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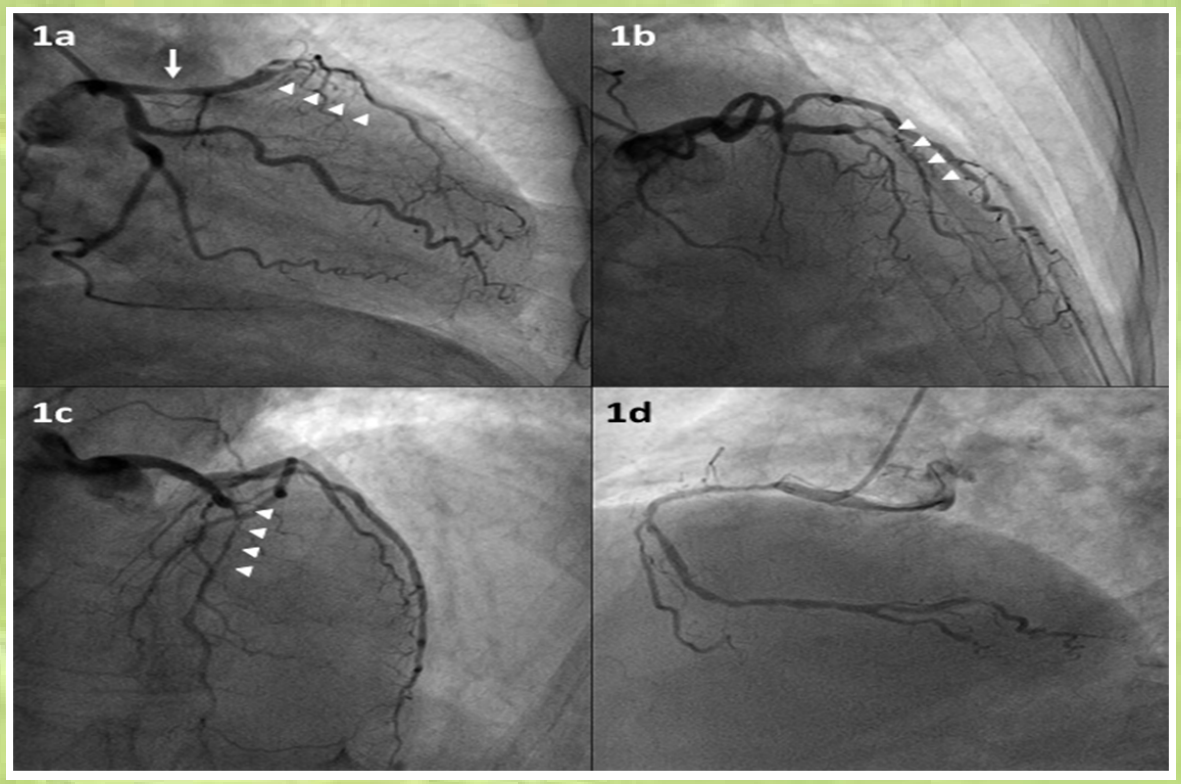

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Association between Aphasia and Acalculia: Analytical Cross-Sectional Study

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Abstract

Acalculia in aphasic patients should be better investigated in order to understand if it is a simple comorbid or if it is influenced by language disorders. This study aimed to compare the performance on EC301 battery calculation tasks between aphasic and normal subjects and sought to verify a possible association between number processing and calculation skills and linguistic changes in aphasic patients, in order to investigate if language disorders interfere with number processing and calculation. Analytical cross-sectional study with a control group, performed of the Department of Speech and Hearing Disorders of a public university, conducted in the city of São Paulo, Brazil. First, to analyze the specific difficulties encountered in numerical processing and calculation tasks among the aphasic group, aphasic and healthy adult's performance in specific calculation tasks were compared. The calculation tasks, which had been badly performed by aphasic patients, were selected. Aphasic patients were also submitted to the language tasks from Montreal-Toulouse Protocol: oral and written comprehension, repetition, reading aloud, naming and dictation. We observed that aphasic individuals showed changes in numerical processing and calculation tasks that were not observed in the healthy population. The most important finding of this study was that aphasic individuals showed changes in numerical processing and calculation that were positively associated to their linguistic performance. The strong associations between battery EC301 and linguistic tasks suggest that language disorders interfere with number processing and calculation.

Keywords

Dyscalculia, Aphasia, Stroke, Disabled Persons, Language

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1. Introduction

Neurological and neuropsychological clinical reports frequently mention acalculia, but specific analyses of acalculia are rather limited. Calculation ability represents an extremely complex cognitive process, which requires multifactorial processes, including verbal, spatial, memory, and executive functions [1] [2]. According to Ardila, Rosselli (2002) aphasia is an impairment of language, affecting the production or comprehension of speech and the ability to read or write. Aphasia is always due to injury on the brain-most commonly from a stroke, particularly in older individuals. On the other hand, acalculia as a complete inability or part of dealing with numbers, and these authors, represents a acalculia commitment in numerical processing and calculation that may result from a brain injury such as strokes, cerebral traumas, dementias and degenerative diseases.

Acalculia following brain injury is not uncommon. Therefore, acalculia in aphasic patients should be better investigated in order to understand if it is a simple comorbid or if it is influenced by language disorders. Studies have shown [3]-[5] that aphasic patients are more likely to present mathematical difficulties, particularly in tests involving numerical transcoding, like reading aloud arabic numbers and writing arabic numbers from an oral dictation. Other studies correlating language and calculation have been performed [3] [6] [7] to investigate whether numerical processing and calculation and language processes are dependent or independent activities. Some studies suggest that numerical and language processings are partially overlapping skills; for some numerical tasks, calculating and linguistic abilities are closely related, and for others, there is no correlation, with some calculation tasks being completed by patients with severe language impairment. These studies were performed involving brain-injured individuals with different types of aphasia, educational levels and professions [3] [5] [6] [8]-[10] and accounted for the variability of each individual and neurological impairment. The pattern of errors for a large sample of aphasic patients was analyzed [9] and the most severe impairment in calculating ability was found in global aphasics [11]. Patients with Broca's and Wernicke's aphasia performed similarly in quantitative terms, while patients with amnestic aphasia exhibited fewer difficulties in making calculations. The authors concluded that although the findings suggested associations between impairments in language processing and numerical tasks, one should be cautious in drawing conclusions regarding the verbal basis of general numerical skills [12].

Many observed numerical processing problems may also arise from other, nonlinguistic impairments frequently found in left hemispheric patients, including attentional deficits, short-term memory problems, or difficulties in monitoring complex sequences. Double dissociations between calculation and language abilities were also observed. The first aspect of dissociation, intact language functions and impaired calculation, has been described [13]. A case study clearly demonstrated the second aspect of dissociation, good calculation abilities despite severe language problems, where the patient correctly performed simple addition, subtraction, and multiplication and performed multidigit operations without problems [14]. Seemingly, this patient neither relied on verbal forms in any operations nor compensated for impaired verbal skills with nonverbal ones. Although this evidence supports the functional independence of language and some numerical skills, the authors nevertheless assumed that linguistic functions mediate other numerical abilities (e.g., counting, writing numerals) or preferentially support them (e.g., multiplication tables). They thus assumed that the systematic association of linguistic and numerical deficits may be informative and contribute to our understanding of the numerical difficulties observed in different clinical populations.

Despite a large number of case reports, it is important to conduct studies with groups of patients to verify whether associations and/or dissociations between language and calculation truly exist while also considering cultural and educational variability [15] [16].

The battery EC301, which was used in this study for evaluation calculation, was developed in 1994 by Delouche *et al*. to the adult assessment with involvement of the calculation and processing numerical after brain injury. This battery includes three systems answers to numbers: arabic digit, spelling and oral.

Thus, considering all the findings described above, this study aimed to verify a possible relationship between numerical processing and calculation difficulties and language disorders in aphasic patients.

2. Materials and Methods

The study was conducted in the outpatient clinic of disturbances acquired neurological speech and ginguagem and other outpatient clinics of the Speech Therapy Department at UNIFESP, São Paulo, Brazil. The sample size was calculated according to the number of patients seen at the clinic, that met the sample inclusion criteria.

To analyze the specific difficulties encountered in numerical processing and calculation tasks among the aphasic group, we first compared aphasic and healthy adults in specific calculating tasks. Then, just were selected for this study the calculation tasks that were badly performed by aphasic patients. Then, the performance of the aphasic patients in calculation tasks was correlated to their performance in language tasks.

Control Group: Forty-four volunteers were selected (74% female). The average education duration was 8.5 ± 4.1 years, and the average age was 40.6 ± 16.0 years. Aphasic Group: Thirty-two patients (37% female) who had suffered a single left hemisphere stroke were evaluated. The mean age of the patients was 51.4 ± 13.7 years, and the mean education duration was 8.0 ± 5.2 years.

The general inclusion criteria were as follows: no history of alcoholism or drug use; no use of psychotropic medications, except for atypical neuroleptics; and the absence of visual or auditory impairments that might affect the outcome of the tests. The control group consisted of individuals who were accompanying patients, family members or friends.

This study included patients who had suffered a single left hemisphere stroke, while illiterate patients were excluded. Patients that presented motor difficulties that prevented them from performing the tasks were excluded from this study. All patients selected for the study were right-handed. All subjects were evaluated by a neurologist and underwent magnetic resonance imaging of the brain, with the following results: of the patients, 10 (32%) had a lesion in the left parietal region, 9 (28%) in the frontal-temporal-parietal, 3 (9.4%) in the left temporal, 6 (18.5%) in the left frontal-temporal, 3 (9.4%) in the left temporal-parietal-occipital, and 1 (3.15%) in the left parietal-occipital. Among the patients with aphasia, 16 (50%) had anomic aphasia, 3 (9.4%) had conduction aphasia, 4 (12.5%) had Broca's aphasia, 1 (3.15%) had transcortical sensory aphasia, 5 (16%) had mist aphasia, and 3 (9.4%) had global aphasia.

The data analyzed in the current study were collected in accordance with the Research Ethics Committee of UNIFESP, protocol No. 0346/04. All patients signed informed consent forms prior to participation.

All patients underwent an evaluation of their calculation skills using the EC301 calculation battery [17]. This battery is referred to be the most used to assess calculation abilities in adults [18] and contains 13 different tasks. Each task is domain specific and is made up of more than one subtask. Thirty-one is the total of subtasks. It was not necessary to adapt the number of stimuli of EC301 battery.

1) Counting (3 subtasks, C1, C2, C3). The subject must use different codes (phonological, Arabic, and orthographic) to produce a somewhat automatic sequence of numbers, backwards and forwards, according to different ratios (by ones, by threes, by tens).

2) Dot Counting (5 subtasks, C4, C5, C6, C7, C8). This task evaluates the capacity to compute the cardinality of a set of discrete elements (dots) with different spatial arrangements. The subject is required to point to the dots while counting aloud.

3) Transcoding (7 subtasks, C9, C10, C11, C12, C13, C14, C15). These subtasks correspond to the six possible transcodings between phonological, Arabic, and orthographic codes and number repetition. Items were selected to make their lexical-syntactical structure directly comparable from one subtask to another.

4) Arithmetic Signs (2 subtasks, C16, C17). The subject is required to name the arithmetic signs and write them as dictated.

5) Number Comparison (2 subtasks, C18, C19). The subject is required to point to the greater of two numbers, presented in Arabic (8 items) or orthographic forms (8 items).

6) Mental Computation (2 subtasks, C20, C21). This task evaluates mental calculation, requiring the subject both to access number fact knowledge and perform simple operations.

7) Estimating the Result of an Operation (1 subtask, C22). Subjects must point to the best approximation (among 4 alternatives) of the correct result of a complex operation.

8) Number Positioning on an Analog Scale (2 subtasks, C23, C24). A vertical line graded from 0 to 100 is shown to the subject, who must place a spoken or written Arabic number on the line, choosing among three possible positions.

9) Writing Down an Operation (1 subtask, C25). The subject is requested to copy a pair of two- or three-digit numbers, placing them in the conventional way for the written operation, corresponding to a given arithmetic sign.

10) Written Calculation (3 subtasks, C26, C27, C28). This task tests the subject's ability to perform addition, subtraction, and multiplication, which involves accessing numerical facts and calculation procedures such as carrying and borrowing.

11) Perceptive Estimation of Quantity (1 subtask, C29). Subjects must estimate the weight, length, or number of objects shown in a picture.

12) Contextual Magnitude Judgments (1 subtask, C30). Given a specific and contextual situation, the subject is asked to give an interpretation of numerical size (*i.e.*, a classroom with nine children, is the number of children too low, average, or too high?).

13) Numerical Knowledge (1 subtask, C31). This task contains questions related to numerical knowledge of specific facts, such as the number of days in a week.

Each item in EC301 was assigned a value of 2 points for a correct response and 0 points for an incorrect response. However, in a few tasks, 1 point could be awarded (*i.e.*, if the patient gives the correct response after requesting item repetition). All subjects completed the battery tests.

In addition to the EC301 battery [4], subjects with aphasia were submitted to a language test to assess their degree of language impairment. The language evaluation used the Montreal Toulouse Protocol-Brazilian version [19] and in this study, we analyzed the performance of aphasic patients on six tests: oral and written comprehension, in which subjects would point at representations of words and simple and complex phrases, verbal and written ordering, repeating and reading words and short and long phrases and picture naming.

Statistical Analysis

In **Table 2**, we calculated the Spearman coefficients to verify the relationship between performance on language tasks in aphasic patients and performance on tests of EC301 battery, and $p < 0.005$ was considered to indicate statistical significance, according to Bonferroni correction. $n = 32$.

All analyses were calculated using the statistical package SPSS (Statistical Package for Social Sciences).

3. Results

There was no statistically significant difference between the control and aphasic groups when comparing their years of schooling (8.5 ± 4.1 versus 8.0 ± 5.2 years, 95% CI = −1.7 to 2.6, t (74) = 0.439, p = 0.662). However, the control group was significantly younger than the aphasic group (40.6 ± 16.0 versus 51.4 ± 13.7 years, 95% CI = −17.7 to −3.7, t (74) = −3.06, p = 0.003) and had a higher proportion of women (74% versus 26%, X^2 = 8.90, P = 0.003).

The performance of patients with aphasia was significantly worse than that of the control group on subtests C1, C2, C3, C9, C10, C11, C12, C13, C14, C15, C16, C17, C19, C20, C21, C22, C26, C27, C28, C29, and C30 from the EC301 battery test, data previously reported (De Luccia, Ortiz, 2014).

Table 2 correlates the findings of the EC301 battery with the language results of the aphasic subjects.

The performance of patients with aphasia was significantly worse than that of the control group on subtests C1, C2, C3, C9, C10, C11, C12,C13, C14, C15, C16, C17, C19, C20, C21, C22, C26, C27, C28, C29, and C30 from the EC301 battery test, data previously reported (De Luccia, Ortiz, 2014).

4. Discussion

We observed that aphasic individuals showed changes in numerical processing and calculation tasks that were not observed in the healthy population. Although the groups were different according to age and gender, we do not believe that this difference interfered with the results, since there were no elderly subjects in our sample and there is no evidence of cognitive changes in adults [20]. As far as gender is concerned, there is a report of a little advantage of males [4] but in most studies, including one that was done with the EC 301 battery, no statistically significant difference between the performance of males and females was found [21] [22], **Table 1**.

The most important finding of this study is the result that aphasic individuals showed changes in numerical processing and calculation tasks that were positively correlated with linguistic task performance.

Concomitant deficits in language and calculation processing proved evident, as discussed below.

The latest research attempting to correlate language processing with numerical processing and calculation has not yet been able to clearly demonstrate an association between these two cognition domains. Questions persist regarding how language skills relate to numerical processing and calculation.

We found strong associations between oral and written comprehension tests and all calculation tests, as shown in **Table 2**. **Table 2** shows the correlations between language and calculation tests. In general, the results of the

Table 1. Demographic characteristics between the control group and the aphasic group.

	Control	Aphasic
Subjects	44	32
Age	40.6 ± 16.0	51.4 ± 13.7
Schooling	8.5 ± 4.1	8.0 ± 5.2
	Female	Male
Gender	56	20

Age and schooling-values expressed as mean/years and DS.

Table 2. Correlation between performance on the EC301 battery and language tests for aphasic patients.

	Test 1	Test 3	Test 4	Test 5	Test 6	Test 7	Test 10	Test 11	Test 12
Oral comprehension									
r	0.73	0.68	0.67	0.52	0.64	0.46	0.55	0.57	0.73
p	<0.001*	<0.001*	<0.001*	0.003*	0.000*	0.008*	0.001*	0.001*	<0.001*
Repetition									
r	0.81	0.87	0.84	0.76	0.81	0.56	0.68	0.57	0.72
p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	0.001*	<0.001*	0.001*	<0.001*
Reading									
r	0.59	0.72	0.57	0.44	0.68	0.48	0.53	0.54	0.35
p	<0.001*	<0.001*	0.001*	0.012	<0.001*	0.006*	0.002*	0.001*	0.051
Graphic comprehension									
r	0.81	0.86	0.86	0.73	0.87	0.75	0.78	0.68	0.63
p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
Naming									
r	0.74	0.79	0.72	0.47	0.76	0.50	0.55	0.62	0.59
p	<0.001*	<0.001*	<0.001*	0.006	<0.001*	0003 *	0.001*	<0.001*	<0.001*
Dictation									
r	0.86	0.91	0.78	0.63	0.83	0.51	0.75	0.63	0.56
p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	0.003*	<0.001*	<0.001*	0.001*

$p < 0.005$ indicates statistical significance after Bonferroni correction. n = 32; 1: Counting (C1, C2, C3); 3: Transcoding (C9, C10, C11, C12, C13, C14, C15); 4: Arithmetic Signs (C16, C17); 5: Number Comparison (C18, C19); 6: Mental Computation (C20, C21); 7: Estimation of the Result of an Operation (C22); 10: Written Calculation (C26, C27, C28); 11: Perceptive Estimation of Quantity (C29); 12: Contextual Magnitude Judgment (C30).

EC301 battery tests, on which aphasic patients performed significantly worse than the control subjects, were strongly associated with the language test results.

A association between oral comprehension, oral assessment, written comprehension, and written computation tests, based on tests with sentences and arithmetic sums, has been found in patients with lesions in the left cerebral hemisphere [23]. From these findings, these authors hypothesized that written and oral language comprehension may be involved in written and oral calculation performance, respectively. For example, when an individual fails the oral comprehension test and has difficulty pointing at the correct figure after a verbal or written command like “show where the girl drinks water”, he also fails to perform the oral calculation “thirteen minus

eight" or the written calculation "13 − 8".

In this work, strong correlations were found. We can observe (**Table 2**) that oral comprehension was strongly correlated with the counting, transcoding, arithmetic sign, and contextual magnitude judgment tests. Written comprehension and repetition tests also correlated strongly with all calculation tests. We observed a strong correlation between reading and counting and transcoding and mental computation tests. Comparing the naming and calculation tests, we observed strong correlations with the counting, transcoding, arithmetic signs, mental computation, perceptive quantity estimation, and contextual magnitude judgment tests. For dictation, the strongest correlations were found with counting, transcoding, arithmetic signs, number comparison, mental computation, written calculation, and perceptive quantity estimation tests.

The only test that showed no correlation with the oral comprehension test was test 7-estimated operation result. However, we observed that in both the aphasic and control groups, the estimated result test (C22) was the most difficult. We can assume that the test is already mathematically complex for this study group, *i.e*., the performance may not be specifically related to not understanding the task. A association between the language tests evaluated in this study and some EC301 battery tests was observed. We can consider that the direct or indirect involvement of skills such as oral counting, alphabetic or orthographical number writing, oral and written comprehension, and repetition is necessary for tests 1—numeric sequence, 3—transcoding, 4—sign recognition, and 6—mental calculation.

In accordance with the international literature, strong correlations were observed between oral and written comprehension tests and oral and written calculation (**Table 2**), suggesting that oral and written linguistic comprehension interferes in understanding the calculation to be performed. The inability, due to linguistic changes in aphasia, to understand task instructions or to give a verbal or written response may thus lead to an interdependency between language and numerical processing and calculation.

We believe that these correlations may be present due to the reduction of language processing resources and that oral comprehension may interfere in the oral calculation test (C20), the magnitude judgment test (C29), and the estimating result and quantity test (C30), (**Table 2**). The best example of the possible changes in oral calculation tests (C20) was the difficulty encountered by aphasics in performing even simple multiplication and subtraction calculations. We believe that these difficulties arose due to gaps in lexical access, both for numerical memory when executing the calculation and for arithmetical rules themselves. Both failure in the phonological loop and the inability to recall prior knowledge may have generated difficulties relating to "multiplication tables". In contrast, the magnitude judgment and result estimation tests (C29 and C30) may suggest more complex changes from the comprehension perspective, as they evaluate a change in the context and/or semantic judgment interpretation. This would imply failure of the quantifier, the role of which is to quantify observations and experiences, translating them into numbers by counting and measuring and thus being a base for mathematics and language [24] [25].

In the aphasic group, arithmetic rule memory was also correlated with the language tests (**Table 2**). These changes can be explained by lexical-semantic failures, based on symbol names and meanings. When analyzing aphasic patient performance, we found that most patients recognized the numbers but had difficulty recognizing or naming some arithmetic signs (+, −, ×, =). Furthermore, we observed that many individuals performed calculations orally when the evaluator mentioned the sign. We believe that this failure could also be attributed to difficulty in graphically recognizing each symbol. Although able to visually recognize symbols, the aphasic cannot establish relationships between them because the graphical symbol is merely a meaningless pictorial representation for these subjects. The difficulty of asymbolic acalculia suggests that arithmetic signs are symbols of a different semiotic system in comparison to written language and numbers and can thus be considered an ideographic note, as each sign has a restricted and independent value [26]. Individuals who had difficulties naming and writing arithmetic signs (*i.e.*, addition, multiplication, division, and equal signs) were also more likely to have the possible diagnosis of asymbolic acalculia (in some cases) and lexical-semantic disruption (in others).

Although some evidence, mainly in single case reports, has upheld the notion of the functional independence of language and number processing and calculation, we believe that some tasks, such as those involving counting, transcoding, and mental and written calculations, depend on linguistic processing. Counting serially requires learned numerical concepts [3]. Numerical transcoding abilities depend on a central component that performs all transcoding and calculations [10]. For numerical comprehension or calculations, mechanisms are necessary to translate numerical inputs, whether in orthographic, arabic, or verbal form. Moreover, numerical production mechanisms have implications that require translation and abstract representations of the appropriate forms of

output for each notation system (verbal, orthographic, or arabic). To complete this process, a distinction must also be made between lexical and syntactic numerical processing, in which a series of verbal, orthographic, and arabic transformations in numeric comprehension are performed.

During the transcoding tests in this study, lexical and syntactical errors were observed in most cases, and misspellings and omissions were observed for all types of aphasia. According to the results found for tasks involving language and arithmetic skills, such as transcoding tests, a relationship was observed between language and mathematics.

The semantic models proposed by McCloskey, 1992, suggest that transcoding produces a semantic representation of numerical processing. For this to occur there must be a full abstract internal representation, which provides information for the arabic, verbal, and orthographical representations of each number, which allows for transcodification from one code to another. The numerical input would be transformed into abstract internal representations, and the output would be converted into another code. Considering the many skills involved in this process, we might expect that patients with brain injuries would encounter major difficulties in these tasks, as such relationships seem stronger in apparently similar skills, including transcoding between different representations of numbers and graphophonetic decoding. This raises questions about the status of the numerical transcoding ability bearing in mind that its mathematical components are the concept of numbers and a sense of the positional value of digits (*i.e.*, ones, tens, hundreds, and thousands). This ability also involves essentially linguistic components, including phonological and syntactic aspects [27]. From this perspective, the numerical transcoding ability is central to the discussion regarding the relationship between language and mathematics, as it seems to be a skill that combines mathematical and linguistic components. The involvement of linguistic and mathematical components suggests the importance of further investigating the specific nature of the numerical transcoding task to observe not only its connections with strictly linguistic skills, such as graphophonetic decoding.

Written calculations were more difficult for aphasic patients. Changes in understanding and lexical and written difficulties, both commonly found in aphasics, may also affect performance on these tasks [28]. In this regard, the systematic association of linguistic and numerical deficits may be informative and contribute to our understanding of numerical difficulties in different clinical populations. The acalculia pattern found in the aphasia group can be explained as follows: both calculation routes may have been damaged, leading to deficiencies in tasks that require semantic number comparison.

This study has some limitations: despite the fact that 50% of our sample comprised anomic aphasics, the remaining was heterogeneous. This did not allow us to perform an analyses considering aphasia type/severity. Also, duration of being aphasic in the aphasic group they were not raised.

Further investigation could consider the severity of language and calculation disorders in specific groups of aphasic patients.

5. Conclusions

Despite that our findings show correlations between losses language and numerical processing and calculation, it is not possible to state as a whole that the calculation is directly related to language. The individual variability in processing linguistic or numerical information and calculation must be considered. Furthermore, the hypothesis that the language and calculation systems are independent, but interdependent in its activity is the most accepted in this study.

As we consider the aphasic in the general population, as was done in this study, terms evidence that the language skills of listening and graphics, repetition, reading and writing are directly related to the difficulties found evidence of oral and graphic calculation and transcoding evidence who are involved reading assignments, writing and repetition, suggesting that linguistic processing is involved in the performance of various mathematical tasks.

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The Role of Risk Assessment at Antenatal Care Clinics in the Prediction of Pre-Eclampsia in a High Altitude Area

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Abstract

Background: Hypertensive disorders are common causes of maternal and fetal mortality and morbidity. Objective: This study aimed to examine the prognostic value of risk assessment at level of antenatal care clinics in predicting pre-eclampsia at a high altitude (3133 m above sea level). Methods: This cross-sectional study, carried out in Abha Maternity and Pediatric Hospital (AMPH), Saudi Arabia, between January and June 2013, included 176 patients (88 pre-eclamptic women and 88 with normal pregnancies). Patient data including age, parity, blood pressure, body mass index, and complete blood count components were recorded. Results: Physical examination of systolic blood pressure, diastolic blood pressure and BMI between two groups showed high statistical significance with a *P* value of <0.001. Also, parity was found to be statistically significant with a *P* value of <0.05. The mean hemoglobin among pre-eclamptic women was 12.27 ± 2.01 g/dL versus 11.92 ± 2.43 g/dL in the control group (*P* = 0.291). Mean plasma hematocrit levels in the study and the control groups were 38.49% ± 4.32% and 37.92% ± 7.04%, respectively; this was not found to be statistically significant (*P* = 0.518). Although there was an increase in laboratory blood tests of maternal hematocrit and hemoglobin levels, both parameters failed to show any statistical significance. Conclusion: Risk assessment at level of antenatal care clinics can be considered as valuable prognostic tool for prediction of preeclampsia. Any pregnant lady with abnormal physical examination findings of: BMI, systolic and diastolic blood pressure and obstetric history following risk assessment in antenatal care clinics should be observed for possibility of pre-eclampsia.

Keywords

Pre-Eclampsia, BMI, Systolic, Diastolic, Pregnancy, Screening, Altitude, Saudi Arabia

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1. Introduction

Pre-eclampsia is a pregnancy-specific disorder that refers to the onset of hypertension (blood pressure [BP] of ≥140/90 mmHg) and proteinuria (24-hour urinary protein excretion of >300 mg) after 20 gestational weeks in a previously normotensive female [1] [2]. Pre-eclampsia is a major cause of maternal and fetal mortality and morbidity [3] [4]. The incidence of this condition is 2% - 10%, depending on the population studied and the definition of pre-eclampsia [5]. A study in the Aseer region of Saudi Arabia observed that hypertensive disorders were present in 2.4% of all pregnancies, with a higher prevalence in high altitude areas than in those at sea level [6].

Numerous clinical and biochemical tests for the prediction or early detection of pre-eclampsia have been proposed [7]. However, most of these methods remain unrealistic for general use in the majority of developing countries. At present, there is no single reliable and cost-effective screening test for pre-eclampsia which can be recommended for use in developing countries [7]. As part of an antenatal care strategy, the World Health Organization (WHO) recommend screening for pre-eclampsia during the patient's third antenatal visit at 32 gestational weeks [8]. In contrast, strategies for risk assessment in developing countries should be based on the obstetric and medical history of the patient as well as a clinical examination. Pregnant women should be assessed at their first antenatal clinic for known risk factors of pre-eclampsia, such as young age, nulliparity, first pregnancy after the age of 35 years, obesity prior to the current pregnancy, multifetal gestation, a previous history of pre-eclampsia, diabetes mellitus and/or hypertension [9].

Studies have shown that the existing clinical, biophysical and biochemical tests for the diagnosis of pre-eclampsia in high-risk women, in addition to being expensive, have little predictive value in making an early diagnosis [10]-[12]. Haemoconcentration is a shared phenomenon in both pre-eclampsia and among those living in high altitude areas. In women who have hypertensive disorders of pregnancy, particularly those with pre-eclampsia, blood volume does not increase at the same proportion as it does in a normotensive pregnancy which results in a relatively higher hemoglobin concentration [10]-[12]. In a number of studies, maternal hemoglobin (Hb) concentration and plasma haematocrit (HCT) levels have been investigated as an early predictive test for pre-eclampsia [13] [14]. These two measurements are routinely taken in antenatal care clinics in order to form a predictive model for pre-eclampsia. This is achieved by combining the results of these tests with any risk factors apparent in the patient's history and physical examinations, including body mass index (BMI) and BP.

Abha, the capital of the Aseer region, is situated 3133 m above sea level in the mountains of south-western Saudi Arabia. It has the lowest average annual temperature and has highest level of rainfall among the regional areas [15]. This study aimed to examine the prognostic value of risk assessment at level of antenatal care clinics in predicting pre-eclampsia among women living in this high altitude area.

2. Methods and Materials

This study was carried out at Abha Maternity and Pediatric Hospital (AMPH), a tertiary care center, between January 2013 and June 2013. There were a total number of 6700 admissions during the study period. We enrolled 88 pre-eclamptic women and 88 pregnant women who were normotensive at the time of delivery (control group). Patients in their second trimester, after 20 gestational weeks on their first visit at the AMPH antenatal clinic were included in the study. Any patients with a history of pre-existing medical diseases and with incomplete medical records were excluded from the study.

Participants were then divided into two groups. The first group was comprised of pre-eclamptic women diagnosed at the time of the study and the second group was a control group of normotensive women with uncomplicated pregnancies. A diagnosis of pre-elampsia was defined as the excretion of >300 mg of urinary protein over 24 hours and a systolic and diastolic BP of more than 140 mmHg and 90 mmHg, respectively.

Patients in both groups were compared for the following factors: maternal age, parity, physical risk factors—BMI and BP and blood investigations (including maternal Hb concentration and HCT levels). Blood and urine investigations were performed on all women and noted in their clinic records. Among the pregnant women, these investigations were carried out before 20 gestational weeks.

Data were analyzed using SPSS software package version 15.0 (SPSS, Chicago, IL. USA). Descriptive data was presented by mean ± sd. Unpaired T-test was used for comparison of characteristics between the pre-eclampsia and control groups. Two sided *P* values of less than 0.05 were considered statistically significant.

Approval for this study was granted by the Ethical Committee of King Khalid University and the AMPH Director. All patients gave consent prior to their enrollment in the study.

3. Results

A total of 176 patients were enrolled during the study period, including 88 pre-eclamptic women and 88 normotensive women in the control group. The mean age of the pre-eclamptic group was 28.64 ± 7.40 years, while the control group of non-pre-eclamptic women had a mean age of 27.66 ± 6.43 years ($P > 0.05$). No statistically significant difference existed between the two groups with respect to age. The mean parity between the cases (1.54% ± 0.661%) and the controls (1.79% ± 0.667%; $P < 0.05$) respectively. Significant differences exist between the cases and controls with respect to the characteristics of parity, systolic BP, diastolic BP and BMI ($P < 0.05$) in **Table 1**. The mean systolic BP in the pre-eclamptic group and the control group was 160.3 ± 17.6 mmHg and 121.1 ± 11.4 mmHg, respectively, while the mean diastolic BP was 103.9 ± 11.0 mmHG and 74.8 ± 7.8 mmHg, respectively. BMI was significantly higher among the pre-eclampic group as compared to the control group ($P < 0.001$).

Mean maternal Hb concentration in the study and control groups were 12.27 ± 2.01 g/dL and 11.92 ± 2.43 g/dL, respectively while the mean plasma HCT levels in the study and control groups were 38.49% ± 4.32% and 37.92% ± 7.04%, respectively. No significant difference is found in the mean maternal Hb concentration and mean plasma HCT levels between the two groups (with and without preeclampsia) ($P > 0.05$) [**Table 2**].

4. Discussion

The aim of this study was to set a model for the prediction of pre-eclampsia in a high altitude area through a combination of simple routine laboratory investigations and risk assessment via patient history and physical risk factors. Significant differences were observed between the pre-eclamptic study group and the control group in terms of BMI and BP measurements as well as parity. The results indicate that high altitude was associated with pre eclampsia and this is positively correlated to BMI and BP.

Given the high degree of concern with regards to this disorder, many studies have been carried out to evaluate the risk factors for pre-eclampsia—including nuliparity, advanced maternal age, race, genetic and environmental factors (e.g. high altitude), obesity, chronic hypertension and multiple pregnancies—all of which are considered

Table 1. Characteristics of pregnant patients in their second trimester with and without pre-eclampsia at an antenatal clinic in Abha, Saudi Arabia (N = 176).

Characteristic	Mean ± SD		*P* value
	Pre-eclamptic group (n = 88)	Control group (n = 88)	
Age in years	28.64 ± 7.400	27.66 ± 6.432	0.348
Parity	1.54 ± 0.661	1.79 ± 0.667	0.013
Systolic BP in mmHg	160.3 ± 17.651	121.17 ± 11.408	<0.001
Diastolic BP in mmHg	103.90 ± 11.021	74.88 ± 7.856	<0.001
BMI in kg/m^2	29.621 ± 6.7809	24.582 ± 4.4895	<0.001

SD = standard deviation; BP = blood pressure; BMI = body mass index. *A *P* value of <0.05 was considered statistically significant.

Table 2. Mean maternal HB concentration and Hct values of pregnant patients in their second trimester with and without pre-eclampsia at an antenatal clinic in Abha, Saudi Arabia (N = 176).

Value	Mean ± SD		*P* value*
	Pre-eclamptic group (n = 88)	Control group (n = 88)	
Hb in g/dL	12.279 ± 2.0167	11.921 ± 2.4311	0.291
Hct in %	38.497 ± 4.3221	37.923 ± 7.0447	0.518

*A *P* value of <0.05 was considered statistically significant.

contributory [16] [17]. A previous study also carried out in Abha found that the incidence of pre-eclampsia and eclampsia was high in both nulliparous women and those aged 20 - 29 years [18]. Siddiqui *et al*. reported that the average age of pre-eclamptic women in their cohort of women in Riyadh, Saudi Arabia, was 29.0 ± 6.1 years [19]. The mean age of the pre-eclamptic group in the current study was therefore consistent with those of the aforementioned studies.

It has been found that the maternal risk of pre-eclampsia rises with an increasing degree of obesity; this risk persists even after other potential confounding factors have been accounted for [20]. Other reported risk factors include a previous history of pre-eclampsia, family history of hypertension and a high BMI [21]. The findings of the current study support the validity of BMI as a risk factor as the mean BMI was observed to be significantly higher in the pre-eclamptic group than in the control group.

The measurement of BP is a routine practice in antenatal care clinics worldwide. Sibai *et al.* found that higher systolic and diastolic BP measurements at the first antenatal visit were associated with an increased incidence of pre-eclampsia (3.8% in women with diastolic BP of <55 mmHg and 7.4% in those with diastolic BP of 70 - 84 mmHg) [22]. Pre-eclamptic women in the present study had significantly higher systolic and diastolic BP measurements than those in the control group. This was consistent with the findings of Siddiqui *et al*. who reported a mean systolic BP of 143.1 ± 7.8 mmHg versus 125.1 ± 19.6 mmHg and mean diastolic BP of 94.3 ± 4.9 mmHg versus 78 ± 13.3 mmHg in the pre-eclamptic and control groups, respectively ($P < 0.05$) [19].

Timely treatment is vital in preventing the development of severe and possibly life-threatening pre-eclampsia. The only conclusive treatment for this condition is to deliver the fetus [23]. When making treatment decisions, physicians should consider the severity of the condition, the potential for maternal complications, the length of the pregnancy and the possible risks to the fetus [23]. Currently, there are few recommendations to prevent pre-eclampsia. There is some evidence to suggest that regular low-dose aspirin and calcium supplements, taken both before and during early pregnancy, may help to prevent the development of this condition in some women [24]. The WHO recommends that low-dose aspirin be initiated before 20 gestational weeks to prevent pre-eclampsia in high-risk women [24]. Calcium supplementation (at least 1 g per day) is also recommended during pregnancy as it prevents pre-eclampsia where dietary calcium intake is low, especially for those at high risk. Magnesium sulfate is preferential to anticonvulsants for the prevention of eclamptic seizures in women with severe pre-eclampsia [24]. In general, thorough prenatal care should be made available to all pregnant women in order to minimize pre-eclampsia-related deaths.

Effective screening tests for pre-eclampsia should be simple, safe, rapid, inexpensive and reproducible. They should also provide intervention opportunities to prevent the development of pre-ecclampsia or, at a minimum, result in a better outcome [13]. The investigations for maternal Hb concentration and plasma HCT levels used in the current fulfill these criteria. Mean maternal Hb concentrations and plasma HCT levels were found to be higher in pre-eclamptic women than among women with normal pregnancies. Although, the results of maternal Hband HCT levels between the groups were not statistically significant.

However, a number of other studies, including that of Siddiqui *et al*., did not observe significant differences between HCT levels in pre-eclamptic women and women with normal pregnancies [19] [25] [26]. Pregnancy at high altitudes, compared to sea level, is characterized by an increased blood viscosity as a result of increased HCT and plasma viscosity [27]. Some evidence suggests that the plasma volume in patients with pre-eclampsia is lower than normal [28] [29]. Decreased plasma volume induces a high Hb concentration [12]. These factors contributed to high maternal hemoglobin levels in our study, the question around absence of statistical significance can be answered by increased Hb and HCT levels in control group as well as the low sample size.

This fact, combined with the results of this study, signal the need for specific pre-eclampsia screening programmes tailored to different locations. These programmes may be different even within a single country, due to the effect of altitude on this condition.

The results of this study should be considered in view of the following limitation. Clear cut-off values for HCT and Hb could not be defined. This resulted in the overlapping of values between the control and study groups and wider standard deviations.

5. Conclusion

The importance of risk assessment at level of antenatal care clinics in prediction of preeclampsia is stressed in our study. Findings of high BMI, systolic and diastolic BP measurements could be the base for a predictive

model for pre-eclampsia in high altitude areas when combined with other risk assessments. Using a predictive model may help achieve timely interventions according to the WHO recommendations for the prevention of pre-eclampsia. However, more studies on this topic are necessary.

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Are Tuberculosis Patients Managed According to the National Guidelines in Lagos State Nigeria?

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Abstract

Setting: Private and public tuberculosis (TB) treatment centers in Lagos State, Nigeria. Objective: To compare adherence of private and public providers of directly observed treatment short course (DOTS) in the Lagos State TB control program, Nigeria (LSTBLCP) with the national TB guidelines. Design: A retrospective review of treatment cards of TB patients managed within the first and second quarter of 2012 in 34 DOTS facilities {23 public, 7 private for profit (PFP), and 4 private not for profit (PNFP)} involved in the private public mix of the LSTBLCP. Results: Of the 1896 treatment cards reviewed, 1524 (80.4%), 132 (7.0%) and 240 (12.6%) were from public, PFP and PNFP DOTS facilities, respectively. About 19%, 25% and none of the patients managed at the public, PNFP, and PFP DOTS facilities were treated in full adherence with the national guidelines respectively. A significantly higher proportion of adults and sputum smear positive TB patients were treated in full adherence with the national guidelines ($p < 0.05$). Treatment success was associated with full adherence with the national guidelines. Conclusion: There is a need to reorient health care providers in public and private health facilities in Lagos State Nigeria to ensure full adherence with the national TB guidelines.

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Keywords

Adherence, National Guidelines, Health Workers, DOTS Facilities

1. Introduction

Tuberculosis (TB) is still a serious public health issue in Nigeria accounting for 46,000 deaths (27 per 100,000 populations) per year [1]. The 2012 national TB prevalence survey showed that the prevalence of smear positive TB in Nigeria (318 per 100,000) was about twice as much as the previous World Health Organization (WHO) estimate, which was based on routine surveillance data [2]. This recent figure placed Nigeria 4th among the 22 high burden TB countries [3]. Another WHO report showed that 2.9% (2.1% - 4.0%) of TB cases in Nigeria present with multi drug resistant TB [4]. Despite the efforts of the National TB and Leprosy Control Program (NTBLCP) since the commencement of directly observed treatment short course (DOTS) for management of TB in 1993, inadequate skills and poor knowledge of health care providers and programme officers at the DOTS centers were some of the barriers of TB control in Nigeria [1] [5].

Adherence to the national TB guidelines is therefore necessary in ensuring that TB patients get quality service irrespective of the service point. Thus, successful treatment could be achieved if the healthcare workers (both public and private medical practitioners) follow the national guidelines for the treatment of TB.

This present study compared adherence of private and public DOTS providers with the national guidelines and the factors associated with adherence with the national guidelines in the Lagos State TB control program, Nigeria.

2. Methods

2.1. Study Setting

Lagos state is one of the 36 states in Nigeria and the population is estimated to be 21 million.

Health care services in Lagos State are provided by both the public and private sector. In the public sector, services are organized at primary, secondary and tertiary care. There are 27 secondary, 215 primary and 1984 registered health care facilities (1925 private for profit and 59 private not for profit) in the state.

The Lagos State TB and leprosy control programme (LSTBLCP) commenced operation in 2003 in collaboration with some international organizations. In 2008, private sector participation in DOTS management of TB was introduced. To be eligible, private providers were expected to offer TB services free of cost to patients and undergo training on DOTS management of TB based on the national guidelines [6].

Based on capacity and interest, private health provider (PHP) were engaged either to refer presumptive TB patients (scheme one), provide DOTS management only (scheme two), serve as microscopy center only or serve both as treatment and microscopy center (scheme three). After training and completion of the necessary formalities, PHP were provided with recording and reporting materials, drugs and other consumables to commence TB services. The patient's treatment card was one of the recording materials provided to the PHP; it contained patients' relevant information and also served as a tool to monitor patient's treatment. Sputum microscopy results, weight measurements and drug intake were recorded on the treatment card.

TB activities in Lagos State were coordinated by the state TB control officer. At the local government level, the state TB control officer was assisted by local government TB supervisors. There are 20 TB supervisors in Lagos State, one in each LGA. They assist the state TB control officer to plan, organize and conduct training programmes, keep an up-to-date and accurate record of activities of TB control activities in the LGA. The supervisors were assisted by TB focal persons in each DOTS facility. Records of patients registered in each DOTS facility were sent to the LGA supervisors monthly and they in turn forward the records of TB patients managed in the LGA to the state control officer quarterly.

The DOTS facilities at the primary health centers (PHCs) were coordinated by Community Health Officers and nurses whereas the medical officer coordinates DOTS facilities at the secondary, tertiary, private and the military health facilities. Any health care worker could initiate treatment for smear positive TB patients; how-

ever children and presumptive TB clients with smear negative results were referred to health facilities manned by doctors for diagnosis.

Management of TB at PHP facilities is free; however they were allowed to charge for consultation and service charge for sputum AFB microscopy because reagents and consumables for sputum AFB were freely supplied by the LSTBLCP. The PHP could also charge for investigations such as chest X-ray, erythrocyte sedimentation rate (ESR), etc. required to diagnose smear negative patients. The duration of treatment was eight months. The treatment regimen consisted of two months intensive phase of Rifampicin, Isoniazid, Pyrazinamide and Ethambutol as fixed dose combination and six months continuation phase of Rifampicin and Isonizid as fixed dose combination. Drugs were prescribed based on patient's weight and recorded on the treatment card.

According to the national guidelines, each presumptive TB patient were offered HIV testing. The HIV rapid test kit used in accordance with the national HCT policy was Determine (determine HIV-1/2 Alere Determine™, Japan 2012) and Uni-Gold™ (Trinity Biotech PLC, Wicklow, Ireland 2013) in parallel algorithm. A concordance result was regarded as positive. In the event of discordant result, STAT-PAK® was used as tie breaker. TB/HIV co-infected patients were offered CPT along with anti-TB drugs and commenced on ART within 8 weeks of anti-TB medications.

At the end of 2011, the LSTBLCP had 130 TB treatment facilities offering DOTS services. Of these, 99 were public and 31 private health care facilities (20 Private for Profit (PFP) and 11 Private not for Profit (PNFP) or missionary hospitals).

2.2. Study Design

A retrospective review of patients' treatment cards managed for pulmonary TB during the first and second quarter of 2012 was conducted.

A sampling frame of 130 DOTS facilities provided by the Lagos state programme officer (99 public and 31 private DOTS facilities) was used to select, 34 DOTS facilities (23 public, 7 PFP and 4 PNFP DOTS facilities) that served as both microscopy and treatment centers and were involved in DOTS programme for at least 2 years prior to the study. All treatment cards of patients managed for pulmonary TB during the first and second quarter of 2012 in the selected DOTS facilities were assessed for adherence with the national guidelines [6]. Treatment cards with wrong or missing data were not included for analysis.

2.3. Evaluation of Compliance of Health Workers with the NTP Guidelines

Adherence of public and private DOTS providers to the national guidelines was based on the following [6].

- Performance of smear microscopy before DOTS treatment.
- HIV test done for patients.
- Specification of patients treatment category.
- Weight measurement of patient before commencement of treatment.
- Weight measurement at least 3 times (2^{nd}, 5^{th} and 7^{th} month of treatment).
- Three follow up sputum results at 2^{nd}, 5^{th} and 7^{th} month of treatment.
- Correct recording of sputum results.
- Correct charting of drugs.
- Correct dosages in line with the weight of the patient.
- Correct filling of treatment cards.
- Specification of the treatment outcomes.

In this study, performance of the entire task stated above was regarded as full adherence to national guidelines while incomplete performance was regarded as partial adherence.

2.4. Definition of Treatment Outcomes

- Treatment success was defined as the sum of the cases that were cured and that completed treatment [6].

2.5. Data Analysis

Data was analysed using the Statistical Package for Social Sciences (SPSS) version 19. Mean and standard deviation were calculated for numerical data while percentages were calculated for both numerical and categorical

data. Chi square and Fishers' exact test was used to compare categorical data as appropriate. The confidence interval was set at 95% for all statistical tests. Microsoft excel was used to draw charts.

2.6. Ethical Approval

As data for this study were retrieved from secondary data routinely collected by the LSTBLCP, no ethical clearance was required.

3. Results

Treatment cards of 1896 TB patients were reviewed out of which 1524 (80.4%), 132 (7.0%) and 240 (12.6%) were from the public, PFP, and PNFP DOTS facilities respectively (**Figure 1**). The mean age of TB patients treated at the public and private DOTS facilities were respectively 34.3 ± 13.4 and 32.2 ± 12.4. More males were treated for TB at the public DOTS facilities (M:F ratio = 1:076) while at the private DOTS facilities the M:F ratio was almost equal (M:F = 1:0.98). Over 60% of the TB cases reviewed were sputum smear positive. However, a significantly higher proportion of the sputum smear positive cases were managed at the private DOTS facilities ($p < 0.001$). Of the patients that had HIV test done, 15.3% and 11.8% were HIV positive from the public and private DOTS facilities respectively as shown in **Table 1**.

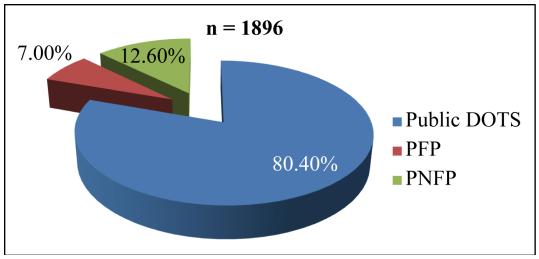

Figure 1. Proportion of treatment cards reviewed in different DOTS facilities.

Table 1. Socio demographic characteristics of TB patients at the public and private DOTS facilities.

Variable	Public DOTS n = 1524 (%)	Private DOTS n = 372 (%)	χ^2	p
Age group				
Less than 15	36 (2.4)	8 (2.2)		
15 - 24	322 (21.1)	96 (25.8)		
25 - 34	528 (34.6)	136 (36.6)	6.723	0.151
35 - 44	328 (21.5)	72 (19.4)		
45 and above	310 (20.3)	60 (16.1)		
Mean ± SD	34.3 ± 13.4	32.2 ± 12.4		
Gender				
Male	860 (56.4)	188 (50.5)	4.200	0.040
Female	654 (43.6)	184 (49.5)		
Pulmonary TB				
Smear positive	938 (61.5)	280 (75.3)	24.503	<0.001
Smear negative	586 (38.5)	92 (24.7)		
HIV status				
Negative	1154 (75.7)	254 (68.3)		
Positive	208 (13.6)	34 (9.1)	2.28	0.131
Not done[#]	162 (10.6)	84 (22.6)		

NB: [#] = Not included in the analysis. All participants in this study were Negros.

Table 2 shows the management pattern of patients treated at the public and private DOTS facilities. HIV test was not done for a significantly higher proportion of patients managed (22.6% vs 10.6%) at the private DOTS facilities ($p < 0.001$). A slightly higher proportion of TB patients treated at the public DOTS facilities (2% vs 0.5%) did not do smear microscopy before commencement of treatment and also did not do three follow up

Table 2. Management practices of at the public and private DOTS facilities.

Variable	Public DOTS n = 1524 (%)	Private DOTS n = 372 (%)	χ^2	p
Smear microscopy done before treatment				
Yes	1494 (98.0)	370 (99.5)	3.690	0.055
No	30 (2.0)	2 (0.5)		
Had three follow up sputum				
Yes	698 (45.8)	177 (47.6)	0.381	0.537
No	826 (54.2)	195 (52.4)		
Number of follow up sputum done				
None	104 (6.8)	34 (9.1)	2.38	0.123
Once	401 (26.3)	70 (18.8)	9.00	0.003
Twice	321 (21.1)	90 (24.2)	1.73	0.189
Thrice	698 (45.8)	178 (47.8)	0.51	0.477
Second month smear microscopy				
Done	1410 (92.5)	334 (89.8)	3.03	0.082
Not done	114 (7.5)	38 (10.2)		
Fifth month smear microscopy				
Done	921 (60.4)	251 (67.5)	6.28	0.012
Not done	603 (39.6)	121 (32.5)		
Seventh month smear microscopy				
Done	805 (52.8)	193 (51.9)	0.11	0.745
Not done	719 (47.2)	179 (48.1)		
Weight measurement during treatment				
None	6 (0.4)	0 (0.0)	1.47	0.604[x]
Once	318 (20.9)	122 (32.8)	23.88	<0.001
Twice	390 (25.6)	96 (25.8)	0.01	0.932
At least thrice	810 (53.1)	154 (41.4)	16.52	<0.001
HIV test conducted				
Yes	1362 (89.4)	288 (77.4)	31.82	<0.001
No	162 (10.6)	84 (22.6)		
Treatment category				
Specified	1514 (99.3)	372 (100.0)	2.454	0.117
Not specified	10 (0.7)	0 (0.0)		
Drug dosage according to patient's weight				
Correctly done	1380 (90.6)	264 (71.0)	99.50	<0.001
Wrongly done	144 (9.4)	108 (29.0)		
Monitoring of treatment				
No drug interruption	1176 (77.2)	280 (75.3)	0.603	0.437
Drug interruption	348 (22.8)	92 (24.7)		

Note: X = Fisher's exact test.

sputum (54.2% vs 52.4%) ($p < 0.05$) during the entire treatment duration. However, more of the patients treated at the public DOTS facilities did weight measurements (53.1% vs 41.4%) and had the correct dosage of TB drugs based on weight (90.6% vs 71.0%) compared with those managed at the private DOTS facilities ($p < 0.001$). A higher proportion of patients managed at the private DOTS facilities interrupted treatment (22.8% vs 24.7%) compared with those managed at the public DOTS facilities ($p = 0.437$).

Recording of sputum smear results (7.7% vs 14.0%), treatment outcome (43.8% vs 53.8%) and filling of the treatment cards (22.6% vs 32.8%) were poorly done for significantly higher proportion of patients managed at the private DOTS facilities compared with those treated at the public DOTS facilities as shown in **Table 3**. **Table 4** shows the proportion of patients treated in full adherence with the national guidelines at the public and private DOTS facilities. Overall, the proportion of patients treated full adherence with the national guidelines was low in both the public and private DOTS facilities. About 19%, 25% and none of the cases seen at the public PNFP and PFP DOTS facilities were treated in full adherence with the NTBLCP guidelines respectively ($p < 0.001$). **Table 5** shows that a significantly higher proportion of adults, smear positives and those that had successful treatment were treated in full compliance with the national guidelines ($p < 0.05$).

4. Discussion

One of the goals of the public private mix (PPM) for TB is to provide rational and standardized treatment to TB patients especially those managed at the private sector, thereby reducing the spread of TB within the community and emergence of multi drug resistance TB. Routinely, the NTBLCP and the LSTBLCP organizes training and retraining programs for health care workers at the public DOTS facilities and private sector involved in the PPM. This training is expected to facilitate adherence with the national guidelines. This study however shows that the proportion of patients managed in full adherence with the national guidelines at the public and private DOTS facilities was low. Particularly striking was the fact that none of the patients managed at the PFP facilities were managed in full adherence with the national guidelines. Studies from Nigeria and other high TB burden countries have shown that private practitioners and health care workers from the public sector do not comply with the National Tuberculosis Programme (NTP) [7]-[10].

Many reasons have been shown to be responsible for the poor adherence of health care workers at the TB treatment centers. Some studies found that insufficient knowledge of health workers at the public and private

Table 3. Record keeping practices of public and private DOTS facilities.

Variable	Public DOTS n = 1524 (%)	Private DOTS n = 372 (%)	χ^2	p
Recording of smear results				
Correctly recorded	1406 (92.3)	320 (86.0)	14.25	<0.001
Wrongly recorded	118 (7.7)	52 (14.0)		
Treatment outcome				
Documented	856 (56.2)	172 (46.2)	11.88	0.001
Not documented	668 (43.8)	200 (53.8)		
Treatment card				
Correctly filled	1180 (77.4)	250 (67.2)	18.88	<0.001
Not correctly filled	344 (22.6)	122 (32.8)		

Table 4. Compliance to national guidelines in the different types DOTS facilities.

Type of health facility	Compliance		χ^2	p
	Partial n = 1544 (%)	Full n = 352 (%)		
Public	1232 (80.8)	292 (19.2)	37.02	<0.001
Private for profit	132 (100.0)	0 (0.0)		
Private not for profit	180 (75.0)	60 (25.0)		

Table 5. Factors associated with compliance of healthcare workers to the NTP guidelines.

Variables	Compliance with NTP		χ^2	p
	Partial freq (%)	Full freq (%)		
Age group				
Children	42 (95.5)	2 (4.5)	5.856	0.010[x]
Adults	1502 (81.1)	350 (18.9)		
Gender				
Male	868 (82.8)	180 (17.2)	2.994	0.084
Female	676 (79.7)	172 (20.3)		
Type of TB				
Smear Positive	866 (71.1)	352 (28.9)	240.61	<0.001[x]
Smear Negative	678 (100.0)	0 (0.0)		
Treatment outcome				
Treatment success	1218 (77.6)	352 (22.4)	89.75	<0.001[x]
No treatment success	326 (100.0)	0 (0.0)		
HIV status	**n = 1298 (%)**	**n = 352 (%)**		
Positive	196 (81.0)	46 (19.0)	0.91	0.339
Negative	1102 (78.3)	306 (21.7)		

sector about the guidelines was responsible for the poor adherence [8] [11]. Other studies also showed that some health workers refused to comply despite their awareness of the NTP guidelines although reasons for their refusal were not highlighted [12] [13].

Health care workers are usually trained before they were allowed to provide TB services. However, maintaining trained staff has been a major challenge in the TB programme especially in a cosmopolitan city like Lagos. The high staff turnover experienced in the private sector maybe due to poor job satisfaction and/or job insecurity. In addition, the regular redeployment and poor distribution of trained staff within the public health sector is a cause of concern in the sustainability of public health programmes in developing countries like Nigeria [14]-[16].

Sputum microscopy is the main diagnostic tool for pulmonary tuberculosis and all presumptive TB clients should have sputum microscopy as the first diagnostic tool. In this study almost all the patients managed at the public and private DOTS facilities did sputum smear microscopy before commencement of anti-TB treatment. This is similar to findings from studies from Nigeria and elsewhere [9] [11] [17] [18]. However other studies from another part of Nigeria and Ethiopia showed that some of the patients treated at the public and private DOTS facilities did not do smear microscopy before initiation of anti-TB treatment [7] [19]. Less than half of the patients managed at the public and private DOTS facilities had three follow up sputum smear microscopy in this study. This may be due to shortages of laboratory equipment and supplies in some of the DOTS laboratories in Lagos [20] which has also been reported in other studies from Ghana, India and Ethiopia [19] [21] [22] and the incessant strike action by health care workers in Lagos Nigeria also contributed to failure of patients to do follow up sputum microscopy.

There is a strong synergy between TB and HIV/AIDS and the WHO recommends HIV testing for TB patients to reduce the burden of TB/HIV [23]. In this study, a high proportion of patients managed at the public and private DOTS facilities did HIV test which was consistent with findings from another study from another part of the country [6] but higher than what was reported in a similar study from Ethiopia [19]. Majority of the Anti retrovital therapy (ART) centres in Lagos state are located at the public health care facilities. This may explain why a significantly higher proportion of patients managed at the public DOTS facilities did HIV test compared with those managed at the private DOTS facilities.

One of the goals of the NTBLCP was to increase the success rate of TB patients [1]. In order to achieve this target, TB patients must be treated in full adherence with the NTP guidelines. This study shows that none of the

patients that had unsuccessful treatment at the public and private DOTS facilities were treated in full adherence with the NTP guidelines. A WHO report documented that within 10 years of DOTS implementation, 16 million people globally were cured [24] in addition to the reduction in incidence of TB in most region of the world except the sub Saharan Africa. For the NTBLCP to achieve the global targets, it is paramount that patients are treated according to the NTP guidelines irrespective of their age, gender, sputum smear results and HIV status.

Limitation

The study was a retrospective review of treatment cards and as such did not consider other factors such as training of health personnel at DOTs facilities, availability laboratory equipment and supplies and provision of logistic necessary to track patients lost to follow which could affect adherence to the national guidelines.

5. Conclusion

Majority of the patients treated at the public and private DOTS facilities in Lagos State were not treated in full adherence with the national guidelines. There is an urgent need for the LSTBLCP to reorient health care providers in public and private health facilities to ensure full adherence with the national guidelines on the management of TB in Nigeria.

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Competing Interests

Authors have declared that no competing interests exist.

Authors Contribution

OAA conceived the study, involved with data collection, data analysis and discusssion. OJD wrote the methodology and was involved in the writing process, MD was involved in reading the manuscript and literature search, ENA was involved in data collection and proff reading the manuscript. EOJ and OEI were involved with data collection and literature search while OOO supervised the research.

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Hemoglobin Subunit Beta Gene Polymorphism rs33949930 T>C and Risk of Sickle Cell Disease—A Case Control Study from Tabuk (Northwestern Part of Saudi Arabia)

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Abstract

Background: Sickle cell disease and sickle cell trait are common erythrocyte disorders that are most often caused by a point mutation (rs334, designated HbS) in the hemoglobin beta gene (HBB); however of this fact, there is extreme variability in occurrence and clinical presentation of sickle cell disease which may be explained by some other genetic changes associated with the gene. In the present study we examined the association between HBB gene polymorphism rs33949930 T>C in the occurrence of sickle cell disease in Saudi Arabia population. Materials and Methods: A case control study of 100 sickle cell disease patients and 100 healthy controls from Tabuk, Saudi Arabia. HBB gene rs33949930 T>C polymorphism was analyzed using Allele specific polymerase chain reaction technique. Results: It was observed that the genotype percentages TT, TC and CC among the patients with sickle cell disease were 63.0%, 35.0% and 2.0% and healthy controls were 68.0%, 27.0% and 5.0% respectively. Allele frequency for T allele was observed to be fT = 0.20 and fT = 0.19, where as for C allele was fC = 0.80 and fC = 0.81 among cases and controls respectively (p = 0.29). Compared to the TT genotype, the odds ratio of 1.4 (95% CI 0.76 - 2.57), risk ratio of 1.2 (95% CI 0.86 - 1.65) and risk difference of 8.4 (−6.66 - 23.38) for heterozygous genotype of HBB rs33949930 T>C was observed in relation to sickle cell disease. In addition, some difference in the laboratory values was observed among sickle cell disease patients with the different variants of HBB gene rs33949930 T>C polymorphism, especially the carriers of heterozygous TC genotype;

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however, the difference doesn't reach to statically significant number. Conclusion: Present study suggested that there was not any significant association between HBB gene rs33949930 T>C polymorphism and occurrence of sickle cell disease. However, the heterozygous TC genotype of the polymorphism showed some higher ratios among cases as compared to healthy control group.

Keywords

Hemoglobin Subunit Beta (HBB), Sickle Cell Disease (SCD), Tabuk-Northwestern Part of Saudi Arabia

1. Introduction

Sickle cell disease (SCD) is a global public health disorder that affects millions of people across the globe. It is a monogenic disorder caused by an A-to-T point mutation at the sixth codon of the hemoglobin beta gene on chromosome 11p15.5. The mutation leads the substitution of a valine amino acid for a glutamic acid that produces abnormal hemoglobin S (Hb S), which polymerizes in the deoxygenated state, resulting in physical deformation or sickling of erythrocytes. Globally sickle cell disease is the most common genetic disorder [1] with highest prevalence in Middle East, Mediterranean regions, Southeast Asia, and sub-Saharan Africa especially Nigeria [2]. As per the World Health Organization published global prevalence map of sickle cell disease, about 20 - 25 million individuals globally suffer from homozygous sickle cell disease [3]. SCD was first reported by Lehmann *et al*. in the eastern province of Saudi Arabia [4] and latter studies have reported that SCD is the most common genetic disorder in Saudi Arabia with the highest frequency in the eastern and southwestern province of Saudi Arabia [4]-[8]. Sickle cell disease carriers in Saudi Arabia ranged from 2% to 27%, and in some areas up to 1.4% had sickle cell disease [4]-[8]. In addition to environmental factors it has been observed that various genetic determinants may be involved in the risk of developing sickle cell disease [9].

Rapid advances made in understanding the molecular genetics of SCD in the early part of the 20th century have not been matched by comparable progress towards understanding its clinical complications, and developing effective therapies. Many studies have investigated the effect of genetic variants in the BCL11A, the HMIP (HBS1L-MYB intergenic polymorphism) locus, in addition to the HBB locus, which is known to be associated with fetal hemoglobin (HbF) levels, a major modulator of the disease phenotype. Genotyping was performed for the BCL11A rs11886868 and rs34211119; HMIP rs9399137, rs189600565, rs7776196, rs34778774, and rs53293029; HBG2 Xmn1 polymorphism rs7482144; and −68C>T HBD promoter polymorphism. All the 3 quantitative trait loci were associated with HbF levels in Indian patients with SCD [10]-[13].

The highest difference was seen in the Xmn1 single-nucleotide polymorphism, which accounted for 11% of the trait variance, the BCL11A rs11886868 for 3.65%, whereas the HMIP rs9399137 for 3.8%. Several studies indicated that the BCL11A, HMIP, and β-globin regions were associated with increased HbF levels in different populations; and investigation of these genotypes with respect to pain crisis is warranted in different population, which may help in prognostication, as also a genome-wide association study, which may help uncover new loci controlling HbF levels [14] [15].

Multiple SNP variants in these gene regions are associated with higher levels of FH and a milder course of disease. Together with the SNPs in the γ-globin region of the β-globin cluster, these loci account for more than 20% of the variance in FH levels among SCA patients in the United States and Brazil. SCD is prevalent in Saudi Arabia and is probably underestimated. The variable genetic origin and variable clinical phenotype of SCD between the East and West parts of Saudi Arabia make it possible to further pursue research on genetic, clinical, and environmental modifiers of SCD [16] [17].

Hemoglobin subunit beta gene polymorphism rs33949930 T>C may be associated with sickle cell diseases. In this polymorphism of Hemoglobin Subunit beta 1, the amino acid valine is replaced by acetylalanine or thiamine to cytosine. Kamel *et al*. [18] investigated that a Qatari family with an electrophoretically fast-moving hemoglobin that they found contained an abnormal beta chain with the sequence met-glu-his-leu at the NH_2-end. Substitution of glutamic acid for valine at beta 1 apparently prevented removal of the initiator methionine. The methionine was blocked by a molecule not completely identified. No clinical consequences were observed in heterozygotes. This variant was numbered based on the first amino acid of the mature protein. In the gene-based

system of counting, this variant is VAL2GLU.

There is a need for systematic, prospective studies that document the prevalence, molecular and clinical epidemiology of SCD in different areas of Saudi Arabia to help predict disease severity, risk stratify patients to receive early intensive care or continued symptomatic care, and describe the problems currently faced by patients affected with SCD in Saudi Arabia. In order to understand the biological basis of various diseases especially the genetic disorders, single nucleotide polymorphisms (SNPs) are being intensively studied with promising conclusions.

In the present study we examined the association between *HBB gene polymorphism rs*33949930 *T>C* in the occurrence of sickle cell disease in Saudi Arabia population. To the best of our knowledge, no information is available concerning the association between *HBB gene polymorphism rs*33949930 *T>C* and sickle cell disease.

2. Material and Methods

The study was conducted in the Division of Cancer Molecular Genetics, Prince Fahd Bin Sultan Research chair, University of Tabuk, Saudi Arabia. The study was approved by the ethics Committee, University of Tabuk. The samples were obtained from the hospital stored at −30˚C and were collected from the patients visiting hospital for routine checkup.

Study population: The study included 100 clinically confirmed Sickle cell disease cases and 100 healthy controls. The samples were also collected from the healthy controls visiting Hospital for routine checkup. The description of the laboratory characteristics for patients with sickle cell disease are summarized in **Table 1**.

2.1. Genotype Analysis

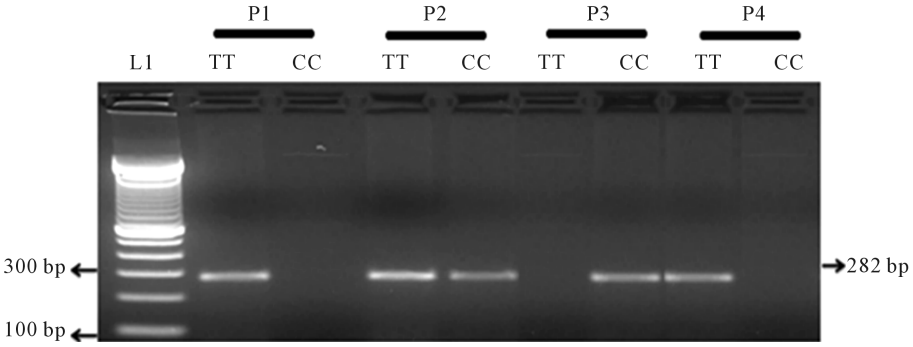
Peripheral blood sample (3 - 5 mL) from each participant was drawn into an EDTA vial and genomic DNA was extracted by following manufacturer's protocol of DNA extraction kit (Qiagen). ASO-PCR was performed to determine the (rs33949930) T>C HBB gene among sickle cell disease patients and compared with healthy controls. PCR was performed in a final volume of 25 µL containing 5 µL of 50 ng genomic DNA, 12.5 µL of PCR-master mix (Promaga), 0.25 µL of 25 pmol/L of each primer (**Table 2**) and remaining nuclease free ddH_2O. PCR program was started with an initial denaturation at 95˚C for 5 minutes, followed by 40 cycles of denaturation at 95˚C for 30 s, annealing at 60˚C for 45 s, extension at 72˚C for 45 s, and completed with a final elongation step at 72˚C for 5 minutes. Negative control with ddH_2O instead of DNA template was included in each PCR run. A blind case/control analysis was performed with approximately more than 10% random samples were selected for confirmation and the results were 100% concordant. PCR products were visualized on ethidium bromide stained 2% agarose gel (**Figure 1**).

Table 1. Laboratory characteristics of patients with sickle cell disease.

Variables	Mean ± SD	Range (min - max)
Hb	13.97 ± 1.87	12.70 (5.50 - 18.20)
WBC	7.17 ± 1.66	13.31 (3.69 - 17.00)
RBC	5.11 ± 0.74	3.72 (3.40 - 7.12)
HCT	39.95 ± 5.08	34.80 (18.00 - 52.8)
MCV	79.91 ± 6.06	27.00 (65.00 - 92.00)
RDW	12.7090 ± 1.22	7.00 (11.00 - 18.00)
PLT	303.90 ± 66.86	266.00 (190.00 - 456.00)
HbA1	63.44 ± 13.44	94.50 (3.10 - 97.60)
HbA2	4.06 ± 3.85	23.90 (2.30 - 26.20)
HbF	0.7680 ± 1.75	14.80 (0.00 - 14.80)
HbS	36.40 ± 7.44	57.40 (25.00 - 82.40)

Table 2. Primer sequences for HBB rs33949930 (T>C) polymorphism.

Primers	Sequence	AT	Product size
F1 (Forward primer 1)	5'-ACGGCAGACTTCTCCTCAGGAGTCAGATGCAC-3'	63˚C	282 bp
F2 (Forward primer 2)	5'-ACGGCAGACTTCTCCTCAGGAGTCAGATGCAT-3'	63˚C	282 bp
R (Common reverse)	5'-TATCTTAGAGGGAGGGGCCTGAGGGTTT-3'		



L1-100 bp DNA ladder
SCD patient - P1 → Positive for TT genotype and negative for CC genotype - homozygous for TT allele
SCD patient - P2 → Positive for both genotypes TT and CC - heterozygous
SCD patient - P3 → Negative for TT genotype and positive for CC genotype - homozygous for CC allele
SCD patient - P4 → Positive for TT genotype and negative for CC genotype - homozygous for TT allele

Figure 1. Agarose gel electrophoresis of PCR amplification of hemoglobin subunit beta gene polymorphism rs33949930 T>C in sickle cell disease.

2.2. Statistical Analysis

Statistical analysis was performed using SPSS 16.0 software. Assessment of the correlations between genetic carrier status and HBB gene polymorphism was carried out using the Chi-Square or Fisher Exact test. HBB gene variants and risk of sickle cell disease were estimated by computing the odds ratios (OR), risk ratio (RR) and risk difference (RD) with 95% confidence intervals (CIs) from multivariate logistic regression analysis. Student t test was used to calculate Mean ± SD and allele frequencies among cases as well as controls were evaluated by using Hardy-Weinberg equilibrium test. A p value < 0.05 was considered significant.

3. Results

3.1. Case-Control Genotype Distribution

In the present study while analyzed the HBB gene rs33949930 (T>C) polymorphism (70599 T>C) polymorphism, it was observed the genotype percentages TT, TC and CC among the patients with sickle cell disease were 63.0%, 35.0% and 2.0% and healthy controls were 68.0%, 27.0% and 5.0% respectively. While calculating the allele frequency, a slight non statistically significant difference (p = 0.29) was observed with T allele frequency of fT = 0.20 and C allele frequency of fC = 0.80 among cases and T allele frequency of fT = 0.19 and C allele frequency of fC = 0.81 among healthy controls (**Table 3**).

3.2. HBB Gene rs33949930 (T>C) Polymorphism and Risk of Sickle Cell Disease

Logistic regression was used to estimate associations between the genotypes and risk of SCD (**Table 4**). Compared to the TT genotype, the odds ratio of 1.4 (95% CI 0.76 - 2.57), risk ratio of 1.2 (95% CI 0.86 - 1.65) and risk difference of 8.4 (−6.66 - 23.38) for heterozygous genotype of HBB (70599TC) was observed in relation to sickle cell disease among population of Saudi Arabia.

3.3. HBB Gene rs33949930 (T>C) Genotypes and Laboratory Characteristics

Upon correlating the laboratory findings of sickle cell disease patients with the different variants of HBB gene

rs33949930 (T>C) polymorphism, it was observed that there was some difference among the values but the different doesn't reach to statically significant number (**Table 5**). In relation to rs33949930 (T>C) genotypes (TT, TC and CC) the mean HbA1 and HbA2 levels were 63.26, 63.80, 62.50 and 4.1, 4.03, 2.90 respectively. While comparing the HbF level with the HBB rs33949930 (T>C) genotypes CC homozygous carriers showed lower level of HbF compared to patients with TT homozygous and TC heterozygous genotypes; however HbS level was almost similar in association with different variants of rs33949930 (T>C) polymorphism (**Table 5**).

4. Discussion

The HBB gene in humans located on chromosome 11p15.5 encodes for a protein called beta-globin, which is an important subunit of hemoglobin. In adults, hemoglobin normally consists of four protein subunits: two subunits of beta-globin and two subunits of another protein called alpha-globin, encoded by HBA gene. Among these subunits several changes at nucleotide level has been observed and has been found to be associated with various genetic disorders like sickle cell disease.

Homozygosity for a single β-globin gene mutation (β6GAG $\rightarrow$ GTG) has been found to be the main cause of sickle cell disease, however of this fact, there is extreme variability in occurrence and clinical presentation from

Table 3. Genotype frequencies of rs33949930 (T>C) HBB among SCD cases and healthy controls.

Genotype	TT genotype (%)	TC genotype (%)	CC genotype (%)	T allele frequency	C allele frequency	p value
Cases (n = 100)	63 (63.00)	35 (35.00)	2 (2.00)	0.20	0.80	0.29
Controls (n = 100)	68 (68.00)	27 (27.00)	5 (5.00)	0.19	0.81	

Table 4. Risk of developing SCD associated with HBB rs33949930 (T>C) genotypes.

Genotypes	Control (n = 100)	Cases (n = 100)	OR (95% CI)	RR (95% CI)	RD (95% CI)	p value
TT (reference)	68	63	1.00			
CC	5	2	0.43 (0.08 - 2.31)	0.73 (0.44 - 1.19)	−19.5 (−54.04 - 15.0)	0.54
TC	27	35	1.4 (0.76 - 2.57)	1.2 (0.86 - 1.65)	8.4 (−6.66 - 23.38)	0.35

Table 5. rs33949930 (T>C) polymorphism with respect to laboratory parameters of SCD patients.

Variables	Mean ± SD			p value
	TT	TC	CC	
Hb	13.78 ± 1.94	14.33 ± 1.76	13.90 ± 0.57	0.380
WBC	5.04 ± 1.78	7.02 ± 1.45	7.32 ± 1.73	0.128
RBC	5.01 ± 0.72	5.29 ± 0.76	4.92 ± 0.32	0.190
HCT	39.31 ± 5.09	41.02 ± 5.04	41.25 ± 3.18	0.264
MCV	79.94 ± 6.18	79.65 ± 6.02	83.25 ± 1.76	0.718
RDW	12.74 ± 1.29	12.65 ± 1.10	12.50 ± 0.707	0.912
PLT	3.00 ± 60.47	3.10 ± 75.33	3.09 ± 143.54	0.781
HbA1	63.26 ± 14.96	63.80 ± 10.75	62.50 ± 6.64	0.978
HbA2	4.11 ± 3.91	4.03 ± 3.89	2.90 ± 0.42	0.909
HbF	0.99 ± 2.17	0.38 ± 0.21	0.20 ± 0.00	0.230
HbS	36.61 ± 8.95	35.99 ± 3.82	36.85 ± 2.75	0.922

asymptomatic to a very severe of the patients with sickle cell disease [19] [20]. The reason for this variability may be explained by some other genetic changes, especially the SNPs in other positions of the HBB gene. In the present study we examined the association between HBB gene polymorphism rs33949930 (T>C) in the development of sickle cell disease in Saudi Arabia population.

Structural analysis of a fast-moving hemoglobin variant, present in three members of a Qatari family, identified a Val → Glu substitution at position 1 (NA1) of the β-chain. The introduction of this glutamic acid residue prevents the removal of the initiator methione, thus extending the N-terminus by one residue to Met-Glu-His-Leu-Thr. The methionine residue is blocked by an as yet not completely identified molecule. The presence of the variant in a heterozygote does not have clinical consequences [21]. Four hemoglobin variants had previously been described that involve the first codon of the HBB gene: Hb Doha (141900.0069), Hb South Florida (141900.0266), Hb Niigata (141900.0471), and Hb Raleigh (141900.0233). Although none of these variants cause any significant clinical problems, mutations of the first codon are of interest because of their potential interference with cotranslational modification at this site during beta-globin synthesis. In eukaryotes, the translation of all peptide mRNAs starts at an AUG codon, producing methionine at the beginning of the nascent peptide chain. Fisher *et al*. [22] identified a new Hb variant, Hb Watford, in which a GTG-to-GGG substitution caused a change of the first amino acid of the beta-globin chain from methionine to glycine, mimicking the gamma-globin chain. The proband was a 48-year-old female of Jewish extraction who was evaluated for chronic mild anemia. Another mutation was found in cis with the val1-to-gly mutation: Cap+36G-A.

In the present study we observed the similar distribution of the genotype percentages among cases and controls; accept slight difference in the heterozygous TC genotype of HBB gene polymorphism rs33949930 (T>C), which was higher among cases than controls. Allele frequency evaluation revealed non-significant distribution among the study groups. Odds and risk ratios were higher for heterozygous TC genotype with respect to the normal homozygous TT genotype of HBB gene polymorphism rs33949930 (T>C) among population of Saudi Arabia. When we correlated the laboratory findings of the sickle cell disease patients with the different variants of HBB gene rs33949930 (T>C) polymorphism observed some differential values; especially in HbA2 and HbF levels which were lower among patients carrying the heterozygous TC genotype; however HbS level was almost similar in association with different variants of HBB rs33949930 (T>C) polymorphism.

5. Conclusion

Our data suggest that there was not any significant association between HBB gene rs33949930 (T>C) polymorphism and occurrence of sickle cell disease. However, the heterozygous TC genotype of the polymorphism showed some higher ratios among cases as compared to healthy control group. The findings of the present study are limited due to smaller sample size under study groups; the importance of the heterozygous TC genotype of HBB gene rs33949930 (T>C) polymorphism in sickle cell disease can be validated by large sample size studies.

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Competing Interests

The authors declare that they have no competing interests.

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Clinical Effects of Hydrogen Administration: From Animal and Human Diseases to Exercise Medicine

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Abstract

Here we review the literature on the effects of molecular hydrogen (H_2) on normal human subjects and patients with a variety of diagnoses, such as metabolic, rheumatic, cardiovascular and neurodegenerative and other diseases, infections and physical and radiation damage as well as effects on aging and exercise. Although the effects of H_2 have been studied in multiple animal models of human disease, such studies will not be reviewed in depth here. H_2 can be administered as a gas, in saline implants or infusions, as topical solutions or baths or by drinking H_2-enriched water. This latter method is the easiest and least costly method of administration. There are no safety issues with hydrogen; it has been used for years in gas mixtures for deep diving and in numerous clinical trials without adverse events, and there are no warnings in the literature of its toxicity or long-term exposure effects. Molecular hydrogen has proven useful and convenient as a novel antioxidant and modifier of gene expression in many conditions where oxidative stress and changes in gene expression result in cellular damage.

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Keywords

Anti-Oxidant, Hydrogen Therapy, Gene Regulation, Gamates, Inflammatory Disease, Neurodegenerative Disease, Rheumatic Disease, Infections, Aging, Exercise, Metabolic Disease, Ischemia, Cardiovascular Disease, Neuromuscular Disease, Radiation, Skin, Sepsis

1. Introduction

Hydrogen (H) is the lightest and most abundant element in the universe; in its molecular form H_2 is a colorless, odorless, tasteless non-toxic nonmetallic gas [1]. Although hydrogen can burn at temperatures above 570˚C, at normal temperatures and partial pressures (at concentrations below 4%), it is a harmless gas that can act as a cellular antioxidant [1]-[3]. Hydrogen was first used as a medical gas in 1888 by Pilcher [4]. It was infused as a gas into patients' rectums to identify colorectal perforations in order to avoid unnecessary surgery [4]. Until recently hydrogen was thought to be physiologically inert [2], but in 2007 it was reported that hydrogen could ameliorate cerebral ischemia reperfusion injury and selectively reduce strong cytotoxic oxygen radicals, including hydroxyl radical (•OH) and peroxynitrite ($ONOO^-$) [2] [5]. This followed from experiments by Christensen and Sehested where molecular hydrogen was found to neutralize hydroxyl radicals in aqueous solutions at 20˚C [6].

The formation of oxygen and nitrogen radicals, as seen under conditions of oxidative stress, is thought to be an important if not an essential element contributing to the formation of a number of diseases, such as cardiovascular, rheumatic, gastrointestinal, neurodegenerative, metabolic, neoplastic and other diseases [2] [5] [7]-[10]. It is also important in tissue injury and aging [1] [2] [5] [7]-[11]. In this process, free radicals, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), are generated as by-products of oxidative metabolism. When in excess over endogenous antioxidants, ROS/RNS can induce casual and cumulative oxidative damage to cellular macromolecules, eventually resulting in cellular dysfunction, cell death and in some cases, leading to the development of various diseases [12] [13].

Mitochondria appear to be closely involved in oxidative stress and the aging process [7] [12]-[14]. They are the main intracellular source of free radical superoxide anion, as well as the initial target of oxidative damage [11]-[14]. Under physiological conditions, low concentrations of ROS/RNS are generated indirectly by the electron transport chain in the inner mitochondrial membrane, and these ROS/RNS are normally neutralized by cellular antioxidants [5] [7] [14] [15]. However, excess ROS/RNS generated under pathological conditions cause progressive oxidative damage to mitochondrial membranes, proteins and mitochondrial DNA and eventually other cellular constituents [16]-[19].

Mitochondrial dysfunction caused by excess concentrations of ROS/RNS is found in essentially all chronic diseases [17] [20]-[22]. Cell death is an important consequence of mitochondrial dysfunction, and the demise of cells can occur via a number of pathways that are initiated in mitochondria and involve apoptosis, autophagy and necrosis [20] [23].

Under normal physiological conditions ROS/RNS exist at low cell concentrations that do not cause excessive cellular damage. The levels of these potentially dangerous free radicals are kept in check by endogenous antioxidant systems that include superoxide dismutase, catalase, glutathione peroxidase and various vitamins [15] [20]-[24]. However, when the concentrations of ROS/RNS exceed the endogenous capacity to neutralize them, oxidative stress and cellular damage can occur. Excess production of ROS/RNS can occur due to a variety of exposures, from irradiation to chemical exposure or by physical stress [25]-[27].

2. Hydrogen Acts as a Cellular Antioxidant and Gene Regulator

Although historically hydrogen (H_2) was considered inert and nonfunctional [28], Ohsawa *et al.* [5] found that H_2 could act as a therapeutic antioxidant by selectively reducing cytotoxic ROS/RNS. We now know that H_2 can act as a cytoprotective anti-oxidation agent in isolated cells in culture, as well as in animals and patients [1] [2] [4] [5] [29].

H_2 acts by reducing the most reactive ROS and RNS oxidants, hydroxyl radical (•OH) and peroxynitrite ($ONOO^-$), but not the most plentiful ROS and RNS oxidants, hydrogen peroxide (H_2O_2) and nitric oxide (NO),

in cells and tissues [1] [2] [5] [29]. Thus H_2 can reduce oxidative stress and readjust the redox status of cells [30]. As a result of its mild but efficient antioxidant properties, H_2 can cause multiple effects in cells and tissues, including anti-apoptosis, anti-inflammation, anti-allergic and metabolic effects, in most cases by reducing oxidative stress and excess amounts of ROS/RNS [1] [2] [5] [29].

Hydrogen can also affect gene regulation that is modified or initiated by ROS/RNS, such as gene regulation by p53, AP-1 and NF-*κ*B [30]-[36]. Hydrogen has the ability to modify signal transduction. Using a rat liver DNA microarray the effects of hydrogen were examined on general gene expression [35]. After drinking H_2-enriched water for 4 weeks, the DNA microarray was used to show that 548 genes were up-regulated and 695 genes were down-regulated in hepatic liver gene microarray. The genes encoding oxidoreduction proteins were enriched in the up-regulated genes. Thus hydrogen can have both specific and general effects on cells and tissues.

3. Methods of Hydrogen Administration

Hydrogen has some distinct advantages as an antioxidant. Since it is a gas, it can be given by a variety of methods, and as a gas or as hydrogen dissolved in fluids, H_2 has extraordinary penetration and tissue distribution properties. Hydrogen as a gas dissolves in physiological fluids and distributes rapidly. It can easily penetrate cellular membranes and enter intracellular compartments [1] [2] [29]. Most antioxidant supplements are limited in their cellular distributions and are poorly taken up by organelles like mitochondria [37] [38], but hydrogen has the ability to effectively penetrate biomembranes and infiltrate into organelles, such as mitochondria and the nucleus. In contrast to many antioxidants, H_2 also has the advantage of being able to penetrate the blood-brain-barrier [39].

Inhalation of H_2 gas is the most straight-forward but not the most convenient method of medical hydrogen administration [1] [29]. At concentrations below 4% H_2 can be inhaled via mask, nasal cannula or ventilator. When inhaled at these concentrations, H_2 does not affect blood pressure [2] [4], and H_2 concentrations have been monitored in animal models by inserting hydrogen electrodes directly into tissues [40]. Inhalation of H_2 has been used in organ transplantation to reduce intestinal and pulmonary transplant injury and prevent organ inflammation [41]. Exposure to 2% H_2 gas also significantly improved gastrointestinal transit, reduced lipid peroxidation and blocked production of several pro-inflammatory cytokines [41].

In a mouse sepsis model H_2-treatment improved the survival rate and organ damage by reducing blood and tissue levels of early and late pro-inflammatory cytokines [42]. This same group investigated the effects of H_2 on survival, tissue damage, and cytokine responses in a zymosan-induced multiple organ damage and inflammation model [43]. They found that H_2 treatment reduced levels of oxidation damage, increased activities of antioxidant enzymes, and reduced the levels of pro-inflammatory cytokines in serum and tissues [43].

Hydrogen has also been administered as an injectable saline solution [44]. For example, Cai *et al.* [44] used H_2 in saline injected intraparatoneally into neonatal rats as a model of hypoxia-ischemia to demonstrate the neuroprotective effects of hydrogen. Using an Alzheimer's disease model in rats H_2-saline injections decreased oxidative stress and inflammation markers, and prevented memory and motor disturbances [45].

By far the easiest, most practical and effective method of H_2 administration is oral ingestion of hydrogen water [29]. Hydrogen dissolved in water is a convenient and safe means of delivering H_2 [46]. For example, H_2 can be dissolved in water at up to 0.8 mM at normal atmospheric pressure and room temperature, and it does not add taste, color or change in any way the characteristics of water. Once ingested, hydrogen-infused water passes quickly into the blood [47].

4. Safety of Hydrogen

Hydrogen has been used for years, without incident, in deep diving gas mixtures to prevent decompression sickness and arterial gas thrombi [48]. Even at relatively high concentrations, H_2 has been reported to have no toxicity [48]-[50].

The safety of H_2 in humans has been well documented in gas mixtures. For example, Hydreliox, a gas mixture used for deep diving, contains 49% hydrogen, 50% helium and 1% oxygen. Hydreliox was shown to be essential in preventing nitrogen narcosis and to prevent decompression sickness in working dives at great depths [48] [51]. In other deep diving studies H_2 was used during compression at 20 ATM to reduce bradycardia and other nervous and psychosensorimotor symptoms (high pressure nervous syndrome) without any long-term safety issues

[52]. Although a mild narcotic effect of hydrogen was detected from breathing hydrogen-helium-oxygen mixtures at high pressure, it was reversed upon return of the divers to ambient pressures [51].

Hydrogen in other forms, such as H_2-water, has not demonstrated any toxic or safety issues [1] [2]. For example, rats were fed H_2-water (0.19 mM hydrogen) or degassed water *ad libitum* for one year, and there were no reported changes in morbidity or mortality between the H_2 and control group of animals. There was, however, reduced periodontal damage in the H_2 group [53]. In clinical studies there were no reported toxic effects of ingesting H_2 [54]. Thus hydrogen is a safe and non-toxic substance when used at relatively low concentrations under normal conditions of pressure and temperature.

5. Hydrogen as a Therapeutic or Preventive Agent in Models for Human Diseases

Animal models of human disease have been used to test the therapeutic effectiveness of H_2 administration. This area has been amply covered in various reviews [1] [2] [29] [54]-[56]. For example, Ohno *et al.* [55] reviewed the effects of hydrogen in 63 animal models of human disease. They found multiple successful studies in animals where hydrogen had been administered as a gas (21 publications), by saline injection (27 publications) or as H_2 water (23 publications) [55]. Other publications have used ocular solutions containing H_2 [57], hydrogen-rich water baths [46], or direct instillation of H_2 solutions into the stomach or other organs [56]. Although most studies have used rodents as models, other animal models have also been used, such as rabbits or pigs [55] [56].

The first studies on the biology of hydrogen used hydrogen-producing algae and bacteria [59] [60]. Hydrogen has been found to promote growth of plants and regulate plant hormones and cytokines [61] [62]. Clinically, hydrogen has been used for a variety of conditions (**Figure 1**). Some of the most beneficial clinical uses of hydrogen will be discussed in this review.

6. Hydrogen and Ischemia/Reperfusion Injury

Many animal studies on the effects of hydrogen have used models for ischemia/reperfusion injury. Ischemia reperfusion injury is a phenomenon that is found clinically and can be attained experimentally. It is described as lack of oxygen supply to cells and tissues due to diminished perfusion, followed by local, and sometimes remote, inflammation due to acute reperfusion of the ischemic cells and tissues that may aggravate the original ischemic failure [2] [4] [29] [40] [41] [44] [62]. Several mechanisms have been proposed to explain ischemia/reperfusion, such as activation of redox signaling pathways, changes in mitochondrial permeability, autophagy, innate immunity, and other mechanisms [63]-[68]. Mitochondria appear to play an essential role in the process of ischemia/reperfusion [67]-[69].

Molecular hydrogen has been proposed as a possible protective molecule in ischemia/reperfusion [1] [2] [4] [29] [54]-[56]. In addition, recent evidence suggests that hydrogen might influence gene expression, possibly as a molecule that can counteract gene expression changes that occur during chronic adaptation responses to tissue damage [70].

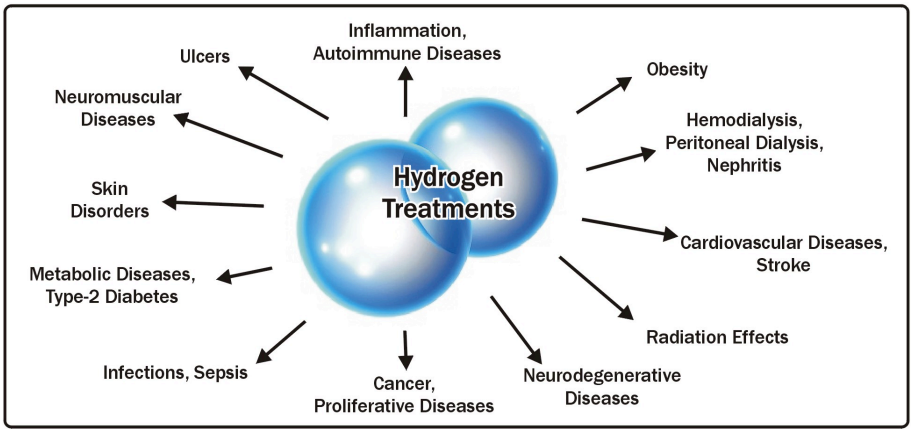

Figure 1. Hydrogen therapy and some of its uses in various acute and chronic clinical conditions.

While ischemia/reperfusion can occur in several organs, it is frequently observed in heart, brain, kidney, liver, retina, lungs, and the gastrointestinal tract [71] [72]. Molecular hydrogen has been used as a prophylactic and therapeutic agent for acute or chronic ischemia/reperfusion in those organs [1] [2] [54] [73]. As ischemia-reperfusion injury might play a significant role in organ transplantation, the effects of molecular hydrogen could also be important in organ transplantation [73]-[76].

The beneficial effects of hydrogen in ischemia/reperfusion models in animals have been extensively reviewed [29] [55]-[77]. Several possible uses of H_2 in humans has been mostly extrapolated or hypothesized from a relevant series of ischemia/reperfusion experiments performed in animals [1] [2] [29] [54]-[56] [73] [78].

Focusing on one organ, the heart, it has been shown that molecular hydrogen administered as a gas in rodents can improve the functional performance of the heart after cardiac arrest [39]. It does so with an efficacy comparable to hypothermia [39]. Hydrogen was proven to be better when H_2 gas was inhaled at 2% concentration, synchronized with the beginning of cardio-pulmonary resuscitation and continued for a minimum of two hours, reducing the increment of damage caused by ROS/RNS free radical reactions related to cardiac arrest.

Molecular hydrogen treatment has been shown to dramatically reduce the size of heart infarcts in rat models of myocardial ischemia/reperfusion injury [40]. It has been hypothesized that this is related to the rapid diffusion of molecular hydrogen gas, which is even faster than that of the coronary reperfusion after an ischemic incident, and its ability to interact with cellular free radicals [5]. ROS/RNS species seem to play a central role in ischemia reperfusion injury mechanisms, and the rapid diffusion of molecular hydrogen and its ability to counteract ROS/RNS species, especially hydroxyl radicals (•OH) and peroxynitrites ($ONOO^-$) [5], has been proposed to significantly reduce damage during ischemia/reperfusion injury. Thus, the experimental infarct size in rats can be significantly reduced with H_2 gas treatment [40].

A similar effect was reported using hydrogen in a saline solution; hydrogen saline protected against the damage produced by free radicals released during ischemia /reperfusion injury [79]-[82]. Hydrogen-saline has been also reported to improve heart failure produced by doxorubicin treatment in rats [83]. Combining H_2 with nitric oxide in a gas mixture also reduced free radicals, as well as protecting from heart damage and reducing infarct size in experiments done on murine hearts [84].

Cardioprotection by ischemic preconditioning or post-conditioning is an important approach to reducing ischemic/reperfusion cardiac injury [85]. Cardioprotection has been defined as “all mechanisms and means that contribute to the preservation of the heart by reducing or even preventing myocardial damage” [86]. Ischemic preconditioning is the protection conferred to ischemic myocardium by preceding brief periods of sublethal ischemia separated by periods of reperfusion [87]. Ischemic post-conditioning is the reduction of infarct size produced by several cycles of coronary occlusion/reperfusion after a sustained ischemia capable of producing an infarct [88]. In this process the opening of mitochondrial pores inhibited by ATP K^+ channels (mKATP) has been implicated in cardioprotecion, as their opening inhibits the permeability of the mitochondrial permeability pore (mPTP), a crucial event for ischemic reperfusion damage to take place [89] [90].

The administration of molecular hydrogen as a gas has been recently shown to activate mKATP, inhibiting mPTP, and thus acting as a cardioprotective agent in mice, rats and pigs [91]-[93]. More recent experiments performed in H9c2 cells in culture have shown that the induction of expression of antioxidant enzymes, such as heme oxygenase-1, by molecular hydrogen is another mechanism by which hydrogen prevents damage during ischemia/reperfusion injury [94].

In another variation of the use of hydrogen to reduce heart transplant damage, organ grafts for transplants were found to show enhanced preservation by submerging them in cold, H_2-supplemented water [54] [91]. For example, in rat heart transplants the grafts were better preserved in cold, H_2-supplemented water baths [91] [92]. Indicators of cardiac injury, such as the release of myocardial creatin phosphokinase and troponin I in serum, diminished significantly in grafts maintained in a cold, hydrogen bath [58] [95] [96]. Addition of hydrogen to HTK (histidine, tryptophan, ketoglutarate) solutions has also been reported to be a major improvement for preserving grafts during heart transplantation in rats [97]. The proposed mechanism is that molecular hydrogen prevents ROS and RNS production after excision of tissue and temporary ischemia and during grafting for transplantation.

Ischemic/reperfusion injury can also occur in gastrointestinal tissues where it can result in dysmotility, inflammation, and finally organ failure in grafts and transplants. Inhaled molecular hydrogen, or applied in H_2-enriched saline solutions, has been tested as a protective agent for gastrointestinal tract transplants in animal models in order to reduce oxidative stress in the grafts [98]-[101]. A recent report using rats has shown that it

has significant beneficial effects in small bowel transplants, when administered to the luminal side as an enriched hydrogen solution [102]. The antioxidant effects of molecular hydrogen, especially its attenuation of hydroxyl radicals, was proposed to play a significant role. Intestinal ischemia/reperfusion injury results in the release of a group of pro-inflammatory agents, such as tumor necrosis factor-α and interleukin-1β, plus neutrophil infiltration and peroxidation of membrane lipids. This damage, potentiated by the production of ROS, can be diminished by hydrogen-rich saline solutions in rats [103].

The lungs can be involved in ischemia/reperfusion injury, especially during lung transplantation, or cardiac bypass, by mechanisms that are not fully understood [104]. Lung ischemia/reperfusion injury is characterized by diffuse alveolar damage within the first few hours after transplantation. It seems to be related to multiple mechanisms comprising production of ROS, but also alterations in intracellular calcium, the Na-K pump and the production of pro-inflammatory factors [105]. Hydrogen has been applied in rats either by inhalation or hydrogen-rich saline solutions to prevent damage in lung grafts [41] [106]-[110]. Although part of the explanation in the improvement of lung grafts with hydrogen application is likely due to its effects in preventing ROS damage [102] [111], there is evidence that it also ameliorates the damage produced by pro-inflammatory agents and protecting against lipopolysaccharide induction damage [112] [113]. It has been also reported that the administration of hydrogen can protect against damage through the expression of proteins related to the production of surfactants, ATP synthases and stress-response molecules [113]. Recent experiments show that the combination of the administration of hydrogen and nitric oxide seems to be even more beneficial than hydrogen alone [114]. It is interesting that in this case, the protective effects of hydrogen are not fully correlated with its protection against oxidative damage, suggesting that hydrogen can also reduce adverse effects by other mechanisms [1] [2].

Retinal ischemia/reperfusion injury is associated with several diseases such as glaucoma, diabetes and several vascular disorders [115]-[117]. In all these cases, one of the damage mechanisms, is the production of ROS species leading to the oxidation of lipids, DNA and protein synthesis disorders, leading to cell death [118] [119]. Studies performed in rats have shown that hydrogen in eye drops, as a gas and in saline solutions, can protect the retina against oxidative and inflammatory damage produced by retinal ischemia reperfusion injury [57] [120]-[123].

Ischemia in the brain leads to temporary or permanent functional deficits. It has been reported that the immediate reperfusion of the brain to stop ischemic damage paradoxically can lead to additional damage due to a change in mitochondrial inner membrane potential and an excess of production of ROS [124]. This has been proposed to constitute the primary basis of brain ischemic reperfusion injury. Examples that can lead to brain ischemia with subsequent ischemia/reperfusion injury are stroke, trauma, and inflammation [125]. Inhalation of hydrogen or using hydrogen-saline solutions has proven to be beneficial for brain damage produced by traumatic injury in rats [126] [127]. Hydrogen-rich saline solutions have been effective after rat brain ischemic damage produced by cardiac arrest or vascular causes [128] [129]. Finally, the protective effects of inhalation of hydrogen gas in mice have been also observed in damaged brains after inflammation [130].

The use of hydrogen in humans has thus far only been tested for acute brain ischemia [131]. This safety study attempted to determine the equivalent concentrations of hydrogen in humans that can reproduce the results obtained in animal studies. The authors concluded that inhalation of 3% hydrogen for 30 minutes in humans is safe and that it might yield a similar hydrogen concentration in blood that have been shown to be useful in animals to treat or prevent this condition. However, studies of inhalation of hydrogen in humans can becomplicated, in part, because of variable hydrogen concentrations achieved in blood, and thus the results have lacked consistency. Because of this, the clinical use of hydrogen in acute brain injury needs further development [131].

7. Hydrogen and Metabolic Diseases

The most common metabolic disease is metabolic syndrome (MetSyn), which is a health condition characterized by a high increment for a group of risk factors that occur together (obesity, insulin resistance, dyslipidemia and hypertension). Collectively these factors increase the risk for coronary artery disease, stroke and type 2 diabetes mellitus (T2DM) [132]-[135]. In the 1980s the important role of insulin resistance in a number of diseases was determined, and this condition along with a group of risk factors was first named syndrome X but is now termed MetSyn [136]-[138]. Major clinical components of MetSyn are abdominal obesity, dyslipidemia with excessive flux of fatty acids, elevated blood pressure, and insulin resistance without glucose intolerance. When found together in the same patient, the long-term outcome is potentially life threatening, and in particular, there is a significant increase in risk for cardiovascular disease [135]. Also, MetSyn-linked T2DM and Alzheimer's disease

are interconnected with overlapping oxidative stress pathways. Though these pathways are not exactly the same in these two diseases, they can have a synergic combination of detrimental effects [139]. The incidence and prevalence of MetSyn strongly increases with age, and it is often found in older populations, especially in men, in association with sex hormones changes during aging [11] [140].

Oxidative stress is a major component in the pathogenesis of MetSyn [141] [142]. The levels of ROS/RNS are significantly increased in MetSyn, along with abdominal obesity and insulin resistance [143] [144]. The increased production of ROS/RNS free-radicals, which attack and oxidize polyunsaturated fatty acids in a process known as lipid peroxidation, is particularly prevalent in MetSyn. Peroxidized lipids are eventually converted into lipid hydroperoxides, such as conjugated dienes and malondialdehyde (MDA) [145] [146]. These peroxidized lipids are elevated in patients with obesity, MetSyn, and T2DM [147]. In addition to cellular lipids, ROS/RNS free radicals can also attack and modify carbohydrates, proteins and DNA [148] [149]. These ROS/RNS modified biomolecules have been used as oxidative stress markers [12] [14]. Peroxidation and ROS/RNS damage are particularly relevant in mitochondria dysfunction, but the loss of mitochondrial function can be prevented with antioxidant and phospholipid supplements [148].

Hydrogen-enriched water has been used to treat rat models of MetSyn. For example, SHR-ND rats are genetically modified rats that develop MetSyn, and they have hyperinsulinemia, hyperglycemia, hyperlipidemia with increased oxidative stress and inflammation [149]. Treatment of SHR-ND rats for sixteen weeks with hydrogen-rich water improved renal function (creatinine clearance increment by 22%, $p < 0.05$) [149]. Kidney damage as a result of glomerular sclerosis was also improved (reduced by 17%, $p < 0.05$). Finally, the total plasma antioxidant capacity as determined by Biological Antioxidant Potential or BAP, measured by standard reactions of Fe^{3+} to Fe^{2+}, was also improved by 22% ($p < 0.05$) [149]. The beneficial effect of hydrogen-enriched water has also been studied in rat myoblasts L6 cells in culture, where glucose uptake was reported to be dramatically increased [150].

Obesity is an important element and major risk factor of MetSyn, and it can constitute a pathological condition in which there is excessive accumulation of body fat along with a reduction in life expectancy and/or increased health problems [151]. Obesity is commonly related to an imbalance in the amounts and ratios of lipids in cells, tissues, and body fluids. In addition, during the development of obesity oxidative stress has been found to increase [152]. Drinking hydrogen-enriched water has been found to reduce hepatic oxidative stress in Db/db mice, which are lacking the leptin receptor [153]. It was found that H_2-enriched water enhanced the hepatic expression of the hormone fibroblast growth factor 21, which is involved in pathways of fatty acid and glucose expenditure in the body, leading to the stimulation of energy metabolism. In humans consumption of H_2-enriched water (1.5 - 2 L/day produced by reaction with magnesium sticks), over a period of 8 weeks, promoted antioxidant capacity in patients with MetSyn [154]. This was quantified by measuring the increased expression of antioxidant enzymes like superoxide dismutase (increased by 39%), and reduction of oxidative substances like MDA in urine (reduced by 43%, as measured by reaction with thiobarbituric acid). Dyslipidemia was also improved, since HDL increased by 8% and the ratio of cholesterol/HDL diminished by 13% [154]. No changes were observed in the fasting blood glucose levels for this time period [154]. Another study conducted in subjects with potential MetSyn found that the consumption of hydrogen-enriched water (1 L/day) for a period of about 10 weeks promoted a reduction in total cholesterol and in LDL-cholesterol serum levels [155]. In addition, HDL levels improved, as measured by different tests, indicating protection against LDL oxidation by an average of 31% ($p < 0.05$). The explanation for the changes in cholesterol and LDL lipoproteins were related to a reduction in apolipoprotein B10 and E. HDL was increased by several different mechanisms, such as protection against oxidation, and this resulted in inhibition of inflammatory cell adhesion and the effects of tumor necrosis factor-α in endothelia. It also resulted in stimulation of cholesterol efflux from macrophages [155].

Alterations in lipids or dyslipidemias, whether associated or not with obesity and MetSyn, are also improved by the use of hydrogen [156]. One of the ways hydrogen promotes such an action is by a reduction of the expression of the fatty acid translocase CD36, which diminishes excessive fatty acid uptake by human liver cells and promotes hepatic steatosis [156]. In animal models of dyslipidemias it was shown that lipid deposition in arteries is reduced upon drinking hydrogen-enriched water [157]. Also, improvements in plasma lipoprotein profiles, such as reductions in LDL-C, apoB and apoE by approximately 30% ($p < 0.05$), were observed in MetSynt apo-E knock-out mice. This was seen after four weeks of intraperitoneal injection of H_2-enriched saline solutions [158]. The same effect was observed in hamsters fed with a high-fat diet. The results suggest that H_2 has an important anti-atherosclerotic effect [158].

An important transport component in plasma membranes is the ATP-binding cassette transporter ABCA1, also known as the cholesterol efflux regulatory protein. The amounts of this membrane component have been associated with high-density lipoprotein deficiency [159]. It has been recently established in patients with hypercholesterolemia that the consumption of one L/day of H_2-enriched water for a period of 10 weeks can activate the ATP-binding cassette transporter A1-dependent cholesterol efflux system. This improved the function of HDL lipoproteins in patients with hypercholesterolemia in a double-blind, placebo-controlled clinical trial (47% reduction, $p < 0.05$) [160].

Another element of MetSyn is insulin resistance, a physiological condition in which cells fail to respond to the normal actions of the hormone insulin, leading to temporary or permanently increased blood glucose levels [136]. Insulin resistance is a major landmark property in the development of T2DM, which is characterized by elevated fasting glucose concentrations and dyslipidemia [136] [137] [161].

Oxidative stress also plays an essential role in insulin resistance [141]. In T2DM patients ROS damage accumulates in MetSyn and also acts at the level of trophic factors, impairs tolerance to glucose, activates pathways of apoptosis and autophagy, causes tissue remodeling, stimulates changes in the homeostasis of cellular energy, and modifies vascular biology [9]. In models for T2DM H_2-water has proven to be beneficial for treating diabetic symptoms in high-fat diet-fed animals [158]. Furthermore, in subjects with insulin resistance, as well as in T2DM patients, the addition of hydrogen to drinking water has proven to be beneficial for normalizing lipid profiles and glucose levels [162]. In this study thirty T2DM patients drank an average of one L/day of hydrogen-enriched or pure water for a period of 8 weeks, and then several biomarkers of oxidative stress, insulin resistance, and glucose metabolism were compared before and after the 8-week period. T2DM patients that consumed hydrogen-rich water showed a significant decrease in the levels of modified low-density lipoprotein (LDL, 15.5% decrease, $p < 0.01$) cholesterol, small dense LDL (5.7% decrease, $p < 0.05$), and urinary 8 isoprostanes (6.6% decrease, $p < 0.05$) [162]. H_2-water intake was also associated with a reduction of insulin-resistance and oxidative stress biomarkers, such as serum concentrations of oxidized LDL (5%, $p < 0.05$) as well as increased adiponectin (2%, $p < 0.1$) and increased extracellular-superoxide dismutase (2%, $p < 0.05$). In 4 out of 6 patients who consumed H_2-enriched water for 8 weeks, hydrogen normalized the glucose tolerance test ($p < 0.01$), while it also improved the secretion of insulin (56%, $p < 0.05$) [163]. Hydrogen may also be beneficial in type 1 diabetes, since it improves glycemic uptake by skeletal muscle in a type 1 diabetic animal model [163].

Since MetSyn can affect endothelial and smooth muscle cells, and these effects can be attenuated by hydrogen, H_2 may also be useful for reducing blood pressure. The beneficial effects of hydrogen on hypertension are described in the next section. Also, the atherogenic susceptibility due to dyslipidemia in blood vessels can be diminished by exposure to hydrogen, and this has been tested in transgenic (apo E−/−) mice [164]. Treatment of these mice with intraperitoneal injections of H_2-enriched saline for 8 weeks diminished the levels of the atherogenic apolipoprotein B (apoB) by 50% - 75%, in addition to other anti-inflammatory responses (suppression of proinflammatory interleukin-6 and tumor necrosis factor-α by 20% - 40%, $p < 0.05$). Lipid deposits in the arterial walls were also reduced significantly in the aortic root upon hydrogen administration (20% - 40% reduction, $p < 0.05$) [164].

Neointimal hyperplasia and advanced glycation in endothelial cells leads to apoptosis. Both can be prevented by applying molecular hydrogen or H_2-enriched saline in rats [165]-[167]. Thus, the use of hydrogen and H_2-enriched water should be very useful in preventing or delaying the appearance of MetSyn and associated diseases.

8. Hydrogen and Cardiovascular Diseases

The vascular system, including endothelial cells, surrounding matrix and smooth muscle and other cells, heart and lung tissues and blood circulatory contents, constitute the cardiovascular system. As mentioned in section 7, the vascular system can be involved in pathogenic changes, including dyslipidemia, protein changes, hypertension and other determinants of cardiovascular diseases (CVD). Most CVD, including stroke, myocardial infarction, peripheral artery disease, among others, involve circulatory plaque formation or atherosclerosis caused potentially by hypertension, obesity, diet, dyslipidemia, smoking, alcohol consumption, metabolic syndrome, diabetes and other factors [168].

Animal models have been established for studying the effects of various procedures and therapeutic agents on CVD, and hypothermia models have been used to assess the effects of temperature on the physiological effects that occur after cardiac arrest. These models mimic the sequelae of effects (often called post-cardiac arrest syn-

drome) that take place, such as neurological dysfunction, cardiac damage, systemic inflammation, among other problems [169]-[171]. For example, hypothermia has been used to protect neurons, cardiac cells, and reduce systemic inflammation in animals [169] [171] [172].

Using a hypothermia treatment model Hayashida *et al.* [40] compared the effects of H_2 gas, with or without hypothermia or hyperthermia alone, on cardioprotection in isolated, perfused rat hearts. They found that H_2 gas enhanced the recovery of left ventricular function following anoxia-reoxygenation, and reduced the infarct size without altering hemodynamic parameters. Hydrogen gas also prevented left ventricular remodeling [40]. This group later compared H_2 gas with therapeutic hypothermia in rats by examining the functional outcome of cardiac arrest, followed by mechanical ventilation (MV) and treatment in four groups of rats (group 1, controls; group 2, MV with 2% H_2-98% O_2 at normal temperature; group 3, MV with 2% N_2-98% O_2 at hypothermia temperature; group 4, MV with 2% H_2-98% O_2 at hypothermia temperature). After return of spontaneous circulation, group 4 animals showed better improvements in survival and neurological deficit scores. They also demonstrated increases in left ventricular end-diastolic pressures measured by a transducer catheter and increases in serum interleukin-6 levels in the H_2-treated animals [39]. One day later, the hearts were removed, fixed, and prepared for histological examination. Consistent with the end-diastolic pressures, water content in the lung as an indicator of edema was similar in control and H_2-treated but not the other groups. Standard histology revealed less severe perivascular and interstitial fibrosis, less inflammatory cell infiltration and other changes on the endocardial side of the myocardium in the H_2-treated groups. Using monoclonal antibodies against 4-hydroxy-2-nonenal to assess lipid peroxidation and antibodies against 8-hydroxy-deoxyguanosine to assess nucleic acid oxidation, Hayashida *et al.* [39] found that there were fewer positive cells in rats administered hydrogen gas, suggesting that inhalation of H_2 gas reduced oxidative myocardial injury.

The rat model of cardiac arrest has also been used to demonstrate that H_2 gas inhalation improves brain function and neurological outcome [173]. After cardiac arrest and resuscitation for 2 hours and after return of spontaneous circulation, the ventilated rats were randomized into four groups: group 1, 26% O_2 at normal temperature (control group); group 2, 1.3% H_2-26% O_2 at normal temperature; group 3, 26% O_2 and hypothermia; and group 4, 1.3% H_2-26% O_2 and hypothermia. The survival rates were as follows: group 1, 38.4%; group 2, 71.4%; group 3, 71.4%; and group 4, 85.7% (group 1 versus group 4, $p < 0.05$). Neurological deficit scores based on consciousness, breathing, cranial nerve reflexes, motor function, sensory function and coordination were scored after 24, 48, 72 hours and 7 days after arrest and resuscitation [174]. Neurological scores were significantly better in the H_2 group 2 ($p < 0.05$) and even more improved in the H_2 + hypothermia group 4 ($p < 0.01$). Neurological scores were also better in H_2 + hypothermia group 4 compared to hypothermia alone (group 3) ($p < 0.05$). A Y-maze test was used to assess motor activity and spatial memory at 7 days. Motor activity was significantly lower in the control group 1 ($p < 0.01$) and hypothermia group 3 ($p < 0.05$) compared to the hydrogen groups, whereas differences in spatial working memory at 7 days were not significantly different [174].

In other models for atherogenesis, such as the apolipoprotein E knockout mouse (apoE [−/−]), feeding H_2-saturated water *ad libitum* prevented development of atherosclerosis. Lesions stained by Oil-red-O in histological sections were significantly reduced ($p < 0.0069$) in the H_2-water group of mice at 6 months, and there was also a reduction in macrophages in the lesions in the H_2 fed mice [175].

The effects of hydrogen on hypertension have also been studied using animal models. For example, using a rat model based on monocrotaline-induced hypertension He *et al.* studied the effects of hydrogen water on pulmonary blood pressure, right ventricle weight and hypertrophy and pulmonary inflammation [176]. They found that all of these parameters were increased in the monocrotaline-treated groups, but oral or injected H_2 was found to prevent the development of hypertension and hypertrophy. They also utilized immuno-histochemistry to assess whether hydrogen prevented the monocrotaline-induced increase in 3-nitrityrosine and intercellular adhesion molecule-1-staining cells in the H_2-treated animals. Hydrogen treatment reduced the chronic inflammation in monocrotaline-treated animals [176].

In clinical studies, H_2-enriched water has been proposed to improve vascular health [177]. To assess vascular function and health a flow-mediated ultrasound dilation test was developed based on a pressure cuff on the brachial artery that was inflated to 50 mm mercury above systolic blood pressure for 5 minutes and then released [178]. After measurement of the brachial artery diameter and flow-mediated dilation at baseline, subjects drank H_2-enriched water or placebo water, and measurements were taken immediately or after a 30-min interval and flow-mediated dilation determined. In the H_2-enriched water group of 8 adult males and 8 adult females flow-mediated dilation increased from 6.80% ± 1.96% to 7.64% ± 1.68%, whereas in the 8 + 8 placebo group

flow-mediated dilation decreased from 8.07% ± 2.41% to 6.87% ± 2.94%, indicating a significant improvement ($p < 0.05$) [177].

The above studies indicate that H_2-water could be very useful in improving vascular health. Although long-term hydrogen-water studies on human subjects and hypertension and CVD have not yet been executed, this remains a viable area for further clinical research.

9. Hydrogen and Neurodegenerative Diseases

Neurodegenerative diseases are caused by the progressive loss of nerves or nerve function by cell death or dysfunction [179]. Neurodegenerative diseases include: amyotrophic lateral sclerosis or ALS, Parkinson's disease, Alzheimer's disease, Huntington's disease, among others, and these diseases are associated in that there are some similarities in the roles of genetics, neurotransmitters, protein misfolding and accumulation of toxic proteins, degradation pathways, membrane damage, and mitochondrial dysfunction that lead to nerve cell dysfunction and death [180]-[182]. Important among these parameters are the dysfunction of mitochondria and excess oxidative stress that can result in programmed cell death [180] [181]. Treatment of neurodegenerative diseases has generally not been successful, but one approach points to the potential of antioxidant agents for the treatment of neurodegenerative disorders [182]-[184].

Chronic oxidative stress has been proposed to be important in Parkinson's disease (PD) [185] [186]. Models of PD have been developed that show many of the neuropathological features of the disease, such as the degeneration of the nigrostriatal dopaminergic neural circuitry controlling motor function, the presence of cytoplasmic structural abnormalities in nerve cells, and other features [186]-[188]. Alternatively, animal models of PD have been used that are based on chronic physical restraint stress that show brain oxidative stress and learning and memory impairments [46] [187]. They also show suppression of neural proliferation in the dentate gyrus of the hippocampus [189]. Nagata *et al*. [46] used mice fed hydrogen water to suppress the oxidative stress associated with chronic physical restraint stress and showed that hydrogen prevented cognitive impairment. The proliferation of nerves in the dentate gyrus was also restored with treatment [46].

In PD the most characteristic feature is chronic dopaminergic cell loss in the *substantia nigra*that is associated with mitochondrial dysfunction and excess oxidative stress [190]. Using a rat model of PD that is based on 6-hydroxydopamine-induced nigrostriatal degeneration Fu *et al.* [191] placed H_2 in the drinking water of rats before and after sterotactic surgery and found that hydrogen prevented the development and further degeneration of substantia nigra in the central nervous system. In another model for PD mice can be given acute or chronic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine to stimulate oxidative stress and dopaminergic cell loss [192]. Feeding mice H_2-water (0.08 - 1.5 ppm [w/w] H_2) significantly reduced the loss of dopaminergic neurons in this chemically-induced mouse model of PD. Hydrogen in the drinking water also reduced the cellular accumulation of 8-oxoguanine, a marker of oxidative DNA damage, and 4-hydroxynonenal, a marker of lipid peroxidation, in the nigro-striatal dopaminergic pathway [193].

Recently, a pilot clinical trial was initiated to study the effects of hydrogen water on the progression of PD in Japanese patients [194]. The trial was a randomized, placebo-controlled, double-blind, parallel-group trial studying the effects of H_2 water in levodopa-medicated PD. Participants drank one liter per day of hydrogenated water or placebo for 48 weeks. Examining the Unified Parkinson's Disease Rating Scale scores in the placebo group ($n = 8$) showed that the PD worsened (mean score = 4.1 ± 9.2; median 4.5), whereas scores in the H_2 water group improved (mean −5.7 ± 8.4; median −1.0) over the course of the trial. In spite of the fact that the patient numbers were small and the short duration of the trial, the difference between the H_2 water group and placebo group was significant ($p < 0.05$). The results indicated that hydrogen water was a safe and well-tolerated treatment that yielded significant results in this short-term trial [194]. Follow-up will be necessary to see if the results hold-up for longer time periods, but the preliminary results in this pilot trial were encouraging.

10. Hydrogen and Neuromuscular Diseases

Neuromuscular diseases are represented by a heterogeneous group of disorders of the muscle, nerve or neuromuscular junction. They commonly lead to progressive muscle wasting and ultimately to premature death [195]. The most common neuromuscular diseases are Duchenne muscular dystrophy (DMD), spinal muscular atrophy, and congenital muscular disorders, comprising a large group of congenital muscular dystrophies or myopathies [196].

Although animal models for neuromuscular diseases exist and have been very useful for studying the genetics and other aspects of neuromuscular diseases [197]-[199], they have not been utilized to study the effects of hydrogen on these disorders. Instead, pilot clinical trials on the use of H_2-enriched water in neuromuscular diseases have been attempted. First, a preliminary open-label clinical trial was conducted on 15 patients (5 with DMD, 4 with polymyositis/dermatomyositis [PM/DM] and 5 with mitochondrial myopathies [MM]) [200]. Each patient was given one liter of hydrogen-enriched water per day in 10 - 12 divided doses for 12 weeks, and clinical signs and symptoms as well as 18 serum parameters and urinary 8-isoprostane were examined every 4 weeks. Although objective clinical signs and symptoms remained the same and did not improve (most symptoms) or improved somewhat (fatigue and myalgia), other symptoms worsened (floating sensations, diarrhea) in some patients. There were also some significant changes in laboratory parameters. For example, there was a significant decrease in lactate-to-pyruvate ratio in MM and DMD patients ($p < 0.05$), a decrease in fasting glucose in DMD patients ($p < 0.01$), and a non-significant decrease in serum triglycerides in PM/DM patients [200].

A randomized, double-blind, placebo-controlled, cross-over clinical trial was then conducted with 22 patients (10 DMD, 12 MM patients) that consumed 0.5 liter per day of H_2-enriched water or placebo water daily in 2 - 5 divided doses for 8 weeks [200]. Between the 8-week arms of the trial was a 4-week washout period. During the trial, signs and symptoms as well as 18 laboratory serum measurements were determined every 4 weeks. Throughout the study there were non-significant objective improvements or some worsening of clinical signs and symptoms. One DMD patient reported subjective improvements in fatigue and reductions in diarrhea on H_2-water, one MM patient complained of increased diarrhea but only initially on H_2-water, whereas another DMD patient reported improvements in myalgia on H_2-water. One MM patient had hypoglycemic episodes only on H_2-water, but the episodes subsided after the insulin dose was decreased. Only serum lactose levels were significantly decreased in MM and DMD patients ($p < 0.05$) in the H_2-enriched water arm. There were also some non-significant decreases in MM patients in the H_2-enriched water arm, such as serum lactate/pyruvate ratios, matrix metalloproteinase-3 and fasting glucose levels [200].

Although the clinical trials on the use of H_2-enriched water in neuromuscular patients were mixed in terms of results, longer, more robust trials with additional patients numbers seem to be warranted.

11. Hydrogen in Infections and Sepsis

The lack of a proper response to infections can eventually result in widespread tissue and organ systemic infectious damage or sepsis that can result in fatal outcome. Sepsis remains one of the most common causes of death in critically ill patients in hospital settings [201]. It is a complex continuum of systemic immune failure against proven or probable infections of bacterial, viral, or fungal origin [201]-[203]. An important factor in the complex process of the development of sepsis is oxidative stress and failure of antioxidant systems, resulting in mitochondrial failure, apoptosis, and activation of inflammatory, immune, hormonal, metabolic and bioenergetic responses [204] [205]. Also important is the loss of intestinal barrier and translocation of bacteria and endotoxin into the circulatory system [205]. Treatment strategies include fluid administration, antimicrobials (antibiotics, antivirals, antifungals), neuroendocrine, coagulation and cytokine normalization, and maintaining or restoringorgan function [201]-[204]. Recently, Xie *et al.* [42] [205] [206] have reviewed the possibility that hydrogen can be used in the treatment of sepsis.

To address the possibility of H_2 treatment for sepsis hydrogen treatment was developed in animal models of sepsis [42] [130] [205]-[207]. Using a mouse model initiated by cecal ligation and puncture initiation of 2% H_2 gas inhalation at 1 or 6 hours after puncture significantly improved the survival rate of septic mice [42]. Combining H_2 therapy with hyperoxia improved the survival rate even further and reduced sepsis markers, such as proinflammatory cytokines, and decreased histological damage to organs [206]. In a more elaborate series of experiments, inhalation of hydrogen gas was found to reduce neuroinflammation, oxidative stress, and neuronal apoptosis caused by sepsis.

Histopathologic changes in brain hippocampus were reduced, along with reductions in brain water content, inflammatory cytokines, and increases in brain antioxidant activities [130].

The protective effects of hydrogen gas on sepsis in mice were proposed to be due, in part, to the activation of heme oxygenase-1 (HO-1) and its upstream regulatory nuclear factor-erythroid 2 p45-related factor 2 (Nrf2) [207]. To demonstrate this, mice were subjected to sepsis by cecal ligation and puncture and were administered H_2 gas as above, and additionally, one hr prior, some mice received a zinc protoporphyrin HO-1 inhibitor. Se-

rum and organs were homogenized and protein and mRNA levels of Nrf2, HO-1 and HMGB1 were measured at 6, 12 and 24 hours. Hydrogen gas reduced the level of inflammatory cytokine HMGB1 and increased HO-1 and Nrf2 levels in septic mice. The protoporphyrin inhibitor eliminated the protective effect of H_2 on septic lung injury, indicating that hydrogen protection is partially mediated through the activation of HO-1 [207].

In a rat model sepsis was induced by cecal ligation and puncture, and hydrogen was introduced in saline intraperitoneally [208]. The researchers measured survival, cognitive function, ROS, malondialdehyde and caspase 3 levels, and superoxide dismutase activities were measured in the hippocampus to determine oxidative stress and apoptosis. Organ damage was assessed by histology. Cecal ligation and puncture resulted in poor survival rates, alterations in brain histology, and cognitive dysfunction. However, administration of the H_2-enriched saline solutions reversed these changes in a dose-dependent manner [208]. Hydrogen-enriched saline also reduced lung injury as indicated by increased gas exchange, reduced water retention in the lung, reduced nitrotyrosine content, maintenance of superoxide dismutase activities, and reduced histological changes in the rat lung tissue caused by sepsis [209]. The H_2-enriched saline also significantly inhibited activation of p38 and NF-κB and suppressed production of several pro-inflammatory cytokines. The authors concluded that the effects of hydrogen were likely due to the antioxidant and anti-inflammatory properties of hydrogen [205] [210].

As of this review, clinical studies on the effects of hydrogen on sepsis have yet to be reported [205]. However, the properties of hydrogen, including its rapid penetration of tissues and cells and ability to modulate oxidative effects without interfering with metabolic and signaling systems makes hydrogen a potentially useful treatment for sepsis [205].

In terms of hydrogen use in the treatment of infections, there is a clinical report on the effects of hydrogen water on viral load, oxidative stress, and liver function in patients with chronic hepatitis B infections [210]. Sixty patients were randomized to two treatment groups with or without H_2 water. The H_2 water group consumed 1.2 - 1.8 liter per day for 6 consecutive weeks, and serum oxidative stress, liver function and hepatitis B virus DNA levels were determined before and after routine treatment for hepatitis. Although there were no differences in the levels of alanine aminotransferase and viral load in both groups, significant differences were found in the activities of superoxide dismutase, glutathione S transferase and xanthine oxidase and levels of malondialdehyde that indicated excess oxidative stress in the control group not receiving hydrogen water ($p < 0.01$) [210]. Of note were the increases in superoxide dismutase and glutathione S transferase in the H_2 water group above controls, indicating the antioxidant properties of hydrogen water ($p < 0.01$). When the patients received treatment for their hepatitis B infections, liver function, monitored by the levels of alanine aminotransferase and total biliary acid, remained unchanged in the H_2 water group, but increased in the H_2 control group ($p < 0.01$ and $p < 0.05$), indicating the hydrogen protected the liver from treatment damage [210]. Since the activities of superoxide dismutase and glutathione S transferase in the H_2-water group remained higher than in the controls after treatment, the hydrogen treatment increased the antioxidant status of the hepatitis patients. Previous studies showed that hepatitis patients show increased oxidative stress and reduced antioxidant capacities [211]. Thus hydrogen-enriched water was able to improve oxidative stress status in the chronic infection patients and reduce some of the adverse effects of hepatitis treatment.

12. Hydrogen Effects on Radiation and Cancer Treatment

Hydrogen exhibits beneficial effects on tissues in organ transplantation and in the treatment of cancers and skin diseases, among other uses [111] [212]. For example, during cancer radiotherapy ionizing radiation causes damage to normal tissues, especially lung, heart and other organs [91] [111]. These radiotoxic effects are mainly due to production of hydroxide (•OH) and to a lesser degree other radicals [213] that damage DNA, proteins, lipids and carbohydrates [213]-[215]. Since hydrogen can neutralize free radicals, such as •OH and other ROS/RNS, this suggested that hydrogen might be useful as a novel protection agent for irradiated tissues [212] [216]-[218].

Experimentally, hydrogen has been used to protect against various types of radiation damage in a variety of animal tissues [212]. Some examples are: skin [219]-[221], intestine [216], lung [111] [212], heart [91] [216], brain [212] [222], bone marrow [216] [223] [224], testis [218] [247], and other tissues [212]. Of special clinical importance is the radioprotection of radiation-sensitive tissues, such as bone marrow, because these are the most likely to be damaged by radiation [212].

The radioprotective effects of hydrogen were also found when human cells and tissues were examined [212].

For example, treatment of human intestinal crypt cells, with or without hydrogen-rich phosphate-buffered saline before exposure to gamma radiation (up to 8 Gy), resulted in significant reduction of radiation-induced apoptosis and an increase in viability in the H_2-enriched phosphate-buffered saline-treated cells [224]. In contrast, if the cells were treated with the H_2-phosphate-buffered saline after radiation exposure, then the protective effects of hydrogen were not seen [224].

Hydrogen has also been used in cancer therapy. Direct treatment of skin cancers with hydrogen was first proposed by Dole *et al*. [225]. They used hyperbaric hydrogen to treat hairless albino mice with cutaneous squamous cell carcinomas. The mice were exposed to a 97.5% hydrogen-2.5% oxygen gas mixture at pressures of 8 atmospheres for periods up to two weeks to see if the gas mixture could cause regression of the skin tumors. They found that the skin tumors regressed and proposed that hydrogen might be useful for the treatment of other types of tumors by suppressing free radical production [225]. Later, Roberts *et al*. [226] examined the responses of five established transplantable mouse tumors and one mouse leukemia to hyperbaric hydrogen and found that H_2 gas could suppress the growth of tumor cells. The actions of molecular hydrogen were established as antioxidant (and therefore anti-oxidative stress), anti-inflammatory, and anti-apoptotic in animal systems [1] [2] [40] [56] [82] [91] [111] [212]-[216].

In clinical studies on the radiation treatment of liver tumors Kang *et al.* [227] studied the effects of hydrogen-rich water on quality of life (QOL) parameters. Acute radiation-induced side effects often include fatigue, nausea, diarrhea, dry mouth, hair loss, skin sores, loss of appetite, changes in taste, and depression [228]. To test if H_2-enriched water reduced adverse effects of radiotherapy and improved QOL scores, these investigators enrolled 49 patients (33 men and 16 women) with hepatocellular carcinomas in a clinical study. The participants were randomized into H_2-water and placebo water groups, and each patient received 5040 - 6500 cGy of radiotherapy over a 7 - 8 week period. During the course of therapy each patient consumed 1.5 - 2.0 liters of H_2-water or placebo control water each day. At the end of the treatment period all of the patients were evaluated for clinical response of their carcinomas to the radiotherapy and QOL determinations were made. Although the responses to radiotherapy were unchanged by the intake of H_2-water, overall QOL scores were significantly improved in the H_2-water group. For example, there were significant reductions in appetite loss and tasting disorders, but no differences in sleep parameters, diarrhea and vomiting [228]. During the course of the trial radiotherapy resulted in significant increases in serum hydroperoxide in the control group that were not seen in the H_2-water group, indicating reductions in oxidative stress in the patients drinking H_2-water. There were no differences found in liver function tests or blood tests, indicating that the H_2-water was a safe and effective means of improving QOL in patients receiving radiotherapy [228].

In order to prevent or retard human skin damage after UV exposure hydrogen-enriched water has been directly applied to the dermis [229]. The H_2-water application prevented UV-induced ROS/RNS and the induction of damage-associated mRNAs for MMP-1 and COX-2, as well as proinflammatory cytokine mRNAs for interleukin-6 and interleukin-1β tissue. The application of H_2-wateralso increased the expression of collagen genes [231]. When elderly human subjects were examined, their constitutive levels of expression of MMP-1, interleukin-6, and interleukin-1β were higher but could be significantly reduced ($p < 0.01$) with application of hydrogen-enriched water. The local application of H_2-water also increased the expression of procollagen mRNA [229]. These studies indicated that H_2-water has a radioprotective effect on skin, and H_2-water can also reduce the increased expression of skin inflammatory cytokines.

An interesting potential use of hydrogen is its ability to protect against graft-versus-host disease (GvHD) [230]. Hematopoietic stem cell transplantation has been successfully used for the treatment of certain leukemias and other malignant and non-malignant hematologic diseases [231]. However, GvHD is a severe complication that can limit its application. Qian and Shen [230] have proposed that hydrogen therapy be used to reduce ROS/RNS important in the development of GvHD and reduce the levels of inflammatory cytokines that play a role in the development of GvHD.

13. Hydrogen in Skin and Aging

One of the most visible signs of aging is a change in the appearance of skin. Some of the hallmarks of aging skin include increased fragility and diminished collagen production, resulting in loss of elasticity and wrinkles. These negative characteristics are caused primarily by exposure to ROS/RNS that damage cellular proteins, membranes and DNA [224] [232].

The ROS concentrations in skin are among the highest of any other organ because of exposure to extrinsic environmental factors, such as ultraviolet light, ionizing radiation and pollutants. Cosmetic interventions to improve skin appearance—including pharmaceutical, surgical, and topical approaches—are considered temporary solutions, unless they deliver antioxidants to skin tissue and prevent ROS/RNS damage. Antioxidants effective in reducing ROS/RNS have been proposed to hold promise in improving skin structure and appearance [224] [233].

Antioxidants have been delivered to skin in lotions, creams and oils, and by bathing [232] [234]. For example, molecular hydrogen is considered a novel antioxidant for combating oxidative damage in skin and promoting a youthful appearance, and it has been used in water for bathing. By bathing daily for 3 months in H_2-water (0.2 - 0.4 ppm H_2), Japanese subjects showed significant improvements in neck wrinkles at the end of the 90-day bathing sessions [235]. This same publication examined the ability of H_2-enriched water to stimulate production of type-1 collagen in skin fibroblasts and keratinocytes after UVA-exposure. They found that type-1 collagen synthesis was increased over 2-fold after 3 - 5 days in the H_2-enriched water samples compared to controls [235].

Another approach has been to drink H_2-water. Using healthy four month-old rats fed H_2-enriched or control water the effects of H_2-water have been examined in aging periodontal tissues [236]. The animals fed H_2-water and control water were examined after 16 months. At this time, the animals were examined for the expression of inflammation-associated genes. Although the expression of interleukin-1β was not different between the two groups of animals, the H_2-water fed group was found to have activated Nod-like receptor protein 3 inflammasomes in periodontal tissue. In addition, oxidative damage was determined in periodontal tissue by measuring the levels of 8-hydroxydeoxyguanosine (8-OHdG) as a marker for DNA damage. Over time 8-OHdG levels increased in the control group ($p < 0.05$), but in the H_2-water fed group the levels of 8-OHdG were significantly lower than the control animals ($p < 0.05$). Also, the serum levels of 8-OHdG were examined. In the control group the serum levels of 8-OHdG increased in an age-dependent manner, whereas in the H_2-water fed group the serum levels of 8-OHdG did not change during aging [236].

When periodontal tissues were examined histologically in the H_2-water and control water animals, the linear distances between the cemento-enamel junction and the alveolar bone crest were significantly lower in the H_2-water fed group than in the control water group ($p < 0.05$). These authors also examined the level of alveolar bone loss for the medial root region of the first molar, but significant differences were not found. In addition, the numbers of TRAP-positive osteoclasts were lower in the experimental H_2-water group than in the control group ($p < 0.05$), but there were no significant differences in the ratios of interleukin-1β-positive cells to total cells between the two groups [236]. Interestingly, examination of gene expression in H_2-water and control animals revealed that the expression of inflammasome NLRP3-associated caspase-1, ASC and interleukin-1β in periodontal tissues was higher in the H_2-water group ($p < 0.05$), whereas expression of NF-κB was significantly lower in the H_2-water group ($p < 0.05$). Thus although drinking H_2-water decreased oxidative damage to DNA, it did not suppress inflammatory reactions in aging periodontal tissue [236].

The protective effects of hydrogen have also been examined in animals exposed to cutaneous burns [237]. Rats were divided into sham, burn plus saline, and burn plus H_2-enriched saline groups and analyzed at various times (6, 24 and 48 hours) after burning by contact with a hot metal comb for 20 seconds. Indexes of oxidative stress, apoptosis and autophagy were measured in each group, and the zone of stasis was evaluated using immuno-fluorescence staining, ELISA, and Western blot analysis. H_2-enriched saline, but not control saline, attenuated the increases in apoptosis and autophagy seen in burn wounds, as measured by the expression of TUNEL staining and the expression of Bax, Bcl-2, caspase-3, Beclin-1, and Atg-5 proteins. Additionally, H_2-saline treatment lowered the level of myeloperoxidase and expression of inflammation markers tumor necrosis factor-α, interleukin-1β, and -6 in the zone of stasis while augmenting interleukin-10. The elevated levels of Akt phosphorylation and NF-κB p65 expression post-burn were also down-regulated by H_2-saline treatment [237]. The results indicated that H_2-enriched saline treatment reduces the inflammation associated with cutaneous burns.

When skin is burned, there are typically changes in the epidermis and dermis tissue. Sections of the skin from H_2-saline and control saline treated animals were examined. The interspaces between two burn wounds in saline control animals gradually narrowed and had a tendency to merge following the burn, whereas the interspaces remained relatively stable at various time points in the H_2-saline treated skin. Certain characteristics, such as severe epidermis layer thinning, epithelium nuclei elongation, and dermis layer swelling with collagen alterations, could be observed in the normal saline-treated animals, whereas in the H_2-enriched saline-treated animals these

changes were alleviated over time [237].

Lipid peroxidation was also examined in the animals after burning their skin [237]. Skin tissue homogenates from the burn wounds reacted with thiobarbituric acid-reactive species (TBARS), a method that has been used to determine the malondialdehyde (MDA) levels. Tissue superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) activities were also evaluated to determine the oxidative stress status in the skin tissues of burn wounds. The burn-induced increases in MDA were reduced in the animals treated with H_2-saline, while the activities of endogenous antioxidant enzymes were significantly increased. The results indicated that H_2-enriched saline treatment attenuates burn-induced oxidative damage in the burn-wounded tissue of rats by inhibiting oxidative stress and increasing the activities of endogenous antioxidant enzymes [237].

Pressure ulcers are a routine problem in long-term hospitalization of aged patients. Li *et al.* [238] examined the effects of H_2-water in 22 elderly Japanese patients (mean = 86.7 ± 8.2 years) with pressure ulcers. The purpose of this study was to clarify the clinical effectiveness of H_2-water given by tube feeding. All patients received routine care treatments for pressure ulcers in combination with H_2-water (600 mL per day) for partial moisture replenishment. Routine care included: ointment, gauze dressing, wrapping, and bed-pad use after washing by the acidic water disinfection. Pressure relief modalities and nutritional support were also employed. The 22 patients were divided into two groups: an effective care group (EG, n = 12) and a less effective group (LG, n = 10) according to the outcomes of endpoint evaluation and healing criteria. Pressure ulcer hospitalization days in EG patients were significantly shorter than in LG (113.3 days versus 155.4 days, $p < 0.05$), and the reduced rate was approximately 28.1% less. In both the EG and LG groups the wound size reductions (91.4% and 48.6%, respectively) were statistically significant with the intake of H_2-water ($p < 0.05$). The results demonstrated that H_2-water intake via tube feeding reduced wound size in hospitalized elderly patients with pressure ulcers [238].

14. Hydrogen in Reproductive Tissues, Pregnancy, Neonatal Development and Newborns

For successful reproduction healthy gametes (haploid cells) are necessary. The process of gamete formation takes place in the male and female gonads following meiotic cell divisions in the testis and ovaries. Oxidative stress during gamete formation is a potential risk and can lead to problems in fertility [239]-[243]. Bearing this in mind, hydrogen treatment has been used experimentally to reduce oxidative stress in both sexes.

Experiments performed in animal models have shown that H_2-enriched saline can protect rats and mice testis against oxidative stress that occurs during ischemia/reperfusion injury or oxidative stress induced by nicotine [244]. Ischemia/reperfusion injury in testis can also be produced by torsion-detorsion movements, which causes irrigation to testes to be diminished. Administration of H_2-enriched saline (5 ml/Kg) by intraperitoneal injection immediately after the injury reduced the testis levels of several oxidative markers, such as superoxide dismutase and MDA, compared to those animals in which no treatment was given [244].

Cigarette smoking and nicotine exposure, a common worldwide problem, also increases oxidative stress. By mechanisms that are still not clear, it has been shown that long-term nicotine exposure related to cigarette smoking increases oxidative stress in testis [245]. Mice with nicotine-induced oxidative damage in testis treated with long-term H_2-enriched saline (6 ml/Kg) showed reduced damage in their gonads [244].

Other factors can also damage sperm. For example, sperm cells in gonads are especially susceptible to radiation. One of the mechanisms implicated in radiation damage to testes is the production of hydroxyl radicals [246]. Use of H_2-saline pretreatment in mice exposed to ionizing radiation resulted in diminished radiation damage, such as reductions in lipid peroxidation, oxidation of proteins, and DNA damage, in testicular tissue. The number and quality of sperm after hydrogen treatment was also improved, and this has been related to reduced oxidative damage [247]. For example, the production of •OH in sperm, as monitored by spin-trapping methods, was diminished up to 80% by suspending them in media containing 0.8 mM H_2. In addition, apoptotic morphological modifications as well as chemical changes characteristic of apoptosis (measured by TUNEL terminal deoxy-nucleotidyl-transferase-mediated dUTP nick-end labeling) were reduced by 40% after H_2 treatment. In addition, daily sperm production and their quality can be evaluated by staining with WR-2721 [*S*-2-(3-aminopropylamino)ethyl phosphorothioic acid], and high-quality sperm could be increased up to 30% after exposure to ionizing radiation and treatment with hydrogen compared to radiation alone [247].

An essential factor in male fertility is sperm motility. Sperm motility, which can be assessed by Computer

Assisted Sperm Analysis, can be used to predict male fertility [248] [249]. Oxidative stress can diminish both sperm motility and fertility [250] [251]. However, after hydrogen exposure, the forward motility of human sperm was found to increase from 17.5% to 40% after treatment with hydrogen for 30 minutes. This increase in motility of the hydrogen-treated sperm was also preserved in treated and frozen sperm, in comparison with nitrogen-treated controls. In agreement with this finding, exposure to hydrogen also restored and improved mitochondrial potential, as evaluated by fluorescent redox dyes, indicating that hydrogen could be a promising new therapy for male infertility [252].

During embryo development and pregnancy, oxidative stress can lead to different tissue alterations and newborn diseases [253] [254]. The use of hydrogen as a possible therapeutic approach for diseases during pregnancy has been tested *in vitro* with trophoblast cell lines (JAR, JEG-3) [255]. This is important, as treatments with antioxidant vitamins (C and E) have been shown to be detrimental on placental function, as determined by decreased cell viability, decreased secretion of hormones and increased production of tumor necrosis factor-*α*. In contrast to vitamins, hydrogen does not cause any detrimental effects. Moreover, molecular hydrogen promoted the secretion of human chorionic gonadotrophin (hCG) by these cells, suggesting that hydrogen might be a suitable antioxidant for the management of diseases, such as preclampsia during pregnancy [255]. The administration of hydrogen to pregnant rats has been reported to be beneficial for ischemia/reperfusion injury and hippocampal damage in fetal rats [256]. In these animals, ischemia/reperfusion injury was performed by transient occlusion of the bilateral utero-ovarian arteries. Two days before the operation to evaluate fetal damage and placental status rats drank H_2-enriched water. When H_2-enriched water was administered to the rats, their placenta showed less evidence of oxidative damage, and in the fetal tissues less neuronal damage was found in CA1 and CA3 hippocampal regions. Oxidative stress markers were also ameliorated when H_2-enriched water was given to the rats. These studies suggest that hydrogen intake by pregnant mothers may prevent hippocampal damage produced by ischemia/reperfusion injury in the offspring [256].

Newborns have a high risk of excess oxidative stress during birth and in the first previous and post-partum months due to an increased rate of hypoxia or ischemia [257]. With that rationale in mind, several experiments in animal models have been performed in neonates. The first series of experiments performed in 2009 showed that hydrogen gas is not effective when there is moderate or severe hypoxia and ischemia in neonatal rats [258]. If there is any asphyxia during birth, neurovascular dysfunction is seen shortly after in an event known as delayed neurovascular dysfunction [259]. Hydrogen-treated newborn pigs showed less cerebrovascular reactivity of pial arterioles compared to those that received no treatment after asphyxia. Thus hydrogen had a neuro-protective effect during these accidents of birth [259].

Hemorrhage of the germinal matrix (GMH) is a neurological disease associated with low-weight premature birth leading to hydrocephalus, cerebral palsy, and mental retardation [260] [261]. The occurrence of the disease is related to oxidative stress [262]. The inhalation of hydrogen gas early after neonatal GMH reduced the incidence of cerebral palsy and mental retardation in treated rats [263]. This was evaluated at juvenile stages. Brain atrophy, splenomegaly and cardiac hypertrophy were also normalized one month after injury. These results suggest that the inhalation of hydrogen gas in low-weight premature neonates could be an important method for reducing GMH and its consequences [263].

Finally, necrotizing enterocolitis (NEC, inflammation and death of intestinal tissue) can also be observed after premature birth, leading to increased mortality [264]. In rat models of this disease it has been shown that the administration of hydrogen-rich saline to neonates is an effective way to protect premature neonates from NEC, which usually takes place two weeks after premature birth [265]. NEC can be induced in neonate rats by formula feeding plus asphyxia and cold stress. In this animal experiment the neonates were administered hydrogen intraperitoneally with H_2-enriched-saline (10 ml/Kg) or normal saline before asphyxia was induced twice daily in 10 min periods. Monitoring of rat neonates continued up to 96 hours after birth and then several indicators of injury by NEC, such as body weight, histological NEC score, survival time, malondialdehyde anitoxidant capacity, inflammatory mediators, and integrity of the mucoses, were evaluated. On average, H_2-enriched saline pretreatment reduced the damage scores by an average of 40%. With hydrogen pretreatment the survival rate was increased by 172% (from 25% to 68%) [265].

15. Hydrogen in Inflammatory Diseases

Inflammation is an innate cellular and humoral response that takes place in a multicellular organism after an in-

jury in an attempt to restore the organism to its preinjury state by removing one or more injurious agents, repairing injured tissue, or both [266]. More than the timing of the response, it is the nature of the inflammatory cells that are immediately involved in the inflammatory response and its resolution after an injury to tissue that classifies inflammation as either acute or chronic. An early marker of an acute inflammatory response is the adhesion of neutrophils to the vascular endothelium or blood vessels, a phenomenon known as "margination." Acute inflammation is almost totally resolved by tissue response to the injuring agent. In contrast, chronic inflammation is characterized by its persistence or lack of resolution when the response is unable to overcome the effects of the injuring agent [266]. Both inflammatory processes are important mechanisms of defense against injuries, and they are associated with increased levels of ROS and RNS generated by the respiratory bursts of immune cells related to the inflammatory response. The increase of ROS/RNS species has two consequences: 1) oxidative/nitrosative modifications of biomolecules, and 2) the reversible triggering of ROS/RNS signaling cascades that strongly modulate the inflammatory response [267] [268]. Inflammation can also be classified with regard to the nature of the injuring agent. For example, it can be the result of a biological or non-biological event, such as an infectious inflammation or a sterile inflammation (trauma, chemicals, ischemia/reperfusion) [267].

Hydrogen has been used to treat both biological and non-biological inflammation. For example, studies with zymosan treatment or using sepsis as a model have utilized hydrogen treatment. Zymosan is a glucan polysaccharide usually found in fungus, and it has been used to promote generalized inflammation in animal models. The inhalation of 2% H_2 gas in mice for 1 - 6 hours post-zymosan injection improved the survival rate at day 14 after the injection from 10% to 70% [43]. The organ damage, as monitored by multiple biomarkers, such as aminotransferases, urea, and creatinine, as well as histopathological organ studies, was significantly reduced in all cases after H_2 gas inhalation. Additionally, it was found that inhalation of H_2 gas decreased oxidative products and proinflammatory cytokines, while it increased antioxidant levels [43]. Another form of acute inflammatory response, sepsis, has been discussed in another section of this review. It is interesting that H_2-enriched saline stimulates recovery of generalized organ function in rat models of polymicrobial sepsis, resulting in decreased proinflammatory responses, oxidative stress, and apoptosis [269].

General Inflammation can be observed during allogeneic hematopoietic stem cells transplantation in hematological diseases undergoing acute-graft-versus-host disease (aGvHD). This complication is often lethal, and it decreases the efficacy of therapy and worsens prognosis in these patients. Inflammatory agents, such as cytokines, including interleukin-6 and ROS (such as hydroxyl radicals), play critical roles in GvHD. As previously discussed in this review, hydrogen lowers the expression of pro-inflammatory agents and acts as a powerful scavenger for hydroxyl radicals. Experiments done with bone marrow transplantation in mice with the complication of GvHD showed that exposure to hydrogen-rich saline solutions after transplantation results in an increase in the survival rate and improvements in all biomarker scores used for monitoring GvHD [270].

Autoimmune disorders occur when the immune system of an organism attacks and destroys healthy body tissue by mistake. More than 80 types of autoimmune disorders have been described [271]. Patients may have several autoimmune disorders at the same time. Though the ultimate causes of autoimmune disorders remain unknown, it is believed that this disorder is related to certain individuals as well as unique antigens from bacteria, viruses, and fungi that can confuse the normal responses of the immune system and result in recognition of self as foreign. The results of this can be an immune attack on self-antigens and promotion of inflammatory reactions, leading to destruction of body tissue, changes in organ function or abnormal growth of tissues [272].

Among the more common autoimmune conditions, rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by joint dysfunction. The joints affected are usually symmetrical, involving hands, knees and other joints, with symptoms often being the most severe in the morning. RA causes pain, swelling, and stiffness in the joints, and in time this may cause severe joint damage, loss of function, and eventual disability. The disease can last from months to a lifetime, and symptoms may improve and then worsen over time. At variance with osteoarthritis, which is limited to the joints, RA is a systemic disease that involves other body systems. Over time, both forms of arthritis can coexist in the same patient [273]. As an example of a systemic manifestation, RA is also associated with an increased risk of atherosclerosis and cardiovascular disease [274].

Chronic inflammation and increased ROS/RNS production with a central role for the hydroxyl radical in RA have been proposed to explain the destruction of bone and cartilage, two of the most common sites of damage in most types of RA. Clinical groups in Japan have been pioneers in studying the possible therapeutic use of hydrogen in RA patients. For example, a pilot study performed in Japan with 20 RA patients showed that drinking daily 530 ml of H_2-enriched (5 ppm) water for a total of 8 weeks resulted in the reduction of biomarkers of RA

damage, such as urinary 8-hydroxydeoxyguanine (8-OHdG) and DAS28 (C-reactive protein), of 14.3% and 21.1%, respectively, after one month ($p < 0.05$). After two months of consuming hydrogen-enriched water, urinary 8-OHdG was stable and DAS28 showed a further decline of 20.41% ($p < 0.05$). Interestingly, after hydrogen treatment in 5 of the patients with early manifestations of RA, 4 of them showed complete remission of the disease, becoming symptom-free with no further evidence of elevations in biomarkers of the disease [274] [275]. Treatment with hydrogen also proved useful in preventing RA-related atherosclerosis [274].

Additional clinical trials with RA patients were also demonstrative. A double-blind, placebo-controlled trial in 24 patients receiving 500 ml/day of H_2-enriched (1 ppm) saline intravenous infusions for 5 days during a 4-week period showed the impact on biomarkers for RA, such as urinary 8-OHdG, DAS28, tumor necrosis factor-α, interleukin-6, and matrix metalloproteinase-3. There was a 30% reduction in DAS28 in the hydrogen treatment group compared with placebo after a 4-week period of treatment ($p < 0.05$). The placebo-control patients showed no change in the levels of DAS28. Additionally, interleukin-6, matrix metalloproteinase-3 and urinary 8-OHdG were also reduced by 37.3%, 19.2% and 4.7%, respectively, in the hydrogen treatment group ($p < 0.05$). In contrast, the levels of interleukin-6 and matrix metalloproteinase-3 increased in the placebo group by 33.6% and 16.9%, respectively ($p < 0.05$). Tumor necrosis factor-α levels did not change significantly in the H_2-saline or placebo groups [276].

There are also other conditions associated with RA. For example, skin lesions like psoriasis are often observed in RA patients. The administration of H_2-enriched (1 ppm) saline by intravenous infusion, drinking H_2-enriched water (5 ppm), or using 3% H_2 gas inhalation over a period of 4 weeks improved all of the symptoms of psoriasis (psoriasis area severity index, PASI, or biomarkers DAS28 and interleukin-6) by an average of 20% in three patients ($p < 0.05$). The psoriatic lesions almost disappeared in all of the patients treated with hydrogen [277].

Hydrogen treatment has also been tested in molecular and general models of inflammation in animals. It has been shown that hydrogen interferes with nitric oxide (NO) pathways that have been implicated in the generation of peroxynitrites. In particular, the lipopolysaccharide/interferon-γ-induced NO production in murine macrophage RAW264 cells was reduced upon hydrogen exposure. This result, in turn, was associated with a reduction of the inducible isoform of nitric oxide synthase (iNOS). Treatment with H_2 inhibited lipopolysaccharide/interferon-γ-induced phosphorylation of the apoptosis signal-regulating kinase 1 (ASK1) and its downstream signaling molecules, such as p38 MAP kinase, JNK, and IκBα. Drinking H_2-water also ameliorated the levels of anti-type II collagen antibody-induced arthritis in mice (an animal model for human RA) [278].

Injection of carrageenan polysaccharides into mice paws can generate acute inflammation with edema, presence of lipopolysaccharide-activated macrophages, secretion of tumor necrosis factor-α by macrophages and infiltration of neutrophils [279]. All these parameters were mitigated by an average of 40% after 4 hours of injection of H_2-enriched saline (2.5 to 10 ml/Kg) ($p < 0.05$) [279]. Consumption of H_2-water by mice also improved lipopolysaccharide-induced neuroinflammation [280]. Molecular hydrogen reduced the symptoms promoted by lipopolysaccharide injection. It was also associated with promotion of anti-inflammatory gene expression, such as down-regulation of tumor necrosis factor-α, up-regulation of interleukin-10 and general regulation of cytokine expression towards anti-inflammatory profiles. The results showed that in addition to its role in reducing the oxidative stress during inflammation, hydrogen is also beneficial in promoting changes in the expression of the modulatory agents of inflammation [280]. In cell culture, H_2 is also able to promote the expression of the gene heme-oxygenase-1 (HO-1). This result demonstrated that H_2 contributes to the anti-inflammatory effect in lipopolysaccharide-stimulated macrophages (RAW 264.7) by inducing the expression of anti-inflammatory molecules [281].

During inflammation the endothelia of blood vessels suffer dramatic changes, such as leukocyte conglutination and endothelium permeability. In lipopolysaccharide-treated vein endothelial cells it was observed that H_2-rich media promoted reductions in vascular cell adhesion protein (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and E-selectin levels, as well as trans-endothelial electrical resistance. This indicated that hydrogen can reduce the increased permeability of endothelial cells found during lipopolysaccharide-induced inflammation. In addition, the expression of VE-cadherin, which diminishes the permeability of the endothelium, was also increased [282]. Consistent with these results, it was shown recently that H_2 is able to inhibit the adhesion of monocytes and polymorphonuclear neutrophils to endothelia, while the expression levels of the pro-inflammatory marker Rho-associated coiled-coil kinase (ROCK) were diminished by the hydrogen-enriched medium [283].

The role of hydrogen in reducing inflammation in specific organs has also been studied in detail in several animal models. At the gastrointestinal level, it has been reported that hydrogen-rich saline has a protective effect, for example, in ulcerative colitis in rats induced by acetic acid [284]. Intraperitoneal administration of hydrogen in 10 - 20 ml/Kg of H_2-enriched saline solutions for a period of 2 weeks diminished various macroscopic and microscopic indicators of colonic mucosal damage. An interesting aspect of this work is that it also demonstrated that, in addition to its antioxidant role, hydrogen inhibited the expression of vascular endothelial growth factor [284]. In neonatal rat models, it has been shown that hydrogen-rich saline reduced the incidence of necroziting enterocolitis (NEC) from 85% to 54.5%, while increasing the survival rate from 25% to 68.2% ($p < 0.05$) [265]. Hydrogen-rich saline also inhibited the expression of pro-inflammatory mediators, such as iNOS, tumor necrosis factor-α, interleukin-6 and lipid peroxidation, while enhancing the total antioxidant capacity [265]. Hydrogen-rich saline and water were also effective in reducing gastric inflammation induced by aspirin or acute peritonitis in rats [285] [286].

Inflammation of critical glands for digestion and metabolism, such as the liver and pancreas, have also been reported to be treatable with hydrogen. Regarding the liver, it is well established that reducing oxidative stress improves the prognosis in acute and chronic hepatitis [287]. Studies with hydrogen have also been performed in humans infected with hepatitis B [288]. In one study, 30 patients drank H_2-enriched water (1200 - 1800 ml/day, twice a day), for 6 weeks. After that period of time oxidative stress markers were measured and the values were compared with those before treatment and with patients who drank normal water. All of the oxidative stress markers were significantly reduced in all the patients who drank H_2-enriched water ($p < 0.01$). However, the authors reported that the results relating to liver function and hepatitis B virus DNA levels were inconsistent, especially after long-term treatments [288]. Biomarkers of acute pancreas inflammation in rats, induced by taurocholate, were significantly improved by intravenous injection of hydrogen-rich saline ($p < 0.05$). Some of the markers explored were Serum tumor necrosis factor-α, interleukin-6, and interleukin-18 [289]. In the pancreas, H_2-enriched saline reduced the histopathological score as well as the levels of malondialdehyde (MDA), myeloperoxidase (MPO) contents and the expression of tumor necrosis factor-α and intercellular adhesion molecule-1 mRNAs [289]. It has also been reported in mice that hydrogen-rich saline inhibits the activation of the NLRP3 inflammasome, while ameliorating acute pancreatitis [290].

At the cardiorespiratory level, hydrogen has been used to treat specific inflammation of the heart and lungs. Inflammation in the heart, as a result of ischemia/reperfusion injury, has been discussed in the corresponding section in this review. It has also been tested in rat models of regional myocardial ischemic reperfusion injury. Hydrogen-enriched saline solutions diminished the levels of biomarkers of inflammation, such as neutrophil infiltration, 3-nitrotyrosine, myeloperoxidase activity, tumor necrosis factor-α, interleukin-1β and the expression of ICAM-1. Consistent with this, heart function parameters also improved in rats treated with hydrogen-enriched saline [82].

Recently, it has been reported that hydrogen-rich saline alleviates symptoms in rats with severe burns and inflammation with delayed resuscitation [291]. The mortality rate, cytokine levels, and oxidative stress biomarkers were all improved after treatment with H_2-enriched saline. A likely intermediary signal in this process was the inhibition of the nuclear factor NF-κB [291].

Oxidative stress also plays a key role in chronic obstructive pulmonary diseases (COPD). It has been hypothesized that the inhalation of hydrogen may improve lung function in COPD [292]. It has been shown in murine models of asthma that H_2-enriched saline reduces airway remodeling and inflammation via inhibition of the NF-κB transcription factor pathway [293].

Regarding urinary tract inflammation, hydrogen solutions have been used in patients with interstitial cystitis and painful bladder syndrome (IC/PBS). In this study, 30 patients were mostly female, of average age 64 years, with stable clinical scores for IC/PBS lasting more than 12 weeks. They were treated with H_2-enriched water or placebo for 8 weeks. Although hydrogen intake did not change significantly the IC/PBS clinical scores during the study, there were improvements in pain perception in 11% of the patients [294].

Maternal inflammation is a critical determinant in preterm births, yielding respiratory malfunction in premature infants. This has been studied in pregnant rats where maternal inflammation was induced by intraperitoneal injection of lipopolysaccharide. Hydrogen-enriched water administered 24 hours before lipopolysaccharide injection diminished the biomarkers related to inflammation, oxidative damage, and apoptosis in comparison with those rats that had liposaccharide-induced inflammation without hydrogen treatment [112].

It has also been reported that use of H_2-enriched saline in mice protects immune system function and spleen

inflammation induced by radiation. Biomarkers of oxidative stress, inflammation, apoptosis and immune response capacity were all improved after hydrogen-enriched saline administration [295].

16. Hydrogen in Injuries

Injuries can cause damage to the body, organs, tissues, or cells, and can be produced by physical, chemical or biological means. An important cause of injury worldwide is body trauma, leading to disability or death [296]. Hydrogen has been experimentally used as an adjuvant to treat injuries in various organs of the body, particularly in the brain, lungs, kidney, retina, and glands, such as liver and pancreas.

Traumatic brain injury (TBI) is a major cause of mortality and disability among the young, and a major problem for modern society. Brain edema, blood-brain-barrier breakdown, and neurological dysfunction can be observed in TBI. In addition, acute TBI can be transformed into a chronic injury, and this is a risk factor for neurodegenerative diseases, such as Parkinson's and Alzheimer's diseases [296]. Experimental exposure of rats with TBI to inhalation of 2% H_2 gas from 5 min to 5 hours after the injury (or surgery to treat the damage) resulted in significant reductions in oxidative stress biomarkers and brain edema, blood-brain barrier breakdown and neurological dysfunction [126] [127]. Mice with TBI induced by controlled cortical impact were also given H_2-enriched water. Hydrogen-rich water reversed the brain edema by about one-half, blocked tau expression, attenuated the expression of inflammatory cytokines and restored the expression and activity of matrix metalloproteinase-2 and matrix metalloproteinase-9. In addition, ATP levels were restored, suggesting the hydrogen-enriched water could be of benefit as a preventive agent to avoid neurodegenerative changes associated with acute TBI [297].

Brain injury can also be observed after hemorrhages, such as subarachnoid hemorrhage (SAH). This condition is associated with neuronal apoptosis triggered by the nuclear transcription factor NF-κB. In such cases, early brain injury plays a key pathogenic role for the development of SAH. Intraperitoneal injections of hydrogen-rich saline in rabbits significantly reduced post-SAH apoptosis, diminishing NF-κB activity and other apoptosis biomarkers, such as Bcl-xL and caspase-3 [298]. Results obtained in rats treated with hydrogen-rich saline suggest that a protective role for hydrogen in SAH apoptosis could be explained using the Akt/GSK3β pathway [299]. A recent report emphasizes the critical role in SAH apoptosis due to the suppression of inflammatory responses through NF-κB and NLRP3 inflammasomes [300]. Additional benefits of hydrogen treatment have also been reported in animal models for other types of brain injury, such as those promoted by cardiac arrest and cardiopulmonary resuscitation [128], survival of retinal ganglion neurons after optic nerve crash [301] and neuroinflammation by sepsis [130].

The lungs can be injured by a variety of mechanisms. Hydrogen-rich saline has been shown to reduce lung injury promoted by intestinal ischemia/reperfusion in rats. Hydrogen-rich saline treatment diminished neutrophil infiltration, lipid membrane peroxidation, NF-κB activation, and increases in pro-inflammatory cytokines (interleukin-1β and tumor necrosis factor-α) in lung tissues, compared with controls without hydrogen treatment [103]. Lung injury produced by extensive burns [109], irradiation [111], or lipopolyscharide treatment [113] are also ameliorated by hydrogen treatment in animal models (rats and mice). Acute lung injury (ALI) can also be observed during sepsis. Cecal ligation and puncture is a model for producing ALI. Hydrogen-rich saline treatment in this model for ALI improved significantly lung function and gas exchange, and diminished oxidative stress and inflammation biomarkers [302]. Early fluid resuscitation and hydrogen inhalation in rats injured by sepsis have also been shown to have less lung and intestinal injury [303]. Interestingly, nitric oxide inhalation has been used to treat ALI with moderate success. However, the adverse effects of production of some toxic free radicals (ROS and RNS) remain. Since hydrogen is a scavenger for ROS/RNS species, combination therapy with NO plus H_2 in mice with ALI has been used to improve on the results of inhalation of NO alone [114].

Cigarette smoking is the leading cause of chronic obstructive pulmonary disease (COPD) and a major cause of lung injury worldwide. Oxidative stress is a key factor that determines abnormal mucus production in lung diseases promoted by cigarette smoking. Hydrogen-enriched saline pretreatment of rats by intraperitoneal injection before exposure to cigarette smoke diminished oxidative stress and proinflammatory biomarkers at the level of the pulmonary epithelia and lungs [112]. Histopathological measurements also indicated that hydrogen-enriched saline pretreatment inhibits the cigarette smoke damage that induces abnormal mucus production and pulmonary epithelial injury. This effect was partially explained by the antioxidant properties of hydrogen and its ability to inhibit the expression of proinflammatory agents [304]. ALI produced by cigarette smoking is also at-

tenuated by intraperitoneal injection of hydrogen-saline in rats, inhibiting the pro-apoptotic pathways as well [305]. Finally, the gaseous emissions of laser printers and photocopiers have been associated with pulmonary health problems in chronic fatigue syndrome patients because of lung injury. A series of metals and volatile organic compounds in toners are thought to be the cause. It has been hypothesized that hydrogen treatment could be of benefit in this occupational exposure [306].

Kidney injury is a fairly common event and can be produced by a variety of mechanisms, such as chemotherapy associated with cancer, ureteral obstruction, hypertension, rhabdomyolysis, severe burns, contrast liquids for imaging techniques, pancreatitis, among other events. In chemotherapy, cisplatin is a widely used drug, but its application is limited by nephrotoxicity. Hydrogen-enriched water and hydrogen inhalation has been shown in mice to reduce kidney damage by cisplatin, without impairing the anti-tumor properties of cisplatin [47]. Carcinogens like ferric nitrilotriacetate promote the apparition of tumors in the kidney after renal injury. Consumption of hydrogen-enriched water by rats after intraperitoneal injection of ferric nitrilotriacetate alleviated kidney injury and the early promotion of tumors in the kidney. This effect was evaluated with histological and functional biomarkers for the kidney as well as biomarkers for oxidative stress and inflammation. The oxidative stress markers were all diminished by hydrogen consumption [336]. Hydrogen-rich saline (5 ml/Kg for ten days) also reduced renal injury scores promoted by unilateral ureteric obstruction in rats [307].

Hypertension is a major cause of kidney injury worldwide. Oxidative stress is an important factor in hypertension and renal disease. In spontaneously hypertensive rats, drinking H_2-enriched water for 3 months alleviated renal injury by diminishing oxidative stress, as measured by the reduction of oxidative stress biomarkers. In addition, the pro-inflammatory biomarkers were also reduced by hydrogen ingestion. The results from this report suggest that the ingestion of hydrogen-enriched water is a promising strategy to reduce renal injury in hypertensive patients [308] [309]. Another major cause of acute kidney injury (AKI) is rhabdomyolysis, which occurs with increased renal oxidative stress and inflammation [310]. Rhabdomyolysis can be induced in rats by intramuscular glycerol injections, and the effects of hydrogen can be assessed by measuring creatine-kinase levels. In this rat model renal function and histology were monitored by serum creatinine, urea and histologic analysis. Biomarkers for oxidative stress and pro-inflammatory responses were also measured in the kidney. The pretreatment of rats with either high or low doses of hydrogen-rich saline improved renal health and reduced the systemic biomarkers for oxidative stress and inflammation, suggesting a protective role for hydrogen in injury produced by rhabdomyolysis [310].

AKI can also be found in severely burned patients or animals. Hydrogen-enriched saline treatment reduced the appearance of AKI in severely burned rats through the combined reduction of oxidative stress, proinflammatory cytokines, and apoptotic agents, as measured by a reduction of various biomarkers after H_2-enriched saline administration [311]. AKI can be observed by the use of contrast media and image analysis. In rats injected with loversol and inhibitors of prostaglandin and nitric oxide synthesis, AKI can be induced and monitored using the above methods. In this rat model, inhalation of hydrogen reduced the biomarkers of apoptosis and oxidative stress and reduced the induction of AKI [312]. AKI is also found during pancreatitis. A rat model for acute pancreatitis was developed by taurocholate injection. Using the pancreatitis model rats were injected with hydrogen-rich saline, and this was found to diminish oxidative stress and proinflammatory biomarkers in the kidney compared to untreated animals [313].

In diabetic patients the retina can be injured causing diabetic retinopathy. Diabetic retinopathy is a major cause of blindness in developed countries [314]. Using rats a disease similar to Type I diabetes can be obtained by injection of streptozotocin. Hydrogen-rich saline (5 ml/Kg, 4 weeks) was intraperitoneally injected into streptozotocin-induced diabetic and control rats. Retinal apoptosis and vascular permeability biomarkers were assessed after hydrogen treatment and were reduced by the gas. The results suggested a potential use of hydrogen for the treatment of diabetic retinopathy [315].

Inflammation in the retina also produces tissue injury. Lipopolysaccharide-induced retinal microglia activation was explored, with and without hydrogen gas treatment in rats. Proinflammatory biomarkers were markedly reduced in the injured retina by the treatment of the rats with hydrogen gas, suggesting a role for the gas in the control of the expression of pro-inflammatory and pro-apoptotic agents [121]. Light can also promote retinal injury and degeneration through promotion of oxidative stress, and this damage can be monitored via electro-retinograms and histological scores [316]. Intraperitoneal injection of H_2-enriched saline into rats improved retinal function and morphology after light-induced retinal damage [63]. Glaucomatous neurodegeneration is another major cause of retinal injury. Oxidative and nitrative processes play a key role in the pathogenesis of this dam-

age. Using cultured adult rat retinal cells that were exposed to increased oxidative stress by introducing the nitric oxide donor S-nitroso-N-acetylpenicillamine, the response of retinal cells could be examined in the presence or absence of hydrogen. In this experiment, hydrogen diminished oxidative stress damage in the cultured retinal cells and diminished the loss of mitochondrial inner membrane potential and reduced apoptosis, presumably through a scavenging role of peroxynitrite. This *in vitro* experiment suggested that hydrogen might be of use in treating and preventing retinal injury induced by glaucoma [122].

Finally, hydrogen has been used to treat liver, pancreas, and heart injuries, or moderate damaged to cells from these organs in tissue culture. Liver injury is often observed after the use of common drugs like acetaminophen. Intraperitoneal H_2-enriched saline (5 ml/Kg), administered to mice, diminished the liver lesions induced by acetaminophen; oxidative and proinflmmatory liver biomarkers were also reduced in the acetaminophen-injected animals. This suggested a liver-protecting role for hydrogen and protection against drug-induced hepatotoxicity [317]. Pancreatitis can be also induced by trauma. Hydrogen-saline has been used to lower oxidative stress biomarkers and reduce the severity of trauma-induced pancreatitis in rats [318]. In cultured heart cell lines (H9c2), hydrogen has been reported to reduce the injury induced by glucose and serum deprivation mediated through the NF-E2 related factor-2 (Nrf2)/heme oxygenase 1 signaling pathway [319].

17. Hydrogen in Exercise and Sports Medicine

Intense exertion during acute physical exercise results in an increased concentration of ROS/RNS in skeletal muscle. Such oxidative stress in skeletal muscles can lead to muscle weakness and fatigue, microinjury, and inflammation. Oxidative stress-induced pathogenic changes in skeletal muscle may include DNA mutations, lipid peroxidation, mitochondrial dysfunction, and apoptosis/necrosis [320] [321].

Most studies on the effects of hydrogen on physical stress and exercise involve the use of hydrogen-enriched saline or water [322]-[324]. The benefits of hydrogen-rich saline in ischemia-reperfusion of skeletal muscle have been examined in a rat model. Ischemia was induced in rats by application of a hind limb tourniquet for 3 hours, followed by 4 hours of reperfusion. Three experimental groups of male Sprague-Dawley rats were used: sham control (group 1); I/R treated with normal saline (group 2); and I/R treated with H_2-enriched saline (group 3) [322]. Normal saline or H_2-enriched saline (1.0 mL/100g) was administered intraperitoneally 10 min before reperfusion, and muscle and serum samples were analyzed for the levels of myeloperoxidase (MPO), superoxide dismutase (SOD), malondialdehyde (MDA), and hydroxyl radical (•OH) at various times in the model [322].

In the rat muscle ischemia/reperfusion model the wet/dry ratio increased significantly in the I/R group compared with that in the sham group ($p < 0.01$) and decreased significantly in the hydrogen-rich saline group ($p < 0.01$). Muscle tissues and serum of the I/R group showed significantly increased levels of MPO, MDA, and •OH content and decreased SOD activities, compared with the sham control group. The activity of SOD in the I/R group treated with H_2-enriched saline was greatly elevated compared to the I/R group ($p < 0.01$), whereas the levels of MPO, MDA, and •OH content were clearly reduced in muscle tissues and serum. The integrated optical density of positive amethyst staining increased significantly in the I/R group, compared to the sham control group, and this decrease was significantly in group 3 (I/R with H_2-enriched saline) compared with group 2 (I/R without H_2-enriched saline). Muscle tissues of group 2 (I/R group) rats had significantly increased levels of the following: BCL2-Associated X Protein (BAX); cytochrome c, a component of the electron transport chain in mitochondria; and LC3B antibody content. There were also decreased levels of BCL2 activities compared with those in the group 1 (sham control) animals. The activity of BCL2 in the group 3 (I/R with H_2-enriched saline) rats was significantly elevated, compared with I/R without H_2-enriched saline, whereas the levels of BAX, cytochrome c, and LC3B content were reduced ($p < 0.01$). The authors concluded that H_2-enriched saline was an effective agent for attenuating I/R injury in rat skeletal muscle "via its antioxidant, anti-apoptosis, and anti-autophagy effects" [322].

Another animal study was designed to identify changes in oxidative stress and antioxidant levels in five treadmill-exercised Thoroughbred horses (3 to 7 years old) [323]. The BAP (Biological Antioxidant Potential) test was utilized to estimate antioxidant markers in the blood, and diacron-reactive oxygen metabolites (d-ROMs) were used to determine the total amount of free radicals in the blood and cerebrospinal fluid. Both are indicators of oxidative stress. To study the effects of H_2-enriched water, animals were given nasogastric hydrogen-rich water or placebo water preceding the treadmill exercise. Each horse was subjected to a maximum exhausting level of treadmill exercise and blood samples were taken at various times. In all horses, d-ROMs tended to ele-

vate, starting immediately after the treadmill exercise; however, there were significant differences between the horses given H_2-enriched compared to placebo water. The BAP values increased in all horses post-exercise, and there were significant differences between the placebo and H_2-enriched water trials.

The results revealed that significant elevations of both oxidative stress and anti-oxidative functions occurred simultaneously in all of the intensively exercised horses, but the increase was less in the horses given H_2-enriched water, suggesting that H_2-water has useful antioxidant-mediated effects during exercise [323].

In injured athletic subjects who were given hydrogen interventions, the efficacy of hydrogen for increasing skeletal muscle injury recovery has been examined. The first study involved a two-week pilot investigation of the effects of hydrogen on inflammation and recovery from acute soft tissue injury in male professional athletes. Thirty-six professional athletes were examined by a certified sports medicine specialist within the first 24 hours after sustaining injury, and they were then allocated to 3 randomly assigned groups in a single-blinded clinical trial [324]. The control group received a traditional treatment for soft tissue injury throughout the study, which consisted of a protocol (RICE) during the first 48 hours (rest, ice packs for 20 minutes every 2 hours, compression with elastic bandage, elevation of the injured area above the level of the heart at all possible times) and a sub-acute protocol thereafter (passive stretching 3 times per day for 90 sec, isometric strength exercise with 3 sets with 15 repetitions, and 30 min of pain-free weight-bearing exercise).

Injured subjects in the first experimental group followed the same protocol as the control group, but with the addition of oral consumption of 2 g of hydrogen-producing tablets per day. Subjects in the second experimental group also received the control group procedures and were given both oral H_2-producing tablets (2 g per day) plus topical hydrogen-rich packs (6 times per day for 20 minutes each). Participants were evaluated at the time of the injury and at 7 and 14 days after baseline testing. The oral-topical hydrogen treatment group showed a decrease in plasma viscosity, when compared with the control group, and this group also showed a faster return to normal joint range of motion for both flexion and extension of the injured limb, when compared with the control group [324].

In the next clinical study ten male soccer players (aged 20.9 ± 1.3 years old) were examined twice for peak torque and muscle activity in a double-blind, crossover trial [325]. The subjects were given either hydrogen-rich water (HW) or placebo water (PW) for one-week intervals. Subjects were provided with three 500 ml bottles of drinking water and instructed to place two magnesium sticks in each bottle 24 hours prior to drinking, and participants were asked to drink one bottle at 10:00 PM of the day before the test, one at 5:00 AM, and one at 6:20 AM on the day of examination. Subjects were given meals between 9:00 PM and 10:00 PM the day before experiments, and they fasted overnight. The subjects were first required to rest in a sitting position for 30 minutes before the exercise test. The exercise test consisted of the following: 1) Maximal progressive exercise to define maximal oxygen uptake (VO_2 max); 2) cycling an ergometer for 30 minutes at approximately 75% VO_2 max (Exercise-1); and 3) performing 100 maximal isokinetic knee extensions at 90˚ per second (Exercise-2). Blood samples were collected just before and after Exercise-1, immediately after Exercise-2, and 30 and 60 minutes after Exercise-2. Oxidative stress markers and creatine kinase in the peripheral blood were monitored during the trial [325].

Although acute exercise resulted in increased blood lactate levels in the subjects given placebo water, oral intake of H_2-enriched water prevented an elevation of blood lactate during heavy exercise. Blood lactate levels in the athletes given placebo water significantly increased immediately after exercise, compared to the levels at pre-exercise, but H_2-water significantly reduced blood lactate levels post-exercise, using a bicycle ergometer ($p < 0.05$). Peak torque of the subjects treated with placebo but not H_2-enriched water also significantly decreased during the initial 40 - 60 contractions by approximately 20% - 25% of the initial values, followed by a phase with little change [325]. This study revealed that adequate hydration with hydrogen-enriched water prior to exercise decreased blood lactate levels. The intervention with H_2-enriched water also improved exercise-induced decline of muscle function.

Since hydrogen therapy has been shown to be highly beneficial for the treatment of inflammation, ischemia-reperfusion injury, and oxidative stress in muscle tissue, H_2-water may be of benefit in enhancing performance, as well as shortened injury times for athletes.

18. Miscellaneous Uses of Hydrogen

There are a variety of miscellaneous uses of hydrogen. For example, hydrogen-rich saline has been used to atte-

nuate neuropathic pain. A useful rat model of neuropathic pain has been developed that is induced by spinal nerve ligation. Ge *et al.* [326] introduced H_2-rich normal saline into the rat spinal cavity of rats with spinal nerve ligation and found that hydrogen relieved mechanical allodynia and thermal hyperalgesia. They also found that preemptive treatment with hydrogen-rich saline prevented development of neuropathic pain behavior, and analysis of brain slices revealed that the H_2-rich saline treatment significantly attenuated the increase of 8-hydroxyguanosine-immunoreactive cells in the ipsilateral spinal dorsal horn induced by spinal nerve ligation. Isolation, fractionation, and Western blot analysis of tyrosine-nitrated spinal proteins indicated that the hydrogen treatment resulted in increased expression, but not over-expression, of tyrosine-nitrated Mn-containing superoxide dismutase (MnSOD) in the spinal cord. The infusion of H_2-enriched normal saline also had an analgesic effect that was associated with decreased activation of astrocytes and microglia, along with decreased expression of interleukin-1β and tumor necrosis factor-α within the spinal cord [326].

By introducing hydrogen in their drinking water, Kawaguchi *et al.* [327] were able to reduce neuropathic pain in mice caused by partial sciatic nerve ligation. They showed that, while repeated intra-peritoneal or intra-thecal injections of strong antioxidants were ineffective in reducing neuropathic pain, the introduction of hydrogen into the drinking water reduced neuropathic pain, as assessed by mechanical allodynia and thermal hyperlgesia. When mice were allowed to continuously drink H_2-enriched water *ad libitum* after spinal ligation, allodynia and hyperalgesia were alleviated. The pain symptoms were also reduced when H_2-enriched water was given only during the induction phase from day 0 to 4, but only hyperalgesia was reduced when H_2-enriched water was given during the maintenance phase from day 4 to 21 [327]. Using immunochemistry staining for oxidative stress markers 4-hydroxy-2 nonenal and 8-hydroxydeoxyguanosine, Kawaguchi *et al.* [327] demonstrated that oxidative stress induced by spinal ligation could be reduced by drinking hydrogen-rich water.

Hyperalgesia has been induced experimentally by remifentanil administration in animals and humans [328]. Since MnSOD nitration and inactivation is caused by ROS, and activation of N-methyl-D-aspartate (NMDA) receptors are known to be involved in the induction and maintenance of central neuropathic pain, hydrogen has been used to selectively reduce ROS, remove superoxide and reduce neuropathic pain. Thus, Zhang *et al.* [328] used intra-peritoneal H_2-enriched saline in a remifentanil-induced post-surgical hyperalgesia rat model of neuropathic pain to demonstrate that hydrogen can significantly attenuate mechanical and thermal hyperalgesia. In this rat model, remifentanil causes dose-dependent long-term hyperalgesia associated with increased expression of NR2B molecules and trafficking from the cytoplasm to the cell surface, as well as MnSOD nitration. Separately, they used spinal cord tissue slices and an *in vitro* clamp system to confirm the role of membrane trafficking of NR1 and NR2B subunits in controlling the amplitude and frequency of NMDA receptor-induced current [329]. Pretreatment of rats with intra-peritoneal H_2-enriched saline reduced the effects of remifentanil and attenuated mechanical and thermal hyperalgesia. The authors concluded that H_2-enriched saline might reverse remifentanil-induced hyperalgesia by regulating NR2B-containing NMDA receptor trafficking and by controlling MnSOD nitration and activity [329].

Hydrogen has also been proposed as a treatment for acute carbon monoxide poisoning [5]. Hydrogen is thought to exert its effects on CO poisoning by reducing oxidative stress, free radicals, neuronal nitric oxide synthesis, and inflammation [330]-[332]. These effects occur slowly after CO poisoning. Rats exposed to 1000 - 3000 ppm CO in air eventually lose consciousness, and after resuscitation, they can be injected intraperitoneally with H_2-enriched saline repeatedly over 3 days to reduce the delayed effects of CO, including tissue inflammation, cognitive dysfunction, and cell death [330]. Within one week after CO poisoning, rats show increased levels of degraded myelin basic protein, ionized calcium-binding adapter molecule one (iba1), DNA oxidation, and increases in inflammatory proteins in the cortex and hippocampal tissues, compared to normal controls. However, hydrogen-rich saline injections improved the histological appearance of brain tissue and reduced the CO poisoning markers listed above. Importantly, the H_2-enedriched saline-injected CO-poisoned animals showed improved memory, and cognition in the Morris water maize test compared to CO-poisoned untreated controls [332]. Examining brain damage in the CO-poisoned rat model, Shen *et al.* [333] found that injection of H_2-enriched saline reduced lipid peroxidation products and the numbers of apoptotic cells found after CO-poisoning, while increasing the levels of endogeneous cellular antioxidants in the brain cortex and hippocampus.

Another use for hydrogen-enriched saline is in reducing the effects of hemorrhagic shock, which causes low perfusion of visceral organs, ischemia and tissue hypoxemia, along with generation of ROS and multiple organ dysfunction [334]. Using a rat model for uncontrolled hemorrhagic shock caused by arterial bleeding and tail amputation, Du *et al.* [334] studied the effects of intra-peritoneal and intravenous injection of H_2-enriched saline

on survival and production of plasma interleukin-6, tumor necrosis factor-α, superoxide dismutase and malondialdehyde. Although the survival rates were similar among the groups of animals, there were significant differences in the levels of oxidative and inflammatory blood markers. Intravenous injection of H_2-enriched saline was superior in its anti-inflammatory and anti-oxidative effects compared to intra-peritoneal injection of H_2-enriched saline ($p < 0.01$), although both provided protection against release of inflammatory mediators and increased antioxidant enzymes [334]. In a follow-on study, Du *et al.* [335] compared the protective effects of three H_2-enriched fluids (H_2-enriched Ringer's solution, H_2-enriched hydroxyethyl starch and hypertonic H_2-enriched hydroxyethyl starch) on hemorrhagic shock in their rat model. They found that all of the H_2-enriched solutions were more effective than their non-hydrogen counterparts in reducing inflammatory mediators and increasing antioxidant enzymes ($p < 0.01$), and reducing polymorphonuclear neutrophil accumulation in alveoli, capillary leakage and edema ($p < 0.01$) [335].

Hydrogen has also been used to protect nerves from the effects of mechanical trauma and light-induced damage. For example, traumatic optic neuropathy is one of the more common causes of visual loss and blindness [336]. Using a rat model for optic nerve trauma, optic nerve crush, Sun *et al.* [301] examined the protective effects of using daily treatments of H_2-enriched saline on nerve function and markers of tissue damage. Optic nerve function was measured by visual-evoked potentials and pupillary light reflexes. Tissue damage was assessed by examining tissue sections for the presence of toxins and gamma synuclein. Deoxynucleotidyl transferase-mediated dUTP nick and labeling (TUNEL) staining were used to measure nerve cell apoptosis. The animals receiving H_2-enriched saline daily were shown to have significantly less optical nerve damage in terms of gamma synuclein staining and apoptosis assessed by TUNEL staining. They also had lower levels of tissue malondialdehyde ($p < 0.01$ and $p < 0.05$, respectively). The H_2-enriched saline animals showed significant improvements in optic nerve function, compared to saline controls ($p < 0.05$). The results indicated that H_2-enriched saline had a significant protective effect on optic nerves after mechanical trauma [301].

Light-induced damage to the retina has also been ameliorated with the use of H_2-enriched saline [316]. Intense light can damage photoreceptors in the retina, and this is associated with excess oxidative damage [337]. To examine the effects of H_2-enriched saline on light-induced retinal damage, Tian *et al.* [316] used intense light to expose the right eye of a rat, while the left eye was used as a control. Animals were untreated or treated with intra-peritoneal injections of H_2-enriched saline before (prevention group) or daily after light exposure for five days (treatment group), and then electroretinography (ERG) recordings were obtained and the animals' eyes prepared for sectioning and light microscopic examination. Light damage could be assessed by ERG, and both H_2-enriched saline groups of animals were significantly less damaged, as assessed by less of a reduction in ERG amplitude. For example, light damage resulted in 70% reduction in ERG amplitude, whereas the prevention group showed 50% reduction in amplitude ($p < 0.001$) and the treatment group showed only a 30% loss ($p < 0.001$). Histology indicated that the light damaged animals had significant losses in retinal pigment epithelium, but the retinal epithelium in the H_2-enriched saline treatment group was almost normal, and the H_2 pretreatment group was intermediate between the untreated and the H_2-enriched saline-treated animals. The losses in retinal pigment epithelium were almost entirely due to light damage to retinal photoreceptors [337].

The use of hydrogen in emergency medicine has been reviewed by Shen *et al.* [80]. They report that administration of hydrogen has a number of uses in emergency trauma and other critical situations. After discussing the different modes of hydrogen delivery, they conclude that drinking H_2-enriched water may be the most practical way for consuming hydrogen in daily life.

Finally, it has been proposed that mental diseases, such as bipolar disorders and schizophrenia, be treated with hydrogen. The use of hydrogen to treat mental disorders was proposed recently by Ghanizadeh and Berk [338]. Since diseases like bipolar disorders and schizophrenia are associated with increased oxidative and inflammatory stresses, hydrogen therapy might be useful as a novel therapeutic approach [338]. Although clinical trials have not yet been conducted in this area, it is only a matter of time before this is investigated as a potential clinical use of hydrogen therapy.

19. Future Studies and Conclusions

This review and others [1] [2] [29] [55] [56] [73] [77] [79] [212] have documented that the clinical use of hydrogen is quite promising for the treatment of many acute and chronic illnesses and conditions, as well as its utility in support of the maintenance of good health. What started in Japan and the Far East as preliminary re-

sults on the clinical use of hydrogen has now continued there and elsewhere, to the point where there are now a critical number of scientific and clinical studies that support the use of hydrogen as a primary or supportive component of clinical care.

With its potent and unique antioxidant properties, gene regulatory abilities, and rapid rates of diffusion across tissue and cellular barriers, as well as its excellent safety record, hydrogen has many unique characteristics that make it very valuable for utilization in medicine and health. Its systemic properties and excellent penetration abilities allow hydrogen to be effective under conditions of poor blood flow and other situations that limit many other types of systemic treatments.

The clinical justification for hydrogen use is growing because:

1) Redox imbalance and the excess production of ROS and RNS (increasing oxidative stress) have been implicated in many, if not all, pathophysiological mechanisms leading to a wide variety of medical conditions and diseases. Hydrogen is useful because of its potent free-radical scavenging properties that significantly reduce strong cellular oxidants, but it does not affect important signaling pathways that depend on mild cellular oxidants.

2) Hydrogen is effective in reducing signs and symptoms and improving quality of life in a wide variety of clinical conditions. Because most of its effects are often indirect, such as reducing excess oxidative stress, hydrogen is useful for many apparently unrelated clinical conditions that are linked to redox imbalances. Often these conditions do not have definitive treatments that eliminate the illness. In such cases, hydrogen can be used in conjunction with less than effective therapies to improve clinical outcomes.

3) Perhaps its most useful property is that hydrogen does not interfere with the underlying mechanisms of most clinical treatments. Thus, its real value may be in adjuvant therapy, along with standard treatments for many clinical conditions.

4) An important factor is the safety of hydrogen and that no adverse effects of hydrogen have been described. This is also very relevant, since many drugs are limited in their use because of toxicity, adverse reactions, and unfavorable dose-response characteristics. Hydrogen does not have these problems.

5) The ease of hydrogen administration is a useful characteristic. This is where H_2-enriched water has an advantage over other methods of hydrogen delivery. Drinking H_2-enriched water can be done on a long-term basis without any special requirements for administration.

Basic and clinical research on the use of hydrogen for acute and chronic illnesses will continue to improve our understanding of the mechanism of action of hydrogen therapy:

1) Hydrogen is able to promote changes in the expression and levels of particular proteins by regulating gene expression. Of particular importance is that hydrogen can inhibit or change the expression patterns of pro-inflammatory, pro-allergic, pro-apoptotic and pro-oxidative proteins. Many, if not most, of these proteins are over-expressed in a variety of chronic and acute illnesses. How hydrogen changes the expression of particular proteins remains an important question that is currently a topic of research in several laboratories.

2) The cellular receptors for hydrogen and the mechanisms of hydrogen action at the level of cellular membranes, enzymes, protein synthesis, and gene regulation will have to be investigated. Little is actually known about these molecular interactions involving hydrogen inside cells and tissues. This will have to also be investigated first in simple *in vitro* models in order to eventually understand more complex *in vivo* environments.

3) Hydrogen is able to quickly penetrate into tissues and cells. Further investigation is needed to monitor the actual levels of hydrogen in the microcirculation and target tissues, especially when hydrogen is administered for long periods of time. We do not yet know the levels of hydrogen administration which provide steady state and effective concentrations of hydrogen in various tissues and cells.

4) The clinical uses of hydrogen must be further investigated. Most of the published research on hydrogen has utilized animal models. Although this has been extremely useful, it is now time to shift the focus of research to patients with acute and chronic clinical conditions.

5) There are some advantages and disadvantages in the various ways hydrogen is administered, and this should be further investigated. Although inhalation of H_2 gas has an advantage in that it is easy to administer; it also has some disadvantages, such as reproducibility of delivering the same dose of H_2 in different patients because of variations in the amounts that effectively reach the microcirculation and tissues. It also requires high-pressure containers and pressure regulators to deliver the required amounts of hydrogen gas, and the patient must use a mask or nasal insert. On the other hand, H_2-enriched water can be easily and accurately delivered without any special apparatus. With any delivery method there is the problem of knowing the effective concen-

trations of hydrogen that reach the target tissues, and this will remain an important research topic.

6) The increased use of controlled, randomized clinical trials will improve our knowledge of the benefits of hydrogen for various acute and chronic conditions. Until recently few clinical trials have used rigorous criteria for evaluation of clinical effects. Many trials have been open label in design, and this is expected for initial clinical investigations. In the future it is expected that more carefully designed (and more expensive!) placebo-controlled, blinded, randomized clinical trials will be necessary to confirm the clinical benefits of hydrogen.

Finally, the use of hydrogen for acute and chronic medical conditions is rapidly being eclipsed by the use of hydrogen for health maintenance, exercise and physical performance, as well as aging. These areas of hydrogen use will continue to grow and will ultimately dwarf the current clinical uses of hydrogen in our society.

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Abbreviations

8-OHdG—8-hydroxydeoxyguanosine;
AKI—Acute kidney injury;
ALI—Acute lung injury;
BAP—Biological antioxidant potential;
CO—Carbon monoxide;
DMD—Duchenne muscular dystrophy;
ERG—Electroretinography;
GMH—Germinal matrix;
GvHD—Graft-versus host disease;
H—Hydrogen;
HDL—High-density lipoprotein;
HO-1—Heme oxygenase-1;
IC/PBS—Interstitial cystitis/painful bladder syndrome;
I/R—Ischemia-reprofusion;
LDL—Low-density lipoprotein;
MDA—Malondialdehyde;
MetSyn—Metabolic syndrome;
mKATP—ATP-potassium channel;
MM—Mitochondrial myopathies;
NEC—Necrotizing enterocolitis;
Nrf2—Nuclear factor-erythroid 2 p45-related factor;
mPTP—Mitochondrial permeability pore;
mKATP—ATP-potassium channel;
MMP—Matrix metalloproteinase;
mPTP—The opening of mitochondrial pore;
MPO—Myeloperoxidase;
NMDA—N-methyl-D-aspartate;
NO—Nitric oxide;
PD—Parkinson's disease;
PM/DM—Polymyositis/dermatomyositis;
QOL—Quality of life;
RA—Rheumatoid arthritis;
RNS—Reactive nitrogen species;
ROS—Reactive oxygen species;
SOD—Superoxide dismutase;
T2DM—Type 2 diabetes mellitus;
TBI—Traumatic brain injury;
TUNEL—Deoxynucleotidyl transferase-mediated dUTP nick and labeling.

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Posturodynamic 6 Test: A New Scoring Method for Effective Communication of Results

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Abstract

Background: Posturodynamic 6 (PDN-6) is a clinical assessment of posture that merges the Clinical Posturodynamic Test and the Pelvic Maintain Test. Current scoring system does not fulfill all our needs and requirements mostly because the same numeric score might reflect 28 different possible combinations of postural dysfunction in terms of anatomic region and laterality. Objective: We propose a new scoring method for the PDN-6 that would not change the clinical methods for the PDN-6 assessment. Specifically, new scoring method would clearly indicate specific patterns of postural dysfunction while still enabling statistical analyses. Methods: We developed a new scoring method for the PDN-6 without changing the instrument's clinical procedures. We qualitatively assessed the validity of the new scoring system to detail specific patterns of postural dysfunction in terms of anatomic region and laterality. Results: New scoring method successfully deals with limitations of the previous scoring method. The new method enables clinicians to differentiate among 2 or more patients who might have very different patterns of postural dysfunction while still having the same numeric score using the previous scoring. The new scoring method provides quantitative data that are easily translated in terms of anatomic region and laterality for the postural dysfunctions that are present. Patient behavioral improvements are quantified, documented and interpreted with a change in score, and the exact nature of the improvements can be determined in terms of anatomic location and laterality. Rash analyses can also be used for statistical analyses. Conclusion: PDN-6 new scoring method provides quantitative data that provide more specific information about a patient's postural deficits and any changes in their postural dysfunction over time without changing the clinical assessment methods.

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Keywords

Posture, Clinical Evaluation, Spine, Balance, Balance Function, PDN

1. Introduction

The Posturodynamic 6 (PDN-6) is a novel method of rating posture using 6 assessment items and a simple measurement scale. The purpose of the PDN-6 is to assist in the evaluation of standing posture, but not to replace other more involved clinical assessments of standing posture. The PDN-6 represents a combined modification of two clinical instruments for postural evaluation: the Clinical Posturodynamic Test (CPT) and the Pelvic Maintain Test (PMT). The traditional method for scoring the PDN-6 has several limitations in terms of not being able to identify the anatomic region of dysfunction and the inability to communicate areas of improvement or exacerbation over time. The purpose of our report is to describe these limitations in detail. We also propose a new scoring system for this instrument that will enable the specific identification of postural deficits and will communicate clearly any positive or negative changes in postural function.

2. The CPT and PMT Instruments

The CPT is a clinical test with 4 evaluation items (cervical, thoracic, lumbar, and pelvis) for the right and for the left sides of the body [1]. This instrument assesses the postural response of each spinal section and pelvis during a lateral flexion movement [1]-[3]. The practitioner observes the result of the physiological response of the spine range of motion [4]. The quality of the postural response is quantified through spinal lateral flexion and pelvic translational maneuvers. The practitioner positions their hands successively on the four anatomical regions that are evaluated: cervical spine, thoracic spine, lumbar spine, and pelvic girdle. For the first three anatomic regions, the patient is instructed to laterally flex in the frontal plane: “slowly slide your hand down the side of your leg to the left and then to the right”. The normal physiologic response of the lower thoracic and lumbar regions is characterized by a slowly progressive contra-lateral rotation. Ipsilateral rotation should occur in the cervical region and upper thoracic regions of the spine because of the orientation of the articular facets. When the practitioner induces a lateral movement of the pelvic region to the right or left, the normal physiological response is a slow but progressive contra-lateral rotation (*i.e.* if the lateral movement induces by practitioner is on the right, the physiological response is pelvic rotation on the left) [1] [2] [4]. Previous research indicates that the CPT, in general, has 80% intra-examiner reliability and that intra-examiner reliability varies depending on the anatomic location [2] [3]. In clinical practice, however, we recommend that CPT measurements be made by the same clinician to improve the reliability of repeated measurements. Observations made during the CPT assessment are reported on a clinical data sheet (**Figure 1(a)**). If the response of the anatomic region is not a normal physiologic response, the practitioner records an “X” on the clinical data sheet and nothing if the patient’s response is a normal physiological response [1] [2]. With 2 possible observations for each of the 8 assessments, 30 possible combinations of overall performance on the CPT are possible. The CPT is scored numerically by replacing every “X” with the number “1” and then adding all of the recorded 1’s for a total score on the CPT. In the examples given in **Figure 1**, the CPT score is 0 for **Figure 1(a)** and the score is 6 for the CPT depicted in **Figure 1(b)**.

The PMT combines the Trendelenburg’s Sign (TS) [5] and the Single-Limb Stance (SLS) test [6]. The TS and the SLS test are timed measures that assess postural steadiness as the patient holds a static position. The popular belief is that better postural steadiness is reflected by longer standing time on a single leg. Little evidence is available, however, regarding how postural steadiness during one-legstance changes over time. The TS and SLS tests evaluate the patient’s response during singleleg standing. The clinician stands behind the patient for the PMT and places his hands on the patient’s iliac crests and his thumb’s on the patient’s posterior superior iliac spines. The subject stands on one lower extremity, slightly bending the knee of the non-stance limb. The normal response for the TS test should be that the patient’s trunk remains erect and the contralateral iliac crest should either remain level with the iliac crest of the stance limb, or should elevate slightly higher than the stance limb’s iliac crest. If the non-stance limb’s iliac crest falls inferior to the level of the stance limb’s iliac crest, then the

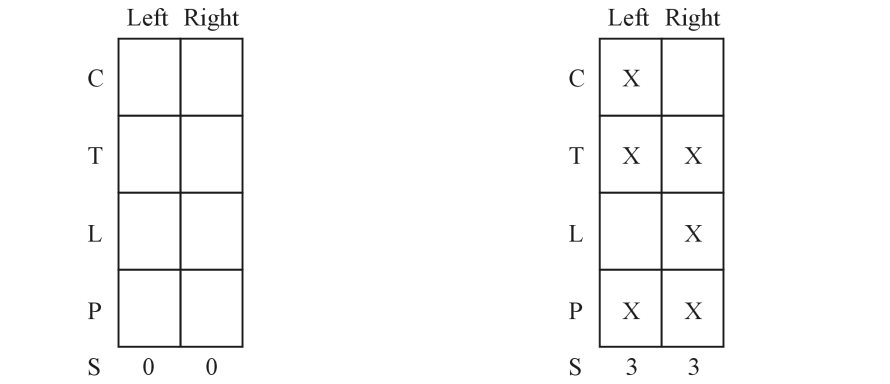


Figure 1. Datasheet for the clinical posturodynamic test.

examiner records a positive TS test result. A positive test result suggests reduced strength or activation of the hip abductor muscles of the stance limb [6]-[9]. A positive test result on the TS test is recorded as an "X" on the clinical data sheet.

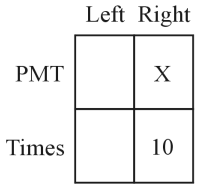
The normal physiologic response for the 30 second SLS test involves the patient's spine being maintained in a vertical position, while the iliac crests remain level with no compensatory movements made by the non-weight bearing lower extremity or the upper extremities for the duration of the test. Any deviation from these normal positions is recorded as an "X" on clinical data sheet and the time from the beginning of the test to the postural fault is recorded in seconds [10] [11]. The test is conducted and responses are recorded for both right and left stance limbs (**Figure 2**).

The combined TS and SLS scores for the PMT allow the clinician to identify stability problems that may exist for issues such as proprioceptive dysfunction. The PMT is positive (abnormal response) when the patient cannot maintain a level pelvis for 30 seconds without compensatory balancing movements by the upper extremities or the contra-lateral lower extremity. An abnormal response for the PMT is recorded on the data sheet with an "X" if the patient cannot maintain a level pelvis for 30 seconds, and the clinician indicates the elapsed time in seconds that the patient was able to maintain normal alignment until the postural fault occurred (**Figure 2**).

As previously stated, the PDN-6 represents the union of the CPT and PMT instruments. The PDN-6 datasheet reflects a scoring method for the 2 tests combined (**Figure 3(a)**) and provides both quantitative and qualitative information. The qualitative information reflects the postural responses of the patient during movement and static positioning tasks. Abnormal responses are recorded as an "X" on the data sheet (**Figure 3(b)**). The quantitative information is the time in seconds (up to a maximum of 30 seconds) that the patient was able to maintain normal pelvic position in the frontal plane during the static standing tests (**Figure 3(a)**, **Figure 3(b)**). The score of each of the two example datasheets represented in **Figure 3** is 6.

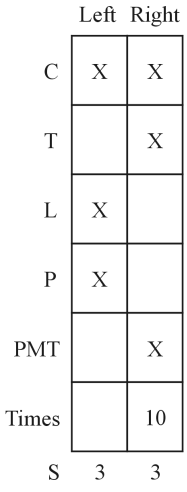
3. Limitations of the Current Scoring System for the PDN-6

All of this information leads to a discussion of the benefits and limitations of the scoring method that has just been described. One benefit of this scoring method is that the quantitative scores derived from the PDN-6 are available for statistical analysis procedures. An examination of the sample data sheets in **Figure 3**, however, reveals the limitations of this scoring method. Both data sheets in **Figure 3** have the same quantitative score of 6, but a close examination of the data sheets indicates that the 2 patients have very different anatomical regions of dysfunction. The quantitative scoring method, therefore, does not have the ability to discriminate different dysfunction profiles. Statistical analysis, therefore, may not be able to discriminate dysfunction among patients, despite the presence of real clinical differences. Extreme scores may be able to differentiate between widespread postural dysfunction and normal postural responses, but the scoring system may not be sensitive to intermediate scores and nominal variations between individuals. We propose, therefore, a new scoring system for the PDN-6 to address these limitations.



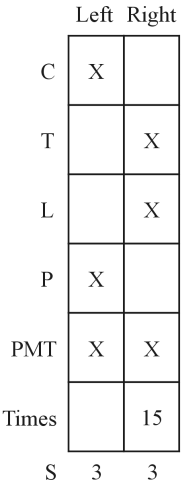
(X: no physiological responses; PMT: Pelvic Maintain Test;
10: only ten seconds maintained on one leg stance)

Figure 2. Datasheet for the pelvic maintain test (PMT).



(X: no physiological responses; C: cervical; T: thoracic;
L: lumbar; P: pelvis; S: score; PMT: Pelvic Maintain Test;
S: score; 10: only ten seconds maintained on one leg stance)

(a)



(X: no physiological responses; C: cervical; T: thoracic;
L: lumbar; P: pelvis; S: score; PMT: Pelvic Maintain Test;
S: score; 15: only fifteen seconds maintained on one leg stance)

(b)

Figure 3. Datasheet for the posturodynamic 6 (PDN-6).

4. Proposed New Scoring System for the PDN-6

We propose a new scoring strategy for the PDN-6 to address the previously described limitations. In doing so, we hope to retain the quantitative and qualitative information and still have a scoring method that will enable valid statistical analysis methods. Therefore, we propose a new scoring method without changing the clinical testing procedures and the data sheet format. The new scoring method simply involves numerical score transformations of the observed behavioural responses by the patient.

For the original scoring method, an abnormal physiologic response was always recorded as an "X" and then transformed to a numerical "1" for the purpose of adding a total score for the test. We now propose that abnormal responses be scored as: cervical = 1, thoracic = 2, lumbar = 4, pelvic = 10, and PMT = 100. The clinician would still record the time that the patient was able to maintain a level pelvis during the PMT test if the patient could not maintain a level pelvis for 30 seconds.

This method of scoring allows some differentiation among the various anatomic segments with the quantitative score. We also propose that separate total scores should be computed for the right and left sides, and that these scores be registered at the bottom of the data sheet and separated by a comma to assist in describing the laterality of any dysfunctions that exist. Sample scores are provided using this method in **Figure 4**, **Figure 5(a)**, and **Figure 5(b)**.

The sample data sheet in **Figure 5(b)** demonstrates how the new quantitative scoring method also provides qualitative information regarding the anatomic locations where postural dysfunction has been detected. A numerical score of "5" to the left of the comma can immediately be translated into cervical and lumbar dysfunction on the left side without examination of the data sheet. A numerical score of 112 to the right of the comma must coincide with thoracic and pelvic postural dysfunction as well as a positive PMT. This scoring method will still

	Left	Right
C	1	1
T	2	2
L	4	4
P	10	10
PMT	100	100
Times		10
S	117,	117

(C: cervical; T: thoracic; L: lumbar; P: pelvis;
S: score; PMT: Pelvic Maintain Test; S: score;
10: only ten seconds maintained on one leg stance)

Figure 4. New scoring in function of anatomical situation.

	Left	Right
C	1	1
T	2	2
L	4	4
P	10	10
PMT	100	100
Times		10
S	117,	117

(C: cervical; T: thoracic; L: lumbar; P: pelvis;
S: score; PMT: Pelvic Maintain Test; S: score;
10: only ten seconds maintained on one leg stance.
Score is 117, 117)

	Left	Right
C	1	
T		2
L	4	
P		10
PMT		100
Times		
S	5,	112

(C: cervical; T: thoracic; L: lumbar; P: pelvis;
S: score; PMT: Pelvic Maintain Test; S: score;
10: only ten seconds maintained on one leg stance.
Score is 5, 112)

Figure 5. Variation of the new scoring in function according to the anatomical situation.

make possible the use of non-parametric statistical analyses of the quantitative raw scores. Moreover, the quantitative scores will also make possible full communication among clinicians regarding the anatomic location and laterality of any postural dysfunctions that are present.

The previous quantitative scoring method for the PDN-6 conveyed, in general, the degree to which a patient demonstrated postural dysfunction. Greater scores suggested widespread postural dysfunction and lesser scores suggested far less postural dysfunction. The exact locations of dysfunction, however, could not be determined. Reductions in total PDN-6 scores indicated improvement, but did not identify the anatomic region or laterality of the improvement. The new scoring method successfully addresses all of these issues.

Figure 5(a), and **Figure 5(b)** represent pre-intervention (117, 117) and post-intervention (5, 112).

PDN-6 assessments for a hypothetical patient. These scores readily reveal the nature of the therapeutic-

induced changes for this patient. Prior to the treatment, the numeric score of "117, 117" clearly indicates postural dysfunction for all segments as well as the PMT on both the left and right sides of the body. The post-intervention score of "5, 112" indicates that postural faults have been resolved on the left except for the cervical and lumbar regions, and that only the cervical and lumbar postural dysfunctions have been resolved on the patient's right side. All of this information can be determined from the numerical scores without examining the patient's data sheets.

The new scoring method that we have suggested does not change the clinical testing procedures that have been used in the past for the PDN-6 instrument. The patient should begin the test by standing in their relaxed and comfortable stance position with double limb support. The patient's arms should be resting at their side and they should be looking straight ahead. During the assessment, the patient should not turn around toward the clinician since this will significantly affect their posture. The total time to complete all PDN-6 assessment procedures should be approximately two minutes. The clinician needs to have unencumbered access to the posterior aspect of the patient during the assessment, and all procedures for the assessment should be performed by the same clinician as per previous literature recommendations regarding intra-tester reliability [12]. To our knowledge, this is the first attempt to improve the scoring of the PDN-6 by addressing the previously described limitations of the current scoring system.

5. Limitations

Similar to the Foot Posture Index, the PDN6 is a qualitative assessment instrument. The obtained scores will range from 0 to 117 and are not on a ratio scale. Parametric data analyses are not appropriate since the data for this scoring system are not ratio data [13]. Other analysis methods such as Rasch analyses may be more appropriate for larger PDN-6 data sets to consider how the person/item interaction is governed by the difficulty of test forms (examiners + tasks + items) and the ability of the subject to perform the task [14] [15].

6. Conclusion

The new scoring method for the PDN-6 enables rapid and specific translation of a numerical score that indicates both the specific anatomic location and laterality of postural dysfunctions. This capability is possible without changing any of the testing procedures for the PDN-6. The scoring method also helps clinicians document and communicate specific details regarding improvements or exacerbations of a patient's postural function, as well as enables statistical analyses of the data. In the clinical setting, however, the examination procedures for the PDN-6 using this new scoring system are standardized so that data for the PDN-6 should more accurately reflect the patient's status compared with the original scoring system.

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Authors' Contributions

Conceived the transformation between the clinical scale and the scoring for statistical analysis: AJ (senior project researcher, scientific bachelor degree). Initiators of reflection for the transformation of the Posturodynamic score: MJ and PD. Wrote the manuscript MTG MJ (MTG main writer). AJ MTG JB PD MJ participated interpretation and revising the paper. All the authors read and approved the final manuscript.

Competing Interests

The authors declare that they have no competing interests.

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External Ventricular Drainage Infections Rates: Clinic Experiences

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Abstract

In this study, we aimed to evaluate patients who develop ventriculostomy requirements and to evaluate the external ventricular drainage infection rates retrospectively at our clinic. In the study, dates between January 2012 and 2014, patients who were inserted external ventricular drainage with different indications were examined retrospectively. By using the medical record system, patients' demographics (age, sex), diagnostic, ventriculostomy indications and ventriculostomy time had been reached. By accessing laboratory data, the patients' cultures of cerebrospinal fluids biochemical tests were analyzed retrospectively. Within the period of the study, 20 in 117 patients with external ventricular drainage were not included the study because of shunt infection and shunt occlusion of extracted patients. During the treatment period 148 EVD were inserted to 97 patients. The number of male patients was 53; the number of female patients was 44. When the reason of the examined patients' using external ventricular drainage was analyzed, 55% hemorrhagic cerebrovascular diseases, (subarachnoid hemorrhage, intraventricular hemorrhage, intracerebral hemorrhage, cerebellar hemorrhage, traumatic intracranial injury), 24.7% tumor induced use, 7.4% central nervous system infections (menengitis, apse), and 12.4% occlusive cerebrovascular diseases (hydrocephalus or brain edema that were developed after the infarct) were seen. During the course of our study, 23% of the surveyed 97 patients had leukocytosis. CFT culture of 12 patients found positive. In CFT cultures mostly coagulase-negative staphylococcus growth took place. Eventually, when we compare our infection proportions to the international literature, very large differences were not observed. Except for revisioned patients, no other criteria were found that increased the rate of infection. We think that a rigorous pre-operative preparation and a regular maintenance of external ventricular drainage may reduce the rate of infection.

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Keywords

External Ventricular Drainage, Infection, Ventriculostomy, Hydrocephalus

1. Introduction

External ventricular drainage (EVD) is one of the neurosurgery methods frequently used by Brain surgeons. EVD is a surgical procedure that allows the establishment of a connection between cerebral ventricular system and external environment. EVD can be considered as a complementary venture for many neurosurgical enterprises such as intracranial hemorrhage, intracranial tumor, traumatic brain injury, cerebral edema and intracranial pressure measurement. However, as all surgery enterprises during and after the ventriculostomy process some complications may occur. Infection is the most important of these complications. Ventriculostomy connected infection rate is reported 0% - 22% [1] [2]. After the ventriculostomy, so many studies were made for the cause of infection but a common cause has not been found [3]-[8].

In this study, we aimed to evaluate patients who develop ventriculostomy requirements retrospectively and to evaluate their infection rates at our clinic for 2 years.

2. Materials and Methods

In this retrospective study, all ventriculostomy procedures were examined dates between January 2012 and 2014.

When the patient information and operations searched, we received permission from the patients and their families and we used Bizmed (data system of our hospital). All ventriculostomy procedures, dates between January 2012 and 2014, were scanned in the system of Bizmed (health care system). EVDs that were inserted after the shunt removal because of shunt infection were not included in the study.

By using the medical record system, patients' demographic (age, sex), diagnostic, ventriculostomy indications and ventriculostomy time had been reached. By accessing laboratory data, the patients' cultures of cerebrospinal CFTs and biochemical tests were analyzed retrospectively.

We compared our results (ventriculostomy time, revision rate, culture of CFT, infection rates) with published international studies.

Written consent was provided from all of the patients before the surgical intervention and the patients were informed about possible involvement in a scientific study.

The statistical analysis was performed using SPSS 22.0. Median, minimum and maximum values were used as definitive statistical values. The analysis of contingency tables was performed with Fisher's exact test. Statistical significance was accepted as $P < 0.05$.

3. Results

20 in 117 patients with external ventricular drainage were not included the study because of shunt infection and shunt occlusion of extracted patients. 148 EVD were inserted to 97 patients during their treatment period.

All ventriculostomies were performed in the operating rooms according to the procedure defined below:

Under local anesthesia, following proper skin preparation right kocher point was identified. (The intervention was performed from the left kocher point in patients with right frontoparietal paranchymal hemorrhage) A 4 cm incision was made with no 21 scalpel. Periosteum was laterally dissected from the bone. A burr hole was placed with a high speed drill. Dura was cauteried with the bipolar cautery and a 5 mm incision was made with no 15 scalpel. Ventricular catheter was placed into the frontal horn. With the establishment of CSF drainage, the subcutaneous tissue and the skin were properly closed.

Evd catheter that contains antibiotic was not used in any of the patients. Before the surgical incision 1 g sefazolin was made all the preoperatively patients. Intravenous ceftriaxone vancomycin 3 × 2 g and vancomycin 4 × 500 mg therapy were given to the 7 patients with a diagnosis of central nervous system infection (menengitis, abscess) from the moment of diagnosis to the duration of hospital stay.

The patients were followed at the intensive care unit or neurosurgery clinic from the moment of inserting

EVD to the removing it by paying attention to sterilization. 3 times in a week, with using aseptic techniques samples of CFTs were taken by brain surgery doctors. Samples were delivered to microbiology laboratory within 10 minutes. EVDs that were not the requirement of the patients' EVDs were filmed aseptically and skin incision was sutured aseptically.

3.1. Sex and Age

53 patients in the study was male and 44 ones was female. The average age of the patients was 42 - 24 (Standard deviation (SD) ± 25.81). The average age was 49. Our oldest patient that was mounted EVD was 88 years old. Our 2 patients' age was under 1 year and the youngest one was 3 days old. Maximum between 60 - 69 age group patients were inserted EVD and large propotion of our patients was composed of patients between the age of 0 - 9 and 40 - 69.

3.2. Indications for EVD

The majority of the cases we used EVD (55.7%) was due to hemorrhagic cerebrovascular diseases (subarachnoid hemorrhage, intraventricular hemorrhage, intracerebral hemorrhage, cerebellar hemorrhage, traumatic intracranial injury). Tumors were the second most common cause we used EVD (24.7%). In this group we included tumor-caused obstructive hydrocephalus and post surgery EVD use for ICP monitorization patients. Other important indications are CNS infections (7.4%) (meningitis, abcess), obstructive cerebrovascular disorders (12.4%) (post-infarction hydrocephalus or cerebral odema) (**Table 1**).

3.3. EVD Period

We defined this period from applying EVD catheter till the need for EVD passed and to the point where we removed the EVD. We changed the EVD catheter at 14th day in patients with need of EVD more than 14 days. The average time for EVD application was 13.96 days (**Table 2**).

3.4. CSF Culture

Cerebral spinal fluid (CSF) samples were obtained from EVD catheter using aseptic technique. CSF samples were obtained in case of the changes of fever, white blood cell number and mental status were observed and they were sent to the microbiology laboratory for evaluation. CSF samples were sent to biochemistry laboratory in order to determine cell number and typing at the same time within 3 day intervals. During our study, 23 patients (23.7%) developed leukocytosis in 97 evaluated patients (leukocytosis > 10 cells in 100 magnification). Coagulase-negative staphylococci is the most growth in 12 patients' culture who had positive CSF culture.

Table 1. Indications of EVD.

Indications for EVD	No. of cases	Percentage of total
Hemorrhage	54	55.7
Tumor	24	24.7
Infection	7	7.4
Others (infarct, normal peresure hydrocephalus, brain edema)	12	12.4

Table 2. Duration of EVD.

Duration of EVD	Days
Mean	13.96 (SD = 14.439)
Longest	91
Shortest	1

Much growth of staphylococcus epidermidis in culture samples that suggests the possibility of transmission. Other pathogens are shown in the following table (**Table 3**).

3.5. EVD Revision and Infection

Revision was performed in case of situations like EVD occlusion or exceeding 14 days EVD requirements during the treatment of the 30 patients. Leukocytes were observed in 14 of the patients who underwent revision. Leukocytes were observed in only 6 patients who did not undergo revision (Fisher's exact test P = 0.000). The observed rate of leukocytes in patients who underwent revision was statistically regarded significant compared to the patients who did not undergo revision. In 7 of the 30 patients who underwent revision bacteria were detected in their CSF culture. Bacterial growth occurred only in 5 of the 67 patients and from them, 3 patients' bacterial growth was coagulase-negative staphylococci. Bacterial growth was regarded significant in patients who underwent revision with the Fisher's exact test (P = 0.043).

3.6. Bleeding and Infections

EVD was inserted to the 54 in 97 patients because of hemorrhagic cerebrovascular disease. Bacterial growth occurred in 6 among 54 patients. Among these 6 patients, 3 were inserted SAK and the other 3 patients were inserted EVD because of intracerebral hematoma. Because of bleeding EVD inserted patients' infection rate when compared considering other results, it was regarded statistically insignificant (Fisher's Exact test P = 0.761) (**Table 4**).

4. Discussion

Bacterial growth was 12.7% in two years follow-up of patients inserted-EVD (12/97). This infection rate is found to be close to the lower limit in comparison with the literatüre. The average infection rate in a literature study by Lozier and his friends in 2002 was reported to be 8.8% (2.13% - 21.95%) [6]. But the infection rates associated with EVD were evaluated in most of these performed studies. The EVDs which have already inserted due to diagnosed meningitis were not assessed.

In our study, infection rates drop 10% if we exclude meningitis patients. When excluded our patients with the diagnosis of meningitis before, coagulase staphylococcus bacteria occurred in 4 patients among 9 who had bacterial growth in their CFT. In line with these results if we give more importance to the sterilization and EVD

Table 3. The distribution of bacteria in the CSF culture.

CSF culture	No. of cases
No growth	85
Total CSF positive culture	12
Coagulase negative staphylococcus	4
Klebsiella	3
Acinotobacter baumani	2
Pseudomonas argineusa	2
MRSA	1

Table 4. EVD infections rates caused by hemorrhage versus others.

Hemorrhage (infection vs no infection)	Hemorrhage as primary presentation	Hemorrhage not as primary presentation	Total
Infection	6	6	12
No infection	48	37	85
Total	54	43	97

care that we can prevent situations that may occur as a result of skin infections. So that our infection rates will fall to very low levels.

5. Conclusion

In conclusion, it does not show huge differences when we compare our infection rates with international literature. Except for revision patients another criterion was not found that significantly increases the rate of infection. We conclude that a rigorous peri-operative preparation and care of regular EVD may reduce the rate of infection.

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Long Coronary Lesions: Challenging Cases for Percutaneous Coronary Intervention

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Abstract

Long coronary lesions are associated with adverse outcomes after percutaneous coronary intervention since the era of plain balloon angioplasty. Long lesion and long stent length are considered as important predictors of restenosis after percutaneous coronary intervention. With the advent of newer generation drug eluting stents, there has been dramatic reduction in the rates of restenosis and repeat revascularization, even in complex cohort of patients with long coronary lesions. We report one such case of long coronary lesion which was intervened successfully with newer generation, thin strut, biodegradable polymer coated sirolimus-eluting stents.

Keywords

Drug Eluting Stents, Biodegradable Polymer, Percutaneous Coronary Intervention, Treatment Outcome

1. Introduction

The treatment of long and diffuse coronary lesions remains a challenge for interventional cardiologists, since the era of balloon angioplasty. A higher angiographic restenosis rate of 58% has been reported after the intervention of long lesions with balloon angioplasty [1]. Although, the advent of bare metal stents (BMS) was a breakthrough discovery, it was not completely successful in the treatment of advanced coronary artery disease, involving long lesions. Even in the BMS era, implantation of multiple and long stents resulted in higher incidence of diffuse in-stent restenosis [2]. The treatment of resultant diffused in-stent restenosis is again problematic requiring multiple additional PCI's or coronary artery bypass grafting (CABG) [3]. Thus, owing to the challenges and complications involved it became imperative to develop an optimal strategy for percutaneous intervention of long coronary lesions and other complex subsets.

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The introduction of drug eluting stents (DES) enkindled new hopes and paved the path for long term optimum management of long coronary lesion. Initial experience with first DES, Cypher sirolimus-eluting stents demonstrated that despite the reduction of clinical events, deployment of multiple overlapping stents for long lesion, an approach colloquially called as "full-metal jacket", is an approach with some risks [4]. But at the same time, few randomized trials and registries that involve implantation of DES for long lesions proved that it was associated with reduced rates of restenosis and target lesion revascularization, as compared to BMS [5] [6]. With the passage of time, continuous modification in stent design, architecture and material has helped us develop newer iterations of DES, which are equally effective in long coronary lesions. Technical advances in development of new stents such as thin strut, biodegradable polymer coated sirolimus-eluting stents have encouraged manufacturers to fabricate long stent platforms with the aim to increase the success of percutaneous revascularization avoiding multiple overlapping stents.

We report one such case of long coronary lesion which was intervened successfully with a long newer generation, thin strut, biodegradable polymer coated sirolimus-eluting stents.

2. Case Report

A 71-year-old woman was admitted in our centre for an episode of acute chest discomfort that occurred 1 week before. She had type 2 diabetes mellitus and a family history of ischemic heart disease. An electrocardiogram was performed, which showed "rS" pattern in V1-V3 leads and negative T waves in precordial leads. High sensitivity troponin T was within the normal values in two serial measurements. An abnormal echocardiogram by two-dimensional echocardiography revealed non-dilated left ventricle with normal ejection fraction and anterior and anteroseptal mid-apical hypokinesia. A cardiac magnetic resonance with dipyridamole was performed showing necrosis of three segments in the territory of left anterior descending (LAD) artery with severe peri-infarction ischemia and myocardial viability in most segments. Coronary angiography was performed after 48 hours of admission which showed a single-vessel disease with a focal severe stenosis in the proximal segment and large, subocclusive stenosis in the mid-distal segment of LAD (**Figure 1**). An angioplasty was performed using a sheathless 6.5 Fr guiding catheter and a hydrophilic soft guide. After predilatation with two semi-compliant 1.5 × 20 mm and 2.5 × 30 mm balloons (**Figure 2**), two sirolimus eluting overlapping stents were implanted (Aima Plus 3.0 × 48 mm and Aima Plus 3.5 × 22 mm distal and proximal, respectively) (**Figure 3**). Overlapping was limited to 3 mm and located at the proximal zone. At the end of the procedure an optimal anatomical result and distal TIMI 3 flow was achieved (**Figure 4**). At 6-month follow-up the patient is asymptomatic and no adverse events have been registered.

3. Discussion

Coronary stents are customarily used during angioplasty to improve immediate and long term outcomes and overcome loopholes associated with balloon angioplasty. In the infancy stage of its development, coronary stents had several limitations. The then available metallic platforms fabricated stents had stiffer configuration and shorter lengths of 15 to 20 mm. Multiple overlapping stents were used to treat diffuse long narrowings and specially designed wires were needed to navigate the stent and overcome vessel tortuosity.

Approximately, long lesions consist of 20% of all percutaneous coronary angioplasty and long or multiple overlapping stents are continuously being used for its treatment [7]. Implantation of multiple stents is time-consuming and expensive. Moreover, accurate placement of multiple stents is difficult with long overlapping stent segments. Overlapping stents are potential source of neointimal hyperplasia due to physical double layer of stent struts, and inadvertently sometimes a short gap is lapsed out between multiple stents, which increases the risk of acute or sub acute stent thrombosis and restenosis [8].

Pooled analysis of five clinical trials using sirolimus-eluting stents and bare metal stents suggest that overlapping stents increases the risk of late lumen loss and restenosis in both BMS and SES [9]. Contrastingly, a study by Lee *et al.* comparing the outcomes after overlapping versus one long stent in long coronary lesions demonstrated that overlapping can be used with outcome similar to that of one long stent in long coronary lesions [10]. But at the same time it also stated that age (≥65 years old) was an independent risk factor of in-stent restenosis (ISR) (54% vs. 23%; OR = 4.4; P = 0.04).

This was a case of a long severe disease of LAD. In most of the cases, such types of lesions are referred to surgery because traditional PCI is not successful. And still, if PCI is performed three or four stents are necessary

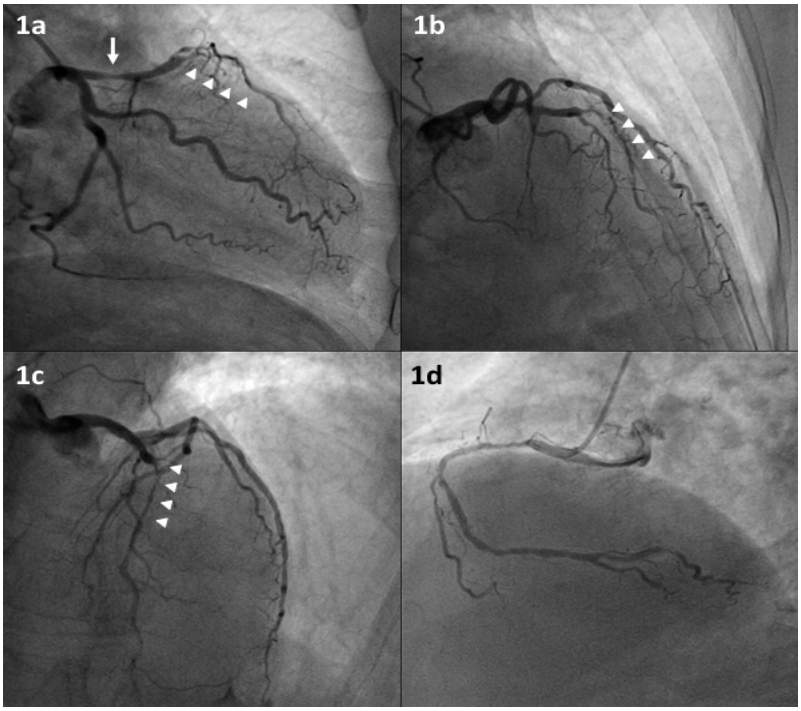

Figure 1. Diagnostic coronary angiography. (a) Left coronary artery, right caudal (spider) projection; (b) Left coronary artery, right cranial projection; (c) Left coronary artery, left cranial projection; (d) Right coronary artery. Severe proximal stenosis (long arrow) and subocclusive mid and distal large stenosis (arrowheads) in left anterior descending artery ((a), (b) and (c)). Diffuse disease in right coronary artery (d).

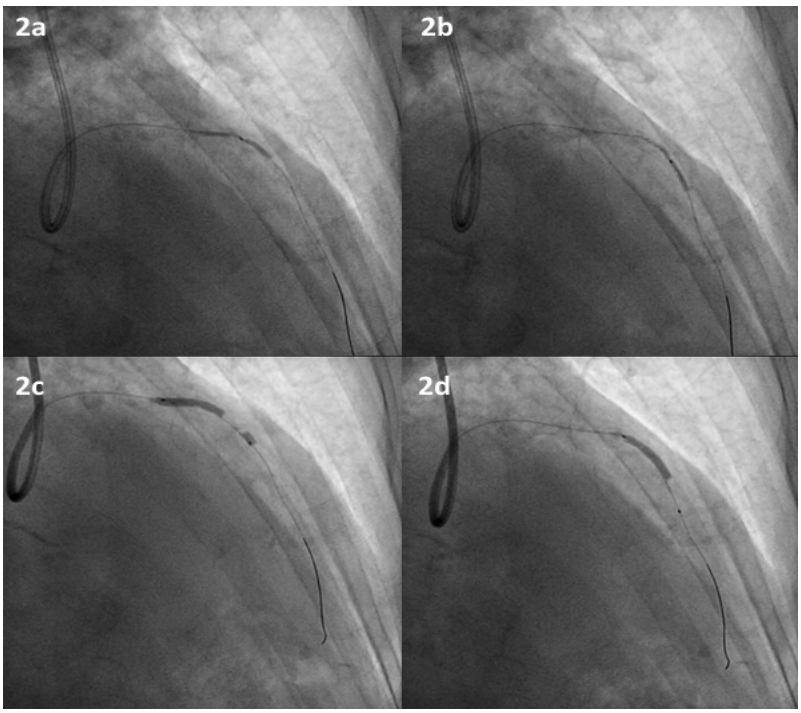

Figure 2. Right cranial projection. Predilatation with 1.5 × 20 mm. (a) & (b) and 2.5 × 30 mm (c) & (d) semi-compliant balloons.

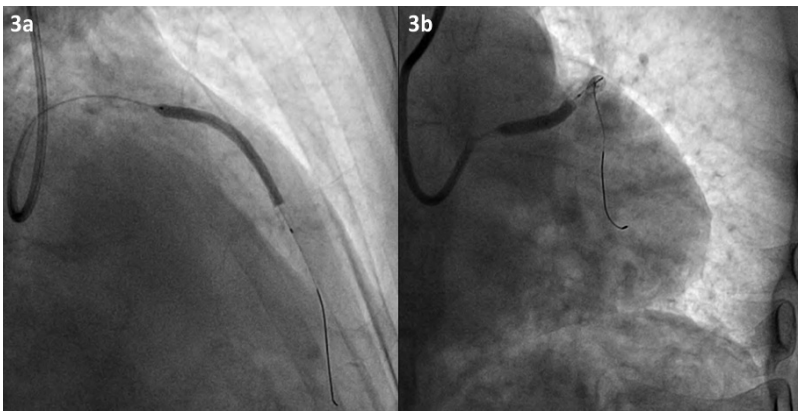

Figure 3. Right cranial projection. Implantation two drug-eluting overlapping stents: AimaPlus 3.0 × 48 mm distally (a) and Aima Plus 3.5 × 22 mm proximally from ostium (b).

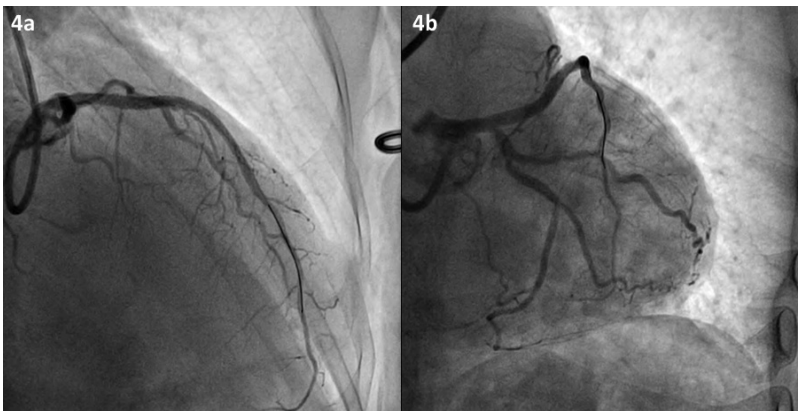

Figure 4. Final result. (a) Right cranial projection; (b) Right caudal (spider) projection.

with high degree of overlapping amongst the stents and this strategy is associated with higher rates of restenosis. Moreover, the patient was elder in age (71 years), which increases the chances of ISR with multiple overlapping stents. Thus the discretion of interventionists to use long stents to minimize the risk of restenosis and adverse outcomes is commendable.

The critical issue in use of long coronary stents is easy navigation and successful deployment to achieve optimal immediate and long-term outcomes. The newer generation biodegradable polymer coated drug eluting stents with thin struts are easy to navigate and deploy. The AIMA plus is one such newer generation biodegradable polymer coated sirolimus-eluting stents that incorporates technical specifications that improve navigability and procedural success. The uniquely designed feature of the AIMA plus is highly flexible S-link to improve four "ability" related aspects; those are deliverability, trackability, pushability, and crossability.

Thus, the newer iterations of DES, with advanced stent design and architecture, can successfully be used for intervention, even in complex cohort of patients with long coronary lesions.

4. Conclusion

Despite of the challenges and complications involved in the percutaneous coronary intervention of long coronary lesions, they can be intervened successfully with newer generation drug eluting stents.

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None.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Video-Assisted Anterior Retroperitoneal Approach to the Lumbar Spine. A Minimal Invasive Technique Improved by the Use of Endoscopic Camera to Treat Lumbar Spine Diseases

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Abstract

The anterior retroperitoneal approach is a technique to treat disc degenerative disease (DDD), spinal deformities, traumas, tumors and infections. It can be used to perform Anterior Lumbar Interbody Fusions (ALIF) or Total Disc Replacements (TDR). Though being a fast procedure that is becoming more often used by spinal surgeons, the anterior approach requires an adequate knowledge of the anatomy of the abdomen to lessen the risks of intraoperative complications. The authors' preferred technique is the left retroperitoneal video-assisted approach, using an endoscope to visualize and magnify the deep anatomical structures, discectomy procedure and hardware implant. In a review of our casuistry from 2010 to 2012, 163 patients underwent an anterior lumbar approach, and 139 of these had a single L4-5 or L5-S1 level treatment. A 3.6% rate of global complications (1.44% of major vascular injuries) has been observed, with a mean operation time of 116.4 minutes and a mean blood loss of 156.1 ml. These values show how the anterior retroperitoneal video-assisted approach for the treatment of lumbar diseases is a fast procedure that implies low blood loss, with a low rate of intraoperative complications.

Keywords

Video-Assisted Anterior Retroperitoneal Approach, ALIF, TDR

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1. Introduction

An anterior approach to the lumbar spine is often required in patients affected by chronic low back pain with or without radiculopathy, non-responsive to of conservative treatment (medication, physical and pain therapy) for a period of at least 6 months. Degenerative disc disease (DDD), degenerative spondylolisthesis, lumbar stenosis with segmental instability, previous unsuccessful posterior lumbar surgery, non-union, restoration of disc height, spinal deformities, trauma or infections are the main indications for anterior lumbar approach.

On the other hand, the main challenges are previous abdominal or gynecological surgery performed by the retroperitoneal or transperitoneal routes (colonic resection, hysterectomy), vascular anatomical variations (low iliac bifurcation: in front of L5-S1, extremely lateral path of the common iliac vein on the left side: laterally in the L4-L5 space) and abdominal diseases (Crohn's disease, ulcerative colitis).

There are different kinds of anterior approaches described in the literature: the eXtreme Lumbar Interbody Fusion (XLIF) is a modification more recently introduced, which involves a lateral incision, a blunt mobilization of the peritoneum and a smooth dissection of the psoas muscle. However, the anatomical limits of this technique, as the height of the iliac crest and lumbar plexus morphology below the psoas muscle could create problems in treating some levels (*i.e.* L4-5) thus limiting the applications of this technique. Huge X-rays exposition is another issue correlated with this technique, because fluoroscopy is mandatory for the correct identification of landmarks and positioning of the hardware and in each step of the procedure. A good option for an anterior approach to the lumbar spine is the anterior retroperitoneal approach, useful to perform a lumbar interbody fusion (Anterior Lumbar Interbody Fusion, ALIF) or a Total Disc Replacement (TDR) [1]. This approach is really minimal invasive for the patient, as the only incisions that are required are those of the skin, the fatty under skin layer and the fascia of the rectus muscles. From there beyond, only the smooth blunt dissection and mobilization of the rectus muscle and of the abdominal structures are required to reach the intervertebral disc. In this paper we will describe the technique we personally use to perform the retroperitoneal anterior approach; it's a video-assisted technique with the use of an endoscope. This tool offers a good magnification and illumination of the surgical field, minimizing the incision and tissue damage and further increasing the precision of the surgery. The use of endoscope improves visualization of spinal anatomy, allowing a complete disc removal. Endoscopic disc decompressions are performed under continuous visualization.

2. Retroperitoneal Anterior Approach to the Lumbar Spine

The retroperitoneal approach is our preferred technique because it respects the anatomy of the abdominal structures. The standard retroperitoneal approach is performed with a midline skin and under skin layer incision, with a left approach to the intra-abdominal structures. Different authors have proposed many variations to this technique. In 2012 a midline approach from the right side was proposed by Edgard-Rosa *et al.* [2]. In this paper the authors proposed a new mobilization technique of the vena cava to perform interbody fusions from L2 to L5, with an approach below the aortic bifurcation to L5-S1 disc. The authors reported four major venous injuries on a total of 469 patients included in the study. However, we prefer the left side approach because the first big vessel found during the approach is an artery (the common iliac artery). This decreases the risk of vascular injury because of the thicker walls of arteries in respect to veins. Authors preferred technique is performed with the patient lying in a supine position, with extended legs. A 3 - 5 cm transversal medial incision (for L5-S1 exposure) or a longitudinal left paramedian incision (for L4-L5 exposure) is performed (**Figure 1**). The anterior sheath of the rectus is cut and the muscle is retracted upward and laterally, to preserve nervous and bloody supply avoiding damages to the inferior epigastric vessels; the peritoneum and the left ureter are then gently pushed from the left to the right side (**Figure 2**) until the psoas muscle is seen. At this point the arcuate ligament is cut (**Figure 3**). This allows the widening of the working space and a better visualization of the deep structures. After the insertion of deep retractors, the big vessels are visualized and mobilized. For the L4-5 disc, aorta, cava and common iliac veins and arteries are retracted laterally from left to right after the ligature of the ileolumbar veins. The L5-S1 disc is located below aorta and cava bifurcation into the two common iliac arteries and veins, respectively. These vessels are then retracted on both sides to expose the disc, and the middle sacral vessels are coagulated with a bipolar forceps or ligated.

After the complete exposure of the disc, the retractors are fixed to the bony surface through dedicated pins. This very stable configuration avoids the risk of soft tissue or vascular injury due to the implant of the pins. A ring is then placed to connect the handles of the retractors, in order to achieve a 360 degrees stability to keep the

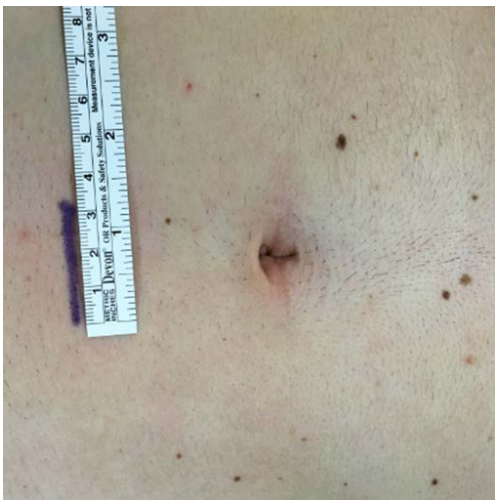

Figure 1. Midlineskin incision below the navel.

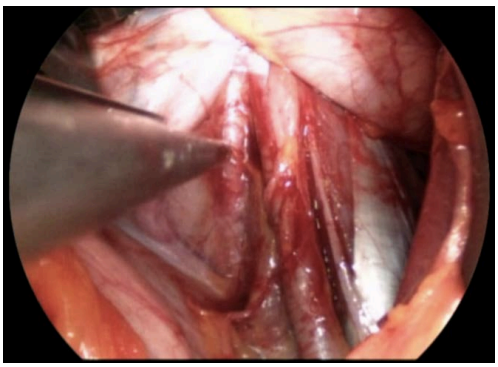

Figure 2. Intraoperative endoscopic view of ureter (indicated with an anatomic claw) and common iliac artery (on the right).

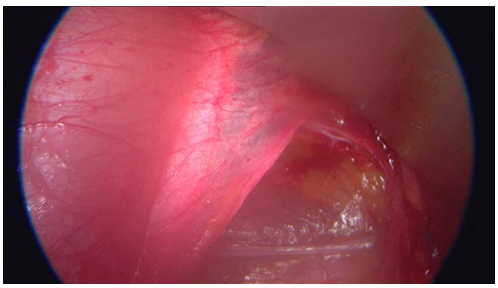

Figure 3. Intraoperative endoscopic view of the arcuate line (also known as linea semicircularis or Douglas' line).

surgical field wide open. Under endoscopic view and magnification, two horizontal cuts are performed with a scalpel on the anterior longitudinal ligament, corresponding to the upper and lower limits of the disc. A vertical incision is then performed on the midline, and the anterior ligament is detached from the midline laterally. This procedure results in two flaps that are preserved to be sutured together after the cage implant. The disc is then removed and the endplates prepared scrapping off the cartilaginous layer (**Figure 4**). Increasing sizes templates are then implanted in the intervertebral space, in order to find the proper fit (**Figure 5**). Finally, the definitive cage is implanted and fixed with an anterior plate with screws (we usually implant a three-screw plate for L5-S1

space, and a four-screw plate for the L4-5 disc) in order to increase primary stability (**Figure 6**). It has been demonstrated that a cage with an anterior plate has the same stiffness than a cage with posterior fixation (four pedicle screws) [3] (**Figure 7**). The whole procedure is performed through a 5 cm incision with the use of an endoscopic camera. This allows the best view on a HD monitor at a higher magnification for all the surgical steps, allowing a lesser invasivity and a better accuracy.

Anterior retroperitoneal approach allows the treatment of the lumbar spine preserving the paraspinal muscles and consequently without disruption of the posterior tension band. Moreover, joint morphology and innervation are preserved, maintaining a better proprioceptive capacity at the treated levels. Furthermore, performing a single anterior approach decreases the morbidity of the patients, and the implant of a wider intervertebral cage is possible without entering the canal. This allows a better restoration of the segmental lordosis and a greater cage-bone contact surface, promoting osteointegration and fusion. Finally, this kind of approach requires only one shot with the C-arm to evaluate the final position of the cage. Since the whole procedure is observed directly

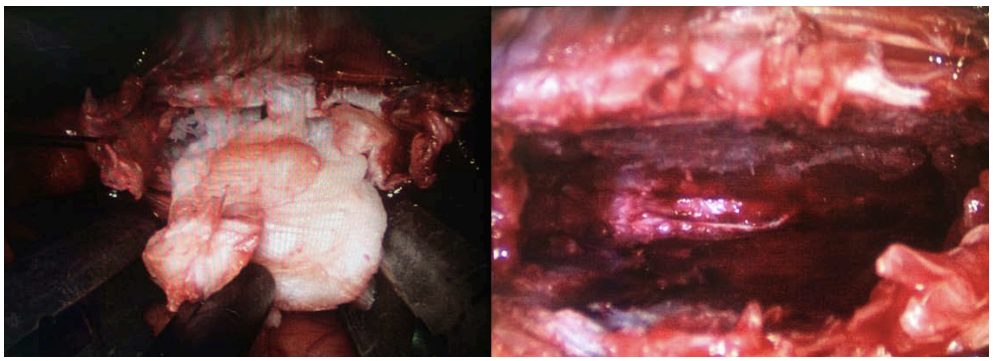

Figure 4. Intraoperative endoscopic view of discectomy and disc space preparation.

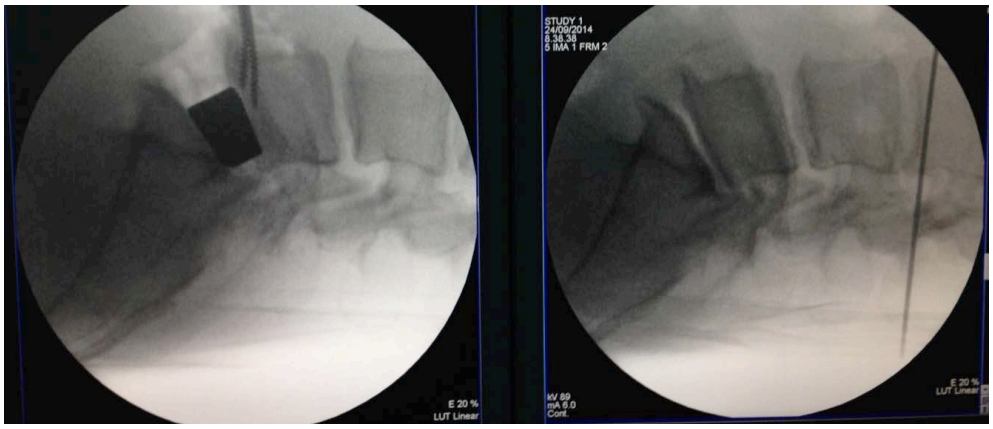

Figure 5. Intraoperative X-ray showing the increase of lumbar lordosis after cage implant.

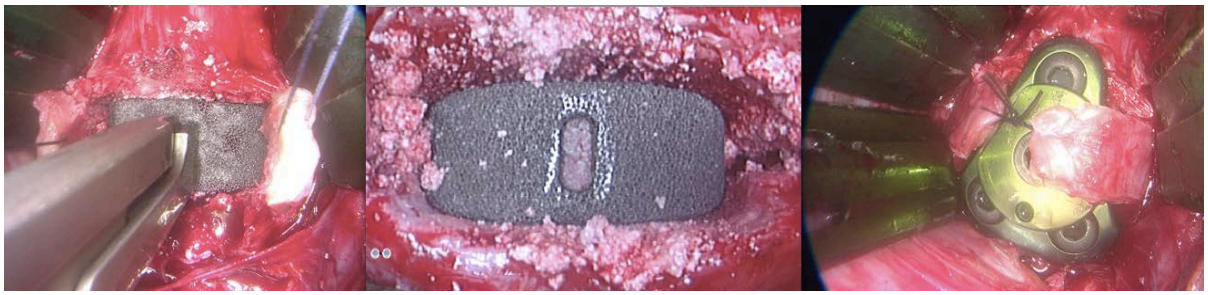

Figure 6. Intraoperative endoscopic view of definitive trabecular metal cage filledwith autologous bone graft and subsequent anterior plate fixation. The two flaps of the anterior longitudinal ligament can now be sutured together.

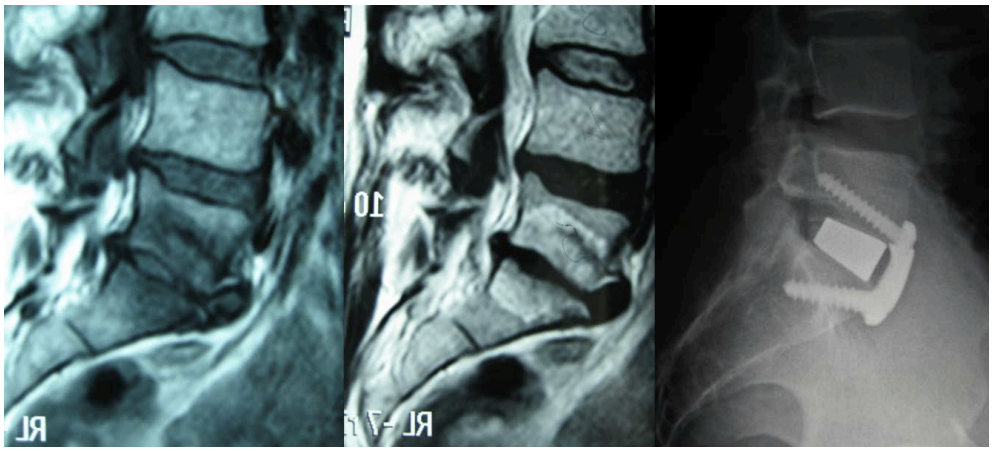

Figure 7. Preoperative sagittal lumbar MRI (T1 and T2 sequences) showing a L5-S1 discopathy (Pfirrmann 5) and postoperative X-ray (lateral view) of a L5-S1 ALIF. The patient has been treated with a tantallum cage and a three-screw anterior plate. This construct has the same stability than an anterior cage with four pedicle screws. It is clearly evaluable how the segmental lordosis have been properly increased after surgery.

from the surgeon and using the endoscope, no intermediate X-rays are needed. This decreases the radiation dose to the patient and to the surgeons in respect to standard posterior procedures, in which fluoroscopy could be performed for each pedicle screw and cage insertion.

3. Complications

The anterior retroperitoneal approach allows the treatment of a wide range of pathologies affecting the lumbar spine, but can be a source of hazardous complications. The most common are: damages to the vascular, urologic, gastrointestinal and neurologic structures. For these reasons, many spinal surgeons need the help of vascular or general surgeons (access surgeon) to perform this procedure.

3.1. Vascular Complications

Mobilization of iliac vessels is a cause of potential lesions. The reported incidence of major vascular injury varies in literature between 2% and 4%, depending on the studies [4]-[10]. The left common iliac vein is the most likely vascular structure to be injured during anterior retroperitoneal approach, especially for the L4-5 disc. Calcified arteries in elderly could represent an increased risk of damage during vessel manipulation. Postoperative bleeding could occur when an incomplete ligature of the ascending ileolumbar vein has been performed during the exposition of L4-5 disc or due to a missed identification of small bleeding arteries. An adequate check should be performed before closure with an increasing blood pressure of the patient. Apart from intraoperative or postoperative bleeding and risk of massive and life threating hemorrhage, venous thrombotic occlusion could occur in the postoperative period. This could happen as a consequence of a prolonged retraction of the vessels. Arterial thrombosis occurs in less than 1% of cases, and is frequently associated with prolonged retraction [5] [6] or due to pathologic calcifications.

3.2. Urologic Complications

The ureter is the main extra peritoneal urologic structure observed during the exposure. It is usually attached to the inferior surface of the peritoneum, and is clearly visible when laterally retracted. It should be pinched with an anatomical claw to be identified observing its typical contraction. Ureteral lesions during primary anterior surgery are really rare, while they happen more frequently during revision surgery, as the scar tissue in front of the instrumentation could encase it [11]. Retrograde ejaculation is a complication that couldoccur in men underwent anterior retroperitoneal approaches, especially to L5-S1. Sterility could result from the inability of the internal vescical sphincter that allows the semen to enter the bladder instead of traveling up the urethra. The superior hypogastric plexus provides innervation to the internal vescical sphincter. This plexus lies on the anterior

surface of the L5-S1 disc, and needs to be mobilized to perform the discectomy. To reduce the risk of this complication it is important to avoid using monopolar coagulation during the deep dissection. The overall rate of retrograde ejaculation varies from 0.42% to 5.9%, according to the literature [12] [13]. A study of Sasso *et al.* published in 2003 [14] demonstrated that patients underwent a lumbar discectomy and fusion through a transperitoneal approach at L4-5 and L5-S1 levels had a 10-times higher incidence of retrograde ejaculation than those who had the same procedures with a retroperitoneal approach.

3.3. Gastrointestinal Complications

The most common gastrointestinal complication observed in the postoperative period in patients underwent an anterior retroperitoneal approach is ileus [11]. The use of anorogastric tube and preoperative bowel preparation are routine measures to decrease the risk of this complication. All the patients are provided with 140 mg of macrogol oral powder in 2 liters of water the day before surgery. The postoperative use of opioids could also increase the risk of constipation.

4. Personal Experience

Analyzing the patients underwent an anterior retroperitoneal lumbar access in our Department from 2010 to 2012, we obtained a cluster of 163 patients, 60 males and 103 females. The mean age was 43 ± 10 years for men and 46 ± 9 years for women. A single level L5-S1 anterior approach was performed in 141 patients (61 men 43.3% and 80 women 56.7%), a single level L4-L5 anterior approach was performed in 12 patients (3 men 25%, 9 women 75%), a two-level L4-L5 L5-S1 was performed in 10 female patients. ALIF was performed in a total of 132 patients (81.0%). A disc prosthesis was implanted in 10 patients (6.1%). An ALIF + TDR was performed in 4 patients (2.5%). 17 patients (10.4%) underwent a double approach surgery in a single stage. If we consider only the single level procedures (L4-5 or L5-S1), 139 patients can be enrolled. The average time of surgery was 116.4 minutes, with 156.1 ml of intraoperative blood loss. These values suggest that anterior approach for single level treatment is a quick procedure with low intraoperative blood loss. If we further consider our rate of complications in the same cluster, we had 3 cases of retrograde ejaculation (2.16%), 2 iliac vein lesions (1.44%) and one immediate revision for hardware malposition (0.72%) (**Table 1**).

5. Conclusion

The anterior approach to the lumbar spine is an effective technique to treat disc degenerative disease, spinal deformities, traumas, tumors or infections. Though being a fast procedure that is becoming more often used by spinal surgeons, the anterior approach requires an adequate knowledge of the anatomy of the abdomen to lessen the risks of intraoperative complications. The retroperitoneal approach has been demonstrated to be safe and less risky than the transperitoneal one, especially regarding the retrograde ejaculation complication [14]. What we suggest and usually perform is a retroperitoneal video-assisted technique that allows a better view with the use of an endoscopic camera, allowing a magnification of the approach-related structures on a monitor. This let the surgeon being less invasive and more accurate in performing this approach and the discectomy for an ALIF or a TDR procedure. In our personal experience we observed how this technique is fast with a very low blood loss in respect to traditional posterior techniques. In our series the rate of complications is lower or similar to those found in literature. Video-assisted anterior lumbar surgery has advantages of little incision, minimal tissue

Table 1. Complication rates on a pool of 139 patients underwent single level surgery (L4-5 or L5-S1).

Complications	Cases	%
Retrograde ejaculation	3	2.16%
Iliac vein lesions	2	1.44%
Hardware malposition	1	0.72%
Revision	1	0.72%

retraction and minimal bleeding with consequent less blood loss, less trauma. Endoscopic video-assisted surgery is a safe, effective method in treating degenerative disease of the lumbar spine. Although, in literature there is no evidence of difference in meanoperative room time, intraoperative complications, or total hospital length of stay, between video assisted and traditionalsurgery [15].

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Benefits of Simple Exchange Transfusion in Sickle Cell Disease (HbSS) with Vaso-Occlusive Crisis Not Responding to Standard Therapy

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Abstract

Sickle cell disease is an autosomal recessive genetic disease. Vaso occlusive crisis (VOC) is frequently seen in such patients. Painful VOC is usually recurrent, of variable severity due to many factors and its management poses important challenge in the clinical practice. Few patients do not respond to standard therapies and continue to suffer severe pain for prolonged period or land to serious life threatening situation. The red cell exchange by aphaeresis is presumed to be one efficient alternative in this situation which can reduce the level of HbS below 40% - 50%. However, it is costly and not available everywhere. Both circumstances are common in our state where incidence of sickle cell disease is quite high. In such situations simple red cell exchange *i.e.* removing 1 unit (350 ml) of blood manually (by phlebotomy) and replacement with one unit normal red cell is effective. All of our four cases of SCA with severe acute VOC, are not responding to standard therapy but responded efficiently to this simple red cell exchange transfusion. Our present observation may pave the way of one simple, affordable, and effective measure to reduce the pain of severe acute VOC not responding to standard therapy. Moderate reduction of HbS by 8% - 14% by simple red cell exchange transfusion was associated with relief of pain of acute VOC; a new observation was reported in all our 4 cases which need to be validated by larger controlled studies.

Keywords

Vaso Occlusive Crisis, Simple Red Cell Exchange Transfusion, SCA (HbSS)

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1. Introduction

Homozygous sickle cell disease (SCD) is an autosomal recessive genetic disease. The fundamental defect in SCD is the substitution of thymine for adenine in the sixth codon of the gene for the β-globin chain, leading to a replacement of glutamic acid by valine at this site. In comparison to normal haemoglobin tetramers, HbS has an altered surface charge that promotes the formation of lengthy polymeric chains (gelation) when in the deoxygenated state. The oxygen affinity of dilute, unpolymerized HbS is similar to that of normal hemoglobin. However, the oxygen affinity of concentrated HbS solutions is decreased [1], thereby representing a further stimulus for molecular polymerization. Vaso-occlusion is the outcome of dynamic combination of abnormalities in haemoglobin structure and function, red cell membrane integrity, erythrocyte density, endothelial activation micro vascular tone, inflammatory mediators and coagulation.

Painful vaso occlusive crisis (VOC) is usually recurrent, of variable severity due to many factors and its management poses important challenge in the clinical practice. Judicious use of IV fluids, efficient use of IV opioids (morphine) and after other potential analgesics, maintaining oxygen saturation more than 95%, prevention of infection, acidosis, exertion, and other precipitating factors constitute the standard of care [2]. Few patients do not respond to these therapies and continue to suffer severe pain for prolonged period or land to serious life threatening situation. Reduction of HbS below 40% to 50% is considered as one of the significant parameters which can inhibit the HbS polymerization and thus prevents VOC [3]. Red cell apheresis is the most efficient method to reduce the HbS level immediately without increasing the viscosity and successfully tried in patients with severe VOC not responding to standard therapies. But this Apheresis procedure is expensive and it needs specialized equipment and set up. Such facilities are not available in most part of Odisha state where a lot of SCD patients live and are treated with such complications. Simple exchange red cell transfusion though less effective than red cell apheresis can reduce HbS level and has not been studied properly in this context.

Red cell exchange can provide needed oxygen carrying capacity while reducing the overall viscosity of blood. Acute red cell exchange is useful in acute infarctive stroke [4], in acute chest and the multi-organ failure syndromes, the right upper quadrant syndrome, and possibly priapism. Our present case has shown impact of simple exchange transfusion on resolution of acute pain episodes.

We report four cases of SCD (homozygous) with severe VOC not responding to standard therapy which were managed successfully with simple red cell exchange transfusion.

2. Case Report

The salient features of all cases are presented in **Table 1**.

2.1. Case 1

A 20 years old male, worked out case of sickle cell anaemia, (SCA) (homozygous) with history of recurrent VOC (four times per year) receiving standard therapy like hydroxy urea (15 mg/kg/day) folic acid (5 mg/day) got hospitalised clinical haematology department of our institution with complains of multiple joint pains, and chest pain with pain score 9 as per Visual Analogue score (pain gradation scale). On examination pulse rate 98/minute, blood pressure 120/82 mmHg, respiratory rate—23/min, and temperature—99.4˚F. On systemic evaluation like

Table 1. Effect of red cell exchange on our cases.

Case	Age (Yr)	Sex	Pretransfusion		Posttransfusion		No. of red cell unit	Pain gradation		Day of relief
			HbS	HbF	HbS	HbF		Before	After	
1	24	M	78	20	70	20	2	9	3	2
2	17	F	82	15	73	14	2	10	3	2
3	22	M	72	25	64	24	2	9	2	2
4	19	M	74	24	68	22	1	8	3	1

cardiovascular, respiratory, gastrointestinal system, and locomatory system etc. were within normal limits. Haemoglobin—9.8 gm% (normal 13 - 16 mg/dL), TLC 6.8 × 10^3 (normal 4 × 10^3 to 11 × 10^3/dL) differential count—N 72 (normal 40 - 70), L—26 (normal 20 - 40), E-2 (normal 1 - 4), TPC—280 × 10^6 (normal 150 × 10^6 to 400 × 10^6) and normal mcv, mch, mchc within normal limit. Hb electrophoresis (Sebia: minicaf) quantified different Hb fraction as: HbS—78%, HbF—24%, hba—4%. Liver function test revealed—Total Bilirubin—0.6 mg/dL (normal up to 1.0 mg/dL), direct 0.1 mg/dL (normal up to 1.0 mg/dL) and indirect 0.5 mg/dL. AST 17 u/L (normal 5 - 40 u/L), ALT 25 u/L (normal 5 - 40 u/L), ALP 77u/L (normal 5 - 40 u/L) and viral marker like HbsAg, HCV, HIV were negative. Renal function test—blood urea 32 mg/dL (normal 10 - 45 mg/dL) serum creatinine 0.8 mg/dL (normal 0.6 - 1.1 mg/dL). Serum electrolyte levels were as—Na^+ 135 meq/L (normal 130 to 150 meq/L), K^+ 4.1 meq/L (normal 3.0 to 5.0 meq/L), Ca^+ 9.1 meq/L (normal 8.0 to 10.0 meq/L). E.C.G. and other test of cardiological evaluation excluded any possibilities of ischemic heart disease. Radiological investigation like X-ray of chest (P-A view) and ultrasound of abdomen and pelvis did not revealed any abnormalities. He was diagnosed as a case of SCA with acute VOC.

He received I.V. fluid (3 liter/24hour), injection soda bicarbonate and intermittent oxygen inhalation besides folic acid and hydroxyurea. Injection morphine was administered by IV root at the dose 1 mg 4 hourly. There was no relaxation from pain even after 48 hours of this treatment, rather the severity of pain increased. IV ketorolac (30/mg/24hour) was administered to alleviate the pain, but no improvement. Thus red cell exchange was planned in this case in Transfusion Medicine Department. Red cell exchange by apheresis facility was not available at that time in our institution, thus the option of simple red cell exchange was considered. About 350 ml blood was removed from the patient by phlebotomy by administration of one pint of 5% DNS and replacement of packed red cell. This procedure was followed for two consecutive days along with continuation of other above mentioned medications. The pain started decreasing after 8 hrs of first procedure, gradually decreased further and subsided completely after 12 hrs of second procedure. Post simple exchange quantification of HbS shows reduction from 78% to 70%. Follow up result showed Haemoglobin 9.7 gm/dL. Patient was discharged from the hospital on the next day of second procedure with advice to continue hydroxyurea, folic acid etc.

2.2. Case 2

Seventeen years old female, fully worked out case of sickle cell anaemia, (SCA, homozygous) receiving standard therapy got hospitalised to the clinical haematology department with the features of acute VOC (multiple joint pain), fever (103.4˚f), and lower respiratory tract infection. Her haematological evaluations were as follows: neutrophilic leucocytosis (ANC = 12.6 × 10^9/L) hb—8.7 gm%, HbS—82%, HbF—15% and other parameters being within normal limits. He received the standard therapy of acute VOC as like that of case 1, in adition to IV antibiotics (injection Ceftriaxone 2 gm IV twice daily and inj. Amikacin 500 mg IV twice daily). Fever subsided on fourth day without any relief from pain. Thus simple red cell exchange transfusion was performed in the Transfusion Medicine Department in similar manner to that of case no 1 for two consecutive days. Pain subsided on day six. Follow up result showed Haemoglobin 9.1 gm/dL. Patient got discharged on next day. Quantification of HbS shows reduction from 82% to 73% post simple exchange.

2.3. Case 3 and Case 4

Another two fully worked out case of SCA, on regular maintenance standard therapy (including hydroxy urea as 15 mg/kg/day) got hospitalised with the features of acute VOC. Haematological evaluation of both the cases revealed: hb—9.2 gm%, TLC—7.6 × 10^9/L, TPC—285 × 10^9/L, HBS—72%, HBF—25% and hb—7.8 gm%, TLC—8.6 × 10^9/L, TPC—310 × 10^9/L, HBS—74%, HBF—22% respectively. Other parameters including biochemical, electrolytes, and systemic evaluation of other systems like cardiovascular, respiratory, gastrointestinal, nervous system etc. were within normal limit. Both cases received standard therapy of acute VOC including IV opioids (morphine). There was no relief of pain after 72 hours, simple red cell exchange transfusion were provided to both the cases on day four as like that of case 1. Pain subsided completely after two procedures on day 6 in case of case 3 and after one procedure on day 5 of case 4. HBS quantification revealed reduction of HbS from 72% to 64% and 74% to 68% & Haemoglobin 9.3 gm/dL and 8.1 gm/dL respectively. Both cases got discharged on day 7 and day 6 respectively with the advice of regular intake of hydroxyurea (15 mg/kg/day), folic acid (5 mg/day) and measures to prevent VOC with regular health check up.

3. Discussion

Sickle cell anaemia patients have higher blood viscosity than normal persons having same haemoglobin level [5]. Sickle blood when deoxygenated has nearly a 10-fold greater viscosity than oxygenated sickle blood at the same hemoglobin level, with the effect being greatest at low shear forces [5] [6]. Vaso-occlusion is the outcome of dynamic combination of abnormalities in haemoglobin structure and function, red cell membrane integrity, erythrocyte density, endothelial activation microvascular tone, inflammatory mediators and coagulation.

Slowing of flow can cause cells to exceed the delay time and initiate red cell sickling in various smaller vessels such as venules. If flow slows it also provides additional opportunities for cell to cell interactions, cell adhesion to endothelium, activation of coagulation systems, and other time dependent processes that may facilitate the piling up of cells that often causes vaso-occlusion [7] [8]. Flow velocity varying with the fourth power of the radius (classic Poiseuille equation for laminar flow) makes such events most likely in the smaller venules, where sickling and vaso-occlusion is well documented [9].

Acute Pain Crisis episodes believed to result from vaso-occlusion, account for the majority of hospitalizations of sickle cell patients in our state Odisha (high incidence of sickle cell disease). Pain crises may involve practically any area of the body but most often are musculoskeletal or soft tissue in origin. Aggravating factors which may be brought on by a variety of initiating conditions, including extreme climatic condition, infection, fever, stress, acidosis, and hypoxia, but frequently there is no identifiable precipitants.

Sometimes, pain crises may be severe and can be unresponsive to high-dose narcotic analgesia. When severe acute pain crisis is unresponsive to the standard therapy of intravenous hydration and analgesia, exchange transfusion designed to lower the HbS to less than 40% to 50% may produce relief [3]. Debilitating cycles of frequent pain crises may be arrested by regular courses of transfusion that maintain the HbS at less than 40% to 50%.

Red cell exchange transfusion is an effective but perhaps underutilized therapy for both acute and chronic complications of sickle cell disease. In a red cell exchange, the patient's red cells are removed and replaced by exogenous normal red cells. The exchange prevents the removed sickle cells from participating in new vaso-occlusive events, reduces haemolytic complications, and provides added oxygen carrying capacity while decreasing the blood viscosity. The above are the possible mechanism associated with reduction of Hbs where we replaced patient's red cell with normal red cells.

The red cell exchange by aphaeresis is although preferred for red cell exchange but not available everywhere. Also it is quite costly. Both circumstances are common in our state where incidence of sickle cell disease quit high. In such situations simple red cell exchange *i.e.* removing 1 unit of blood (350 ml) manually (by phlebotomy) and replacement with one unit normal red cell is useful. Many paper supports and recommend exchange transfusion as part of treatment in VOC [4].

Analysis about the present four cases showed that all of our four cases of SCA with severe acute VOC, not responding to standard therapy but responded efficiently to this simple red cell exchange transfusion. Another two important points to be noted in our case reports that: the pain relief was early *i.e.* within 24 to 48 hour of red cell exchange so that patient got discharged quickly. Secondly even if the HbS reduction was not below 40% to 50%, even moderate reduction of HbS by 8% to 14% could lower the threshold for reduction of pain of VOC especially along with other standard therapy. In addition, the contribution of other factors vis-a-vis HbS in the pathogenesis of VOC could be another reason. This later observation needs validation by larger randomized controlled studies. However our observation may pave the way of one simple, affordable, and effective measure to reduce the pain of severe acute VOC not responding to standard therapy.

4. Conclusion

Simple red cell exchange transfusion may be tried in case of severe acute VOC especially when pain is continuing in spite of standard therapy and when red cell exchange is either not available or not affordable. This procedure appears to be simple, affordable, and effective. However, the exact mechanism and the assessment of minimum reduction of HbS that is effective need to be confirmed by large randomized controlled studies.

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The Effect of Ginger Capsule on Nausea and Vomiting during and after Caesarean Section under Spinal Anesthesia

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Abstract

Objective: To study the efficacy of ginger on the Nausea and Vomiting during and after Cesarean Section under Spinal Anesthesia. Study Design: Double blind randomized controlled trial. Setting: Department of Obstetrics and Gynecology, Ahvaz University, Razi Hospital. Material and Method: From January 2008 to April 2008, 70 pregnant women underwent for elective cesarean section under spinal anesthesia were randomized received coded drug one hour prior section with 30 ml water. The patients were at term, single pregnancy, uterine and abdominal incision transversal, and spinal anesthesia with lidocain 5%. Patients were matched in two groups by these factors: age, height, weight, BMI, cause of cesarean section, gestational age, hypotention during and after cesarean section, duration of operation and nausea and vomiting in pregnancy. All patients were assessed for severity of nausea by visual analog nausea score (VANS). Frequency of vomiting and need antiemetic drug were evaluated during and 0/5, 1, 2, 4, 6 hours after cesarean section. Results: The results demonstrated the statistically significant differences in severity of nausea and vomiting during cesarean section (p = 0/000). Severity of nausea (p = 0/000) and vomiting (0/046) after cesarean section also was lower in ginger group than placebo group. There were statistically significant differences between two groups for need antiemetic drug during (0/000) and after (0/003) cesarean section. This need was lower in ginger group than placebo group. Side effects caused by ginger were not detected. Conclusion: Ginger has efficacy in decrease severity of nausea and vomiting during and after cesarean section under spinal anesthesia.

Keywords

Ginger, Cesarean Section, Regional Anesthesia, Nausea, Vomiting

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1. Introduction

More than 23 million surgical procedures are performed annually [1]. As a major surgical procedure, caesarean section is the most common surgery in most hospitals in the Unites States [2], where it has quadrupled in the last two decades [3]. There are some complications related to cesarean section, among which the complications of anesthesia cause seven percent of maternal mortality [4]. In recent years, spinal anesthesia is used more than general anesthesia [5]. Spinal anesthesia is the most common anesthesia method for elective cesarean section [6], but it also has some complications such as decreased blood pressure, spinal headaches, itching, nausea and vomiting. The latter complication occurs in 46% - 80% of cesarean section under spinal anesthesia [7]. Pan (1992) and Balki *et al.* (2005) reported the prevalence of nausea and vomiting during surgery more than 66% [8] and 80% of cases, respectively [9]. Despite dramatic advances in anesthesia techniques, the prevalence of nausea and vomiting has also remained constant in the past 30 years [10]. Pregnant women undergoing spinal anesthesia are more prone to nausea and vomiting during surgery due to orthostatic hypotension and progesterone-dependent relaxation of smooth muscles and decreased gastrointestinal motility and esophageal sphincter tone [9]. In the United States, the average treatment cost for a patient experiencing nausea and vomiting after cesarean section is 14.94 dollars including personnel wages, supplements, medications, increased hospital stay in post-op care unit, and amounts to about 253,000 - 520,000 dollars in a fiscal year [11]. Postoperative nausea and vomiting can cause dehydration, electrolyte disorders, increased blood pressure, tension on suture lines and bleeding from the subcutaneous tissue [12]. Persistent vomiting can delay discharge from the recovery room for 47 - 60 minutes [13]. Aspiration of gastric contents and undigested foods blocks airways and eventually leads to pneumonia, atelectasis and death [14]. Several studies have evaluated the therapeutic effect of different medications and techniques on nausea and vomiting during and after surgery, including anticholinergics, antihistamines, and serotonin antagonists [12] [15]; but each of these medications have complications such as long recovery time, restlessness, anxiety, dry mouth, increased dizziness after surgery, and increased blood pressure [5] [12] [16]. Many physicians tend to use complementary treatments because of the complications of these medications, the cost of new medications and their incomplete success [17] [18]. Some studies report using non-pharmacological methods such as acupuncture, acupressure, music therapy and herbal therapy [18]-[20]. Ginger root is a common old spice, recognized as a dietary supplement by the Food and Drug Administration (FDA) [21] and without any complications or interaction with other medications as mentioned in Commission E monograph [22].

Granger writes that galanolactone antagonists, 5-hydroxytryptamine-3 receptors and serotonin-3 in ginger may explain antiemetic properties and increased gastrointestinal motility [20]. Gingerol and shogaol in ginger inhibit stomach contractions, increase gastrointestinal motility, stimulate bowel peristalsis and eliminate free radicals that stimulate vomiting [23].

This study was conducted considering the importance of controlling nausea during and after cesarean section because of the mentioned risks and establishing an immediate emotional bond between mother and baby and initiating breastfeeding.

2. Materials and Methods

This study was a double-blind clinical trial conducted in Razi Hospital, Ahvaz (Iran). Of the pregnant women presenting to the hospital for elective cesarean delivery, 70 qualified women with a history of one to three pregnancies, aged between 18 and 35 years and the full-term gestational age were selected (94 women were entered into the research according to Cochran formula and finally 70 women were selected). Pregnant women did not have any gastrointestinal diseases, motion sickness, nausea and vomiting in previous anesthesia experiences, morbid obesity and a history of hyperemesis gravidarum, a history of nausea and vomiting during the 24 hours before surgery, underlying diseases or complications of pregnancy such as pregnancy hypertension. Before surgery, patients received similar training about the use of visual analog scale for nausea and one hour before surgery, the subjects received coded capsules with 30 cc of water. Ginger capsules were manufactured by Goldaru Company and each capsule contained 250 mg of ginger root powder approved by a pharmacognosy expert and placebo capsules contained rice flour. These patients did not receive any medication before surgery.

Patients were hydrated with 500 cc of Ringer's solution in the operating room, and then an anesthetist performed spinal anesthesia with the injection of 50 - 100 mg lidocaine (5%) in L3-4 or L4-5 space using 24 - 25 gaugeneedle. If systolic blood pressure dropped more than 20% as compared with the initial pressure or less

than 100 mmHg, routine treatments were performed including glowering the head of the bed, increasing Ringer's solution infusion rate or injecting 5 - 10 mg intravenous ephedrine. Patients' blood pressure was monitored every minute until it became normal and all women received 5 liters per minute of oxygen until the birth. After the birth of the baby, 40 units of syntocinon was intravenously infused. After the surgery, women were transferred to the recovery room and then to the gynecology ward after their foot sensation was restored and their vital signs and complications were stabilized. The researcher recorded women's nausea and vomiting status and antiemetic medications received during surgery and 0.5, 1, 2, 6, 4 hours after the end of surgery.

Visual analog scale, a 100-mm line with specific beginning, end and range, was used to assess nausea severity, as patients marked their status on it from zero to represent the best situation and the absence of the target complication to 100 to represent the worst situation. This scale was used in several studies on quality of life and its reliability was determined from 40% to 95% [5]. Visual analog scale for nausea severity is a self-report scale. Since nausea is felt by the patient, self-report scale is an appropriate technique to measure its severity. In addition, it can be easily understood by the subject and its usage can be taught simply [24]. Nausea is classified as mild, moderate and severe if it scores, respectively, less than 3.5, between 3.5 and 7, and greater than 7 [25].

The number of retching or vomiting was also used to assess nausea severity, which was rated severe, moderate and severe if retching or vomiting occurred more than 5 times, between 3 and 5 times and fewer than 3 times, respectively [26].

If patients could tolerate nausea, they did not receive any medication, but in case nausea was intolerable, or retching or vomiting occurred, they received 10 mg metoclopramide. It should be noted that all women were transferred in a similar way to the gynecology ward on a stretcher in order to remove the effects of patients' movement on nausea and vomiting. Patients received 2 g of cefazolin simultaneous with baby's shoulder delivery and postoperative antibiotics was 1 g IV infusion of cefazolin as according to the gynecology ward routine. The data obtained were analyzed in SPSS-15 software using t-test, chi-square, and descriptive statistics to determine the frequency and the percentage of data obtained. The significance level was set as $p < 0.05$.

3. Results

Data from the two study groups, including age, weight, height, BMI, gestational age, duration of surgery, duration of preoperative fasting, the ephedrine used during and after surgery using t-test are presented in **Table 1**. The mean duration of surgery was 45.8 min in the trial group and 46.2 min in the control group, with no significant difference between the two groups as confirmed by t-test ($p = 0.368$) (**Table 1**).

The highest frequency of nausea severity during surgery was moderate nausea of 11 women (31.4%) in the trial group and severe nausea of 18 women (51.4%) in the control. The difference in nausea severity experienced during surgery was reported significant between the two groups by t-test ($p = 0.0001$) (**Table 2**).

Seven women (20%) in the trial group and 22 women (62.9%) in the control group vomited during surgery and the Chi-square test reported a significant difference between the two groups in this regard ($p = 0.0001$).

The following results were obtained on the severity of vomiting during surgery: the most frequent vomiting severity during surgery was mild vomiting of six women (17.1%) in the trial group, but moderate vomiting of 11 women (31.4%) in the control group. The difference in the mean frequency of vomiting between the two groups was reported significant by t-test ($p = 0.0001$) (**Table 3**).

The need for metoclopramide during surgery was reported significant by the chi-square test between the groups ($p = 0.0001$). The amount was 22.9% ($n = 8$) in the ginger group and 77.1% ($n = 27$) in the placebo group.

The results on the control of nausea 6 hours after surgery showed that the overall distribution of nausea between the two groups in different hours after surgery was not statistically significant by the Mantel-Haenszel chi-square test ($p = 0.07$). Repeated-measures analysis of variance showed that the mean severity of postoperative nausea was significantly different between the two groups ($p = 0.0001$), and the difference was significant by t-test in half an hour ($p = 0.0001$) and one hour ($p = 0.02$) after surgery (**Table 2**).

The following results were obtained on the control of vomiting 6 hours after surgery: the overall distribution of vomiting between the two groups in different hours after surgery was statistically significant by the Mantel-Haenszel chi-square test ($p = 0.026$). Repeated-measures analysis of variance showed that the mean severity of postoperative vomiting was statistically significant between the two groups ($p = 0.046$), and the difference was significant by t-test in half an hour after surgery ($p = 0.02$) (**Table 3**). None of the women vomited four and six hours after surgery.

Table 1. Comparison of demographic variables and information related to ginger and control groups in terms of mean and standard deviation using t-test.

Variables	Ginger group (n = 35) (mean ± SD)	Control group (n = 35) (mean ± SD)	p value
Age (year)	25 ± 3.6	24.8 ± 3.4	0.775
Weight (kg)	79.1 ± 11.6	74 ± 10.7	0.061
Height (cm)	164.9 ± 6.1	164.9 ± 6.1	0.969
BMI (kg/m^2)	29 ± 4.1	24.7 ± 3.4	0.075
Gestational age (week)	39 ± 0.5	39.2 ± 0.5	0.105
Duration of surgery (min)	45.8 ± 5	46.2 ± 5.2	0.773
Ephedrine used during surgery (mg)	13 ± 8.7	14.1 ± 7.1	0.551
Ephedrine used after surgery (mg)	2.8 ± 1.4	2.9 ± 1.8	0.542
Duration of fasting	8.6 ± 0.4	8.5 ± 0.4	0.296

Table 2. Comparison of nausea severity during and after caesarean section under spinal anesthesia in subjects.

Variables	Ginger group (n = 35) (mean ± SD)	Control group (n = 35) (mean ± SD)	p value
Nausea severity during surgery	2.8 ± 2.8	6.3 ± 3.4	0.000
Nausea severity half an hour after surgery	1.3 ± 0.6	3.1 ± 3	0.000
Nausea severity 0.5 - 1 hour after surgery	1.1 ± 0.5	1.8 ± 1.3	0.27
Nausea severity 1 - 2 hour after surgery	1.2 ± 0.4	1.6 ± 0.58	0.261
Nausea severity 2 - 4 hour after surgery	0.7 ± 0.2	0.8 ± 0.2	0.882
Nausea severity 4 - 6 hour after surgery	1.6 ± 0.03	-	0.321

Table 3. Comparison of vomiting severity during and after caesarean section under spinal anesthesia in subjects.

Variables	Ginger group (n = 35) (mean ± SD)	Control group (n = 35) (mean ± SD)	p value
Vomiting severity during surgery	1 ± 0.5	2.7 ± 2.7	0.000
Vomiting severity half an hour after surgery	0.6 ± 0.3	1.6 ± 0.7	0.022
Vomiting severity 0.5 - 1 hour after surgery	-	0.3 ± 0.06	0.321
Vomiting severity 1 - 2 hour after surgery	-	0.3 ± 0.06	0.321
Vomiting severity 2 - 4 hour after surgery	-	-	-
Vomiting severity 4 - 6 hour after surgery	-	-	-

The need for anti-emetic medication after surgery was reported significant by the chi-square test. Ten women (28.6%) in the control group versus 2 women (5.7%) in the test group needed metoclopramide (p = 0.011).

4. Discussion

This study aimed to evaluate the effect of ginger capsule on nausea and vomiting during and after caesarean section under spinal anesthesia. There is a high prevalence of nausea and vomiting during and after caesarean section, and although metoclopramide is the most common medication used to control or prevent this complication;

its occasional extrapyramidal complications are always a concern for its administration [27]. Various factors affect nausea and vomiting during and after cesarean section, such as age, education, anxiety, weight, height, and BMI, but we did not find a significant difference between the two groups regard these variables.

Since reduced blood pressure can cause ischemia of the brain stem and stimulate vomiting center in the brain stem and also hypotension during and after surgery can lead to bowel ischemia and release of nausea-causing substances such as serotonin [27], this variable was studied in the two groups and there was no difference in two groups in this regard.

As the results showed, ginger led to a decrease in the incidence and severity of nausea and vomiting during and after cesarean section and also the need for anti-emetic medication.

The incidence of nausea during surgery in the placebo group was 80%, Pan (1992) reported the prevalence of nausea during cesarean section 66% [8] and Balki *et al.* (2005) reported the prevalence in the cesarean section under spinal anesthesia 80% [9].

Tongta *et al.* (2006) concluded that ginger reduced nausea and vomiting after surgery. It also reduced the need for anti-emetic medication which is roughly consistent with the present study. Tongta also concluded that the mean severity of postoperative nausea in the hours he investigated (immediately, two and six hours after surgery) was less in the ginger group than in the placebo group. But nausea severity two and six hours after surgery was statistically significant [29].

In the present study, mean severity of postoperative nausea in the hours investigated (half, one, two and four hours after surgery) was less in the ginger group than in the placebo group. But it was statistically significant half and one hour after surgery. It seems that this significant difference in the hours after surgery is due to the type of anesthesia, type of surgery, and duration of surgery. In his study, the type of anesthesia was general anesthesia and the type of surgery was hysterectomy and other gynecology procedures and the average duration of surgery was 127.08 minutes.

Sirrirat *et al.* (2006) also concluded that ginger is effective in preventing postoperative nausea in gynecology surgeries and in preventing postoperative vomiting is nearly significant. In their study, the mean severity of postoperative nausea two and six hours after surgery was less in the ginger group than in the placebo group. But it was statistically significant six hours after surgery [30].

Phillips *et al.* (1992) in their study to determine the effect of ginger with metoclopramide in reducing nausea and vomiting after gynecology surgeries in 120 patients concluded that the antiemetic effect of ginger was significant compared to placebo and also the incidence of postoperative nausea and vomiting was 21%, 27% and 41% in the ginger group, the metoclopramide group and the placebo group respectively. Phillips concluded that the effect of one gram of ginger is equivalent to 10 mg metoclopramide [31].

Grainger writes that galanolactone antagonists, 5-hydroxytryptamine-3 receptors and serotonin-3 in ginger may explainits antiemetic properties and increased gastrointestinal motility [32].

Bone *et al.* (1990) compared the antiemetic effect of ginger with placebo and metoclopramide on postoperative nausea and vomiting and found that the postoperative nausea and vomiting was less in patients who received ginger or metoclopramide compared to patients who received placebo. The frequency of vomiting in groups that had received ginger or metoclopramide was less than that in the placebo group. The need for metoclopramide was also reduced in patients [33]. The results of this study are consistent with the present study.

Nonetheless, Visalyputra (1998) suggested that ginger powder at a dose of 2 g and droperidol at a dose of 1.25 mg, or both were ineffective in reducing nausea and vomiting after laparoscopy [34]. Arfeen (1995) and Leopold (2003) also found negative results in their studies [35] [36] maybe due to the fact that the number of patient evaluations was low by these researchers. Visalyputra evaluated patients once after surgery and once at the time of discharge and Leopold used very low doses of ginger (300 and 600 mg).

Given that 93% of anesthetists believe nausea and vomiting are intractable [5] and in this study ginger capsule could decrease nausea severity to some extent during and after caesarean section under spinal anesthesia, it can be used as a useful medication by anesthesiologists and all personnel performing care during and after cesarean section as the most common surgery.

5. Conclusion

Ginger has efficacy in decrease severity of nausea and vomiting during and after cesarean section under spinal anesthesia.

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