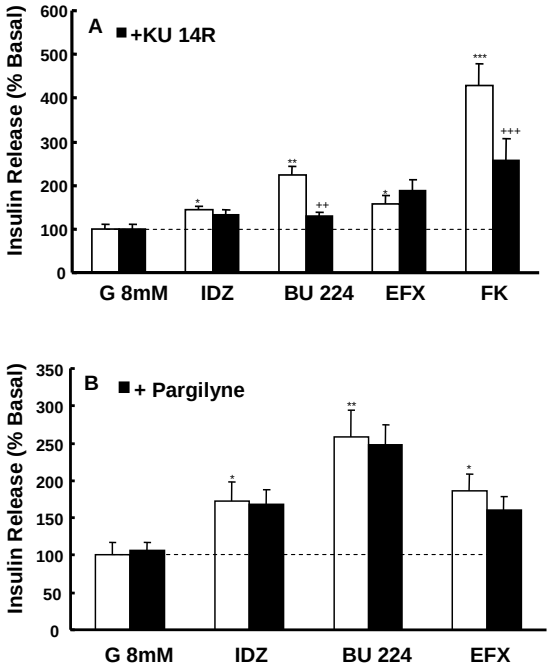

Figure 9. (A): The effect of the compound KU 14R (100 µM) on insulin release induced by the three imidazoline ligands and 10 µM forskolin. (B): The insulin secretory effect of three imidazoline ligands in the absence (□) and presence (■) of the dual MAO inhibitor pargyline (10 µM). Basal insulin secretion when islets were bathed in 8 mM glucose: (A) 7.8±0.8 and (B) 5.6±0.5 µIU ml^{-1} islet^{-1} h^{-1}. **P*<0.05 and ***P*<0.01, significant percentage increase relative to glucose. +*P*<0.05 and +++*P* <0.001, when compared to secretagogue alone. Data were obtained from at least 17 observations.

3.7. Effects in the Presence of Yohimbine, Idazoxan, BU 224 and Efaroxan on Intravenous Glucose Tolerance Test and Plasma Insulin Levels, Studies in Conscious Fasted Rabbits

When infused alone at the equivalent dose of 10 µg kg^{-1} min^{-1}, neither yohimbine [29], nor idazoxan modified basal values of either plasma glucose or circulating insulin (**Figure 10**). Interestingly, after the administration of BU 224 at the same dose, the ligand induced a progressive, persistent and significant increase in plasma insulin (Δ at 45 min=184.40±42.95%, *n*=5, *P*<0.05, *vs.* 1.27±6.75%, *n*=7 in saline treated animals, see **Figure 11**). The pre-infusion plasma glucose level was: 6.25±0.80 mM, *n*=5. These levels were not significantly modified after drug infusion.

Pre-treatment with yohimbine did not modify the responses to a glucose load (10 mg kg^{-1} min^{-1}), (**Figure 10**). Δ in plasma glucose at 30 min in the absence and presence of yohimbine was, respectively, 3.87±0.3 mM, *n*=8,

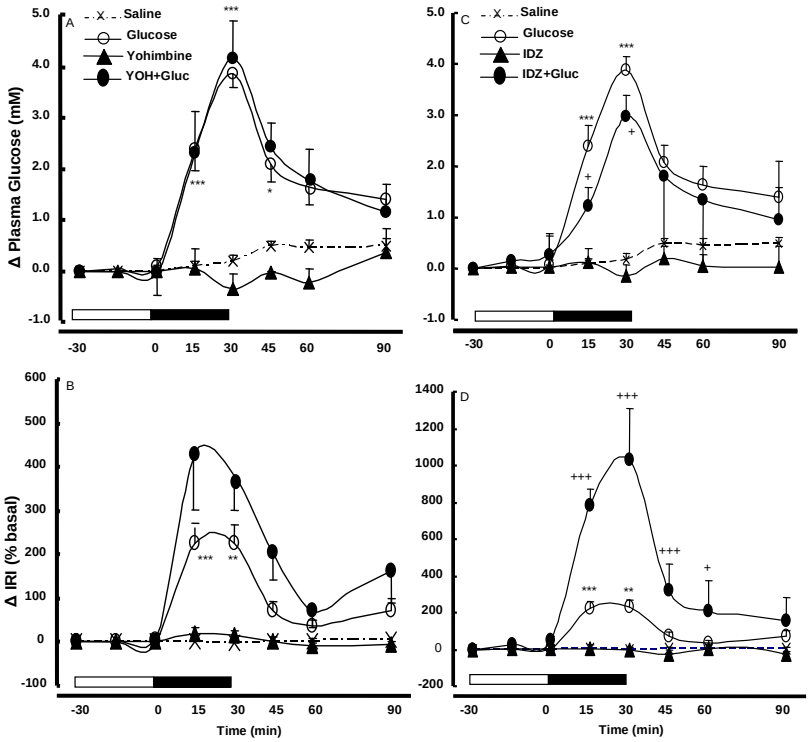

Figure 10. Effects of a glucose load (10 mg kg^{-1} min^{-1}) on plasma glucose (A and C) and circulating insulin levels (B and D) in the absence (-○-) and presence of yohimbine *(left)* or idazoxan *(right)* (-●-) in conscious fasted rabbits. The effects of saline (-**X**-), yohimbine and idazoxan (-▲-, 10 µg kg^{-1} min^{-1}) by themselves on both parameters are also presented; saline or drugs were administered for 30min (white horizontal bar) alone or before a 30min i.v. glucose load (black bar). Ordinate scales, Δ mM plasma glucose refers to the variations from control values. Δ IRI levels are expressed as percentage changes from the control level (control=100%). Each point of any given curve represents the mean±s.e.m. for at least 6 rabbits. Vertical lines indicate s.e.m. ***P*<0.01 and ****P*<0.001, values significantly different between glucose and saline. +*P*<0.05 and +++*P*< 0.001, values significantly different between glucose *vs.* glucose+idazoxan.

vs. 4.17±0.75 mM, *n*=6, N.S. Δ in immunoreactive insulin (IRI) plasma levels, also at 30 min, in the absence and presence of the drug were: 226.55±40.55%, *n*=8, *vs.* 380±102%, *n*=6. No significant difference in the area under the insulin curve was found between glucose and yohimbine pre-treated animals (98.5±27.93 *vs.* 141.06± 31.22 µIU ml^{-1} h^{-1}, *n*=6).

However, in the presence of idazoxan (10 µg kg^{-1} min^{-1}), glucose evoked a greater rise in IRI levels, (**Figure 10**) (Δ at 30 min=1032.90±278.60%, *n*=6, *P*<0.01 when compared to glucose alone). A significant reduction in plasma glucose was also observed (Δ at 15 and 30 min in the presence of the drug being 1.22±0.38 and 2.98±0.4 mM, *n*=6, *P*<0.05).

When the glucose load was assayed in animals pre-treated with BU 224, this ligand induced both a reduction in plasma glucose (Δ at 30 min in the absence and presence of the drug being: 3.40±0.35 mM, *n*=6 *vs.* 2.30±0.45, *n*=6, *P*<0.05) and a greater increase in IRI plasma levels, (**Figure 11**) (similarly, Δ at 30 min was: 116.60±28.10%, *n*=6 *vs.* 325.65±59.05%, *n*=8, *P*<0.001).

Finally, efaroxan alone induced an increase in circulating insulin (Δ at 30 min=196.9%, mean of 2 animals *vs.* -2.90±2.85%, *n*=7, in saline control rabbits) which persisted for 90 min (Δ at 60 min=603.65±260%). When administered before glucose it also enhanced insulin secretion (Δ at 30 min=356.35±60%, *n*=2 *vs.* 116.60± 28.10, *n*=6 in glucose treated animals). However, this insulin secretory effect persisted for as long as 9 h, the animals remaining in hypoglycaemia. Therefore, *in vivo* experiments with this molecule were discontinued.

Pre-infusion absolute values of arterial plasma glucose and circulating insulin for saline, glucose and drug treated animals ranged between 4.56±05 and 6.62±0.25 mM, and 5.65±1.60 and 12.15±2.70 µIU ml^{-1}, respectively.

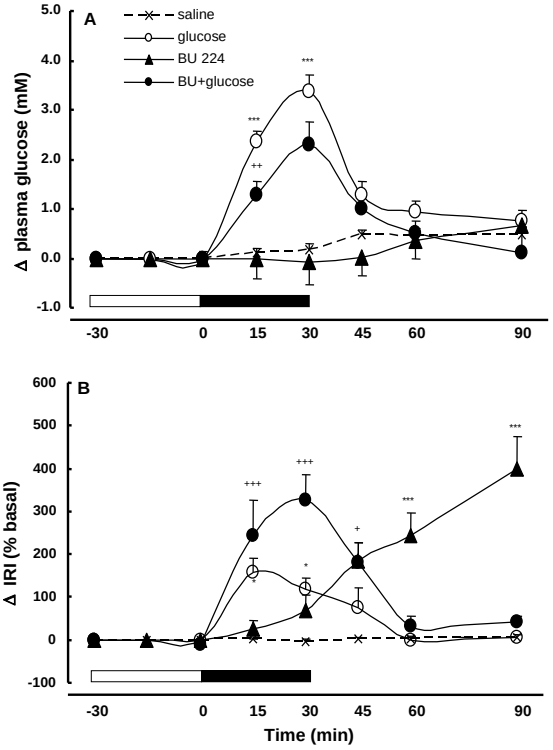

Figure 11. Changes in plasma glucose (A) and in immunoreactive insulin (IRI) (B) levels in conscious fasted rabbits, measured after the i.v. infusion of physiological saline (-**X**-), glucose alone (-○-, 10 mg $kg^{-1}min^{-1}$), BU 224 (-▲- 10 µg $kg^{-1}min^{-1}$) and BU 224+glucose (-●-); the BU 224 was infused for 30 min (open bar) just before a 30min glucose infusion (black horizontal bar). Ordinate scales, Δ mM plasma glucose refers to the variations from control values. Δ IRI levels are expressed as percentage changes from the control level (control=100%). Each point of any given curve represents the mean±s.e.m. for at least 7 rabbits. **P*<0.05 and ****P*<0.001, values significantly different between glucose or BU 224 *vs.* saline. +*P*<0.05, ++*P*<0.01, and +++*P*<0.001, values significantly different between glucose *vs.* glucose+BU 224.

4. DISCUSSIONS

Studies on insulin secretion have mainly been carried out using mouse and/or rat isolated islets. However, there is a lack of experimental data for other animal species, rabbit included. Since rabbit tissues are very rich in imidazoline binding sites [17,24] this animal was chosen for the present work. The results reported in this study assess the validity of our model: insulin release from isolated islets was glucose-dependent and very sensitive to changes in the extracellular concentrations of Ca^{2+} and K^+ ions. Similarly, conventional stimulatory and/or inhibitory responses (i.e., to forskolin, diazoxide) were also confirmed.

Idazoxan induced a very clear dose-dependent insulin secretory response. These are rather paradoxical results, since it has been reported that idazoxan is unable to release insulin in the rat, or it has a weak concentration independent effect in mouse [13,14]. An even more I_2-selective ligand, BU 224 [27], also evoked a dose-dependent secretory response, as did the well known and studied ligand efaroxan [1]. In this model methoxy-idazoxan assayed over a wide range failed to elicit insulin release, thus corroborating its nature as a true α_2-adrenoceptor antagonist. This property was also shared by idazoxan and it was unmasked when tested against the α_2-adrenoceptor agonist brimonidine (UK 14,304). In this way, idazoxan reflected its established dual behaviour. However, no interaction with α_2-adrenoceptors could be found with BU 224.

The mode of action of imidazolines is complex. Classical insulinotropic compounds inhibit K_{ATP} channels in the ß-cell, but, in addition, they exert a direct effect on exocytosis [7,32]. The three ligands studied behaved as K_{ATP} channel blockers. Interaction of efaroxan with K_{ATP} channels could be inferred from perifusion studies and when incubating the islets in the presence of diazoxide, since efaroxan alleviated the suppressive effect on insulin release induced by the potassium channel opener. Similar results have been reported for rat islets [30] using other imidazoline drugs (RX871024, S-22068 [4, 34] and in the present work with the selective I_2-ligand BU 224. However, diazoxide abolished the response to idazoxan, but not to BU 224. It is necessary to consider at this time that in mouse islets idazoxan induces a partial inhibition of K_{ATP} channels, sufficient to depolarize the plasma membrane and to open voltage-dependent Ca^{2+} channels with a subsequent modest increase in intracellular calcium [13]. In the presence of partial channel inhibition, diazoxide, by reducing the binding affinity of the ligand, could easily suppress the response. It is also noteworthy that nimodipine similarly abolished the effect of idazoxan and that just simple reduction in the Ca^{2+} concentration in the extracellular medium severely attenuated the ligand response. An α-1 partial agonist effect of idazoxan has been recently reported [35]. However there is no evidence for a α-1 adrenoceptor involvement in insulin secretion in isolated islets [36]. When rabbit islets were incubated in the presence of the selective α-1 adrenoceptor agonist, amidephrine, no insulin secretory response was found (glucose 8 mM 11.6±1.4 vs amidefrine 10.5±2.2 µIU ml^{-1} $islet^{-1}$ h^{-1}). In the same experimental situations, BU 224 was still able to increase insulin release.

It is also accepted that this kind of imidazoline compounds block the K_{ATP} channel at the level of the Kir6.2 pore [9]. Our experimental results, studying drug interactions among themselves and in the presence of tolbutamide, trend to support this notion. Simultaneous addition of efaroxan and idazoxan, or efaroxan and BU 224, did not lead to synergism or antagonism, probably considering that the three ligands shared a common drug binding site at the pore level [37]. On the other hand, an additive effect was found when BU 224 and tolbutamide were administered together. Indeed, the imidazoline could not displace the

sulphonylurea from its SUR1 receptor. The ensuing enhanced response could result from additive effects at the K_{ATP} channel, or by BU 224 activation of a signal-transduction pathway (see below). Similar results have been reported with other imidazolines [14,38].

It is also well established that insulin secretion in the presence of these compounds exhibited glucose dependency [8,38]. Identical results, expressing the requirement for a high energy state of the cell (high ATP/ADP ratio) have been found with the I_2-ligands used in this work.

Studies with permeabilised islets and HIT T15 cells have revealed a direct effect of imidazolines (RX871024, BL11282) on exocytosis independent of K_{ATP} channel activity. PKA and PKC, with subsequent activation of protein phosphorylation/dephosphorylation steps, would play a central role in the regulation of this process [8]. However, these kinase inhibitors failed to alter the response to efaroxan and idazoxan [12]. Our results, using either PKA/PKC inhibitors or excess K^+, confirm that efaroxan and idazoxan induced insulin secretion is dependent of K_{ATP} channel activity, whereas the effect of BU 224 requires, in addition, PK activation. A synergism between the effect of BU 224 and either forskolin or IBMX on insulin secretion was also evident, suggesting the permissive role of PKA activity on this particular response.

Interestingly, the compound KU 14R, known as an efaroxan antagonist [39], did not alter, in the present work, the response to this ligand, though it blocked the effect of BU 224, significantly attenuating the response to forskolin [40]. A lack of antagonism between efaroxan and KU 14R has also been reported recently in mouse islets [41]. Consequently an association among BU 224-PKA-KU 14R could be inferred in our model. It is noteworthy that at the concentrations used in the present work BU 224 behaved as a reversible inhibitor of MAO A and B, preventing hydrogen peroxidase production in adipose tissue [42]. However, in our model MAO inhibition did not modify glucose or BU 224 mediated insulin release. At this point it is interesting to note that the total capacity of the pancreas to oxidise MAO substrates was limited compared with the overall mass and amine oxidase activities of muscular and adipose tissue [43]. Therefore these results reassess the true nature of BU 224 as an I_2 ligand, though the response under study seems to be independent of MAO binding sites. It has been reported recently that selective I_2-ligands can bind creatine kinase [44,45], a key enzyme important for ATP synthesis. This additional interaction would help to understand the mechanism(s) of BU 224 induced insulin release, considering the importance of ATP for exocytosis even at stages distal to an increase in $[Ca^{+2}]_i$ (see experiments at high concentrations of K^+).

The presence of I_2 binding sites (IBS) mediating the effects of these ligands has not been accepted on the basis of the failure of idazoxan to elicit insulin secretion, lack of data with more selective I_2-ligands and binding studies with methoxy-idazoxan. Results presented in this work refute these premises. In addition the ligand BTS 67582 can bind to the I_2 imidazoline receptor with potency consistent with its effect on insulin secretion [46]. The drug could regulate insulin release by an interaction with the K_{ATP} channel or by exerting a direct effect in the process of exocytosis [46,47]. Curiously indeed, idazoxan and BU 224 also increase insulin release, blocking K_{ATP} channel activity at a site shared by the third imidazoline ligand efaroxan. Therefore, considering that a number of I_2-ligands can bind a common site on the channel, this binding site, independent of MAO activity, might be considered as a variant or subtype of the classic I_2-binding site. It is also known that the presence of I_2 sites on MAO enzyme can not satisfactorily represent the diverse biological targets of I_2-ligands [48,49]. Intracellular binding sites linked to protein kinase(s) activation (I_3-receptor?) would also be involved in the amplifying effect of BU 224.

In vivo studies reassess *in vitro* data. Idazoxan and BU 224, but not yohimbine, enhanced the insulin secretory response to a glucose load. Temporal patterns of insulin secretion when BU 224 was infused alone or in the presence of glucose showed the complex behaviour of this molecule: its glucose dependency as well as its interaction with K_{ATP} dependent and independent mechanisms. When comparing circulating levels of insulin after a glucose challenge in animals pretreated with any of the three drugs, idazoxan was able to induce the maximal response. The dual nature of this ligand should be borne in mind: its ability to block: 1) pre and post-synaptic α_2 adrenoceptors and thus increase plasma catecholamine levels [50], with a subsequent β-adrenoceptor mediated effect; and 2) K_{ATP} channels as an I-ligand (like BU 224). Combined mechanisms would be responsible for such an effect.

Though BU 224 showed a greater antihyperglycaemic effect than idazoxan, the effect was lower than expected considering its ability to release insulin and to restrain lipolysis [42]. However this molecule, being an MAO inhibitor, should prevent metabolic inactivation of endogenous catecholamines, thus enhancing intrinsic sympathomimetic activity. Non-selective blocking of Kir6.2 affecting channels at several locations could also unmask compensatory responses able to attenuate the blood glucose lowering response.

5. CONCLUSIONS

The imidazoline ligands interacting with either I_2 or I_3-binding sites would mediate in vitro, as well as *in vivo*, insulin secretion. However, additional extrapancreatic sites of action would attenuate their antihyperglycaemic effect. In conclusion, the administration of BU 244 in-

duces extended insulin release that would produce potential hypoglycaemia. This could be an adverse effect whether this molecule is used as antidiabetic drug.

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Effect of past and present lifestyle habits and nutrition on calcaneal quantitative osteo-sono index in pre- and post-menopausal females

Masakatsu Nakada[1], Shinichi Demura[2]

[1]National Defense Academy, Kanagawa, Japan; nakada@nda.ac.jp
[2]Graduate school of Natural Science & Technology, Kanazawa University, Kanazawa, Japan

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ABSTRACT

This study is examined the effect of past and present lifestyle habits and nutrition on the osteo-sono assessment index (OSI) in pre- and post-menopausal females. The subjects were 200 premenopausal females (38.8±10.3years) and 156 postmenopausal females (59.2±5.9 years). BMD (Body mineral density) was estimated by right-calcaneal OSI using an ultrasonic transmission method with an AOS-100 device (ALOKA). The number of postmenopau- sal females in the close examination and guidance required groups (80 cases: 51.3 %) (OSI < 2.428) was significantly higher than that of premenopausal females (44 cases: 22.0 %) (χ^2=33.105: P<0.000).

In premenopausal females, the proportion of subjects that had not taken vitamin D in the past (in junior high school and high school) was significantly higher in the close examination-guidance required group (OSI < 2.428) than in the normal group (OSI ≧ 2.428). However, in postmenopausal females, there was no significant difference in past and present lifestyle habits and nutrition between the close examination-guidance required group and the normal group. In premenopausal females, it was determined that the intake of vitamin D during puberty increased the absorption of calcium significantly.

Keywords: Lifestyle and Nutrition Habits, Osteo-Sono Assessment Index, Pre and Postmenopausal Females

1. INTRODUCTION

The occurrence of osteoporosis with bone-thinning and brittle bones is high in elderly people [1-3]. The complications of fractures limit daily activities (ADL) and reduce the quality of life (QOL) of the affected individuals [4-6]. Even when the level of bone loss is below normal (osteopenia), the risk of fractures is high [5]. Females in particular are prone to have osteoporosis and should pay particular attention to the increased risk of a nutritionally deficient diet [6,7].

On the other hand, the significance of healthy eating habits in addition to exercise to maintain and increase BMD has been established [1,8]. In females, bone mass increases during puberty with skeletal growth and peaks from the late teens into the twenties. Afterwards, bone mass is merely maintained from the late thirties to early forties [5,6]. Bone mass density peaks during youth. Increased BMD through proper nutrition, exercise, exposure to sun, etc. are all effective measures for preventing osteoporosis [2]. Hence, it will be necessary to correlate not only present conditions, but also life style habits during youth. Aging is a significant factor which affects BMD. However, the effects of lifestyle habits (the amount of sleep and alcohol consumption, etc.) and nutrition on the bone mineral density in females have been studied specifically in elderly people and young students [1,2,7,9,10]. However, the effects of lifestyle habits should be researched for people of a wider age range. On the other hand, the bone mass of postmenopausal females decreases markedly with a rapid decline in estrogen levels [11]. Hence, studies on the BMD in females should be considered from the onset of menopause. This study examined the effects of past and present lifestyle habits and nutrition on the osteo-sono assessment index (OSI) in pre- and post-menopausal females from age 20 to 70.

2. METHODS

2.1. Subjects

Subjects were 200 premenopausal females (38.8±10.3 years) and 156 postmenopausal females (59.2±5.9 years). **Table 1** shows the number of subjects, and their mean heights and weights, at each age level. Written informed consent was obtained from all subjects after a full ex-

planation of the experimental purpose and protocol.

2.2. Measurement of Osteo-Sono Assessment Index (OSI) and Setting of OSI Group

BMD was estimated by the right-calcaneus using an ultrasonic transmission method with an AOS-100 device (ALOKA). The calcaneal osteo-sono assesment used the osteo-sono assesment index (OSI: TI×SOS^2) by calculating speed of sound (SOS) of ultrasonic transmission in the calcaneus and the transmission index (TI) as described previously [12,13].

The quantitative assessment of bones has generally been performed by Dual X-ray absorptiometry (DXA) and quantitative ultrasound (QUS) [14]. The DXA can measure the total body bone mineral density and is mainly used for precise measurement after screening tests [5]. The QUS is very practical and safe without the effects of radiation [5]. The OSI by AOS-100 has high reliability [14]. Thus, this study used an AOS-100 by QUS. The Japan Osteoporosis Foundation [5] classified females into a close examination (OSI < 80% of an average OSI = 2.158), a guidance required group (2.158 ≦ OSI < 90% of an average OSI = 2.428) and a normal group (OSI ≧ 2.428) based on an average OSI (OSI = 2.698) of females between 20 and 44 years old by osteo-sono assessment criteria. In this study, we combined the former two groups considering a sample size of each age level and compared the close examination and guidance required group (OSI < 2.428) with the normal group (OSI ≧2.428).

2.3. Lifestyle and Nutrition Habits Questionnaire

At present, factors found to be involved in BMD by a large-scale prospective cohort study (Japanese Population-Based Osteoporosis (JPOS) Study) have been examined [15,16]. These prospective cohort studies have the advantage of being prospectively able to measure the predictor, but also require considerable time [17]. The present study is equivalent to a retrospective cohort study [17] and while recall bias may affect the conclusions, useful findings can be identified at an earlier stage.

The study evaluated past and present lifestyle habits and nutrition by questionnaires. The survey was carried out just before a measurement of OSI. Naka *et al*. [2] selected menopause, regular milk consumption, intensity of physical exercise, and awareness of eating habits and physical activity as lifestyle habits. Tomita selected breakfast habits and regular consumption of milk and dairy products, fish and shellfish, meat products etc. during childhood and later years (about 6 – 15 yr) as well as eating habits. Elgán *et al*. [18] selected 10 items (dietary habits (i.e. sugar, fat, fiber, and fruit and vegetables), physical activity, smoking habits, alcohol consumption,

Table 1. Physique of subjects.

		Height (cm)		Weight (kg)	
	N	Mean	SD	Mean	SD
20 s	44	161.0	5.0	53.8	5.5
30 s	61	158.8	5.2	53.4	7.2
40 s	76	158.3	5.2	53.9	8.1
50 s	104	156.6	4.8	54.2	6.5
60 s	62	154.7	5.7	52.8	7.9
70 s	9	152.3	4.2	52.3	6.6

time spent outdoors etc.) as lifestyle habits. The Japan Osteoporosis Foundation [5] coffee, milk, dairy products, fish, meat, soy products, green and yellow vegetables, and natto as meal and articles of taste items for their interview sheet. Referring to the above, this study selected the following 9 items to investigate present eating habits: (1) sleep duration, (2) frequency of alcohol consumption, (3) smoking habits, (4) intervals without meals, (5) regular consumption of dairy products (milk, cheese, yogurt, etc.), (6) intake of calcium supplements, (7) intake of vitamin D (fish, chicken egg, fungi), (8) intake of instant food (instant noodles, instant coffee, etc.), and (9) frequency and length of exposure to sun.

The agreement rates of 9 question items by the test-retest method of 59 subjects ranged from 0.559 – 0.983. Their κ coefficients [19] ranged from 0.287 (P=0.010) - 0.890 (P=0.000) and any value was significant. As stated, the subjects' past lifestyle habit (in junior high school and high school) regarding (1) amount of sleep, (2) intervals without meals, (3) intake of dairy products, (4) intake of vitamin D, and (5) intake of instant food were among the above 9 items surveyed.

2.3.2. Data Analysis

Both groups were classified into pre- and post-menopausal females, cross tabulations of the frequency of past and present lifestyle habits and nutrition were made, and then independent tests were performed. When a significant difference emerged, residual analysis was used. A probability level of 0.05 was indicated statistical significance.

3. RESULTS

Figure 1 show the results of the osteo-sono assessment index (OSI). The number of postmenopausal females in the close examination and guidance required groups (OSI < 2.428) was significantly higher than that (44 cases: 22.0%) of premenopausal females (χ^2=33.105: P<0.000). The number of people in the close examination and guidance required groups increased with age, particularly in people 50 years and older.

Table 2 (premenopausal females) and **Table 3** (postmenopausal females) show cross tabulations by the fre-

Table 2. Present lifestyle and nutrition habits and the OSI of premenopausal females.

Sleeping time		Less than 6 hours	More than 6 hours - less than 7 hours	More than 7 hours - less than 8 hours	More than 8 hours	χ^2	p	ϕ
Result of OSI	CEGR	10(-1.04)	29(2.39)	4 (-2.05)	1 (-0.96)	7.891	0.048*	0.20
	Normal	48(1.04)	71(-2.39)	36 (2.05)	1 (0.96)			
Alcohol intake		No	1 - 3 times a month	1 - 3 times a week	nearly every day			
Result of OSI	CEGR	19	8	6	10	7.76	0.051	0.20
	Normal	50	62	22	21			
Smoking		No	Have a habit	Quit				
Result of OSI	CEGR	35	4	5		0.602	0.74	0.06
	Normal	127	16	12				
Skip a meal		No	Breakfast	Lunch	Supper			
Result of OSI	CEGR	35	5	0	0	1.664	0.645	0.09
	Normal	125	18	2	4			
Intake of dairy prodcts		No	1 - 3 times a month	4 -7 times a week				
Result of OSI	CEGR	3	20	21		0.63	0.73	0.06
	Normal	15	62	79				
Intake of Ca supplement		No	Rarely	Continuous				
Result of OSI	CEGR	33	7	3		0.564	0.754	0.05
	Normal	110	31	14				
Intake of vitamin D		No	1 - 3 times a week	4 -7 times a week				
Result of OSI	CEGR	5	25	14		2.200	0.333	0.11
	Normal	8	94	54				
Intake of instant food		No	1 - 3 times a month	More than once a week				
Result of OSI	CEGR	5	14	25		1.816	0.403	0.10
	Normal	27	56	71				
Sunbathing		No	1 - 3 times a week	More than 4 times a week				
Result of OSI	CEGR	14	15	15		1.557	0.459	0.09
	Normal	39	68	46				

Note)CEGR:close examination or guidance required group, *: P<0.05,
Number shown in parenthese is the Z score of residual analysis.

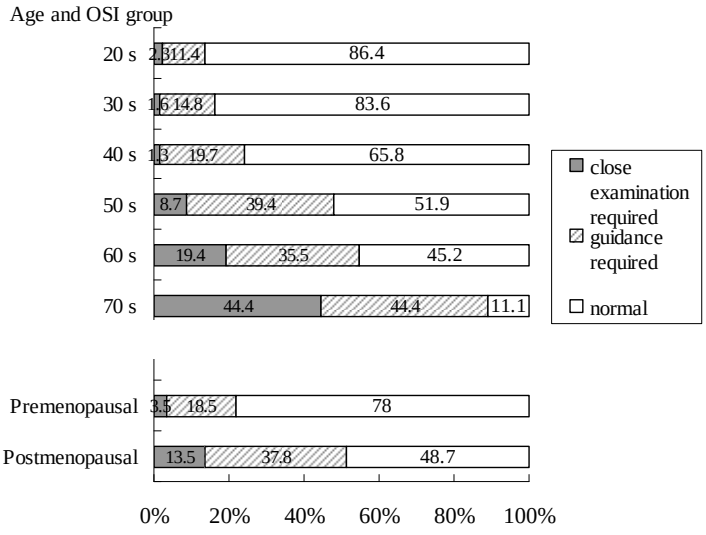

Figure 1. Result of osteo-sono assessment index (OSI).

ency of OSI groups and the frequency of present lifestyle habits and nutrition. An independent test showed sig-

nificant differences in the amount of sleep in the premenopausal group of females. However, the results of residual analysis showed no significant differences in any category. In postmenopausal females, there was no significant difference in any present lifestyle habits or nutrition.

Table 4 (premenopausal females) and **Table 5** (postmenopausal females) show cross tabulations by the frequency of OSI groups and past lifestyle habits and nutrition (in junior high school and high school). An independent test showed significant differences in the amount of sleep and intake of vitamin D in the group of premenopausal females. The results of residual analysis showed significant differences in the intake of vitamin D, and there was a higher proportion of subjects taking no vitamin D in the close examination-guidance required group ($z = 2.77 > 2.64$: $p < 0.05$). In postmenopausal females, there was no significant difference in past lifestyle habits and nutrition.

4. DISCUSSION

The Japan Osteoporosis Foundation [5] set the level for close examination (OSI < 2.158) when using an AOS-100 device (ALOKA) as 0.8 - 1.0 % in people 40-year olds, and 5.2 -11.4 % in 50-year olds. In this study, they were respectively 1.3 % and 8.7 %. Hence, the level for close examination was considered to be standard.

There were no differences in present lifestyle habits and nutrition by OSI level. Nutrition and eating habits in addition to exercise habits are important for maintaining and increasing BMD. Principal minerals for absorption of calcium are magnesium and Vitamin D [7]. We surveyed past and present consumption of dairy products and vitamin D excluding magnesium and present intake of calcium supplements. Lloyd *et al*. [20] reported that by increasing daily calcium intake from 80% of the recommended daily allowance to 110% via supplementation with calcium citrate malate resulted in significant increases in total body and spinal bone density in adolescent girls. The proportion of those test subjects taking no vitamin D (fish, chicken egg, fungi etc) was higher in the close examination-guidance required group than in the normal group. The considerable amount of time that elderly people spend indoors also decreases Vitamin D synthesis through the skin in addition to their intake of vitamin D [5]. Dawson-Hughes *et al*. [21] examined during a long-term (3-year) study that the proper intake of vitamin D with intake of calcium helps reduce the decrease of BMD. Intake of sufficient calcium and vitamin D additionally promotes the absorption of calcium in the small intestines, maintains calcitriol in the blood, and prevents an increased parathyroid hormone (PTH) level. This contributes to a reduction of bone loss [5,21]. The absorption of calcium is supported by the intake of vitamin D during puberty (junior high school and high school age) which increases bone mass with skeletal growth which may be very important for increasing peak bone mass.

On the other hand, there was no difference in the past or present lifestyle habits and nutrition in postmenopausal females between the close examination and guidance required groups and the normal group. Bone mass decreases with age at the rate of about 3 % a year through a lack of estrogen even in normal postmenopausal females [22]. Elderly people may need to increase their daily requirement of calcium as their intestinal calcium absorption decreases [23]. Hence, with increasing age and lack of estrogen, the bone metabolism of postmenopausal females is largely affected, thus obscuring the effect of past and present life habits on bone mass changes. In addition, because of the long interval since puberty for postmenopausal females, subsequent lifestyle habits (eating habits and exercise) may have a greater affect on BMD. Hence, a long-term study should be done considering the BMD of youth.

This study did not examine the effect of exercise stimulus. Sanada *et al.* [24] reportedly showed a significant relationship between calcaneal bone strength and the strength of triceps muscle in postmenopausal females. Bone mass increases by imposing the load of body mass on the lumbar spine. Thus, the bone structure of the lower limbs and thereby the bones of the upper and lower limbs and spine benefit from the mechanical muscle stimulus received from twisting, distortion, and towing. Therefore, BMD is the result of past and present exercise and lifestyle habits.

5. SUMMARY

This study examined the effect of past and present lifestyle habits and nutrition on OSI in pre- and postmenopausal females from 20 to 70 years of age.

1) The number of postmenopausal females in the close examination and guidance required groups (80 cases: 51.3 %) (OSI < 2.428) was significantly higher than that of premenopausal females (44 cases: 22.0 %) ($\chi^2 = 33.105$: $P < 0.000$).

2) In premenopausal females, the number of subjects who had not taken vitamin D in the past (in junior high school and high school) was significantly higher in a close examination-guidance required group (OSI < 2.428) than in the normal group (OSI ≧ 2.428). However, in postmenopausal females, there was no significant difference in past and present lifestyle habits and nutrition between the two groups.

3) In premenopausal females, it was inferred that increased intake of vitamin D during puberty is important to increase the absorption of calcium.

Table 3. Present lifestyle and nutrition habits and the OSI of postmenopausal females.

Sleeping time		Less than 6 hours	More than 6 hours - less than 7 hours	More than 7 hours - less than 8 hours	More than 8 hours	χ^2	p	ϕ
Result of OSI	CEGR	22	37	17	4	1.330	0.722	0.09
	Normal	17	38	19	2			
Alcohol intake		No	1 - 3 times a month	1 - 3 times a week	nearly every day			
Result of OSI	CEGR	44	16	8	11	3.907	0.272	0.16
	Normal	43	8	8	17			
Smoking		No	Have a habit	Quit				
Result of OSI	CEGR	68	7	4		1.028	0.598	0.08
	Normal	63	6	7				
Skip a meal		No	Breakfast	Lunch	Supper			
Result of OSI	CEGR	71	3	3	0	1.942	0.584	0.12
	Normal	66	3	1	1			
Intake of dairy prodcts		No	1 - 3 times a month	4 −7 times a week				
Result of OSI	CEGR	4	29	45		1.001	0.606	0.08
	Normal	2	25	48				
Intake of Ca supplement		No	Rarely	Continuous				
Result of OSI	CEGR	57	11	10		2.349	0.309	0.12
	Normal	60	5	11				
Intake of vitamin D		No	1 - 3 times a week	4 −7 times a week				
Result of OSI	CEGR	7	40	32		0.012	0.994	0.01
	Normal	7	38	30				
Intake of instant food		No	1 - 3 times a month	More than once a week				
Result of OSI	CEGR	21	24	33		2.787	0.248	0.14
	Normal	27	24	22				
Sunbathing		No	1 - 3 times a week	More than 4 times a week				
Result of OSI	CEGR	12	31	30		2.534	0.282	0.13
	Normal	10	23	39				

Table 4. Past lifestyle and nutrition and the OSI of premenopausal females.

Sleeping time		Less than 6 hours	More than 6 hours - less than 7 hours	More than 7 hours - less than 8 hours	More than 8 hours	χ^2	p	ϕ
Result of OSI	CEGR	1(-1.96)	10(-0.89)	15(0.87)	7(2.16)	8.471	0.037*	0.23
	Normal	20(1.96)	48(0.89)	46(-0.87)	10(-2.16)			
Skip a meal		No	Breakfast	Lunch	Supper			
Result of OSI	CEGR	32	5	0	0	1.225	0.268	0.08
	Normal	112	31	0	0			
Intake of dairy prodcts		No	1 - 3 times a month	4 −7 times a week				
Result of OSI	CEGR	4	20	14		1.825	0.402	0.10
	Normal	16	61	72				
Intake of vitamin D		No	1 - 3 times a week	4 −7 times a week				
Result of OSI	CEGR	5(2.77*)	22(0.17)	8(-1.47)		8.712	0.013*	0.22
	Normal	4(-2.77*)	87(-0.17)	51(1.47)				
Intake of instant food		No	1 - 3 times a month	More than once a week				
Result of OSI	CEGR	7	19	8		0.710	0.701	0.06
	Normal	24	79	44				

Note）CEGR:close examination or guidance required group, *：P<0.05,
Number shown in parentheses is the Z score of the residual analysis

Table 5. Past lifestyle and nutrition habits and the OSI of postmenopausal females.

Sleeping time		Less than 6 hours	More than 6 hours - less than 7 hours	More than 7 hours - less than 8 hours	More than 8 hours	χ^2	p	ϕ
Result of OSI	CEGR	4	24	23	9	0.891	0.828	0.09
	Normal	6	20	25	10			
Skip a meal		No	Breakfast	Lunch	Supper			
Result of OSI	CEGR	55	8	2	0	2.527	0.283	0.14
	Normal	52	11	0	0			
Intake of dairy prodcts		No	1 - 3 times a month	4 −7 times a week				
Result of OSI	CEGR	15	39	10		2.937	0.230	0.15
	Normal	13	36	19				
Intake of vitamin D		No	1 - 3 times a month	4 −7 times a week				
Result of OSI	CEGR	7	40	15		1.946	0.378	0.12
	Normal	5	38	23				
Intake of instant food		No	1 - 3 times a month	More than once a week				
Result of OSI	CEGR	25	27	10		0.203	0.904	0.04
	Normal	24	29	12				

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Acute Myocardial Infarction (AMI) and Intermediate Coronary Syndrome (ICS)

E. Stoupel[1,2], A. Tamoshiunas[3], R. Radishauskas[3], G. Bernotiene[3], E. Abramson[4], J.Sulkes[4], P. Israelevich[5]

[1]Division of Cardiology, Rabin Medical Center, Petah Tiqwa, Israel
[2]Sackler Faculty of Medicine, Tel Aviv University, Israel
[3]Institute of Cardiology, University of Medicine, Kaunas, Lithuania
[4]Informatics & Epidemiology Unit Rabin Medical Center, Petah Tiqwa, Israel
[5]Department of Geophysics & Planetary Sciences, Tel Aviv University, Israel

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ABSTRACT

In recent studies it was shown that blood coagulation and inflammation markers are raising at high geomagnetic activity; acute myocardial infarction and all his subtypes, mostly related to atheromatous plaque disruption with higher Cosmic Ray (Neutron) activity. The aim of this study was to explore AMI and ICS differences by concomitant physical conditions, accompanying each of these acute coronary syndromes. The data was a part of MONICA international study in Kaunas, Lithuania in years 2000-2005 (72 consecutive months). 4633 patients with AMI (2461 men) and 961 with ICS (654 men), (age up to 65) were studied. For comparison four indices of Solar (SA), three of Geomagnetic (GMA), Cosmic Ray (CRA) measured by Neutron activity imp/min. were used. Cosmophysical data were from space science institutions in the USA and Russia. Pearson correlation coefficients and their probabilities were obtained.Monthly number of AMI and ICS shows different links with the physical parameters: AMI were significantly inverse related to SA (r=-0.4, p=0.0021) and direct to CRA (Neutron) activity (r=0.23, p=0.048). ICS was not correlated with these two parameters, but show significant links to GMA (r=0.25, p=0.037). Gender differences were evident, men more close related to changes in the mentioned physical parameters.Conclusion: 1. Monthly number of AMI and ICS is different related to fluctuations of environmental physical parameters. 2. The described connections can affect differences in the pathogenesis of these forms of Acute Coronary Insufficiency.

Keywords: Acute Myocardial Infarction; Intermediate Coronary Syndrome; Geomagnetic; Cosmic Ray; Neutron Activity

1. INTRODUCTION

Acute Coronary Syndrome includes many forms of acute coronary insufficiency. Some related to clinical variants connected to myocardial ischemia-blood supply deficit due to, mostly, atherothrombosis, but also coronary artery spasm, blood losses, vasculitis etc. As a result a number of clinical manifestations of coronary insufficiency take place: pain (Anginal pain), cardiac arrhythmia, resulting in many cases Sudden Cardiac Death, Heart Failure etc. These symptoms can be short, resulting in angina pectoris attacks (AP), and longer and more severe, but remaining without significant myocardial damage named Intermediate Coronary Syndrome (ICS) The drop of blood supply and provoked oxygen and other vital agents disballance for a longer time (mostly more than 20 minutes) often lead to necrobiosis in the area of the damaged (culprit) artery with a specific additional complex of symptoms:(clinical, electrocardiographic, enzymatic) and result in later scar formation, or myocardial rupture, life threatening arrhythmias, aneurysm formation, heart failure that is considered as Acute myocardial Infarction (AMI).The ICS takes place between AP and AMI. Recent studied demonstrated significant links between a number of pathogenetic factors involved in ACS development and environmental physical activity [1-9]. The aim of this study was to explore AMI and ICS (code I21-I22 versus I20, ICD 10) by differences in concomitant environmental physical conditions: Geomagnetic, Solar and Cosmic Ray (Neutron) activity.

2. PATIENTS AND METHODS

1) 4633 patients with AMI (2461, 53.12% men) age up

Table 1. Number of stenotic arteries and their maximal stenosis (percentage) in patients with AMI (n-572) and ICS (n-86). Kaunas, Lithuania, 2000-2005.

Code ICD-10	Maximal Coronary Stenosis (%)	1 artery (%)	1 + 2 arteries (%)	3 and more arteries (%)
121-122 AMI	less than 70	46.9	81.3	18.8
	70-99	26.3	51.5	48.5
	100	22.0	51.2	48.8
121-122 Total		24.8	53.0	47.0
120.0 ICS	less than 70	55.0	80.0	20.0
	70-99	17.5	42.5	57.5
	100	23.1	38.5	61.5
120.0Total		27.9	50.0	50.0
Grand Total		25.2	52.6	47.4

to 75y. and 961 ICS (654, 68.05% men)—age up to 65y. treated in Kaunas, Lithuania hospitals at years 2000-2005-72 consecutive months—were included in this study. The registry was part of the international MONICA study registry. In addition, we studied coronary angiography data of 572 patients with AMI (code121-122, ICD 10) and 86 with ICS (code120, ICD 10) comparing the number of stenotic coronary arteries in both groups (1, 1+2 artery and 3 and more artery disease) and degree of coronary artery stenosis: <70%, 70-99% and 100% .

2) The monthly distribution of acute cardiovascular events was compared with concomitant four Solar Activity (SA) indices, three Geomagnetic activity indices (GMA) and their antagonist on Earth action—Cosmic Ray activity indices (CRA) presented by Neutron monitoring at the Earth surface in imp/min. Monthly comparison was chosen because the precise time of the beginning of the studied clinical events is difficult to fix hourly or daily and some differences in time of arrival to the surface of our planet of Solar activity elements- particles and wave energy components.

3) The physical data was obtained from the Space Science institutions in the USA, Russia and Finland. [10-16]

4) Pearson correlation coefficients r and their probabilities p were established for monthly AMI and ICS number and the mentioned cosmophysical indices. Probabilities of 95% and higher were described as significant; of 90%-94%—as a strong trend toward significance. Comparing the number of narrowed coronary arteries in AMI and ICS groups Chi^2 analysis was used, comparing pathology location and severity; 95% and higher probability in differences was accepted as significant.

3. RESULTS

We made some attempts compare the character of coronary lesions resulting in AMI and ICS in this relatively young population group (in accord to MONICA protocol). The age average of the studied patients was 69.12 ± 12.123y. for AMI (64.98 ± 12.21 for men, 73.81 ± 10.17 for women), woman 9 years older and 55.15 ± 7.07y. for ICS (54.1 ± 7.5y. for men and 57.38 ± 5.5 for woman), woman 3 years older.

Table 1 presents the number of affected by narrowing (mostly by atherothrombosis) in both groups. In the AMI group the distribution (in the coronary angiography tested population) was 1 vessel 24.8%, 1-2 vessels - 53.0% and 3 and more vessel disease in 47.0 %. In the ICS group the same distribution included 25.2%; 52.5% and 47.4% of patients. It's difficult to imagine more similar results.

Table 2 presents data about maximal coronary artery stenoses in both groups: less that 70%, 70-99% and 100% occlusion. In the AMI group it was only 32 (5.6%) of maximal stenoses less than 70%. In the ICS (despite not high number of invasive verification) were 20 (23.3%) of 86. **Table 3** presents 70%-100% and 100% ratio in the AMI and ICS groups. 540 of 572 (94.4%) patients in the AMI group had maximal stenoses of 70%-100% versus 76.7% (66 of 86 patients) in the ICS group, Chi^2 =27.85, $p<0.0001$. Comparing total (100%) occlusion in both groups we found 346 (60.5%) in the AMI group and 26 (30.2%) in ICS, Chi^2 =32.04, $p<0.0001$. **Table 4** presents the interrelationship of both groups of acute cardiac events with three groups of monthly space physical activity parameters SA, GMA, CRA. The AMI group was inverse correlated (not strong, but significantly) with SA and related to CRA (Neutron) activity. A different picture was seen in the ICS: absence of significant links with SA and CRA (Neutron) activity and links with GMA in the whole group and men. Absence of such connections in the woman group that was also more than twice smaller, and relatively young.

4. DISCUSSION

AMI and ICS, despite many similarities in the etiology, pathogenesis and natural history have also principal dif-

Table 2. Distribution of maximal coronary stenoses (%) and number of stenotic coronary arteries in patients with AMI (n-572) and ICS (n-86). Coronary angiography data, 2000- 2005, Kaunas, Lithuania.

Code ICD-10	Maximal Coronary Stenosis (%)	1 artery disease	2 artery disease	3 artery disease	4 and more artery disease	Total
121-122 AMI	less than 70	15	11	5	1	32
	70-99	51	49	51	43	194
	100	76	101	84	85	346
121-122 Total		142	161	140	129	572
120. ICS	less than 70	11	5	3	1	20
	70-99	7	10	11	12	40
	100	6	4	4	12	26
120.0 Total		24	19	18	25	86
Grand Total		166	180	158	154	658

Table 3. Differences in maximal coronary stenoses and number of stenotic coronary arteries in patient with AMI (n-572) and ICS (n-86).

Coronary Angiography Data, 2000 - 2005, Kaunas, Lithuania								
Diagnoses	**Maximal Stenosis (%)**	**1 artery disease**	**2 artery disease**	**3 artery disease**	**4 and more artery disease**	**Total**		**Differences & probabilities**
AMI	70 - 99	89.4	93.2	96.4	99.2	94.4	}	**Chi^2=27.85, p<0.0001**
ICS	70 - 99	54.2	73.7	83.3	96.0	76.7		
AMI	100	53.5	62.7	60.0	65.9	60.5	}	**Chi^2=32.04, p<0.0001**
ICS	100	25.0	21.1	22.2	48.0	30.2		

Table 4. Data about AMI and ICS environmental Links. KaunaS, 2000 – 2005 pearson correlation coefficients (r) and their probabilities (p), 72 months data.

	Code 121-122 - AMI			Code 120.0 - ICS		
	Male	**Female**	**Total**	**Male**	**Female**	**Total**
Year	0.3	0.29	0.4	N.S.	N.S.	N.S.
Month 1-12	p=0.019	p=0.013	p=0.0017			
Sunspot Number	-0.3	-0.263	-0.4	N.S.	N.S.	N.S.
	p=0.01	p=0.025	p=0.0021			
Smoothed	-0.3	-0.3	-0.355	N.S.	N.S.	N.S.
Sunspot Number	p=0.02	p=0.015	0.0022			
Solar Flux	-0.3	-0.2	-0.32	N.S.	N.S.	N.S.
2800 MGH 10.7 cm	p=0.01	p=0.096	p=0.0066			
Adjusted	-0.3	-0.22	-0.33	N.S.	N.S.	N.S.
Solar Flux	p=0.01	p=0.067	p=0.004			
GMA Indices:						
Ap.	N.S.	N.S.	N.S.	0.3	N.S.	0.245
				p=0.027		p=0.037
Cp.	N.S.	N.S.	N.S.	0.3	N.S.	0.24
				p=0.028		p=0.043
Am.	N.S.	N.S.	N.S.	0.3	N.S.	0.25
				p=0.01		p=0.037
Cosmic Ray	0.2	0,17	0.23	N.S.	N.S.	N.S.
Activity	p=0.10	N.S.	p=0.048			
Patients Number	2461	2172	4633	654	307	961

ferences. In both types of Acute Coronary Syndrome (ACS) the role of lipid abnormalities, atheromatous plaque formation in the coronary arteries is evident [17–23]. In the last decades the role of inflammation in the development of ACS is widely discussed and mostly accepted [19,21]. The role of thrombosis and endothelial function abnormalities is presented in many studies [21, 20,24].

The cell death of myocytes-necrobiosis and related acute changes in the necrosis area of supplying the culprit artery artery is in 75% provoked by plaque rupture or fissuring with concomitant thrombosis and occlusion, resulting extreme oxygen transport supply disruption and leading to cell death, rise of specific enzyme, if prolonged about 20 minutes or longer are components characteristic for AMI [21,24,25]. In ICS very similar events of atheromatous-thrombothic narrowing in the coronary arteries are accompanied by signs of clinical, ECG, Echocardiography changes related to drop of myocardial oxygen supply and his functioning, but the component of plaque rupture are rare, complete occlusion of the culprit artery (-ies) not so often and cellular changes mostly reversible [21-25].

In recent years many studies confirmed the role of environmental physical factors-solar, cosmic ray — neutron, geomagnetic, proton flux activity on the timing of occurrence, natural history of many cardiovascular pathologies. The mentioned studies included myocardial infarction, sudden cardiac death, cardiovascular deaths, stroke etc. [26]

In addition, some of risk factors of Coronary Heart Disease like arterial pressure, blood lipids and coagulation, C-reactive protein (CRP), life threatening cardiac arrhythmia (ventricular fibrillation, tachycardia), atrial fibrillation, cardiac arrhythmia in patients with AMI were studied in relation to accompanying levels of GMA, CRA, SA, Space Proton flux of different energy levels. [1-9, 26-32].

Summary of this data can be expressed as follows: both blood coagulation and inflammation markers, including such urgent signal reactants like Fibrinogen and CRP, are rising in high GMA.

Also arterial blood pressure monitoring shows similar results.

GMA and SA are inverse related to CRA –Neutron activity ($r=-0.83$-0.84, $p<0.0001$ for SA, $r=-0.60$, $p<0.0001$ for GMA in 216 consecutive months) [31].

AMI occurrence (mostly associated with atheroma-plaque rupture-fissuring [17,18,20,23,22,25] in three separate studies was correlated with CRA-Neutron activity. These findings were confirmed for all subtypes of AMI-STEMI, NSTEMI, Q Wave and Non Q Wave groups. [6,26,9].

CRA was also correlated with SCD as a consequence of AMI, repeated AMI, Myocardial rupture and atherothrombosis without AMI- sudden coronary deaths prior formation of AMI by postmortem data analysis [26].

Life threatening cardiac arrhythmias, detected by implantable cardioverter-defibrillators, mostly in patients with Ischemic Cardiomyopathy also showed significant links with daily CRA-Neutron activity [29]. Similar correlation was found exploring the culprit artery in AMI for lesions predominantly related to Left Anterior Descending (LAD) coronary artery lesions. LAD related AMI were accompanied by higher Neutron activity [28].

Right ventricular infarction in AMI related to Right Coronary Artery (RCA) atherothrombosis, a form of AMI with higher mortality and AMI of any culprit artery complicated by cardiogenic shock (mortality still about 60%) are also linked with higher Neutron (CRA) activity [30]. A recent published study confirmed the changes in enzyme functioning, a key in most metabolic changes accompanying acute coronary events, by elementary particles or radiation [33].

In ICS coronary ischemia event is mostly connected with thrombosis, atheroma and inflammation related vascular narrowing and acute ischemic myocardial event not involving the most critical element- coronary plaque rupture and sudden or additional thrombosis in the ruptured plaque area, resulting acute arterial occlusion and myocyte necrosis-AMI. The presented maximal stenoses levels confirm the predominant total or near total arterial occlusion phenomena in the AMI group compared with the ICS patients. The action of Neutrons is partially explained by their damage to human tissues and cells as they connect free H^+ radicals, and transformation to Protons, attacking, first of all, the cell nuclei and other cellular structures. Neutron activity on the Earth surface reflects the CRA- the Neutrons are remains of crushed by Cosmic Rays atoms in the higher parts of the Universe beyond the SA and GMA terrestrial effects zone. [34-39]

This is the way for an attempt to explain the differences seen in the physical situation concomitant with the two groups of acute coronary syndrome (ACS). It's clear, that many additional risk factors and ingredients of the pathogenesis are involved. But in this study we concentrated our attention on the role of environmental physical activity. Some limitations of this study can be mentioned:

1) Absence of elderly patients, especially woman patients (older 75 in AMI and 65 in ICS groups.

2) Relatively small group of angiography proved coronary lesions in the ICS patients group; despite this limitation, the number of invasive studied in ICS was enough to obtain statistically significant results comparing with AMI patients.

5. CONCLUSIONS

1) Both AMI and ICS patients show significant links by timing to monthly environmental physical activity.

2) ICS patients show correlation with level of GMA. Patients with AMI - with CRA.

3) These differences can be a result to diminished role of plaque disruption and / or fissuring in ICS compared to AMI.

4) Both groups were similar by number of affected coronary arteries, but AMI patients suffered from more critical and total coronary occlusions.

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Cellular responding kinetics based on a model of gene regulatory networks under radiotherapy

Jin-Peng Qi[1,3*], Yong-Sheng Ding[1,2,3*], Shi-Huang Shao[1], Xian-Hui Zeng[1], Kuo-Chen Chou[1,2]

[1]College of Information Sciences and Technology, Donghua University, Shanghai, China
[2]Gordon Life Science Institute, San Diego, USA; qipengkai@126.com, ysding@dhu.edu.cn
[3]Engineering Research Center of Digitized Textile & Fashion Technology, Ministry of Education, Donghua University, Shanghai, China

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ABSTRACT

Radiotherapy can cause DNA damage into cells, triggering the cell cycle arrest and cell apoptosis through complicated interactions among vital genes and their signal pathways. In order to in-depth study the complicated cellular responses under such a circumstance, a novel model for P53 stress response networks is proposed. It can be successfully used to simulate the dynamic processes of DNA damage transferring, ATM and ARF activation, regulations of P53-MDM2 feedback loop, as well as the toxins degradation. Particularly, it has become feasible to predict the outcomes of cellular response in fighting against genome stresses. Consequently, the new model has provided a reasonable framework for analyzing the complicated regulations of P53 stress response networks, as well as investigating the mechanisms of the cellular self-defense under radiotherapy.

Keywords: P53; MDM2; DNA Damage; IR; Oscillations; Radiotherapy

1. INTRODUCTION

Like immunotherapy, chemotherapy, and surgery, radiotherapy is one of the major tools in fighting against cancer. As acute IR is applied, cell can trigger its self-defensive mechanisms in response to genome stresses [1]. As one of the pivotal anticancer genes within the cell, P53 can control the transcription and translation of series genes, and trigger cell cycle arrest and apoptosis through interaction with downstream genes and their complicated signal pathways [2]. Under radiotherapy, the outcomes of cellular response depend on the presence of functional P53 proteins to induce tumor regression through apoptotic pathways [3]. Conversely, the P53 tumor suppressor is the most commonly known specific target of mutation in tumorigenesis [4]. Abnormalities in the P53 have been identified in over 60% of human cancers and the status of P53 within tumor cells has been proposed to be one of the determinant response to anticancer therapies [3,4]. Controlled radiotherapy studies show the existence of a strong biologic basis for considering P53 status as a radiation predictor [3,5]. Therefore, the status of P53 in tumor cell can be considered as a predictor for long-term biochemical control during and after radiotherapy [6-8].

Recently, several models have been proposed to explain the damped oscillations of P53 in cell populations [9-12]. However, the dynamic mechanism of the single-cell responses is not completely clear yet, and the complicated regulations among genes and their signal pathways need to be further addressed, particularly under the condition of acute IR.

Many studies have indicated that introducing novel mathematical and computational approaches can stimulate in-depth investigation into various complicated biological systems (see, e.g., [13-23]). These methods have provided useful tools for both basic research and drug development [24-33], helping understanding many marvelous action mechanisms in various biomacromolecular systems (see, e.g., [21, 34-39]).

Based on the existing models [9-12] and inspired by the aforementioned mathematical and computational approaches in studying biological systems, here a new model is proposed for studying the P53 stress response networks under radiotherapy at the cellular level, along with the kinetics of DNA double-strand breaks (DSBs) generation and repair, ATM and ARF activation, as well as the regulating oscillations of P53-MDM2 feedback loop (MDM2 is an important negative regulator of the p53 tumor suppressor). Furthermore, the kinetics of the oncogenes degradation, as well as the eliminations of the mutation of P53 (mP53) and the toxins were presented. Also, the plausible outcomes of cellular response were

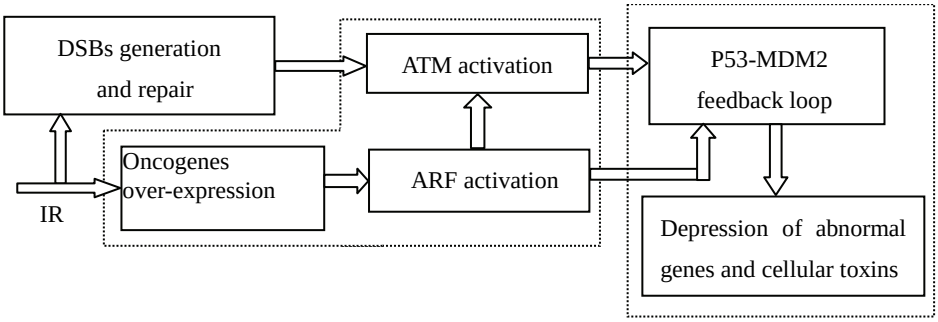


Figure 1. Illustration showing the integrated model of P53 stress response networks under radiotherapy. It is composed of three modules, including DNA damage generation and repair, ATM and ARF activation, as well as P53-MDM2 feedback loop. As acute IR is applied, ARF is activated by the over-expression of oncogenes, and ATM is activated with the cooperation of DSBCs and ARF*. ATM* and ARF* corporately trigger the responding mechanism of P53-MDM2 feedback loop.

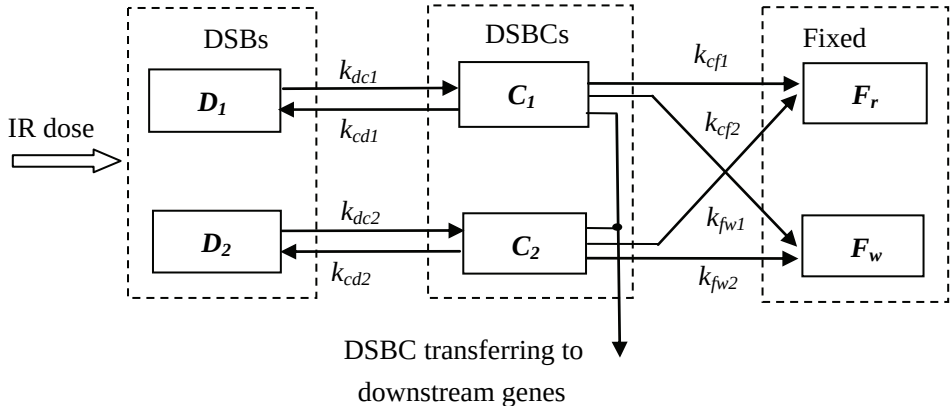


Figure 2. Illustration showing the module of DNA repair process. It includes both a fast repair pathway and a slow one. DSB can be in one of four states: intact DSB (DSB), DBSC, F_r and F_w. Subscripts '1' and '2' refer to the fast kinetics and slow one.

analyzed under different IR dose domains.

It is instructive to mention that using differential equations and graphic approaches to study various dynamical and kinetic processes of biological systems can provide useful insights, as indicated by many previous studies on a series of important biological topics, such as enzyme-catalyzed reactions [18,40], low-frequency internal motions of biomacromolecules [41-46], protein folding kinetics [47,48], analysis of codon usage [49,50], base distribution in the anti-sense strands [51], hepatitis B viral infections [52], HBV virus gene missense mutation [53], GPCR type prediction [54], protein subcellular location prediction [55], and visual analysis of SARS-CoV [7,56].

2. METHODS

2.1. Model Review

Under the genome stresses, many efforts have been made to enhance P53-mediated transcription through some models [9-12,58,59]. However, the interactions in a real system would make these models [60] extremely complicated. Therefore, a new feasible model is needed in order to incorporate more biochemical information. To realize this, let us take the following criteria or assumptions for the new model: 1) only the vital components and interactions are taken into account; 2) all the localization issues are ignored; 3) the simple linear relations are used to describe the interactions among the components concerned; and 4) there are enough substances to keep the system ''workable'' [58].

The new integrated model thus established for the P53 stress response networks under radiotherapy is illustrated in **Figure 1**. Compared with the previous models [9-12], the current model contains more vital components, such as oncogenes, ARF and mP53, as well as their related regulating pathways. In the DSBs generation and repair module, the acute IR induces DSBs stochastically and forms DSB-protein complexes (DSBCs) at each of the

damage sites after interacting with the DNA repair proteins [2,3]. As a sensor of genome stress, ATM is activated by the DSBCs signal transferred from DSBs. Meanwhile, the over-expression of oncogenes prompted by acute IR can trigger the activation of ARF, further prompting the ATM activation [2,7]. The cooperating effects of active ATM (ATM*) and active ARF (ARF*) switch on or off the P53-MDM2 feedback loop [2,7,9], further regulating the downstream genes to control the cell-cycle arrest and the cell apoptosis in response to genome stresses [8]. Here, we use the superscript * to represent the activate state as done in [61].

2.2. DSBs Generation and Repair

Under the continuous effect of acute IR dose, DSBs occur and trigger two major repair mechanisms in eukaryotic cells: homologous recombination (HR) and nonhomologous end joining (NHEJ) [62,63]. About 60-80% of DSBs are rejoined quickly, whereas the remaining 20-40% of DSBs are rejoined more slowly [64,65]. As shown in **Figure 2**, the module of DSBs generation and repair process contains both the fast and slow kinetics, with each being composed of a reversible binding of repair proteins and DSB lesions into DSBCs, and an irreversible process from the DSBCs to the fixed DSBs [62,65]. DSBCs are synthesized by binding the resulting DSBs with repair proteins (RP), which is the main signal source to transfer the DNA damage to P53-MDM2 feedback loop by ATM activation [2].

Due to the misrepair part of DSBs (Fw) having the profound consequences on the subsequent cellular viability and the cellular response in fighting against genome stresses [1,3], we obviously distinguish between correct repair part of DSBs (Fr) and Fw [9,10,12]. Moreover, we further deal the total Fw in both repair processes as a part of toxins within the cell [2,4,11], which can be eliminated by the regulatory functions of P53 during and after radiotherapy, and treated as an indicator of outcomes in cellular response to genome stresses [2].

Some experimental data suggest that the quantity of the resulting DSBs within different IR dose domains obey a Poisson distribution [11]. In accordance with the experiments, we assume that the stochastic number of the resulting DSBs per time scale is proportional to the number generated by a Poisson random function during the period of acute radiation [11]. The DSBs generation process is formulated as follows:

$$\frac{d[\mathrm{DT}]}{dt} = k_t \times \mathrm{Poissrnd}(a_{ir} \times \mathrm{IR}) \quad (1)$$

where [DT] is the concentration of total resulting DSBs induced by IR in both fast and slow repair processes. kt is the parameter to set the number of DSBs per time scale, and air is the parameter to set the number of DSBs per IR dose.

Moreover, we assume that the limited repair proteins are available around DSBs sites, and 70% of the initial DSBs are fixed by the fast repair process. Each DSB can be in one of the four states: intact DSB, DSBC, Fr and Fw [9,10,12]. Thus, we have the following differential equations:

$$\frac{d[\mathrm{D}_1]}{dt} = a_1[\mathrm{D}_t] + k_{cd1}[\mathrm{C}_1] - [\mathrm{RP}](k_{\mathrm{dc1}}[\mathrm{D}_1] + k_{\mathrm{cross}}([\mathrm{D}_1] + [\mathrm{D}_2])) \quad (2)$$

$$\frac{d[\mathrm{D}_2]}{dt} = a_2[\mathrm{D}_t] + k_{\mathrm{cd2}}[\mathrm{C}_2] - [\mathrm{RP}](k_{\mathrm{dc2}}[\mathrm{D}_2] + k_{\mathrm{cross}}([\mathrm{D}_1] + [\mathrm{D}_2]) \quad (3)$$

$$\frac{d[\mathrm{C}_1]}{dt} = k_{\mathrm{dc1}}[\mathrm{D}_1] - k_{\mathrm{cd1}}[\mathrm{C}_1] - k_{\mathrm{cf1}}[\mathrm{C}_1] \quad (4)$$

$$\frac{d[C_2]}{dt} = k_{\mathrm{dc2}}[\mathrm{D}_2] - k_{\mathrm{cd2}}[\mathrm{C}_2] - k_{\mathrm{cf2}}[\mathrm{C}_2] \quad (5)$$

$$\frac{d[\mathrm{RP}]}{dt} = S_{rp} + k_{\mathrm{cd1}}[\mathrm{C}_1] + k_{\mathrm{cd2}}[\mathrm{C}_2] - [\mathrm{RP}](k_{\mathrm{dc1}}[\mathrm{D}_1] + k_{\mathrm{dc2}}[\mathrm{D}_2] + k_{\mathrm{cross}}([\mathrm{D}_1] + [\mathrm{D}_2])) \quad (6)$$

$$\frac{d[\mathrm{F_w}]}{dt} = k_{fw1}[\mathrm{C}_1] + k_{\mathrm{fw2}}[\mathrm{C}_2] \quad (7)$$

where [D], [C], and [Fw] represent the concentrations of DSBs, DSBCs, and Fw in the fast and the slow repair kinetics respectively, kdc, kcd, kcf, and kfw are the transition rates among the above three states; kdc, and kcross represent the first-order and second-order rate constants in both the fast and the slow repair kinetics respectively [65]. Srp is the basal induction rate of repair mRNA, and subscripts '1' and '2' refer to the fast and the slow kinetics.

2.3. ATM and ARF Activation

As a DNA damage detector, ATM exists as a dimer in unstressed cells. After IR is applied, intermolecular autophosphorylation occurs, causing the dimer to dissociate rapidly into the active monomers. The active ATM monomer (ATM*) can prompt the P53 expression further [64]. Meanwhile, ARF, another tumor suppressor, is activated by hyperproliferative signals emanating from oncogenes, such as Ras, c-myc etc., further prompting the ATM activation [2, 7, 10]. Based on the existing model of ATM switch [11], we present an ATM and ARF activation module under IR. Shown in **Figure 3** is the module scheme of ATM and ARF activation, which includes five components: ATM dimer, inactive ATM monomer, ATM*, ARF, and ARF*. Compared with the previous studies in

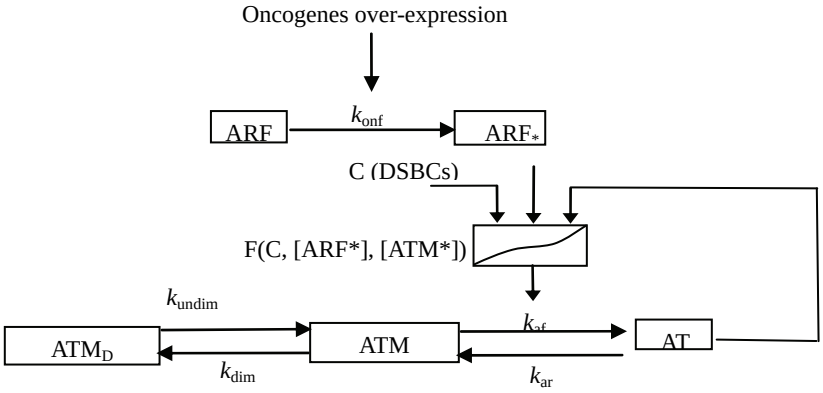


Figure 3. Illustration showing the module scheme of ATM and ARF activation under constant IR. ARF is activated by the over-expression of oncogenes induced by acute IR, and ATM is activated from ATM monomers under the cooperating effects of DSBCs, ARF*, and self-feedback of ATM*.

[9-12], ARF, oncogenes, and the related signal pathways are involved in this module [2,7]. Here, let us assume that DSBCs is the main signal transduction from DSBs to P53-MDM2 feedback loop through ATM activation, and the rate of ATM activation is a function of the amount of DSBCs, ARF* and the self-feedback of ATM*. Furthermore, the total concentration of ATM is a constant, including ATM dimer, ATM monomer and ATM, as treated in {Ma, 2005 #1194].

As a detector of DNA damage, ATM activation plays an important role in triggering the regulatory mechanisms of P53 stress response networks [2,65]. After the acute IR is applied, phosphorylation of inactive ATM monomers is promoted first by DSBCs and then rapidly by means of the positive feedback from ATM*, accounting for the intermolecular autophosphorylation [11]. Meanwhile, under the circumstance of continuous IR dose, ARF, a detector of over-expression of oncogenes is activated by hyperproliferative signals emanating from oncogenes, further prompting the ATM activation [2,7,10], as can be formulated as follows:

$$\frac{d[\mathrm{ATM_d}]}{dt} = \frac{1}{2}k_{\dim}[\mathrm{ATM_m}]^2 - k_{un\dim}[\mathrm{ATM_d}] \quad (8)$$

$$\frac{d[\mathrm{ATM_m}]}{dt} = 2k_{\mathrm{undim}}[\mathrm{ATM_d}] - k_{\dim}[\mathrm{ATM_m}]^2 - k_{\mathrm{af}}\, f[\mathrm{ATM_m}] + k_{\mathrm{ar}}[\mathrm{ATM^*}] \quad (9)$$

$$\frac{d[\mathrm{ATM^*}]}{dt} = k_{\mathrm{af}}\, f[\mathrm{ATM_m}] - k_{ar}[\mathrm{ATM^*}] \quad (10)$$

$$\frac{d[\mathrm{ARF}]}{dt} = S_{\mathrm{arf}} - k_{\mathrm{ad}}[\mathrm{ARF}] - k_{\mathrm{onf}}[\mathrm{Onco}][\mathrm{ARF}] \quad (11)$$

$$\frac{d[\mathrm{ARF^*}]}{dt} = k_{\mathrm{onf}}[\mathrm{Onco}][\mathrm{ARF}] - k_{\mathrm{pad}}[\mathrm{ARF^*}] \quad (12)$$

$$f(C,[\mathrm{ATM^*}]) = a_1 C + a_2[\mathrm{ATM^*}] + a_3 C[\mathrm{ATM^*}] + a_4[ARF^*] \quad (13)$$

where [ATMd], [ATM] and [ATM*] represent the concentrations of ATM dimer, ATM monomer, and active ATM monomer respectively; [Onco], [ARF] and [ARF*] represent the concentrations of oncogenes, ARF, and active ARF respectively; kundim, kdim, kar, and kaf are the rates of ATM undimerization, ATM dimerization, ATM monomer inactivation, and ATM monomer activation, respectively. Sarf, konf, kad and kpad are the rates of ARF basal induction, ARF activation triggered by Oncogenes, ARF degradation, and ARF* degradation, respetively. In addition, f is the function of ATM activation, the term a1C implies the fact that DSBs somehow activate ATM molecules at a distance, a2 [ATM*] indicates the mechanism of autophosphorylation of ATM, a3C [ATM*] represents the interaction between the DSBCs and ATM* [9-12,66], and a4 [ARF*] represents the regulating function of ARF* to ATM activation [1,3,7].

2.4. Regulation of P53-MDM2 Feedback Loop

As shown in **Figure 4**, P53 and its principal antagonist, MDM2 transactivated by P53, form a P53-MDM2 feedback loop, which is the core part in the integrated networks [9-12]. ATM* elevates the transcriptional activity of P53 by prompting phosphorylation of P53 and degradation of MDM2 protein [67]. Also, ARF* can indirectly prompt the transcriptional activity of P53 by inhibiting the expression of MDM2 and preventing P53 degradation [2,7,9]. With the cooperating regulations of ATM* and ARF*, this negative feedback loop can produce oscillations in response to the sufficiently strong IR dose [11].

Especially, the mutation of P53 (mP53) triggered by oncogenes is added in this module, and mP53 is further dealt as another detector of outcomes in cellular response

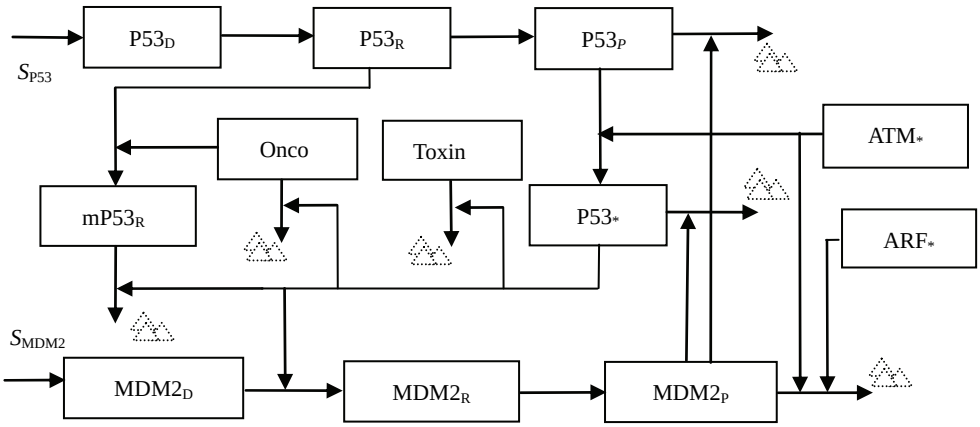


Figure 4. The directed graph of P53-MDM2 feedback loop under radiotherapy. P53 is translated from P53mRNA and phosphorylated by ATM* and ARF*. MDM2 protein promotes a fast degradation of P53 protein and a slow degradation of P53*. In addition, ATM*and ARF* stimulate the degradation of MDM2, and then indirectly increase the regulatory activation of P53* further. Especially, oncogenes, toxins and mP53 are decreased directly by the regulatory functions of P53*.

to acute IR. To account for a decreased binding affinity between inactive P53 and P53*, we assume that MDM2-induced degradation of inactive P53 is faster than that of P53*, and only P53* can induce target genes to depress the over-expression of oncogenes and further eliminate the toxins within the cell [3,4,9-12]. The main differential equations used in this module are as follows:

$$\frac{d[P53_R]}{dt} = S_{P53} - d_{rp}[P53_R] - k_{rp}[P53_R] \quad (14)$$

$$\frac{d[P53_P]}{dt} = k_{rp}[P53_R] + k_{p^*p}[P53^*] - d_{pp}[P53_P] - k_{app^*}[ATM^*]\frac{[P53_P]}{[P53_P]+k_p} - k_{mp}[MDM2_P]\frac{[P53_P]}{[P53_P]+k_d} \quad (15)$$

$$\frac{d[P53^*]}{dt} = k_{app^*}[ATM^*]\frac{[P53_P]}{[P53_P]+k_p} - k_{p^*p}[P53^*] - d_{pp^*}[P53^*] - k_{p^*p}[P53^*] - d_{pp^*}[P53^*] - k_{mp^*}[MDM2_P]\frac{[P53^*]}{[P53^*]+k_{d^*}} \quad (16)$$

$$\frac{d[MDM2_R]}{dt} = S_{mdm2} + k_{p^*m}\frac{[P53^*]^n}{[P53^*]^n + k^n} - k_{mrp}[MDM2_R] - d_{mr}[MDM2_R] \quad (17)$$

$$\frac{d[MDM2_P]}{dt} = k_{mrp}[MDM2_R] - d_{mp}[MDM2_P] - (k_{mat}\frac{[ATM^*]}{[ATM^*]+k_{at}} + k_{mar}\frac{[ARF^*]}{[ARF^*]+k_{ar}})[MDM2_P] \quad (18)$$

$$\frac{d[Onco]}{dt} = k_{onIR}[Onco][IR] - k_{onp}[Onco][P53^*] \quad (19)$$

$$\frac{d[Toxins]}{dt} = k_{tfw}[F_w] - k_{pt}[P53^*][Toxins] \quad (20)$$

$$\frac{d[mP53]}{dt} = k_{mp}[P53_R][Onco] - k_{pmd}[P53_{P^*}][mP53] \quad (21)$$

where [P53R], [P53P], [P53*], [MDM2R], and [MDM2P] represent the concentrations of P53 mRNA, P53 protein, active P53, MDM2 mRNA, and MDM2 protein, respectively; [Onco], [Toxins], and [mP53] represent the concentrations of oncogenes, Fw and mP53, respectively. SP53, and SMDM2 represent the basal induction rates of P53 mRNA and MDM2 mRNA, respectively; k, and d represent the regulation and degradation rates among genes and proteins, respectively. The other parameters are presented in **Tables 1-3**.

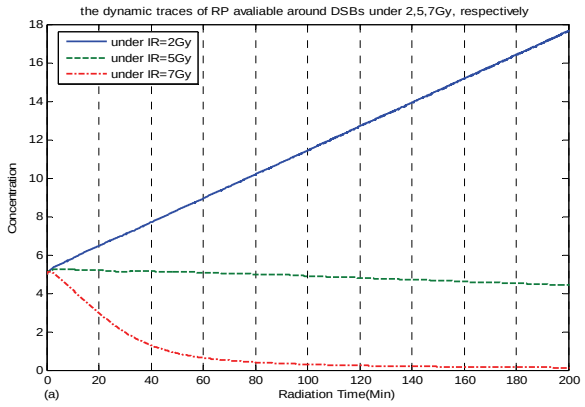
3. RESULTS AND DISCUSSIONS

3.1. Kinetics of DSBCs Synthesizing

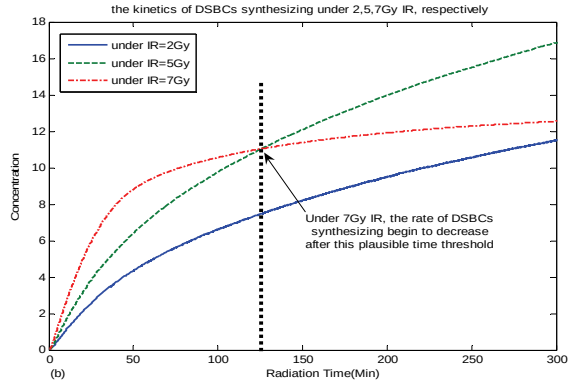
During the simulation process, the continuous 2, 5, and 7Gy IR are applied into a cell respectively. As shown in **Figure 5(a)**, owing to the condition that many DSBs occur and the limited RP are available around damage sites, the concentration of RP begins to decrease as IR dose overtakes 5Gy, and trends to zero versus radiation time. Meanwhile, the kinetics of DSBCs synthe sizing is shown in **Figure 5(b)**. We can see that the rates of DSBCs synthesis keep increasing under 2, and 5Gy IR, whereas, it begins to decrease and trend to constant after about 120min under 7Gy IR dose.

3.2. Kinetics of ARF and ATM Activation

The ARF activation is used to describe the mechanisms in cellular response to the over-expression of oncogenes



(a)

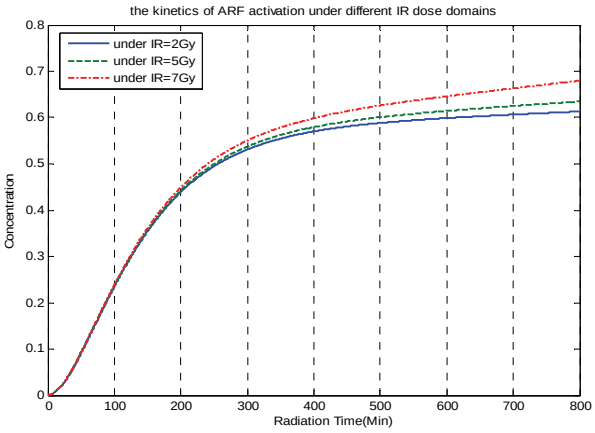


(b)

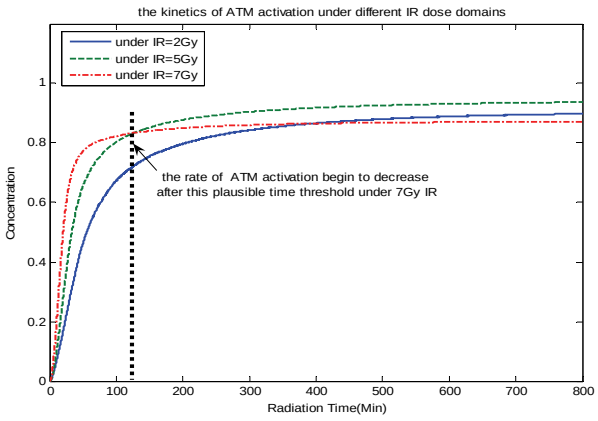
Figure 5. The kinetics of DSBs repairing and transferring under continuous effect of 2, 5, 7Gy IR. (a) The dynamics of RP available around the resulting DSBs under different IR dose domains. (b) The kinetics of DSBCs synthesized by DSBs and RP versus continuous radiation time under different IR dose domains.

induced by acute IR [2,7]. The kinetics of ARF activation is shown in **Figure 6(a)**. Owing to the over-expression of oncogenes without depressing functions of P53*, ARF is activated fast and ARF* keeps increasing followed by trending to dynamic equilibrium versus radiation time.

Meanwhile, the ATM activation module was established to describe the switch-like dynamics of the ATM activation in response to DSBCs increasing, and the regulation mechanisms during the process of the ATM transferring DNA damage signals to the P53-MDM2 feedback loop. Under the cooperative function of DSBCs, ARF*, and the positive self-feedback of ATM*, the ATM would reach the equilibrium state within minutes due to the fast phosphorylation [2,11,67]. Kinetics of ATM activation is shown in **Figure 6(b)**. ATM is activated rapidly and switches to "on" state with respective rates, and then trends to the saturation state. The step- like traces suggest that the ATM module can produce an on-off switching signal, and transfer the damage signal to the P53-MDM2 feedback loop [3]. Furthermore, under the cooperation effects of ATM* and ARF*, DNA damage



(a)



(b)

Figure 6. The kinetics of ARF and ATM activation under 2, 5, 7Gy IR. (a) The kinetics of ARF activation in response to over-expression of oncogenes induced by different IR dose. (b) The switch-like kinetics of ATM activation, ATM* reach saturation and trend to constant state in response to continuous radiation time of different IR dose domains.

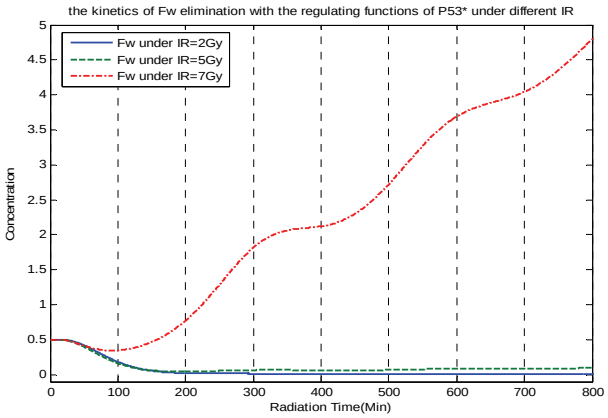
signals can be further transferred to the downstream genes and their signal pathways more efficiently [2,7].

3.3. Outcomes of Cellular Responding Radiotherapy

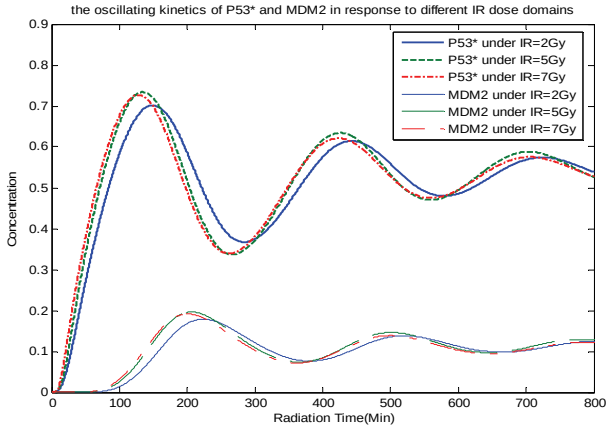
The P53-MDM2 feedback loop is a vital part in controlling the downstream genes and regulation pathways to-fight against the genome stresses [6,67,68]. In response to the input signal of ATM* and ARF*, the P53-MDM2 module generates one or more oscillations. The response traces of P53 and MDM2 protein under continuous application of 2, 5, and 7Gy IR from time 0 are shown in **Figure 7(a)**. Upon the activation by ATM*, ARF* and decreased degradation by MDM2, the total amount of P53 proteins increases quickly. Due to the P53-dependent induction of MDM2 transcription, the increase of MDM2 proteins is sufficiently large to lower the P53 level, which in turn reduces the amount of the MDM2 proteins.

The oscillation pulses shown in **Figure 7(a)** have a period of 400 min, and the phase difference between P53 and MDM2 is about 100 min. Moreover, the first pulse is slightly higher than the second, quite consistent with the experimental observations [2,7,11] as well as the previous simulation results [9,10,12,69].

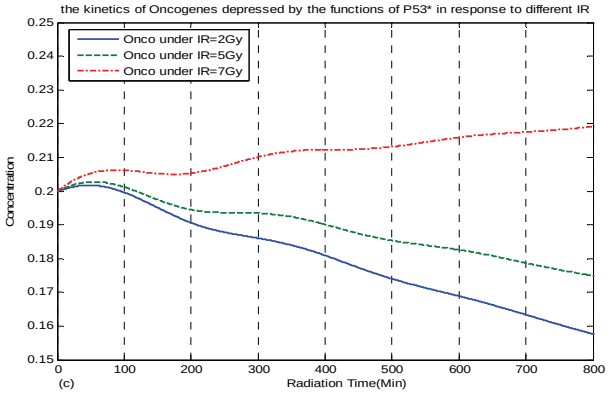
Also, by comparing these simulation results, we can see that the strength and swing of these oscillations begin to decrease as IR overtakes 7Gy, suggesting that the ability of cellular responding genome stresses begin to decrease as IR dose exceeds a certain threshold.



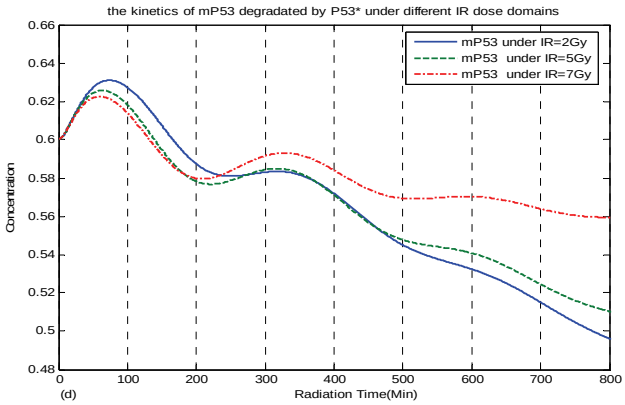
(a)



(b)



(c)



(d)

Figure 7. The outcomes of cellular responding 2, 5, 7Gy IR under radiotherapy. (a) The oscillating kinetics of P53* and MDM2 in response to the cooperative effect of ATM* and ARF* under different IR dose domains; (b) The kinetics of toxins elimination triggered by the functions of P53*; (c) The depressing dynamics of oncogenes over-expression with the regulations of P53*; (d) The kinetics of mP53 elimination triggered by the effect of P53*.

Furthermore, because in the current model the toxins, mP53 and oncogenes can be degraded directly by P53* in this module, we can plot the predictable outcomes of cellular response in fighting against genome stresses under different IR dose domains. As shown in **Figure 7(b)**, Fw remaining within the cell keeps decreasing with respective rate, and trends to zero versus continuous radiation time under 2 and 5Gy IR. Whereas, when IR exceeds 7Gy, Fw begins to increase slightly with some oscillations. Also, the kinetics of oncogenes degrading is plotted in **Figure 7(c)**. As we can see, owing to the negative regulations of P53*, the expression level of oncogenes keeps decreasing after the first climate under 2 and 5Gy IR dose, and then begins to increase slowly under 7Gy IR dose. Meanwhile, as shown in **Figure 7(d)**, quite similar to the results in **Figure 7(b)** and **Figure 7(c)**, mP53 keeps decrease after reaching the first maximum under 2 and 5Gy IR dose, and then begins to increase slowly under 7Gy IR dose. All these results obtained by the above simulations based on the new model indicate that that P53* indeed acts an important role in regulating downstream genes and their signal pathways, whereas its capabilities in cellular responding DNA damage under radiotherapy begin to decrease as the strength of IR exceeds a certain maximal threshold.

4. CONCLUSIONS

A new model was proposed to simulate the P53 stress response network under radiotherapy. It is demonstrated according to our model that ATM and ARF exhibits a strong sensitivity and switch-like behavior in response to

the number of DSBs, fully consistent with the experimental observations. Interestingly, it is shown in this study that after the DNA damage signals transferring, P53-MDM2 feedback loop will produce oscillations, then triggering the cellular self-defense mechanisms to degrade the toxins remaining within the cell, such as Fw, oncogenes, and mP53. Particularly, under different IR dose domains, the new model can reasonably predict outcomes of cellular response in fighting against genome stresses, and hence providing a framework for analyzing the complicated regulations of P53 stress response networks, as well as the mechanisms of the cellular self-defense under radiotherapy.

5. ACKNOWLEDGMENT

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Review of varicella-zoster virus infections in pregnant women and neonates

A. Sauerbrei

Institute of Virology and Antiviral Therapy, Friedrich-Schiller University of Jena, Jena, Germany;Adreas.Sauerbrei@med.uni-jena.de

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ABSTRACT

Even though varicella is rare in pregnancy, the disease can lead occasionally to disastrous illnesses for both the mother and her neonate. By contrast, normal zoster is not associated with special problems during pregnancy and perinatal period. Pregnant women, who contract varicella, are at risk of varicella pneumonia which must be regarded as medical emergency. At any stage during pregnancy, chickenpox may cause intrauterine infection. The consequences for the fetus depend on the time of maternal disease. During the first two trimesters, maternal varicella may result in congenital varicella syndrome which may occur in nearly 2%. Typical symptoms are skin lesions in dermatomal distribution, neurological defects, eye diseases, and skeletal anomalies. Maternal infection near term is associated with a substantial risk of intrauterine acquired neonatal chickenpox in the neonate. If the mother develops varicella rash between day 4 (5) ante partum and day 2 post partum, generalized neonatal varicella leading to death in about 20% of the cases has to be expected. The present paper reviews the clinical consequences and the currently available concepts of prevention, diagnosis, and therapy of varicella-zoster virus infections during pregnancy.

Keywords: Varicella-Zoster Virus Infection; Pregnancy; Neonate; Prevention; Diagnosis; Therapy

1. MATERNAL VARICELLA-ZOSTER VIRUS INFECTIONS AND THEIR CONSEQUENCES

In most industrial countries, chickenpox is a rare disease during pregnancy, as more than 90% of women of childbearing age have virus-specific immunoglobulin (Ig) G class antibodies. The etiological agent, the varicella-zoster virus (VZV), is spread by respiratory transmission or direct contact with infectious lesions. Seronegative persons are at high risk of primary infection manifest as varicella. According to a seroepidemiological study in Germany, the prevalence of VZV-specific IgG class antibodies in women of reproductive age is 96-97% and only 3-4% of women were found to be susceptible to varicella [1]. The average incidence of varicella in pregnant women has been calculated as 0.7-3 per 1,000 pregnancies [2-4]. Information on a positive history of varicella correlates well with serological findings. However, VZV IgG can be detected in 85% of persons with negative history of varicella [5]. Although the clinical course of chickenpox is usually mild, varicella in pregnant women may occasionally lead to serious maternal and fetal diseases (**Table 1**).

Pregnant women who contract varicella are at risk of severe pneumonia associated with life-threatening ventilatory compromise and death. The disease seems to occur more often in the third trimester [6]. At any stage during pregnancy, chickenpox may cause intrauterine infection. Maternal varicella leading to viremia may transmit the virus to the fetus by either transplacental spread or by ascending infection from lesions in the birth canal. The fetal consequences depend on the time of maternal disease. They range from asymptomatic infection to fetal loss especially in case of severe maternal disease. Neonatal varicella can be expected if a mother contracts chickenpox during the last 3 weeks of pregnancy. After maternal varicella between 4-5 days before and 2 days after delivery, generalized neonatal varicella, leading to death in up to 20% of the cases, may occur since these neonates have not acquired protecting antibodies [7].

1.1. Varicella Pneumonia

Although chickenpox is much less common in adults than in children, the infection is associated with greater morbidity, namely pneumonia, hepatitis and encephalitis. Varicella pneumonia during pregnancy must be regarded as a medical emergency. On the basis of retrospective

Table 1. Varicella-zoster virus infections and their potential consequences during pregnancy.

Maternal disease	Timing during pregnancy	Consequences for mother, fetus, term neonate
Varicella	At any stage	Intrauterine death, neonatal or infantile zoster
	5-24th weeks	Congenital varicella syndrome (risk: 2%, mortality: 30%)
	At any stage, especially in the third trimester	Maternal pneumonia (risk: 10-20%, mortality: 10-45%)
	Near term: ≥5 days before delivery	Neonatal varicella at ages 10 (-12) days (risk: 20-50%, mortality: 0%)
	Near term: ≤4-5 days before to 2 days after delivery	Neonatal varicella 0-4 days after birth (risk: 20-50%; mortality: 0-3%); neonatal varicella 5-10 (-12) days after birth (risk: 20-50%; mortality: 20-25%)
Normal zoster	At any stage	No risk for severe maternal, fetal or neonatal infections

hospital-based studies, an incidence of 10-20% among adults with chickenpox has been reported [6,8]. The clinical course is unpredictable and may rapidly progress to hypoxia and life-threatening respiratory failure. Retrospective studies suggest that varicella pneumonia may be more severe, although not more frequent, in pregnant compared to non-pregnant women [9]. Risk factors for the development of pneumonia are smoking and the occurrence of at least 100 skin lesions [10]. The disease usually develops within 3-5 days of the rash and is associated with cough, dyspnea, fever, and tachypnea. Additionally, cyanosis, pleuritic pain in the chest and hemoptysis can occur and secondary bacterial infections are frequent. The chest x-ray findings include a diffuse or miliary/nodular infiltrative pattern often in the peribronchial distribution involving both lungs [11]. Without antiviral treatment, the mortality of the disease may be as high as 45%. However, more recent studies suggest that the mortality has decreased to 10-11% for both non-pregnant and pregnant patients most likely due to the effects of antiviral therapy and better respiratory management [12]. Nevertheless, the risk of fatal course appears to be considerably higher in pregnant than in non-pregnant immunocompetent adults.

1.2 Congenital Varicella Syndrome

Primary VZV infection during first two trimesters of pregnancy may result in intrauterine infection in up to a quarter of the cases [13]. However, the reported rate of spontaneous abortion following acute varicella did not exceed the rate of abortion in pregnant women without chickenpox. Congenital anomalies described as congenital varicella syndrome (CVS) can be expected in about 12% of infected fetuses [14]. Prospective studies in Europe and North America revealed that the incidence of congenital anomalies after maternal varicella infection in the first 20 weeks of pregnancy is about 1-2% [15,16]. The first case of CVS was reported by Laforet and Lynch in 1947 [17]. Since then, more than 130 neonates born with signs of CVS have been described in the English and German literature [13]. Since most of them have been reported during the last 15-20 years, it can be concluded that many cases of this syndrome were formerly not seen in connection with chickenpox during pregnancy. CVS has generally to be expected after maternal chickenpox between the 5^{th} and 24^{th} gestational weeks. Nearly 80% of all cases have been observed between the 9^{th} and 20^{th} weeks of gestation. Before the 5^{th} and after the 24^{th} gestational weeks, the probability of CVS is extremely low. The characteristic clinical symptoms consist of skin lesions in dermatomal distribution, neurological defects, eye diseases, and limb hypoplasia (**Table 2**). Less frequent abnormalities include muscle hypoplasia, affections of the internal organs as well as gastrointestinal, genitourinary, and cardiovascular manifestations [18]. Nearly 30% of neonates born with signs of CVS died during the first months of life. A follow-up report in the literature demonstrated that in spite of initially poor prognosis a good long-term outcome can occur in patients with CVS [19]. On the basis of the segmental distribution of some of the signs, there was postulated that the CVS is not the immediate consequence of intrauterine varicella, but caused by intrauterine zoster-like VZV reactivations with accompanying encephalitis [20]. Immunologic studies suggest that the fetus is not able to mount a VZV-specific cell-mediated immune response [21].

Case report [22]: After a mother had chickenpox with characteristic skin rash between the 13^{th} and 15^{th} gestational weeks, a stillborn girl with typical clinical signs of CVS was delivered by caesarean section at the 34^{th} week of gestation. Postmortal findings included hypoplasia of the left upper and the right lower limb, dermatomally distributed skin lesions in the region of the left anterior thorax, axilla and shoulder as well as on the right lower extremity (**Figure 1**). The autopsy and histological investigations revealed microphthalmia of the left eye associated with opticus atrophia and chorioretinitis pigmentosa. Additional findings were coarcatio aortae of preductal type, Meckel's diverticulum, miliary calcified necroses in the lungs, liver and adrenal glands as well as one-sided cell reduction of the motor anterior column of the cervical and lumbar spinal cord. The eumature placenta showed a focal villous fibrosis,

Table 2. Main symptoms of neonates with congenital varicella syndrome cited in the literature.

Symptoms	Neonates (n=125)	
	n	%
Skin lesions (cicatrical scars, skin loss)	90	72
Neurological defects or diseases (cortical atrophy, spinal cord atrophy, limb paresis, seizures, microcephaly, Horner's syndrome, encephalitis, dysphagia)	78	62
Eye diseases (microphthalmia, enophthalmia, chorioretinitis, cataract, nystagmus, anisocoria, optic atrophy)	66	53
Limb hypoplasia and other skeletal anomalies	55	44
Intrauterine retardation	28	22
Gastrointestinal abnormalities	25	20
Muscle hypoplasia	24	19
Genitourinary abnormalities	15	12
Affections of internal organs	15	12
Developmental delay	14	11
Defects of the cardiovascular system	9	7
Defects of other organs	9	7

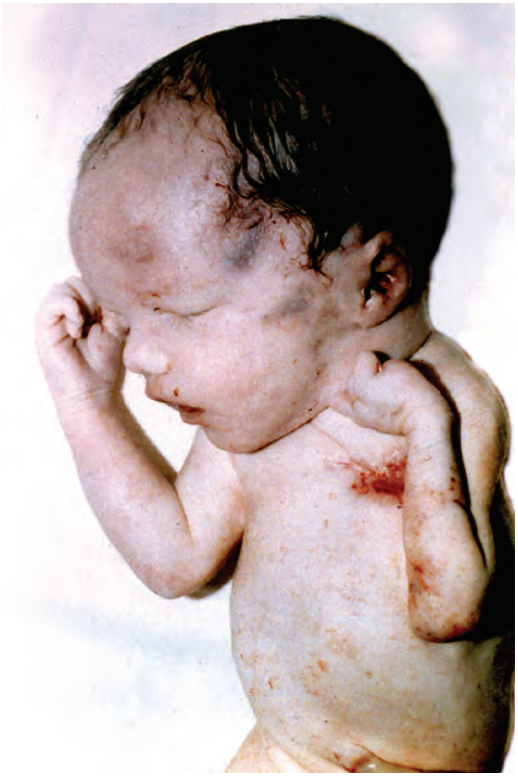

Figure 1. Female stillborn with cicatricial skin lesions involving the left side of chest, axilla, and shoulder as well as hypoplasia of the left upper limb after maternal varicella between the 13th and 15th gestational weeks.

intervillous thrombosis and chronic infarcts. VZV DNA could be detected by polymerase chain reaction (PCR) in the lungs, spleen, adrenal glands, bulbus oculi, and placenta. In addition, VZV DNA/antigens were localized in some organs by means of in situ hybridization/ monoclonal antibodies.

1.3. Zoster During Pregnancy

On the basis of current knowledge, zoster during pregnancy is not associated with birth defects [15,23]. Although there are some reports of neonates with congenital malformations being born to mothers with history of zoster during early pregnancy, no case showed laboratory evidence of intrauterine infection with VZV. In addition, maternal zoster during the perinatal period does not cause problems for neonates [24] as they possess specific maternal IgG class antibodies and there is usually no longer viremic spread of VZV unless the woman is immunocompromised.

2. VARICELLA-ZOSTER VIRUS INFECTIONS IN NEONATES

2.1. Neonatal Varicella

During the perinatal period, maternal varicella can infect the infant by: 1) transplacental viremia, 2) ascending infection during birth, or 3) respiratory droplet/direct contact with infectious lesions after birth. Varicella of the neonate can be expected if a mother contracts chickenpox during the last 3 weeks of pregnancy. Chickenpox occurring in the first 12 days of life is described as intrauterine acquired neonatal varicella. Clinical observations suggest that the incubation period of intrauterine transmitted varicella from the beginning of maternal varicella rash to the onset of rash in the neonate is about 12 days, but it can be reduced to few days [4]. On the basis of these data, neonatal chickenpox occurring after the 12th day of the neonatal period is most likely not transmitted by intrauterine infection, but it is acquired by postnatal VZV infection. Maternal chickenpox few days before or after delivery may cause life-threatening neonatal chickenpox. The disease was first recognized by Hubbard in 1878 [25]. To date, hundreds of cases have been reported [7].

The severity of intrauterine acquired neonatal chickenpox is closely related to the time of onset of maternal infection as transplacentally transmitted antibodies may reduce the severity of symptoms in the neonate. Fetuses

exposed to VZV between 20 and 6 days before delivery may develop neonatal chickenpox however with nonfatal course. These neonates got maternal antibodies and have therefore a lower risk of complications. Generalized neonatal varicella associated with fatal course has to be expected if mothers develop varicella rash between 4-5 days before and 2 days after delivery [7] since these neonates did not acquire protecting maternal antibodies. Additionally, the cell-mediated immune response of the neonate is likely insufficient to retard the hematogeneous dissemination of VZV after transplacental spread [26]. Thus, a fatal outcome has been reported in nearly 20% of these cases (**Table 3**). Furthermore, there is a close relationship between the prognosis of intrauterine acquired neonatal varicella and the onset of disease in the neonate. A fatal outcome is more likely if the neonatal disease occurs between 5 and 10 days after delivery. To our knowledge, 23% of the neonates reported in the literature died from a disseminated and fulminant neonatal infection [7]. In comparison, neonatal varicella within the first 4 days after birth has usually been found to be mild (**Table 3**).

Case report [27]: Two days before delivery, the mother of a female neonate had chickenpox with characteristic skin rash. The girl was transferred from the nursery to the isolation ward and received 1.5 ml varicella-zoster immune globulin (VZIG) Zostergam®. After one week, she was discharged home since she was healthy. On the tenth day, the girl was hospitalized again because of unclear skin lesions appearing on the face. One day later, she developed generalized varicella skin rash. The neonate died from respiratory distress on the 16th day. Autopsy revealed typical varicella rash on the skin (**Figure 2**) and the mucous membrane of the mouth, confluent hemorrhagic-necrotizing pneumonia, necrotizing hepatitis as well as focal necroses of the intestinal mucous membranes, spleen, adrenal glands and brain. Virological investigations confirmed the diagnosis of varicella by detecting VZV DNA in liver tissue using PCR and in situ hybridization. By electron microscopy, intranuclear herpesvirus-like particles could be seen in specimens from skin lesions as well as in necrotic lesions of liver and lungs.

Postnatal VZV infections during the neonatal period have a low morbidity rate [28] as most neonates are protected by maternally derived antibodies. However, premature neonates younger than 28 weeks gestation must be considered to have an increased risk for severe varicella during the first 6 weeks after birth [29,30]. It has been suggested they have got no protecting maternal antibodies because of the reduced gestation period.

2.2. Zoster in Neonates and Young Infants

Nearly 20% of infants with intrauterine acquired VZV primary infection develop neonatal or infantile zoster, usually with uncomplicated course [18]. The disease is thought to represent reactivation of the virus after primary infection in uteri. The relatively short viral latency period may be explained by the immature cell-mediated immune response in young infants.

3. LABORATORY DIAGNOSIS

3.1. Congenital Varicella Syndrome

Most cases of CVS have been reported on the basis of the described main clinical symptoms without laboratory evidence of intrauterine infection. However, the causal relationship between maternal varicella infection and congenital abnormalities can be most convincingly verified by detection of viral DNA or viral antigens in the neonates [22]. Therefore, molecular biological methods should be regularly included in the diagnosis of CVS. In particular, cases presented with rare malformations or after sub-clinical maternal VZV infection need confirmation by virological methods, otherwise the causal relationship between maternal infection and congenital abnormalities remains doubtful [31]. Unlike in cases of intrauterine rubella or cytomegalovirus infection,

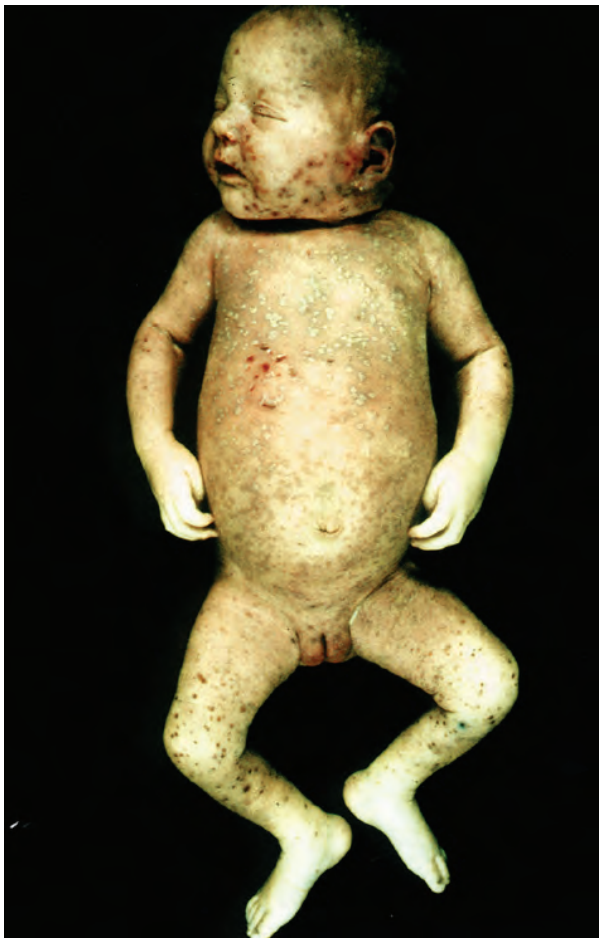

Figure 2. Female neonate with lethal neonatal varicella.

Table 3. Prognosis of neonatal varicella without antiviral treatment in 136 term neonates cited in the literature.

Mother/Neonate	Total number	Day of onset of varicella	Number of cases of neonatal varicella	
			Non-fatal	Fatal
Mother	57	≥5 days before delivery	57 (100%)	0
	79	4 days before to 2 days after delivery	65 (82%)	14 (18%)
Neonate*	35	0-4 days after birth	34 (97%)	1 (3%)
	47	5-10 days after birth	36 (77%)	11 (23%)

* Data of 54 neonates have not been described.

VZV has not been isolated in cell cultures from any neonate with CVS. Usually, the detection of virus-specific antibodies in neonates has been reported to confirm a suspected prenatal infection with VZV. Serologic diagnosis is mostly based on the persistence of VZV-specific IgG class antibodies beyond 7 months of life when maternal antibodies should normally have disappeared [32,33]. The presence of virus-specific IgM has only been described in about 25% of the cases with CVS [23]. The reason for that is primarily the low sensitivity of commercially distributed enzyme immuneo-assays, which are most frequently used to detect VZV-specific IgM. To establish a relationship between maternal VZV infection and congenital anomalies of neonates, the criteria listed in **Table 4** should be used as guideline.

The differential diagnosis of CVS includes congenital infections caused by rubella virus, cytomegalovirus, herpes simplex virus (HSV), coxsackie virus or Toxoplasma gondii [23,34,35] and the specific genetic disorder called MIDAS (Microphthalmus, Dermal Aplasia, Sclerokornea) syndrome [36], whose cardinal symptoms represent congenital skin defects in dermatomal distribution associated with microphthalmia.

3.2. Neonatal Varicella and Varicella Pneumonia

The diagnosis of neonatal varicella is usually based on the typical clinical picture. The characteristic point in time of infection and the maternal history of chickenpox during the last weeks of pregnancy have to be considered to diagnose the intrauterine acquired disease. Clinical findings have been usually confirmed by serological syndrome.methods, but they are not useful for early diagnosis. For laboratory diagnosis of VZV infection, PCR technique should be used as method of choice. Suitable patient materials are skin swabs or biopsies, liquor specimens and tissue samples. HSV and enterovirus infections should be considered in differential diagnosis [37,38]. On suspicion of varicella pneumonia, a laboratory diagnosis is necessary for reason of differential diagnosis. As method of choice, the PCR should be used to detect viral DNA in broncho-alveolar lavage.

(1) Appearance of maternal varicella during pregnancy

(2) Neonate or fetus with
congenital skin lesions in dermatomal distribution and/or neurological defects,
eye diseases,
limb hypoplasia

(3) Proof of intrauterine VZV infection by
detection of viral DNA using polymerase chain reaction and/or presence of specific IgM/persistence of IgG beyond 7 months of age, appearance of zoster during early infancy

Table 4. Criteria used for diagnosis of congenital varicella

4. MEASURES IN MATERNAL VARICELLA-ZOSTER VIRUS INFECTIONS

4.1. Preventive Measures

An effective prophylaxis of chickenpox in pregnant women and neonates is only possible by active immunization of seronegative women before pregnancy. A live attenuated varicella vaccine has been shown to be safe and effective in preventing chickenpox in adults [39]. Varicella vaccine, as all live-attenuated vaccines, is contraindicated in pregnant women and pregnancy has to be avoided for at least 4 weeks following vaccination. The Pregnancy Registry managed by the Merck Research Laboratories (USA) in collaboration with the Centers for Disease Control and Prevention (USA), records women, who exposed to varicella vaccine during pregnancy or within 3 months before conception. Preliminary results showed no hints to any birth defects related to vaccine exposure [40,41]. In few cases, vaccines can develop breakthrough varicella that occurs 42 days after vaccination and represents wild virus infection [42]. Most diseases are very mild, the infectivity is relatively low and there is a low or no risk for complications [43]. Thus, the risk for CVS from breakthrough varicella can be regarded as considerably lower than that for CVS in unvaccinated women with varicella. However, prophylactic and therapeutic measures in women with breakthrough varicella should be considered as in unvaccinated women who develop varicella since data about the risk for CVS after breakthrough varicella are not available to date.

Non-immune pregnant women should be advised to avoid exposure to chickenpox and zoster. If pregnant

women with a negative or indeterminate history of varicella have been exposed significantly to VZV by household contact, face-to-face contact for at least 5 minutes or indoors contact for more than 15 minutes, virus-specific IgG class antibodies should be measured immediately. If the woman is seronegative or there is an indeterminate or unknown status of immunity, she should be regarded as susceptible. Antibodies detected within 7-10 days of contact must have been acquired before exposure. For pregnant women, who were adequately vaccinated with 2 vaccine doses, routine serologic testing cannot be recommended since 99% of persons become seropositive after the second dose of varicella vaccination [44]. Furthermore, most currently used enzyme immunoassays are too insensitive to detect vaccine-induced VZV-specific IgG class antibodies [45] and sensitive procedures such as the fluorescent antibody to membrane antigen assay or tests for the determination of the cell-mediated immune response are too laborious and/or time consuming for daily routine. Recently, a new generation of VZV glycoprotein-based enzyme immunoassays has been shown to have higher sensitivity for the determination of VZV IgG [46].

In case of negative, indeterminate or unknown serologic status, the application of VZIG within 72 (-96) hours has been recommended [47,48] (**Figure 3**) intramuscularly at a concentration of 125 U/10 kg of body weight, up to a maximum of 625 U [29] or 0.5 ml/kg of body weight [30]. A dosage of 1 ml/kg of body weight can be administered intravenously as alternative [30]. Even though the passive immunization does not prevent varicella, it may reduce most likely the risk of severe varicella as well as fetal infection. However, there is no evidence that this prevents fetal viremia or CVS. Thus, the primary reason for VZIG is to prevent severe maternal chickenpox and its complications, such as pneumonia. If there is a definitive past history of chickenpox, it is reasonable to assume that the woman is immune to varicella. Vaccinated pregnant women who were tested VZV IgG-negative should be managed as a seronegative pregnant woman without varicella vaccination. However, in most cases, seronegative vaccinees have most likely acquired VZV-specific cell-mediated immunity.

In the United States, VZV vaccine was licensed in 1995 and decreased the incidence of varicella by 85-90% in the decade following licensure as result of a vaccine coverage rate of about 80% [49]. Even though varicella incidence decreased most significantly in children aged 1-4 years, the incidence also declined nearly 75% among adults despite of low vaccination rates [50]. These data suggest that the universal childhood immunization, introduced also in Germany as the only country in Europe during 2004, has the potential to reduce the occurrence of varicella in pregnant women through herd immunity. However, recent publications demonstrate that varicella during pregnancy is still a problem in many countries which do not offer a routine universal childhood varicella immunization program [51,52]. In Italy, epidemiological data suggest that the risk of acquiring varicella during pregnancy is increasing [53]. Therefore, information programs are recommended to disseminate and to promote screenng and immunization against varicella in susceptible women of childbearing age [54].

4.2. Diagnostic and Therapeutic Measures

Mothers with varicella during the first or second trimester should be carefully monitored since intrauterine infection may lead to CVS. Fetal ultrasound and magnetic resonance imaging at 16 to 22 weeks gestational age or 5 weeks after infection can identify signs of CVS (**Figure 3**) [55,56]. Laboratory investigations for VZV DNA in placental villi, fetal blood or amniotic fluid and for VZV IgM in fetal blood are only indicated if suspicious fetal abnormalities can be detected [55]. However, several studies have shown that the presence of VZV DNA alone does not necessarily correlate with fetal disease [57]. Thus, the question of how severely the fetus is affected cannot be answered definitely. This and the low risk of CVS should be considered in counseling women with varicella in early pregnancy. Termination of pregnancy is only indicated if there are definitive signs of serious fetal abnormalities.

An antiviral treatment has immediately to be introduced at first signs of varicella pneumonia or other disseminated infections. As the only therapeutic agent, aciclovir is indicated in pregnant women. Aciclovir has to be administered orally at a dosage of 5 x 800 mg or intravenously at a concentration of 3 x 10-15 mg/kg for 7-10 days. Zoster during pregnancy should only be treated with aciclovir in severe courses of the disease [58]. To date, there are no controlled studies concerning antiviral chemotherapy in preventing CVS [59].

5. MEASURES IN NEONATAL VARICELLA-ZOSTER VIRUS INFECTIONS

5.1. Preventive Measures

Mothers and neonates suffering from or being at risk of varicella have to be isolated on maternity wards. To reduce the mortality from neonatal chickenpox, the date of delivery may be postponed for several days to allow maternal antibodies to pass the placental barrier (**Figure 3**). However, there is only little experience with successful delay of labor when neonatal varicella must be expected [60,61]. The administration of aciclovir at a dosage of 5 x 800 mg orally or 3 x 10-15 mg/kg intravenously for 7 days has been recommended for pregnant women within 24 hours of the varicella rash when the disease occurs within 4-5 days before delivery. VZIG is indicated for neonates whose mothers have signs and symptoms of varicella between 5 days before and 2 days after delivery

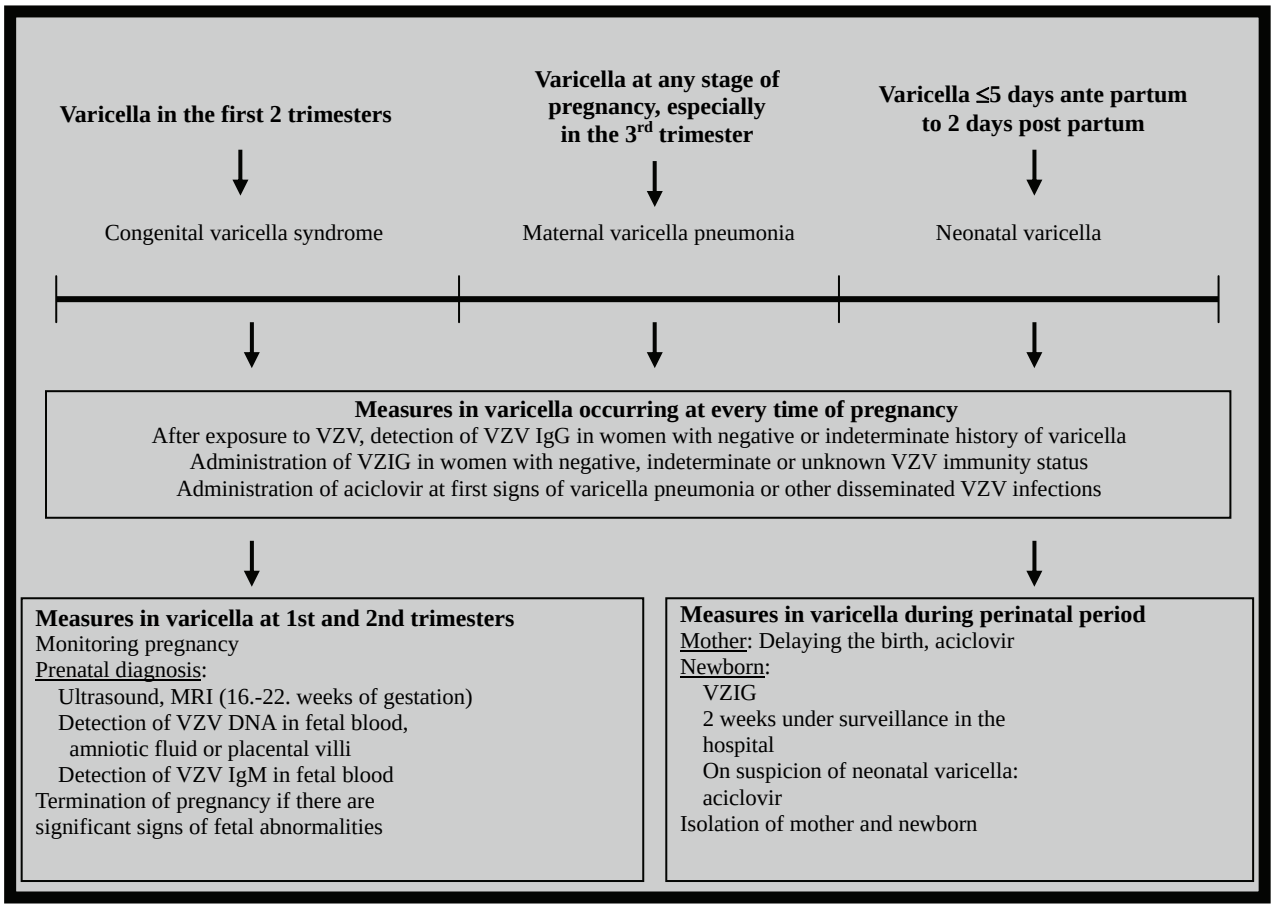

Figure 3. Measures in case of varicella during pregnancy depending on the point of time of infection; MRI: magnetic resonance imaging, VZIG: varicella-zoster immune globulin, VZV: varicella-zoster virus.

Table 5. Administration of varicella-zoster immune globulin (VZIG) and aciclovir in neonates to prevent neonatal varicella.

VZIG after intrauterine exposure to VZV		
Neonates whose mothers develop varicella within 5 days before and 2 days after delivery	Intravenously: 1 ml/kg [30] or intramuscularly: 125 U [29] or 0.5 mg/kg [30]	Immediately after birth or onset of maternal rash
VZIG after postnatal exposure		
Premature neonates with negative varicella history of the mother	Intravenously: 1 ml/kg [30] or intramuscularly: 125 U [29] or 0.5 mg/kg [30]	Within 96 hours after exposure
Premature neonates <28 weeks gestation or <1,000 g birth weight independent of maternal varicella history	Intravenously: 1 ml/kg [30] or intramuscularly: 125 U [29] or 0.5 mg/kg [30]	Within 96 hours after exposure
Antiviral treatment of neonatal varicella		
Suspected neonatal varicella	Aciclovir intravenously: 3 x 10-15 mg/kg	Length of therapy: 5-7 days

(**Table 5**). Passive immunization is probably not necessary for neonates whose mothers have sings of varicella >5 days before or >2 days after delivery [14,62] because those neonates are not at risk of severe neonatal varicella. Hospitalized premature neonates, younger than 28 weeks gestation, who are exposed to VZV, have to receive VZIG, regardless of the maternal history of chickenpox as these neonates may not have acquired maternal antibodies (**Table 5**) [29]. Following treatment, these neonates should be under surveillance in the hospital for 2 weeks, i.e. to the end of incubation period [14,30]. When a neonate who has received VZIG is discharged home, it

should be made clear to the parents that prompt hospital review should be undertaken if the neonate becomes unwell or develops rash. It has been generally accepted that passive immunization of the neonate can modify the clinical course of neonatal varicella but it does not prevent the disease and, although decreased, the risk of death is not completely eliminated [63,64].

5.2. Therapeutic Measures

An antiviral treatment of neonates with CVS has only been described in few cases [65-67]. Clinical observations suggest that aciclovir therapy may be helpful especially to stop the progression of eye diseases or to prevent neurological diseases after VZV reactivations. Suspected cases of neonatal chickenpox should be treated promptly with aciclovir (**Figure 3**, **Table 5**). Prophylactic intravenous administration of aciclovir can prevent neonatal varicella or can reduce the severity of the disease markedly [68]. Well-controlled studies on the use of aciclovir in neonates have not been reported to date [59].

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Is overweight or obesity a perioperative risk factor in total hip replacement?

Mushtaq AlAssaf[1], Michael Kolbeck[2], Wolfgang Hönle[1], Alexander Schuh[2]

[1]Department of Orthopaedic Surgery, Neumarkt Clinic, Neumarkt, Germany
[2]Research Unit Orthopaedics and General Surgery, Neumarkt Clinic, Neumarkt,Germany; Alexander.Schuh@klinikum.neumarkt.de

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ABSTRACT

Introduction: A large proportion of patients who undergo total hip replacement (THR) are obese. Aim of the present study is to investigate the influence of Body Mass Index (BMI) on complications following THR in a single surgeon in the short term follow-up. Material and method: This study was based on the retrospective review of charts and BMIs from 171 patients who had undergone THR between April 2005 and March 2006 at our hospital. All operations were performed by a single surgeon. All patients were followed up 6 weeks after operation. Results: 27 / 171 patients (15.8%) were found to have complications. Systemic minor complications included arrythmia in 1 case, urinary tract infection in two cases, ileus in two cases, renal insufficiency in 3 cases, confusion in 2 cases and anaemia in 14 cases (8.2%) requiring blood transfusion. There was one case of pulmonary embolism as a major systemic complication. Local minor complications included one single dislocation and 1 superficial wound infection. Body mass index ranged from 20.8 to 46.7 with a mean of 28.6. Hospital length of stay ranged from 10 to 42 days with a mean of 13. The length of operation time between obese and non-obese patients varied significantly in our study. There was no increased risk for complications and length of hospital stay. Discussion: We can conclude that there are no economic or medical reasons for excluding obese patients from THR as there is no increased risk for complications and length of hospital stay.

Keywords: Total Hip Replacement, Complication, Obesity, BMI, Bleeding

1. INTRODUCTION

A large proportion of patients who undergo total hip replacement (THR) are obese [17,18]. There has been much concern that obesity is associated with anaesthetic and operative complications after THR. Obese patients have a higher risk of adverse cardiovascular and respiratory events and obesity is an independent risk factor for the development of type II diabetes mellitus, a condition that carries an increased risk of post-operative morbidity. Other studies have demonstrated an increased risk of venous thromboembolic disease following joint replacement surgery in the obese. There is a strong association between obesity and prolonged wound drainage post-operatively which, in turn, is associated with a higher rate of wound infection and blood loss [1,4,20]. Namba *et al.* [15] concluded that obese patients have a 4.2 times higher risk of post-operative infection following THR. Furthermore, obesity has a bearing on health economics, with obese patients having an increased length of hospital stay compared with the general population [3,12]. Recently a weak correlation BMI and the complication rate in THR has been published by Patel A *et al*. [17]. He stated that orthopaedic surgeons should be aware of the slightly higher risk in THR in such patients. On the other hand several authors found no increased post-operative complications rate in obese patients undergoing THR [2,9,11,19]. Aim of this study was to investigate the influence of BMI on complications following THR in a single surgeon.

2. MATERIAL AND METHOD

This study was based on the retrospective review of charts and BMIs from 171 patients who had undergone THR between April 2005 and March 2006 at our hospital. All operations were performed by a single surgeon (WH). Prior to surgery weight and height of the patients were recorded by nursing staff. BMI was calculated as the body weight in kilograms divided by the height in metres squared. Co-morbid conditions were also recorded. Any complications reported by the patient or the medical staff were recorded. Complications were group-

ed into systemic and local, each group being subdivided into minor and major according to Patel [17]. A complication that could be treated with medical and conservative management or that was not a risk to the artificial joint and/or the patient was listed as a minor complication (superficial infection, ooze, pain). Any complication that needed surgical/medical intervention and posed a risk to the joint or the patient was listed as a major complication (dehiscence, deep infection, post- operative stiffness requiring manipulation under anaesthesia, haematoma requiring second operation, cardiac arrest, deep vein thrombosis, cardiovascular accident, congestive cardiac failure, systemic infection, intensive care unit admission, myocardial infarction, pulmonary embolism). There were 75 men and 96 women. Average height was 168 cm (Min: 147, Max: 193). Average weight was 81 Kg (Min: 53, Max: 160). Average age at time of operation was 66 years (Min: 43, Max: 90). We reviewed all BMIs and divided the patients into 4 groups: BMI < 26 (Optimal and under weight), 26 - 30 (Clinically overweight), 31 - 40 (Clinically obese), > 40 (Morbidly obese) [4]. Patient clinical complexity level (PCCL) was assigned. PCCL of women was 0 in 62 cases, 1 in 1 case, 2 in 14 cases, 3 in 12 cases and 4 in 7 cases. PCCL of men was 0 in 55 cases, 2 in 7 cases, 3 in 10 cases and 4 in 3 cases. The indications for THR were dysplastic coxarthrosis (15), primary arthrosis of the hip (124), inflammatory arthritis (2), secondary osteoarthritis (26), rheumatoid arthritis (3), necrosis of the femoral head (1).

On the acetabular side, a standard cementless acetabular cup according to Wagner was used in 148 cases, in 19 cases a cemented full-profile polyethylene cup in 4 cases an acetabular reconstruction ring. On the femoral side, 1 case was treated with the cementless Cone Prosthesis according to Wagner, 154 with a cementless CLS stem and 16 with a cemented Müller Straight Stem. The bearing surfaces were polyethylene/ceramic in all cases. All procedures were performed in an ultra-clean-air theater (with antibiotic prophylaxis). During their stay at the hospital, all patients were treated with low molecular weight Heparin and compression stockings as a prophylaxis against deep vein thrombosis. For the duration of 8 weeks, partial weight bearing of 20 kg with the support of lower arm crutches was required. Average operation time was 65 min (Min: 42, Max: 170).

Data were first fed in electronic format into Excel. Accuracy of the electronic data was confirmed by three independent observers. Data were then analysed for frequencies and Chi-squared test. All patients were followed up by one of us (MA) 6 weeks after operation.

3. RESULTS

Body mass index ranged from 20.8 to 46.7 with a mean of 28.6. 27 / 171 patients (15.8%) were found to have complications. Systemic minor complications included arrythmia in 1 case, acute hypertension in one case, urinary tract infection in two cases, ileus in two cases, renal insufficiency in 3 cases, confusion in 2 cases and anaemia in 14 cases (8.2%) requiring blood transfusion. There was one case of pulmonary embolism as a major systemic complication. Local minor complications included one single dislocation and 1 superficial wound infection. Hospital length of stay ranged from 10 to 42 days with a mean of 13. Average loss of haemoglobin in women was -3.5 (Min: -0.8, Max: -9.2) and in men -3.8 (Min: -1, Max: -7.7) (**Table 1**). **Table 2** shows the different complications and related BMI.

For the purpose of analysis patients were split into 4 groups of BMI (**Table 3**) with a complication rate ranging from 6.2 - 41.5% (17/ 41 (41.5%), 4/64 (6.2%), 5/ 62 (8.0%) and 2/6 (33.3%). When patients were put into BMI groups there was no effect on complication rate with increasing BMI.

Table 1. Distibution of demographic data and BMI.

BMI vs Hemoglobin loss	Mean (g/dl)
Women	
< 26	-3,84
26 - 30	-3,88
31 - 40	-3,68
> 40	-3,30
Men	
< 26	-4,77
26 - 30	-3,72
31 - 40	-4,11
> 40	---

BMI vs. Length of hospital stay	Mean (days)
Women	
< 26	13,5
26 - 30	13,14
31 - 40	13,82
> 40	14,66
Men	
< 26	13,5
26 - 30	13,12
31 - 40	15,62
> 40	---

BMI vs. Duration of operation	Mean (hours)
Women	
< 26	1:08
26 - 30	1:04
31 - 40	1:07
> 40	1:27
Men	
< 26	1:02
26 - 30	1:04
31 - 40	1:09
> 40	---

Table 2. Complications and BMI.

Initials	Complication	Sex	Age	BMI
BE1	Acute hypertension	f	56	42,2
GM1	Acute renal failure	f	80	20,8
NR1	Acute renal failure	m	62	32,0
TG1	Acute renal failure	f	73	30,1
KF1	Postoperative Delirium	m	79	23,4
RM1	Postoperative Delirium	f	90	21,2
KF1	Arrhythmia	m	79	23,4
AE1	Haemorrhage	f	81	25,8
AC1	Haemorrhage	f	66	24,7
GH1	Haemorrhage	f	70	22,6
GM1	Haemorrhage	f	80	20,8
KH1	Haemorrhage	f	77	25,2
KM1	Haemorrhage	f	46	23,7
NR1	Haemorrhage	m	62	32,1
PM1	Haemorrhage	f	55	26,4
RA1	Haemorrhage	f	59	23,4
SB1	Haemorrhage	f	81	25,6
SH1	Haemorrhage	m	76	28,7
TG1	Haemorrhage	f	73	30,1
WC1	Haemorrhage	f	90	24,5
WM1	Haemorrhage	f	82	21,3
KP1	Ileus, not particularly specified	m	68	34,3
SA1	Ileus, not particularly specified	m	75	30,3
GM1	Infection, not particularly specified	f	80	20,8
EG1	Dislocation	m	56	35,9
BE1	Pulmonary embolism	f	56	42,2
BR1	Urinary tract infection	f	78	25,0
KF1	Urinary tract infection	m	79	23,4

Table 3. Correlation between complications and BMI divided into four groups.

BMI groups		Complications	
		No	Yes
< 26	Count	24	17
26 - 30	Count	60	4
31 - 40	Count	57	5
> 40	Count	4	2

4. DISCUSSION

The numbers of patients presenting for THR who are obese is increasing and there have been concerns that these patients have an increased risk of complications and a reduced benefit of surgery in terms of function and pain relief [3,9,13,14,16]. A higher risk for obese patients in respect to deep infection dislocations and revision rate for septic loosening has been postulated [7,8, 10,12,21].

Stickles *et al.* [23] reported a higher rate of orthopaedic complications (infection, dislocation, component failure) with increasing BMI in the first year after THR.

Obese patients are often considered poor candidates for total joint arthroplasty. These patients tend to have longer hospital stays and higher total charges compared with nonobese patients [6]. Ibrahim *et al.* [9] could not found any difference in the length of hospital stay between obese and non-obese patients. This compares favourably with our results. Within the current literature, the effect of obesity on THA remains varied. Some reports have found no difference in the rate of perioperative complications between obese and non-obese patients. Anderson *et al.* [2] and Soballe *et al.* [22] found no relationship between obesity and postoperative complications. Lehman *et al.* [11] showed no difference in the prevalence of perioperative complications in obese pa-

tients with similar gains in pain relief and functional abilities as non-obese patients. Chan and Villar [5] showed no difference in the quality of life in the short term following THA between obese and non-obese patients. With our study results we can follow Andrew *et al*. [3] and Ibrahim *et al*. [9] that there is no association between obesity and the risk of revision surgery or other complications. Especially we could not found any increased risk for hematoma, dislocation rate and infection rate and the rate of blood transfusion in the short term follow up.

Certainly a limitation of this study is the short period of follow-up. This limitation is of particular importance to the rate of revision surgery. A major advantage of our study is a consecutive patient series who were operated by the same orthopaedic surgeon. Patel *et al*. [17] came to the same conclusion in a patient cohort who was operated by seven different orthopaedic surgeons. Interestingly Bowditch *et al*. [4] found a higher rate of blood loss in obese patients. A lack of this study is the small number of 80 Patients operated by four surgeons. To be able to compare different studies we suggest a larger series of patients operated by the same orthopaedic surgeon and the use of the same classification system for obesity. We believe that the lack of consensus regarding the impact of obesity on THR may be explained in part by the different definitions of obesity which have been used [3]. The aim of our study was to investigate the influence of BMI on early complications in THR. The length of operation between obese and non-obese patients varied significantly in our study. Nevertheless we can conclude that there are no economic or medical reasons for excluding obese patients from THR, especially as there is no increased risk for complications and length of hospital stay. We fully agree with Andrew *et al*. [3] who postulated that patients should still be encouraged to reduce their weight prior to surgery but it seems unacceptable for patients to be denied treatment in the form of hip replacement solely on the basis of their BMI.

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Modified rives-stoppa repair for abdominal incisional hernias

Peter Nau, Clancy J. Clark, Mason Fisher, Gregory Walker, Bradley J. Needleman, E. Christopher Ellison, Peter Muscarella

Department of Surgery, The Ohio State University, Columbus, America; Pete.Muscarella@osumc.edu

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ABSTRACT

Incisional hernias are a prevalent problem in abdominal surgery and occur in 11% of patients who undergo laparotomy. Primary suture closure of incisional hernias results in a 31%-58% chance of recurrence. The addition of a prosthetic mesh implant decreases recurrence rates to 8%-10%. Popularized in Europe by Rives and Stoppa, the sublay technique has proven to be very effective, with low recurrence rates (0%-23%) and minimal complications. The purpose of the study was to evaluate the experience of a single surgeon at a large tertiary care center performing a modified Rives-Stoppa repair for abdominal incisional hernias. To do this, the records of all patients undergoing a modified Rives-Stoppa incisional hernia repair between January 2000 and August 2003 were retrospectively reviewed. Outpatient clinic notes, discharge summaries, operative reports, and laboratory data were reviewed for patient demographics, surgical data and postoperative complications. Univariate analysis was performed in order to identify predictors for recurrence. During the study period, 83 patients underwent a modified Rives-Stoppa incisional hernia repair. Nineteen patients were excluded due to incomplete medical records. No patients required postoperative exploration for an intra-abdominal catastrophe. Twenty-five percent (n=16) of patients had a complication as a result of the hernia repair. Only two patients (3.1%) developed recurrent incisional hernias. History of diabetes (p=0.007) and benign prostatic hyperplasia (p=0.000) were the only significant predictors for recurrence. The results presented here confirm that the modified Rives-Stoppa retromuscular repair is an effective method for the repair of incisional hernias. The complication and recurrence rates compare favorably to results for currently popular alternative techniques.

Keywords: Incisional Hernia Repair; Mesh; Rives-Stoppa Repair; Abdominal Wall Defects

1. INTRODUCTION

Incisional hernias are a common problem in abdominal surgery and occur in up to 11% of patients who undergo laparotomy [1]. Complications of incisional hernias include infection, ulceration, incarceration of viscera, and small bowel obstruction [2-4]. Patients also experience discomfort and a cosmetically unpleasing bulge at the incision site. Successful repair of incisional hernias continues to be challenging. Primary suture closure of incisional hernias results in recurrence rates of 31%-58% [5-10]. The addition of prosthetic mesh implants has been shown to decrease the incidence of recurrence to 8%-10% [11-14].

A tension-free prosthetic mesh repair of incisional hernias dates back the to 1940's and 1950's with the introduction of metal wire mesh and polypropylene mesh respectively [15-17]. With the development of new prosthetic materials, advances in minimally invasive techniques, and improvements in open surgical procedures, surgeons continue to debate the appropriate operative technique for the repair of incisional hernias, particularly regarding the anatomic placement and type of prosthetic mesh. Various operative techniques for incisional hernia repair use onlay, sublay (retromuscular or extrafascial), or underlay (intraperitoneal or subfascial) placement of mesh. Popularized in Europe by Rives and Stoppa, the sublay technique has proven to be very effective, with low recurrence rates (0%-23%) and minimal complications [18-25]. Disadvantages include complexity, long operative times, and the possibility of chronic abdominal pain [26].

The experience of one surgeon at The Ohio State University Medical Center suggests that a modified

Rives-Stoppa incisional hernia repair compares favorably to the standard Rives-Stoppa repair as well as to other techniques for addressing incisional hernias. The purpose of this study was to fully characterize the complications and recurrence rates of this surgical technique by conducting a retrospective review of patients who had undergone a modified Rives-Stoppa incisional hernia repair.

2. MATERIALS AND METHODS

Between January 2000 and August 2003, 83 patients in the practice of one surgeon at a large urban academic hospital (The Ohio State University Medical Center) underwent a modified Rives-Stoppa incisional hernia repair. Of the 83 patients initially identified, 19 patients were excluded due to incomplete medical records. Outpatient clinic notes, discharge summaries, operative reports, and laboratory data of 64 patients were reviewed. There were 20 males and 44 females (mean age 50 years, range 27-85). The majority of incisional hernias were midline and supraumbilical, with several (n=3) flank hernias. Forty-five percent (n=29) of the incisional hernias were recurrent (mean 2, range 1-7, S.D.± 1.6). At the time of repair, most incisional hernias were symptomatic and evident on physical exam. Six percent (n=4) were incarcerated at the time of presentation and one patient had an infected abdominal wound **(Table 1).**

The operative technique is a variation of the previously described Rives-Stoppa technique [18,22,27]. All patients received intravenous prophylactic antibiotics (Cefazolin, 1 g) prior to incision. Patients were placed supine for midline hernias, or in lateral decubitus for flank hernias. The old incision scar and hernia sac were removed en bloc using an elliptical incision. Dissection of the hernia sac in all patients required entry into the peritoneum and division of adhesions to the sac, if identified. The fascial plane between the rectus muscle and posterior rectus sheath was dissected as far lateral as possible, typically 5-10 cm, between eight and ten polybutester (Novofil, United States Surgical/Syneture, Norwalk, CT) "U-stitch" anchoring sutures were placed only in the posterior rectus sheath, not penetrating the rectus muscle, anterior rectus sheath or skin. The fascial defect was closed by reapproximating the posterior rectus sheath with running polybutester suture reinforced with simple interrupted polybutester suture **(Figure 1).** Tension was minimal and the posterior rectus fascia was closed in all 64 patients. Below the arcuate line, the peritoneum was carefully reapproximated. Monofilament polypropylene mesh (Bard Mesh Flat Sheets, Davol Inc., Cranston, RI) was cut to size slightly larger (5 cm overlap) than the fascial defect and anchored using the previously placed sutures **(Figure 2).** Cefazolin (1 g) powder was placed on the mesh prior to closure of the anterior rectus sheath **(Figure 3).** In the majority of the patients (n=46), one to two bulb suction drains were placed between the skin and the anterior rectus sheath. The skin was approximated with interrupted, dermal 2-0 polyglycolic acid sutures (Dexon II, Syneture, Norwalk, CT) and staples. Oral prohylactic antibiotics (Cefalexin, 500 mg PO TID) were started postoperatively and continued until drain removal. Hospital discharge typically occurred on post-operative day 3 (mean 5, range 2-29, S.D.± 3.9).

Table 1. Hernia characteristics.

	N (%)
Recurrent	29 (45.3)
Location	
Supraumbilical	42 (65.6)
Umbilical	9 (14.1)
Infraumbilical	8 (12.5)
Paramedian	1 (1.6)
Subcostal	1 (1.6)
Flank	3 (4.7)
Symptomatic	59 (92.2)
Evident on Physical Exam	61 (95.3)
Incarcerated	4 (6.4)
Infected	1 (1.6)
Ulcerated	0 (0)

Follow-up information was available for 61 patients, with mean follow-up of 20 weeks (range 1-144 weeks, S.D.± 30.1) and mean number of follow-up visits of three (range 1-13, S.D. ± 2.9). All patients were examined during follow-up by the surgeon who completed the operation. Three patients had no follow-up information available and their records indicated that another physician is currently following them. Physician correspondence information was reviewed to determine any complications or recurrences in these three patients. One patient died of causes unrelated to the repair of the incisional hernia, and there is no evidence that any of these patients developed a recurrent incisional hernia. This study was approved by the medical center institutional review board. Statistical analysis was conducted using SPSS for Windows (Version 11.5.0, SPSS Inc., Chicago, IL).

3. RESULTS

All 64 patients tolerated the procedure well with no intraoperative complications. There were two (3.1%) perioperative complications. No myocardial infarctions or cases of pneumonia were recorded. One patient suffered a pulmonary embolism and was treated with anticoagulant therapy. The second patient was readmitted for drainage of a rectus sheath hematoma found anterior to the prosthetic mesh. Three (4.7%) patients required perioperative packed red blood cell transfusions. Blood loss was negligible, with a mean post-operative decrease in hemoglobin of 0.8 g/dl (S.D. ±1.3).

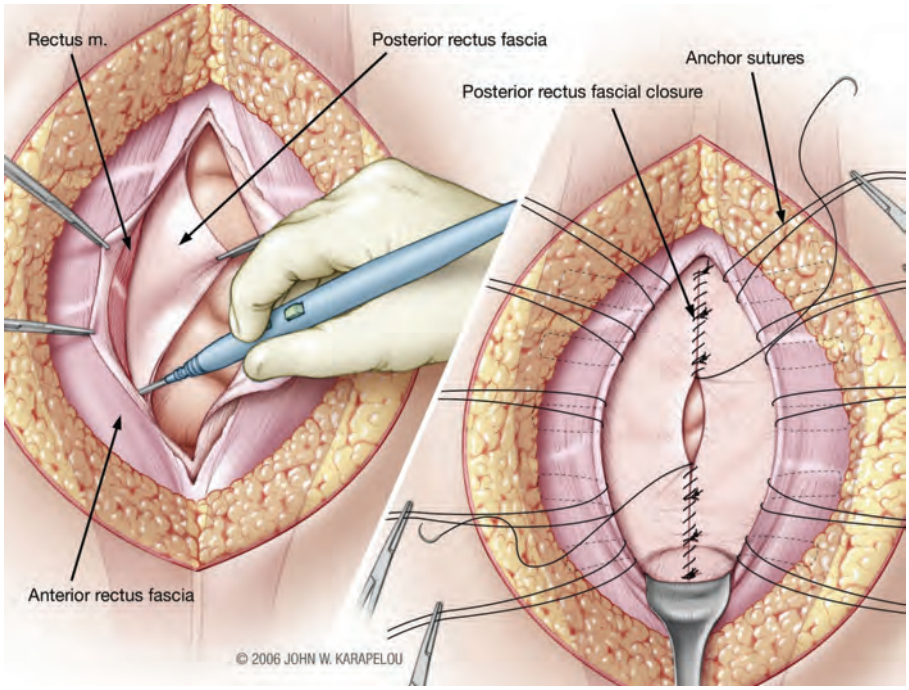

Figure 1. The posterior rectus sheath is dissected away from the overlying rectus abdominus muscle on both sides of the defect using electrocautery. The peritoneum is mobilized below the level of the arcuate line. Care must be taken to ligate and divide perforating blood vessels, as unidentified injury to these structures could result in the formation of rectus hematomas postoperatively. The posterior layer is approximated with running suture and reinforced intermittently with additional suture in order to avoid excess tension and tearing during manipulation.

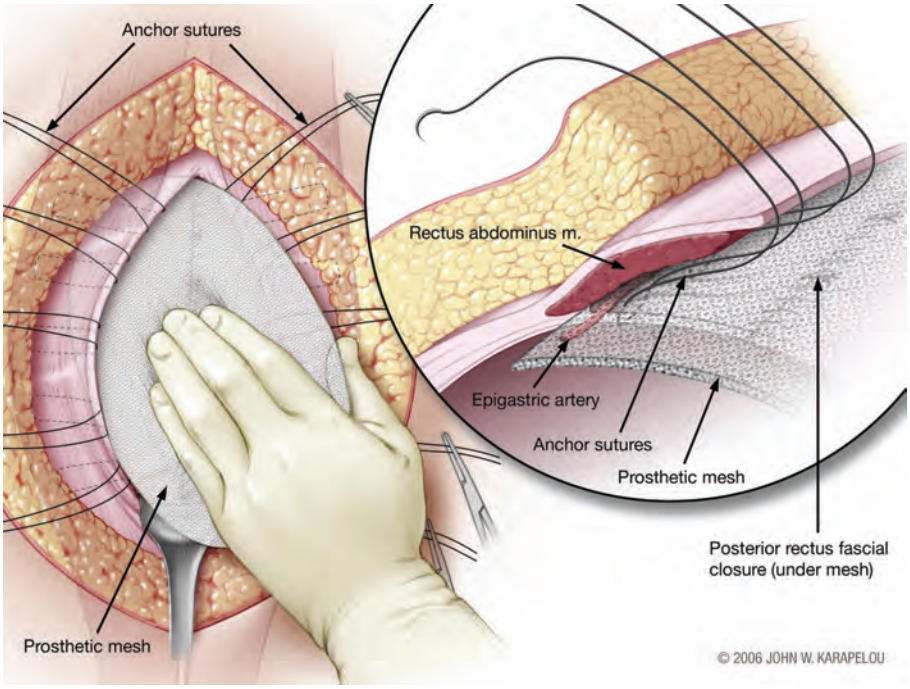

Figure 2. Polypropylene mesh is trimmed and placed into the space behind the rectus muscle. Previously placed anchoring sutures are passed through the mesh and used for fixation.

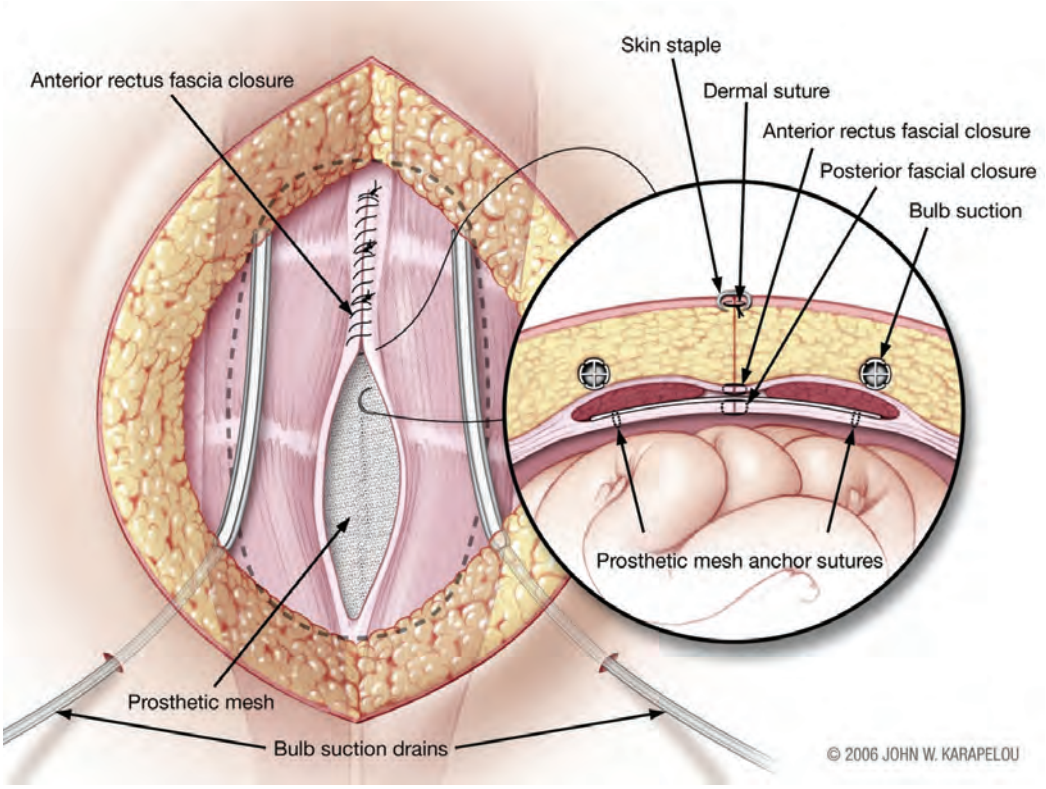

Figure 3. Bulb suction drains are placed into the subcutaneous space and brought out of the skin through separate stab incisions. The skin is approximated with dermal sutures and staples in order to maintain an adequate seal for drain.

There were complications in twenty-five percent (n=16) of patients as a result of the hernia repair. The majority of the complications were minor with only seven patients requiring admission to the hospital for management (15.6%). Six patients (9.4%) had superficial wound or deep mesh infections, defined as purulent drainage or positive wound cultures. None required removal of the prosthetic mesh and all were successfully managed with antibiotics and wound management. One patient developed erythema adjacent to the skin staples, four (6.3%) developed seromas, and three (4.7%) developed wound hematomas. In no case was evidence of infection, including erythema or purulent drainage noted in those patients with seromas. Wound cultures were not performed routinely on suspected seromas in the absence of clinical signs of infection. A hematoma was defined as a fluid collection with bloody drainage. Although no patients developed fistulas, four patients (6.3%) presented post-operatively with partial small bowel obstructions, all of who were successfully treated with nasogastric decompression. Fifteen patients (23.4%) are being managed for chronic abdominal pain **(Table 2).** In the subgroup of morbidly obese patients (n=27), there were two partial small bowel obstructions (7.4%), one seroma (3.7%), one hematoma (3.7%), and two surgical site infections (7.4%).Two patients (3.1%) developed recurrent incisional hernias. The first patient developed a small (1-2 cm) supraumbilical recurrent hernia 10 months (post-operative day 285) following the hernia repair. The patient subsequently developed a second small periumbilical hernia. The second patient was originally treated for a recurrent midline incisional hernia and a primary flank hernia. Recurrence of the flank hernia developed six weeks post-operatively (post-operative day 42). The flank hernia has recurred twice and it is believed that inadequate repair can be attributed to insufficient overlap of mesh and fascia or incomplete coverage of the fascial defect. Both patients had medical histories significant for diabetes and a respiratory disorder. The study population was a typical patient population for recurrent complex

Table 2. Complications.

	N (%)
Infection	6 (9.4)
Seroma	4 (6.3)
Hematoma	3 (4.7)
pSBO*	4 (6.3)
Staple Reaction	1 (1.6)
Fistula	0 (0)
Chronic Pain	15 (23.4)
Recurrence	2 (3.1)

*partial small bowel obstruction.

Table 3. Potential prognostic factors for hernia recurrence.

	N (%)	p
Smoking	18 (28.1)	
COPD*	8 (12.5.)	0.103
Asthma	7 (10.9)	0.072
Diabetes (Type I or II)	14 (21.9)	0.007
Sleep Apnea	12 (18.8)	0.490
Morbid Obesity	27 (42.2)	0.220
Chronic Steroid Use	3 (4.7)	0.750
Benign Prostatic Hyperplasia	2 (3.1)	0.000
Cirrhosis	3 (4.7)	0.750
Malabsorptive Surgery†	30 (46.9)	0.177

*COPD, chronic obstructive pulmonary disease. †Malabsorptive surgery defined as roux-en-Y gastric bypass (25), cystojejunostomy (1), choledochojejunostomy (3), or vertical banded gastroplasty (1).

incisional hernias, with numerous potential risk factors for recurrence **(Table 3).** Forty-two percent of patients (n=27) were considered to be morbidly obese (BMI > 40 kg/m2) and 46.9% of patients (n=30) had previously undergone a surgical procedure that could potentially result in malabsorption (Rouxen-Y gastric bypass, choledocho-jejunostomy, etc.). In the subgroup of morbidly obese patients, no recurrences were observed. With univariate analysis, a history of diabetes (p=0.007) or benign prostatic hyperplasia (p=0.000) were the only significant prognostic factors for recurrence.

4. DISCUSSION

Surgical techniques for the repair of incisional hernias continue to evolve with advances in prosthetic materials and minimally invasive technology. Luijendijk *et al.* conducted a randomized, multicenter study suggesting that mesh repair is superior to suture repair [28]. The optimal technique for mesh placement has not been established and remains controversial. Laparoscopic repair appears to have numerous benefits, including low recurrence rates, decreased hospital length of stay, decreased postoperative pain, and earlier return to work and normal activities. Although a single randomized trial has been designed [29], the results are still pending and no randomized controlled trials are available to prove any benefit of laparoscopic techniques over open repair. A meta-analysis by Goodney *et al.* indicated that laparoscopic ventral hernia repair may have lower complication rates [30]. Initial concern for increased fistula formation and small bowel obstruction with the laparoscopic underlay techniques have also been raised, but appear to be less of concern with recent studies and changes in mesh technology [11]. It has been our experience that catastrophic abdominal complications, such as small bowel perforation, can occur with the laparoscopic repair. Although this does not appear to occur frequently, these types of complications can be disastrous in that they may require multiple surgeries, mesh removal, long-term wound care, and skin grafting procedures. The end result is usually an incisional hernia that is larger than the initial hernia. There are currently no reliable methods for selecting appropriate patients for laparoscopic incisional hernia repair. The data from our study suggests that the technique described here is rarely, if ever, associated with these types of complications.

The separation of components technique, originally described by Ramirez *et al*. in 1990 has recently drawn interest for complex incisional hernia repair. After mobilization of skin flaps, the external oblique muscle is released just lateral to its rectus sheath insertion from the underlying internal oblique muscle. This enables the well vascularized rectus abdominis muscle complex to be advanced medially, allowing for coverage of the hernia defect [31]. Up to a 20 cm defect at the waist, 12 cm in the upper abdomen, and 10 cm in the lower abdomen can be closed using additional relaxing incisions. Advantages include utilization of a dynamic muscle group that remains innervated for closure of the defect, and potentially improved cosmetic results as excess skin is commonly excised prior to closure of the wound. Shestak et al. reported only one recurrence, two superficial wound infections, and one seroma in 22 patients undergoing this procedure [32]. In contrast, another study demonstrated recurrent hernias in 32.0% of patients and complications, including fascial dehiscence, hematoma, seroma, wound infection, skin necrosis, and respiratory insufficiency, in 39.5% of patients [33]. The modified Rives-Stoppa technique allows for cosmetically pleasing results, in that redundant skin is removed en bloc with the underlying hernia sac. Skin necrosis is rare because large skins flaps are not created and careful attention is paid to preserving the perforating blood vessels that supply the remaining skin and subcutaneous tissues. The Rives-Stoppa technique preserves the functionality and integrity of the abdominal wall, factors considered to be crucial for effective repair of abdominal wall defects by proponents of the separation of components technique. Furthermore, the Rives-Stoppa technique employs the use of mesh as reinforcement, and this is generally believed to decrease recurrence rates as described previously.

The data reported here indicate that a modified Rives-Stoppa retromuscular repair results in favorable recurrence and complication rates when compared with the standard Rives-Stoppa repair as well as other techniques. The experience of a single surgeon's practice at The Ohio State University is comparable to previously reported results **(Table 4).** Benefits of this technique include the ability to explore the entire fascial defect and to identify any potentially weak points in the fascia. Fenestrations in the peritoneum can be recognized, and if necessary, selection of a larger mesh implant to cover

Table 4. Studies of the rives-stoppa open mesh repair

	Seroma, N(%)	Infection, N(%)	Recurrence, N(%)
Knight *et al*. 2002 [18]	1 (1.5)	2 (3.1)	0 (0)
Bauer *et al*. 2002 [19]	7 (12.3)	2 (3.5)	0 (0)
Toniato *et al*. 2002 [22]	--	6 (7.8)	2 (2.6)
Luijendijk *et al*. 2000 [21]	4 (4.8)	3 (4)	17 (23)
Balen *et al*. 1998 [26]	2 (4.4)	1 (2)	1 (2)
McLanahan *et al*. 1997 [24]	1 (1)	13 (13)	3 (3.5)
Sugerman *et al*. 1996 [23]	5 (5)	17 (17)	4 (4)
Temudom *et al*. 1996 [28]	3 (6)	6 (12)	2 (4)
Adloff *et al*. 1987 [30]	--	3 (2.3)	6 (4)

these areas of potential weakness can be made. Placement of the mesh between the posterior rectus sheath and the rectus muscle takes advantage of the intra-abdominal pressure to secure the mesh, while minimizing the risk of adhesion and fistula formation. Unique to this series of patients was the surgeon's ability to close the posterior rectus sheath with suture. Extensive dissection of the plane between the posterior rectus sheath and the rectus muscle likely decreases tension when closing this layer. Inability to close the posterior rectus sheath requires bridging the fascial defect and would likely increase the risk of recurrence.

It should be noted that the majority of the complications in this series were minor and easily managed on an outpatient basis. Despite pre- and post-operative prophylactic antibiotics, bulb suction drainage, and direct placement of cefazolin powder on the prosthetic mesh, the infection rate still approached 10%. This may be attributed to patient factors, natural reaction to a foreign material, and/or long-term placement of bulb suction drains. Colonization of drain sites increases with time and they are a potential portal of entry for bacterial infection [34]. Criteria for drain removal generally required that the drain output be less than 30 ml per day in an attempt to minimize the incidence of seroma formation. As a result, drains remained in place for a mean duration of three weeks (range 7-85 days, S.D. ±15.7). Removing drains earlier might help to decrease infection rates. It is also unclear whether continuing oral antibiotics while the drains are in imparts any benefit for the patient, but was performed in order to decrease the chance of infection. The direct application of antibiotics on the mesh at the time of surgery is clearly controversial, but has been described previously [35,36]. The rationale is to improve the local concentration of antibiotics and this may have potential benefits, particularly for obese patients in whom systemic antibiotic therapy may not be as effective. Although post-operative complications were not significant risk factors for hernia recurrence (data not shown), other studies have recognized an increased risk of recurrence following infection [28,37]. The concern is generally that infected mesh must be removed to successfully treat the infection and removal of the mesh results in hernia recurrence. In a study of patients undergoing concurrent incisional hernia repair and elective colon resections, the authors concluded that prosthetic mesh may be employed for incisional hernia repairs in contaminated fields without increasing the risk of complications [38]. In a study comparing different mesh materials, Leber *et al*. concluded that multifilament polyester mesh (Mersilene) has a significantly increased risk of infection as compared to double-filament polypropylene mesh (Prolene) [39]. Infection rates vary among studies, but this is understandable given variations in antibiotic usage, drain management, and types of mesh employed. Considering the current body of evidence, the appropriate prevention and management of wound infection in patients undergoing mesh incisional hernia repair remains to be determined.

Chronic pain was a concern of almost a quarter of study patients, but comparison of pre and post-operative pain was not available in this study. The concern of chronic pain has also been raised in previous studies [12, 20]. McLanahan *et al*. reported that 11% of patients had moderate to severe pain at 12 months after incisional hernia repair [20]. In a 1997 symposium on incisional hernia repair, Schumpelick argued that mesh can limit range of motion and result in a stiff abdomen [21]. Decreased abdominal wall compliance has been confirmed with three dimensional stereography [40]. Given the potentially negative long-term effects of prosthetic mesh repair, data characterizing the quality-of-life, chronic pain, and physical limitations of mesh implants should prove to be helpful.

Although diabetes and benign prostatic hyperplasia were the only identified risk factors for recurrence in this study, previously identified risk factors for recurrence were common in the study population. Considering the size and non-randomized nature of this study, it is possible that we did not have sufficient statistical power to display the significance of these known risk factors. Sugerman *et al*. have shown that severe obesity is a greater risk factor for hernia recurrence than chronic steroid use [41]. In the subgroup of morbidly obese patients we expected to see increased rates of recurrence and infection, however, this was not the case. The majority of the obese patients who underwent hernia repair were actively losing weight from recent gastric bypass surgery. It is possible that weight loss, the presence of redundant skin, and general changes in body habitus facilitated the repair and decreased the risk of recurrence.

The modified Rives-Stoppa retromuscular repair, as described here, appears to be an effective treatment for incisional hernias. These results compare favorably with other published reports for the Rives-Stoppa repair and other techniques. The recurrence rate of 3.2% is clearly

acceptable in this series that includes numerous patients with multiple risk factors, and a considerable number of patients who have failed previous attempts at repair. We also believe that the absence of catastrophic abdominal events following repair is important to note. Wound infection, chronic pain, and persistent abdominal stiffness continue to be problematic, but manageable. Randomized prospective trials are required to determine the optimum technique for incisional hernia repair.

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