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From the Editor

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. In this issue a number of papers deal with theoretical laboratory research and other discussed clinical research. A paper on SLE attempted to determine the difference in serum resistin levels in Systemic lupus Erythematosus (SLE) and Rheumatoid arthritis (RA) patients compared to a control group. This study included three groups of 30 SLE patients, 30 RA patients and 30 apparent healthy volunteers. All patients were subjected to full history taking, clinical examination, and laboratory assessment. Serum samples from all patients and controls were tested for serum resistin levels. The mean of serum resistin levels in SLE (2.86±0.02 ng/ml) and RA (3.002±0.06 ng/ml) were insignificantly higher than controls (2.14± 0.08ng/ml) (p=0.233 and p=0.07respectively) . There was no significant difference between serum resistin levels between SLE and RA patients (p=0.586). The authors concluded that serum resistin levels did not correlate with clinical or laboratory markers except platelet counts in SLE and or RA cases, although its higher level in these diseases compared to the controls.

A cross sectional descriptive study from Nigeria looked at prevention of otologic disorders in primary school children. A Random sample of 1,200 pupils were taken. A semi structured questionnaire was used and all children were examined. Findings showed that out of 802 children studied, only 279(34.8%) were found normal. The common Otologic diseases found among the respondents were impacted cerumen 39.7%, chronic suppurative otitis media 95 (11.8%), debris 55 (6.9%) Otitis media with effusion 28 (3.5%), and acute otitis media 10 (1.3%). The authors concluded that based on the proportion of children identified with otologic problems, there is need for periodic and well coordinated school health programmes.

A paper from Saudi Arabia looked at the Clinicopathologic features of Lichen Planus in Assir area. The diagnostic records of dermatopathology cases received at the Pathology Department, Assir Central Hospitals (2007–2008) were reviewed. The lesions included 51 cases of lichen planus. It was found that lichen planus was more common in males than in females (2 : 1). The average age incidence was 35.8 years, and 35.4 years for males and females respectively. The lower extremities, face and trunk were the most common sites for the lichen planus. The author concluded that in Assir region, Kingdom of Saudi Arabia lichen planus is a common disease. It usually affects middle age populations and has a male sex predilection.

A cross sectional study was conducted in Kirkuk city included 176 pregnant women, and 134 non-pregnant married women (control group). The objective was to estimate the prevalence of HSV-2 antibodies among pregnant women in Kirkuk city. The study revealed 62.48 % pregnant women infected with HSV-2. The highest rate of IgM antibodies was found in 50% of pregnant women aged 18-23, this was also true for both IgM and IgG antibodies together that was found in 41.17% of them. The authors concluded that seroprevalence of HSV-2 was relatively high in pregnant women in Kirkuk city. Primary and re-infection of latency occurred at highest rate in age group 18-23 years old. Primary HSV-2 infection increases the AEC and IL-2 during pregnancy. The highest rate of abortion occurred during the first trimester of pregnancy in women with HSV-2.

A paper from Turkey Hatay looked at the Predictive value of pain intensity in the clinical severity of painful crises in children and adolescents with sickle cell diseases. All hospitalized SCD patients with painful crisis between September 2012 and September 2013 were included into the study. The intensity of pain was assessed at the first visit. Pain scores were obtained using the Faces Pain scale and Verbal Descriptor Scale. Seventy-nine patients under the age of 18 years-old with SCD and 146 episodes of painful crisis were evaluated. Forty-five (57%) patients were women and mean age was 11.5 years. The white blood cell counts, aspartate aminotransferase and C-reactive protein (CRP) were significantly higher while erythrocytes, hemoglobin, hematocrit and albumin levels were significantly lower in the in the severe pain episodes group ($p<0.05$ for all). The number of patients transfused was significantly high in severe pain episodes group than the other two groups (p=0.006, p=0.001). Most of severe pain episodes group had complicated vaso-occlusive crisis (acute chest syndrome 41.6 %, Hepatic sequestration crisis 6.7%), ($p<0.05$). The authors concluded that there may be a direct relationship between prevalence of complicated vaso-occlusive crisis and pain intensity of SCD. Patients with sickle cell anemia should be classified according to their pain scores during hospitalization, and patients with high pain scores should be closely monitored for complications.

A paper from Erbil, Iraq, was designed to evaluate and compare the effects of different doses of omega-3, gemfibrozil and atorvastatin on lipid profile and haematological parameters in hyperlipidemic rats. Forty eight rats were divided into two groups. The first groups included 18 rats; they were subdivided into three subgroups each of 6 rats. The first subgroup served as a control. The second and third subgroups received omega-3 (15 mg/kg) and (30 mg/kg) orally (PO) daily respectively. The second group included 30 rats and received atherogenic diet throughout the treatment period and served as hyperlipidemic rats. At the end of treatment period of all these groups, the rats were subjected to various biochemical and hematological tests. The authors concluded that Omega-3 was effective in controlling lipid profile especially serum (TC, TG and LDL-C). No significant differences were found between the effects of both doses omega-3 and gemfibrozile or atorvastatin on TC, TG, and LDL-C of hyperlipidemic rats. In contrast to omega-3, gemfibrozile and atorvastatin induced a significant raise in the level of HDL-C. Omega-3 was effective in increasing the levels of HB, RBC, HTC and MCH in hyperlipidemic rats.

Resistin, an adipokine, its relation to inflammation in Systemic Lupus Erythematosus and Rheumatoid Arthritis

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ABSTRACT

Objective: To determine the difference in serum resistin levels in Systemic lupus Erythematosus (SLE) and Rheumatoid arthritis (RA) patients compared to a control group. Also, to find the relationship between serum resistin levels and disease activity in SLE and RA patients.

Subjects and Methods: This study included three groups of 30 SLE patients, 30 RA patients and 30 apparent healthy volunteers. All patients were subjected to full history taking, clinical examination, laboratory assessment (ESR, CRP, renal function, urine examination, lipid profile, RF, ANA, anti-dsDNA, ACPA, C3 and C4), X-ray both hands for RA patients for both SLE and RA patients, assessment of disease activity according to SLEDAI for SLE patients and according to DAS 28 score for RA patients and assessment of radiological damage for RA patients using Larsen score. Serum samples from all patients and controls were tested for serum resistin levels.

Results: The mean of serum resistin levels in SLE (2.86±0.02 ng/ml) and RA (3.002±0.06 ng/ml) were insignificantly higher than controls (2.14± 0.08ng/ml) ($p=0.233$ and $p=0.07$ respectively) . There was no significant difference between serum resistin levels between SLE and RA patients ($p=0.586$). There were insignificant correlations between disease duration and all laboratory parameters compared to serum resistin levels in SLE and RA ($p>0.05$) but the platelets had an inverse significant correlation with serum resistin levels in SLE ($p<0.022$). There was insignificant correlation between serum levels of resistin and SLEDAI in SLE ($p=0.180$). Moreover, there was insignificant correlation between and DAS 28 and Larsen score compared to serum resistin levels in RA ($p=0.207$, $p=0.735$, respectively).

Conclusion: Serum resistin levels did not correlate with clinical or laboratory markers except platelet counts in SLE and or RA cases, although it is a higher level in these diseases compared to the controls.

Key words: Resistin, SLE and RA.

Introduction

Systemic Lupus Erythematosus (SLE) is a disease characterized by systemic inflammation with the property of affecting several organs throughout the body (1). Rheumatoid arthritis (RA) is a systemic autoimmune inflammatory disorder of unknown etiology that primarily affects the synovial lining of the diarthrodial joints. It is characterized by symmetric, erosive synovitis and in some cases extra-articular involvement (2); most patients experience a chronic fluctuating course of disease that, despite therapy, may result in progressive joint destruction, deformity, disability, and even premature death (3).

Resistin is a low-molecular-weight adipokine also known as the adipocyte specific secretory factor that was independently identified by three groups (4). It is an adipocyte secreted hormone belonging to a cysteine-rich protein family. It is expressed in white adipose tissues in rodents and has also been found in several other tissues in humans. Insulin, glucose, many cytokines and anti-diabetic thiazolidinediones are regulators of resistin gene expression (5).

The role of resistin in humans has not been fully established (6). It was first proposed to be involved in insulin resistance and type 2 diabetes, but later, it was found to be relevant to inflammation and inflammation-related diseases like atherosclerosis and arthritis (5). There was evidence that resistin has proinflammatory properties, is abundant in inflammatory diseases e.g., RA and Crohn's disease; and is also associated with inflammatory markers in several different populations (7, 8).

Resistin was found accumulated in inflamed joints of patients with RA and had the capacity to induce arthritis in mice. In humans, resistin is expressed in inflammatory cells, leukocytes, and macrophages and has the potency of inducing production of interleukin -6 and tumor necrosis factor-alpha (9, 10).

Aim of the Work

The aim of this work is to determine the level of resistin in the serum of patients with SLE and RA. The aim extends to examine the relationship and possible associations between the serum resistin levels and different markers of disease activity, inflammation, renal function and lipids with RA and SLE patients.

Subjects and Methods

Thirty patients fulfilling at least four of the updated American College of Rheumatology (ACR) revised criteria for the classification of systemic lupus erythematosus (SLE) (11), thirty patients fulfilling at least four of the 1987 Revised ACR Criteria for the classification of rheumatoid arthritis (RA (12) and 30 apparent healthy volunteers matched for age and sex with the SLE and RA were enrolled in this study.

These patients were recruited from the in-patients and out-patients' clinic of the Rheumatology, Rehabilitation and Physical Medicine Department of King Fahad Specialist Hospital Dammam Saudi Arabia. Informed consent was obtained from all participants, and the study was approved by the IRB committee of King Fahad Specialist Hospital Dammam.

Patients with the following conditions were excluded from the study including pre-existing diseases causing nephritis, evidence of malignancy, concurrent infection and diabetes in patients and controls.

All the patients and controls were subjected to complete history taking as well as thorough clinical examination. Assessment of disease activity of SLE was done using Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) (13). Grading of SLE disease activity (SLEDAI) includes Mild activity: 1-10, Moderate activity: 11-20, Severe activity: 21-45, Very severe activity: >45 (13).

Assessment of the Disease Activity Score 28 (DAS28) was done in patients with RA (14). The rheumatoid Disease Activity Score 28 (DAS28) was determined from scores as follows: Remission: DAS 28 $\leq$ 2.6, Low disease activity: DAS 28 >2.6 $\leq$ 3.2, Moderate disease activity: DAS 28 > 3.2 and $\leq$ 5.1, High disease activity: DAS 28 > 5.1. DAS 28 = [0.56 × $\sqrt{}$ (tender 28) + 0.28 × $\sqrt{}$ (swollen 28) + 0.70 × Ln (ESR)] 1.08 + 0.16 (14).

All patients were subjected to the following lab tests as indicated by their disease, using standard laboratory techniques Erythrocyte Sedimentation Rate [ESR] by Westergren method, C-reactive protein (CRP) by latex agglutination slide test, serum creatinine and blood urea, complete urine analysis, complete blood count, C3 & C4 by using a standard nephelometric technique, ANA by using a standard immune-fluorescence technique, Anti double stranded DNA by using ELISA testing and Plasma lipoproteins by using a standard colorimetric reaction.

Serum resistin levels were determined in patients and controls by using a quantitative sandwich Enzyme-Linked Immunosorbent Assay (ELISA). 2 ml venous blood samples are taken after a one-night fast, and serum from these samples will be stored at -70°C until the time of analyses according to a standard ELISA technique using a Quantikine ELISA kit for Human Resistin supplied by R&D Systems USA.

Plain X-ray of both hands and wrists, postero-anterior views, were done for all RA patients. Radiographic damage specific for RA is evaluated by Larsen method (LS) for each of the patients (15).

Statistical analysis was performed using an IBM computer utilizing Statistical Package for Social Science (SPSS) program version 16. Continuous data were expressed in the form of mean ±SD while categorical data were expressed in the form of count and percent. The difference between the two groups was analyzed via student's t-test. One-way analysis of variance (ANOVA) was used to compare more than two groups. Spearman's correlation coefficient (r) was used to assess the degree of association between 2 continuous variables.

Results

Table 1: Demographic characteristics, clinical and Laboratory data in SLE and RA patients and control (ANOVA test)

Mean ±SD	SLE	RA	Controls
Age (years)	40.36±10.55 p1> 0.05	40.24± 10.41 p2> 0.05	38.07±10.01
Sex	All Females	All Females	All Females
BMI kg/m2	32.05±1.07 p1> 0.05	29.48± 1.11 p> 0.05	30.17± 1.01
Disease duration (y)	9.73± 6.78 p1> 0.05	6.1± 3.7 p> 0.05	-
SLEDAI score	4.63 ±0.4 p1> 0.05	-	-
DAS 28 score	-	4.19 ±0.2 p2> 0.05	-
Larsen score	-	1.5 ±0.02 p2> 0.05	-
ESR mm/1st hour	33.76± 7.2 p1> 0.05	47.5± 8.5 P2> 0.05	15.23±5.3
CRP mg/l	10.2± 2.3 p1> 0.05	26.43±2.7 p 2> 0.05	7.9±1.8
HB gm%	10.7± 2.1 p1> 0.05	10.7±2.4 p 2> 0.05	8.1±1.7
RBCs thousands/ mm3	4.76± 0.4 p1> 0.05	4.484± 0.6 p2> 0.05	3.64±0.8
WBCs thousands/ mm3	7.08±2.2 p1> 0.05	7.96 ±2.8 p2> 0.05	5.88±2.2
Platelet thousands/ mm3	294.43 ±74.3 p1> 0.05	366.83 ±63.2 **p 2< 0.05***	265±53.1
Total cholesterol mmol/l	6.18 ±1.7 p1> 0.05	6.71 ±1.9 p2> 0.05	3.69±1.5
TG mmol/l	1.21± 0.08 p1> 0.05	1.224 ±0.05 p2> 0.05	0.4±0.01
Serum create nine µmol/l	160.93 ±10.7 p1> 0.05	71.73±10.4 p2> 0.05	39.34±7.7
Anti-dsDNA IU/ml	80.03 ±13.9 p1> 0.05	-	12.9±3.75
C3 g/l	1.14± 0.01 p1> 0.05	-	0.50±0.08
C4 g/l	0.26±0.05 p1> 0.05	-	0.02±0.009
Serum Resistin , ng/ml	2.86± 0.02 p1> 0.05	3.00±0.06 p2> 0.05	2.41 ±0.08

• Relation between two groups of SLE and RA patients and control group,

• p 1= between SLE patients and control, p 2= between RA patients and controls.

• Non-Significant (NS) p > 0.05; Significant (S) * p < 0.05

The mean of serum resistin levels in SLE (2.866 ng/ml) and RA (3.002 ng/ml) were insignificantly higher than controls (2.14 ng/ml) (p=.233 and p=.233 respectively). There was no significant difference between serum levels of resistin between SLE and RA parients (p=0.098). There were insignificant correlations between disease duration and all laboratory parameters compared to serum resistin levels in SLE and RA (p>0.05) but the platelets had an inverse significant correlation with serum resistin levels in SLE (p< 0.022). There was insignificant correlation between serum levels of resistin and SLEDAI in SLE (p=0.180).

There was insignificant correlation between DAS 28 and Larsen score compared to serum levels of resistin in RA (p=0.207, p=0.735, respectively) (p>0.05). The demographic characteristics, clinical and laboratory findings of all the studied groups are demonstrated in Table 1. Table 2 shows correlations of serum resistin levels with clinical and laboratory data in SLE and RA patients. While, Table 3 and Figure 1 reveal correlation of serum resistin levels in SLE, RA patients and controls.

Table 2: Correlations of serum resistin levels with clinical and laboratory data in SLE and RA patients

Parameter	Serum resistin levels correlation coefficients *(r)* in SLE patients	Serum resistin levels correlation coefficients *(r)* in RA patients
Age	*(r)*-0.176,P=0.617	*(r)*-0.081,P=0.742
Disease duration	*(r)*0.090 ,P=0.779	*(r)*0.576 ,P=0.499
BMI	*(r)*0.213,P=0.476	*(r)*0.145 ,P=0.522
SLEDAI(SLR)	*(r)*0.314 ,P=0.180	-
DAS28	-	(r)0.264, P=0.207
Larsen score	-	(r)0.065, P=0.735
ESR	*(r)*-0.068, P=0.842	(r)0.250 ,P=0.336
C-Reactive protein	*(r)*0.354 ,P=0.271	(r)0.299 ,P=0.233
Red blood cell count	*(r)*0.128 ,P=0.602	(r)0.105,P=0.602
White blood cell count	*(r)*0.050 ,P=0.815	(r)-0.317 ,P=0.128
Platelets count	(r)-0.491 ,P=0.022*	(r)-0.073 ,P=0.731
Triglyceride	*(r)*-0.074 ,P=0.753	(r)-0.180 ,P=0.387
Creatinine	*(r)*0.266,P=0.234	(r)0.426 ,P=0.065
Complement 3	*(r)*-00.207 ,P=0.438	-
Complement 4	*(r)*-0.147 ,P=0.538	-
Ds-DNA antibodies	*(r)*-0.148 ,P=0.503	-
ANA	*(r)*0.230 ,P=0.247	-
Anti- citrullinated protein antibody	-	(r)-0.201,P=0.350

• All clinical and laboratory parameters had insignificant correlations with serum resistin levels. Non-Significant (NS) (p > 0.05).

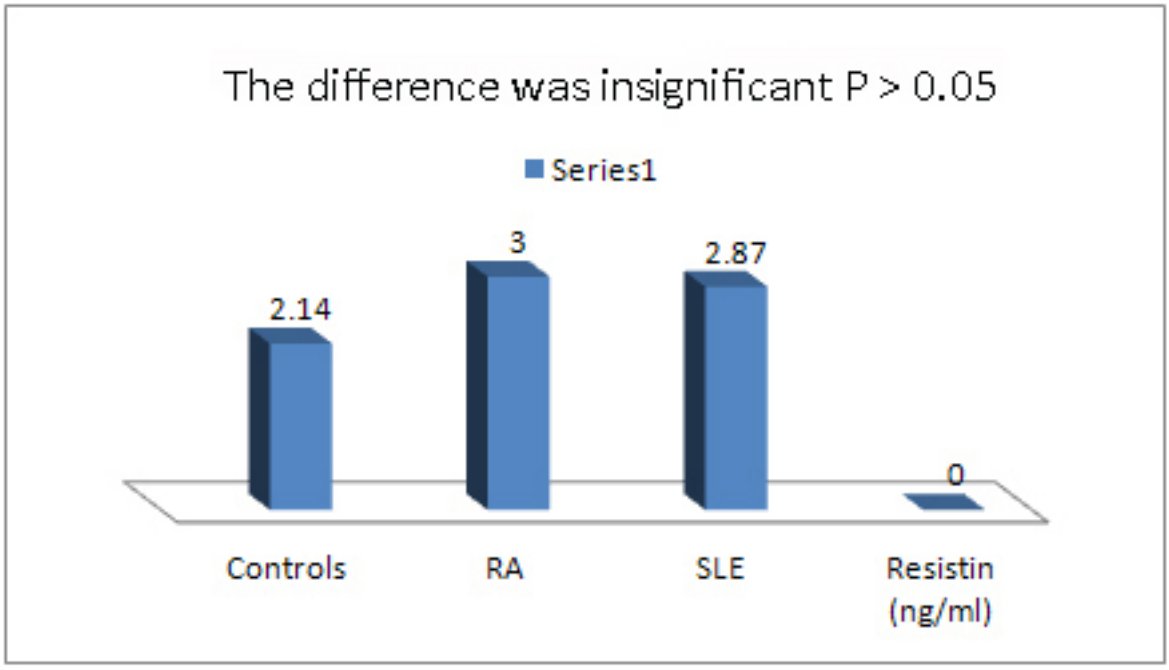
Table 3: Correlation of Serum Resistin levels in SLE, RA patients and control (ANOVA test)

Mean ±SD		SLE	RA	Controls	p value
Serum level,ng/ml	Resistin	2.86± 0.02	3.00±0.06	2.41 ±0.08	p1=0.233 p2=0.233 p3= 0.09

• Relation between two groups of SLE and RA patients and control group,

• p 1= between SLE patients and control, p 2= between RA patients and control, p 3= between SLE patients and RA. Non-Significant (NS) p > 0.05.

Figure 1: Mean of Serum Resistin Levels in SLE, RA and Control groups



Discussion

The immune system requires a proper energy balance for its physiological functions. In the past years, an important connection has been evidenced between that system and metabolism, with the identification of obesity as a predisposing factor for the development of several disorders, such as some immune-mediated diseases. The adipose tissue is not inert, and has been considered an organ with immune and neuroendocrine functions. That tissue produces several mediators, such as resistin, tumor necrosis factor alpha (TNF-a), interleukin 6 (IL-6), interleukin 1 (IL-1), chemokine ligand 2 (CCL2), plasminogen activator inhibitor type 1, and complement components, all participating in the innate immune response as pro-inflammatory mediators (16).

Macrophages are components of adipose tissue and actively participate in its activities. Furthermore, cross-talk between lymphocytes and adipocytes can lead to immune regulation. Adipose tissue produces and releases a variety of pro-inflammatory and anti-inflammatory factors, including resistin, the adipocytokines leptin, adiponectin, and visfatin, as well as cytokines and chemokines, such as TNF-a, IL-6, monocyte chemo-attractant protein-1, and others. Reduced leptin levels might predispose to increased susceptibility to infection caused by reduced T-cell responses in malnourished individuals (17).

Resistin, a novel adipocyte-secreted hormone, has gained attention for its involvement in insulin resistance in obesity and diabetes mellitus. Several groups have reported a close relationship between resistin and inflammation. Resistin increases the production of pro-inflammatory cytokines TNF-a and interleukin (IL)-12, both of which are important for T cell development (18).

In the current study, the mean serum level of resistin was highest in RA patients although there were insignificant differences of its level between SLE patients and controls (p=0.233), RA patients and controls (p=0.07) as well as SLE and RA patients (p=0.586). There is no agreement over the concentrations and function of resistin in SLE, because of a limited number of studies and their inconsistent results.

Data demonstrated by several authors were in agreement with our results. Almehed et al. found that serum resistin levels in controls were similar to those of SLE patients (1). Chung et al. have assessed the concentrations of resistin, visfatin, leptin

and adiponectin in 109 patients with SLE. They did not find statistically significant differences in resistin concentrations among SLE patients and control subjects (p=0.41) (19). Otero et al. (20) and Forsblad et al. (21) also found no difference in resistin concentration between RA patients and healthy controls. Yoshino et al. reported that there were no statistically significant differences in serum resistin levels between the RA patients (22).

Moreover, Yee et al. (23), Heilbronn et al. (24) and Iqbal et al. (25) showed no significant correlation between BMI and resistin levels in normal individuals. Senolt et al. (26) and Canruc et al. (27) found no significant correlation was found between BMI and serum resistin levels in RA patients. Bokarewa et al. did not find a relation between serum resistin levels and disease duration in RA patients. No significant correlations were found between serum resistin levels of SLE or RA patients and their disease duration (28). Bokarewa et al. found no significant difference of resistin levels between RA patients and healthy controls. Resistin levels in blood were neither related to the duration of RA, age of the patients, nor to circulating C-reactive protein levels or white blood cell counts (28).

In addition, Canruc et al. (27) and Kassem et al. (29) found no significant correlation was found between BMI and serum resistin levels in SLE or RA patients. Canruc et al. did not find a relation between serum resistin levels and disease duration in RA patients. They found no significant correlations between ESR or CRP and serum resistin levels in RA patients (27). Bokarewa et al. showed resistin levels in blood were not related to circulating C-reactive protein level in RA patients (28).

Bokarewa et al. (28), Canruc et al. (27) found no significant correlation between serum resistin levels and white blood cell count in RA patients. Elshishtawy et al. found insignificant correlation between serum resistin levels and SLEDAI ($p>0.05$) (30).

On the contrary, Elshishtawy et al. found a highly significant difference in the serum resistin levels of SLE patients compared to the control group ($p<0.0001$) (30). Migita K et al. (31) found serum resistin levels to be significantly higher in RA patients compared to the control subjects ($P= 0.0005$) (1). Also, Yoshino et al. (21) found significant correlation between serum levels of resistin and BMI (1). Zhang et al. (32) and Yannakoulia et al. (33) reported about correlation of resistin levels with BMI in normal individuals where resistin levels correlated significantly with adiposity in obese individuals.

In contrast to our result, Migita et al. (31), Senolt et al. (26) and Kassem et al. (29) found statistically significant correlations between resistin levels in the serum of RA patients and ESR and CRP. However, Senolt et al. (26) found a positive association between serum resistin level and disease duration in patients with RA (1). Gonzalez et al. found a highly significant association between the platelet count and resistin levels in RA patients (34). In our study, we find any significant negative correlation between serum resistin levels with the platelet count in SLE patients ($r = -0.491, p=0.022$), but Elshishtawy et al. had a statistically significant positive correlation between platelet count and serum resistin levels in SLE patients (30).

Almehed et al. found a relationship between serum resistin levels and the severity of inflammation, bone mass density (BMD) and renal function in SLE patients (1). They stated that the association between resistin, ESR, and complement 3 (C3) levels, observed in their study, may reflect disease activity (1).

However, Senolt et al. found a positive correlation between serum resistin levels and disease activity based on DAS 28 in patients with RA. Forsblad et al. (21), Kassem et al. (29) and Rho et al. (35) found a significant positive correlation between serum resistin levels and Larsen score for radiological joint damage in RA patients ($p< 0.05$). In one study, the authors found a relationship between serum resistin levels and the severity of inflammation and renal function in SLE patients (1).

In our study, explanation of serum resistin levels did not correlate with clinical or laboratory markers except platelet counts; it could be due to difference in disease activities, age, BMI, disease duration in SLE and RA patients. Also, this can be explained by the fact that serum resistin may not have a main role in the pathogenesis of these diseases, but other mediators may have a main role in the pathogenesis of SLE and or RA patients.

Conclusion

We conclude that serum resistin levels did not correlate with clinical or laboratory markers except platelet counts in SLE and or RA cases, although it has a higher level in these diseases compared to the controls. In explanation of our results, it could be difference in disease activities, age, BMI or disease duration in SLE and RA patients. Moreover, resistin may not have a main role in the pathogenesis of SLE and or RA patients. We recommended that further longitudinal studies including a greater number of patients are required and further comparative studies are required.

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Lichen Planus: A Clinicopathologic Study at Southern Part of Saudi Arabia

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ABSTRACT

Background: Lichen planus (LP) is a subacute or chronic immunologically mediated dermatosis that involves the skin, mucous membranes, hair follicles, and nails. To date, the clinicopathologic features of these lesions in Assir region, Kingdom of Saudi Arabia are largely unknown.

Materials and Methods: To define these features, diagnostic records of dermatopathology cases received at the Pathology Department, Assir Central Hospitals (2007-2008) were reviewed. The lesions included 51 cases of lichen planus.

Results: Lichen planus was more common in males than in females (2 : 1). The average age incidence was 35.8 3.2 years, and 35.4 2.4 years for males and females respectively. The lower extremities, face and trunk were the most common sites for the lichen planus.

Conclusions: In Assir region, Kingdom of Saudi Arabia lichen planus is a common disease. It usually affects middle age populations and has a male sex predilection.

Key words: Lichen planus, Saudi Arabia

Review of literature

Normal skin

The epidermis is a stratified squamous epithelium composed predominantly of keratinocytes, and of at least three other resident cells: melanocytes, Langerhans cells and Merkel cells.

The keratinocytes differ from the dendritic cells, or clear T-cells, by having intercellular bridges and ample amount of stainable cytoplasm. The epidermis is not only the most cellular but also the most dynamic layer of the skin. As such, it continuously sheds and regenerates itself.

The keratinocytes are arranged in four layers: the basal cell layer (stratum basalis), the squamous cell layer (stratum spinosum), the granular layer (stratum granulosum) and the horny layer (stratum corneum).(18)

The epidermis forms a broadly undulating interface with the dermis. It extends into the dermis as broad folds (the rete ridges) and the dermis projects into the epidermis in finger-like projections (the dermal papillae). (18)

The basal cell layer is formed of a single layer of columnar cells. They lie with their long axes perpendicular to the dividing line between the epidermis and the dermis. They have a more basophilic cytoplasm than cells of the stratum spinosum and contain dark stained oval or elongated nuclei. They often contain melanin pigment transferred from adjacent melanocytes. The extent and distribution of this pigment correlates with skin color. The basal cells are connected with each other and with the overlying cells by intercellular bridges or desmosomes. At their base, they are attached to the subepidermal BM zone by modified desmosomes; termed hemidesmosomes. The basal cells and the overlying squamous cells contain keratin intermediate filaments termed tonofilaments, which form the developing cytoskeleton. Most of the mitotic activity in normal epidermis occurs in the basal cell layer.(1 and 18)

The second layer is the prickle cell layer. It is formed of 5 to 10 layers of polyhedral cells that become flattened toward the surface. The cells are separated by spaces that are transversed by intercellular bridges. The tonofilaments within the cytoplasm of these cells are loose bundles of electron-dense filaments. They are attached to the attachment plaque of a desmosome at one end, and the other end lies free in the cytoplasm near the nucleus. The intercellular cement substance between two adjacent keratinocytes contains glycoproteins. It has a gel-like consistency that explains why it on one hand provides cohesion between the epidermal cells and on the other hand allows the rapid passage of water-soluble substances through

the intercellular spaces. Furthermore, it allows the opening up of desmosomes and individual cell movement. (22)

Recent studies established the molecular basis for cell-to-cell adhesion within the prickle cell layer and other epidermal layers. Cadherins is a key family of adhesion molecules that are derived from multiple genes. Desmosomal cadherins are desmogleins and desmocollins that localize to desmosomes. They are linked to intracytoplasmic intermediate filaments by plakoglobin and desmoplakin. (22)

The third layer is the granular cell layer. It is composed of flattened cells and their cytoplasm is filled with keratohyaline granules that are deeply basophilic. The thickness of the granular layer in normal skin is proportional to the thickness of the horny layer. It is only 1-3 cell layers thick in areas with a thin horny layer. It reaches up to 10 layers in areas with a thick horny layer such as the palm and sole. There is often an inverse relationship between the presence and thickness of the granular cell layer and parakeratosis. For instance in psoriasis, parakeratosis is associated with markedly attenuated or absent granular cell layer. The keratohyaline granules are the precursors of the protein filaggrin that promotes aggregation of keratin filaments in the cornified layer. (15)

The fourth layer is the horny layer. It is composed of multiple layers of polyhedral cells that are arranged in a basket weave pattern. These cells lose their nucleus and cytoplasmic organelles and are composed entirely of keratin filaments. These cells are the most differentiated cells of the keratinizing system. They eventually shed from the surface of the skin. (17) The basement membrane separates the epidermal basal layer from the dermis. It is seen by light microscopy, as a continuous and thin periodic acid Schiff (PAS)-stained layer. Alternatively, by electron microscopy, the basal cells are seen attached to the basal lamina by hemidesmosomes. (15) Ultrastructurally, the basal lamina is composed of four different regions. From the epidermis to the dermis, they are respectively: i) the plasma membrane of the basal cells containing the hemidesmosomes and anchoring filaments (15), ii) the lamina Lucida which represents an electron-lucent area composed of laminin and bullous pemphigoid antigen, iii) the lamina densa, an electron-dense area composed of type IV collagen, and iv) the sublamina densa or lamina fibroreticularis containing the structures that attach the basal lamina to the connective tissue of the dermis. The latter represents extension of the lamina densa, the anchoring fibrils (type VII collagen) and the antigen to epidermolysis bullosa acquista. (22)

The supportive structure of the skin is provided by the dermis, a relatively hypocellular layer of varying thickness. It is composed of a structural collagen matrix, elastin and ground substance. Embedded within the dermis are epidermal appendages, nerve endings, resident cells and vessels. The dermis is divided into two compartments; the papillary dermis and the reticular dermis. The papillary dermis underlies the epidermis and extends around the adenexa (in which location it is also known as the adventitial dermis). It is composed of fine fibers consisting predominantly of type I and type III collagen. It moors the epidermis, interdigitates with the reticular dermis, and surrounds the epidermal appendages. It also contains a delicate branching network of fine elastic fibers, abundant ground substance, superficial capillary plexuses and fibroblasts. (21)

The reticular dermis is thicker than the papillary dermis. It is made up of densely packed coarse-fibered collagen which is predominantly type I. The collagen bundles traverse the dermis in a pattern that has not yet been defined. Associated with these two interstitial collagens are the finely filamentous collagens type V and type VI. (9) Supplementing its protective function, the skin has three specialized redundancies referred to as epidermal appendages or adnexa. These epidermis-derived structures consist of the pilosebaceous apparatus (with its hair, sebaceous and apocrine elements), the eccrine glands and the nails.

Lichen planus

Lichen planus (LP) is a subacute or chronic immunologically mediated dermatosis that involves the skin, mucous membranes, hair follicles, and nails. (6)

Pathophysiology: Although the exact cause of lichen planus is unknown, a cell-mediated immune reaction has been implicated in its pathogenesis. In support, lichen planus is associated with other diseases of altered immunity, such as ulcerative colitis, alopecia areata, vitiligo, dermatomyositis, morphea, lichen sclerosus and myasthenia gravis. In addition an association is noted among lichen planus and hepatitis C infection, chronic active hepatitis and primary biliary cirrhosis. (23,24 and 25)

Immunohistochemical studies show that the infiltrating cells in lichen planus are predominantly T lymphocytes with very few B lymphocytes. The predominant subtypes of T lymphocytes in the infiltrate are of helper-inducer or suppressor-cytotoxic T lymphocytes lineage. Both subsets participate in the immunologic reaction with the suppressor-cytotoxic T lymphocytes being predominant in the epidermotropic response, suggesting a cell-mediated cytotoxic mechanism against the epidermal cells. The basal keratinocytes adjacent to the infiltrate express intercellular adhesion molecule-1, which enhances the interaction between lymphocytes and their epidermal targets, resulting in keratinocytic destruction.(34)

This surface antigen is probably induced by cytokines released by lymphocytes from the infiltrate. In addition, a superantigen may be involved in the pathogenesis of lichen planus. (2,3,5,6,7,8,9,11 and 26)

The number of Langerhans' cells in the epidermis is increased very early in the disease. Immunoelectron studies have shown close contacts of lymphocytes with Langerhans' cells and macrophages. (14) The Langerhans' cells can process and present antigens to T lymphocytes, leading to their stimulation and thus attacking keratinocytes. These cell-to-cell interactions suggest that a cell mediated immune mechanism is operative in lichen planus. (31 and 32)

Incidence: Lichen planus has a worldwide distribution with no significant geographical variation in its incidence. It can occur at any age, with a tendency to affect middle aged and elderly individuals. No sex predilection has been noted. (6 and 7)

Clinical features: The eruption of lichen planus is characterized by small, flat-topped, shiny, violaceous papules that may coalesce into plaques. The papules are polygonal and often show a network of white lines known as Wickham's striae. They vary in size from 1 mm to greater than 1 cm. The disease has a predilection for the flexor surfaces of the forearms and legs. Pruritus is very common, but it varies in severity according to the type of lesion and extent of involvement. Hypertrophic lesions are usually extremely pruritic. The eruption of lichen planus may be localized or generalized, and Koebner's phenomenon is commonly seen. (6 and 7)

In addition to the cutaneous eruption, lichen planus may involve the mucous membranes of the buccal mucosa and tongue (oral LP), genitalia, nails, and scalp.

The oral lesions of lichen planus are common and may occur as a sole manifestation of the disease or be associated with cutaneous involvement. They usually involve the buccal and glossal mucosa in the form of a reticular network of coalescent papules. Besides this reticular type, other lesional patterns have been described in oral lichen planus, such as papular, plaquelike, atrophic, erosive, and bullous. (6 ,28and 30) Genital involvement is common in men with cutaneous lichen planus, usually in the form of an annular configuration of papules on the glans penis. Less commonly, linear white striae may be seen. The nails are involved in about 10% of cases of lichen planus, in the form of roughening, longitudinal ridging, and, rarely, thinning and destruction. (16,29 and 31)

Lichen planopilaris is a type of lichen planus that predominantly affects the scalp with follicular and perifollicular violaceous scaly pruritic papules. It may coexist with typical lichen planus lesions on the skin, mucous membranes, or nails. Progressive hair loss may occur, resulting in the development of irregularly shaped atrophic patches of scarring alopecia on the scalp (pseudopelade of Brocq). The axillae and the pubic region may also be affected. Hyperkeratotic follicular papules may also be seen on glabrous skin. The Graham Little syndrome consists of an association of scarring alopecia of hair-bearing areas and hyperkeratotic papules on glabrous skin. Linear lichen planopilaris of the face resolving with scarring has also been described. (17)

Other variants of lichen planus include hypertrophic lichen planus, atrophic lichen planus, vesicular lichen planus, lichen planus pemphigoides, ulcerative lichen planus, actinic lichen planus, annular lichen planus, linear lichen planus, and guttate lichen planus. The hypertrophic lichen planus is a common variant of lichen planus that usually affects the extensor surfaces of the lower extremities, especially around the ankles. It is a pruritic lesion that consists of thickened, often verrucous plaques that may heal with residual pigmentation and scarring. The atrophic variant of lichen planus is characterized by a few lesions that are often the resolution of annular or hypertrophic lesions. The vesicular lichen planus is a rare variant that shows vesicles situated on some of the preexisting lichen planus lesions.

The lichen planus pemphigoides differs from vesicular lichen planus by its more disseminated eruption and more extensive bullae. In addition, lichen planus pemphigoides may arise from papules of lichen planus and normal-appearing skin.

The ulcerative lichen planus is a rare variant of lichen planus, which shows bullae, erosions, and painful ulcerations on the feet and toes resulting in atrophic scarring and permanent loss of the toenails. It is usually associated with cutaneous and oral lesions of lichen planus, as well as atrophic alopecia of the scalp. The actinic lichen planus (lichen planus actinicus or pigmentosus) usually develops in spring and summer on sun-exposed areas of the skin, particularly the face. It is characterized by annular plaques with central blue to light brown pigmentation and well-defined, slightly raised, hypopigmented borders. Pruritus is minimal or absent.

Three forms of actinic lichen planus have been described: annular, pigmented, and dyschromic. (27 and 32)

The annular lichen planus is characterized by annular lesions with an atrophic center usually found on the buccal mucosa and male genitalia. Lichen planus papules that are purely annular are rare. The linear lichen planus represents a zosteriform lesion or may develop as a Koebner's effect. Finally, the guttate lichen planus develops in the form of discrete lesions which may vary in size from 1 mm to 1 cm. They almost never become chronic. Of note, the hypertrophic and actinic variants of lichen planus are commoner than the other variants. (6)

Histopathologic features: The typical papules of lichen planus show the following histologic features: 1) compact orthokeratosis, which contains very few, if any, parakeratotic cells, 2) wedge-shaped hypergranulosis with the granular cells being increased in number and size, and contain more abundant coarse keratohyaline granules, 3) irregular acanthosis, which affects the spinous layer of the rete ridges as well as the suprapapillary plates. The rete ridges show irregular lengthening, and some of them are pointed at their lower end, giving them a saw-toothed appearance, 4) destruction of the basal cell layer, which is obvious in fully developed lesions. In these lesions, the basal layer has the appearance of flattened squamous cells (squamatization of the basal layer), and 5) a band-like (lichenoid) dermal inflammatory infiltrate, which is in close approximation to the epidermis and is sharply demarcated at its lower border. It is composed mainly of lymphocytes intermingled with macrophages. Melanophages are usually seen in the papillary and upper reticular dermis as a result of destruction of the basal cells with subsequent pigment incontinence. (4)

In addition, apoptotic keratinocytes are present in the lower epidermis and papillary dermis in most cases. They appear in the form of round or oval, homogenous, eosinophilic bodies (colloid, hyaline, or Civatte bodies). Occasionally, small areas of artifactual separation between the epidermis and the dermis are present and are known as Max-Joseph spaces. In some instances, this separation occurs in vivo as a result of extensive damage to the basal cells. Wickham's striae are caused by the focal increase in the thickness of the granular layer and of the total epidermis. (20)

The typical papules of lichen planus show the following histologic features: 1) compact orthokeratosis, which contains very few, if any, parakeratotic cells, 2) wedge-shaped hypergranulosis with the granular cells being increased in number and size, and contain more abundant coarse keratohyaline granules, 3) irregular acanthosis, which affects the spinous layer of the rete ridges as well as the suprapapillary plates. In addition, variants of lichen planus have additional histological changes. In this regard, the hypertrophic lichen planus shows considerable acanthosis, papillomatosis, and hyperkeratosis. The vesicular lichen planus usually shows large Max-Joseph spaces, with subepidermal blisters. The lichen planus pemphigoides that arises from uninvolved skin shows subepidermal bullae with an inflammatory infiltrate that is not band-like and contains eosinophils. The lichen planus actinicus may show histologic features similar to those of typical lichen planus, but with a tendency toward thinning of the epidermis in the center of the lesion. In addition, more evident pigment incontinence and numerous melanophages are usually present in the papillary and upper reticular dermis. The oral lichen planus may show parakeratosis rather that orthokeratosis, with the presence of a granular layer (the buccal mucosa is normally devoid of a granular layer, except in the hard palate). The epithelium is often atrophic, and ulcerations may develop. The lichen planopilaris usually shows a focally dense, band-like perifollicular lymphocytic infiltrate. Vacuolar changes of the basal layer of the outer root sheath and necrotic keratinocytes are often seen. Advanced cases may show perifollicular fibrosis and epithelial atrophy, which may result in scarring alopecia. (10,19 and 33)

Specific Aims

In this investigation, we took an aim at studying the clinicopathologic features of lichen planus in Assir region, Kingdom of Saudi Arabia. To explore this aim and to fill this existing gap in literature, we carried out this investigation. To achieve our goals, we examined clinical and pathological characteristics of these lesions. A total of 51 lesions representing lichen planus were examined.

Materials and Methods

Tissue specimens: The formalin fixed, paraffin embedded tissues were obtained from the Department of Pathology, in Assir region, Kingdom of Saudi Arabia. The total number of specimens was 51 cases, including 51 cases of lichen planus. Clinical data were obtained from the clinical referral reports. They included: age and sex of the patient, type of lesions, and the site, and number of these lesions. All the patients were Saudi (Caucasian). No black individuals were included in this study.

Results

Clinical features of lichen planus: The study group consisted of 51 patients, including 17 females and 34 males. Evaluation of the clinical and histological profiles of the lesions in our locality (Assir region) demonstrated that they usually tend to affect the middle age groups and had male sex predilection.

Clinical features: The study group consisted of 51 patients, including 34 males (34/51, 67%) and 17 females (17/51, 33%). The clinical data were obtained from the referral clinical reports. The clinical characteristics of these lesions were summarized in Tables 1-2 and Figure 2 (next pages).

Pathological features: The typical papules of lichen planus show the following histologic features: 1) compact orthokeratosis, which contains very few, if any, parakeratotic cells, 2) wedge-shaped hypergranulosis with the granular cells being increased in number and size, and contain more abundant coarse keratohyaline granules, 3) irregular acanthosis, which affects the spinous layer of the rete ridges as well as the suprapapillary plates. The rete ridges show irregular lengthening, and some of them are pointed at their lower end, giving them a saw-toothed appearance, 4) destruction of the basal cell layer, which is obvious in fully developed lesions. In these lesions, the basal layer has the appearance of flattened squamous cells (squamatization of the basal layer), and 5) a band-like (lichenoid) dermal inflammatory infiltrate, which is in close approximation to the epidermis and is sharply demarcated at its lower border. It is composed mainly of lymphocytes intermingled with macrophages. Melanophages are usually seen in the papillary and upper reticular dermis as a result of destruction of the basal cells with subsequent pigment incontinence (Figure 3, page 17).

In addition, apoptotic keratinocytes are present in the lower epidermis and papillary dermis in most cases. They appear in the form of round or oval, homogenous, eosinophilic bodies (colloid, hyaline, or Civatte bodies). Occasionally, small areas of artifactual separation between the epidermis and the dermis are present and are known as Max-Joseph spaces. In some instances, this separation occurs in vivo as a result of extensive damage to the basal cells (Figure 3).

Discussion and Conclusions

Interface dermatitis encompasses a wide range of lesions characterized by lichenoid and vacuolar changes at the dermoepidermal junction i.e. interface zone. The former is characterized by lichenoid infiltrate and basal cell keratinocytes damage (LID). In the vacuolar subtype (VID), vacuolation of the basal cell keratinocytes is a characteristic feature. Although these lesions are thought to be autoimmune in nature, their exact pathogenetic causes are still unknown. In this vein, ID lesions are multifactorial in origin; may be

Table 1: Clinical characteristics of lichen planus in males

Case #	Age (Y)	Site	Clinical presentations
S08-0171	12	F	well-defined violaceous scaly papules
S08-2418	20	F	well-defined violaceous scaly papules
S08-0802	25	F	well-defined violaceous scaly papules and plaques
S07-2438	31	F	well-defined violaceous scaly papules and plaques
S07-0151	37	F	well-defined violaceous scaly papules and plaques
S07-3997	52	F	well-defined brownish scaly plaques
S08-0237	56	F	well-defined brownish scaly papules and plaques
S07- 0079	66	F	well-defined scaly plaques
S07-1797	17	UL	well-defined violaceous scaly plaques
S07- 0253	22	UL	well-defined violaceous scaly plaques
S08-2419	5	LL	well-defined violaceous scaly papules
S08-0005	12	LL	well-defined violaceous scaly papules
S08-0002	16	LL	well-defined violaceous scaly plaques
S07-0251	19	LL	well-defined violaceous scaly papules
S07-2147	23	LL	well-defined violaceous scaly papules and plaques
S08-0559	26	LL	well-defined brownish scaly papules and plaques
S08-1944	29	LL	well-defined brownish scaly papules and plaques
S07-2046	30	LL	well-defined purplish scaly papules and plaques
S07-0959	32	LL	well-defined violaceous scaly papules and plaques
S07-0876	33	LL	well-defined violaceous scaly papules and plaques
S07-0977	33	LL	well-defined grayish scaly plaques
S07-3183	37	LL	well-defined grayish scaly papules
S08-0877	50	LL	well-defined brownish scaly plaques
S08-0382	51	LL	well-defined grayish scaly plaques
S07-2360	55	LL	well-defined grayish scaly papules and plaques
S07-3427	57	LL	well-defined grayish scaly papules
S07-2228	60	LL	well-defined brownish scaly papules and plaques
S07-1530	71	LL	well-defined brownish scaly plaques
S08-1688	87	LL	well-defined brownish scaly papules and plaques
S08-1525	19	T	well-defined violaceous scaly papules and plaques
S08-1725	27	T	well-defined grayish scaly papules
S07-0254	28	T	well-defined violaceous scaly plaques
S08-0178	31	T	well-defined purplish scaly papules
S08-0470	51	T	well-defined brownish scaly papules and plaques

F : Face
LL : Lower Limb
UL : Upper Limb
T : Trunk

Table 2: Clinical characteristics of lichen planus in females

Case #	Age (Y)	Site	Clinical presentations
S07-0562	17	F	well-defined grayish scaly papules
S08-0294	30	F	well-defined violaceous scaly plaques
S07-1534	34	F	well-defined grayish scaly papules and plaques
S07-1800	50	F	well-defined brownish scaly plaques
S07-4347	20	UL	well-defined violaceous scaly papules
S07-0325	31	UL	well-defined violaceous scaly papules and plaques
S07-2077	50	UL	well-defined violaceous scaly plaques
S07-4451	24	LL	well-defined violaceous scaly papules
S08-0347	30	LL	well-defined brownish scaly papules and plaques
S07-0911	32	LL	well-defined violaceous scaly papules
S07-3572	33	LL	well-defined violaceous scaly plaques
S07-0147	36	LL	well-defined brownish scaly papules and plaques
S08-1853	38	LL	well-defined violaceous scaly papules and plaques
S08-0674	39	LL	well-defined brownish scaly papules
S07-0584	50	LL	well-defined violaceous scaly plaques
S07-1778	41	T	well-defined brownish scaly papules and plaques
S07-1571	47	T	well-defined brownish scaly plaques

F : Face
LL : Lower Limb
UL : Upper Limb
T : Trunk

induced by drugs; or by complex environmental, genetic and life style factors. (28)

Interestingly, a new association between these lesions and chronic liver disease has emerged. In this respect, Harman and his colleagues found a close association between LP and hepatitis C infection. (13) Also, anti hepatitis C antibodies were seen in patients with lichen planus. (13)

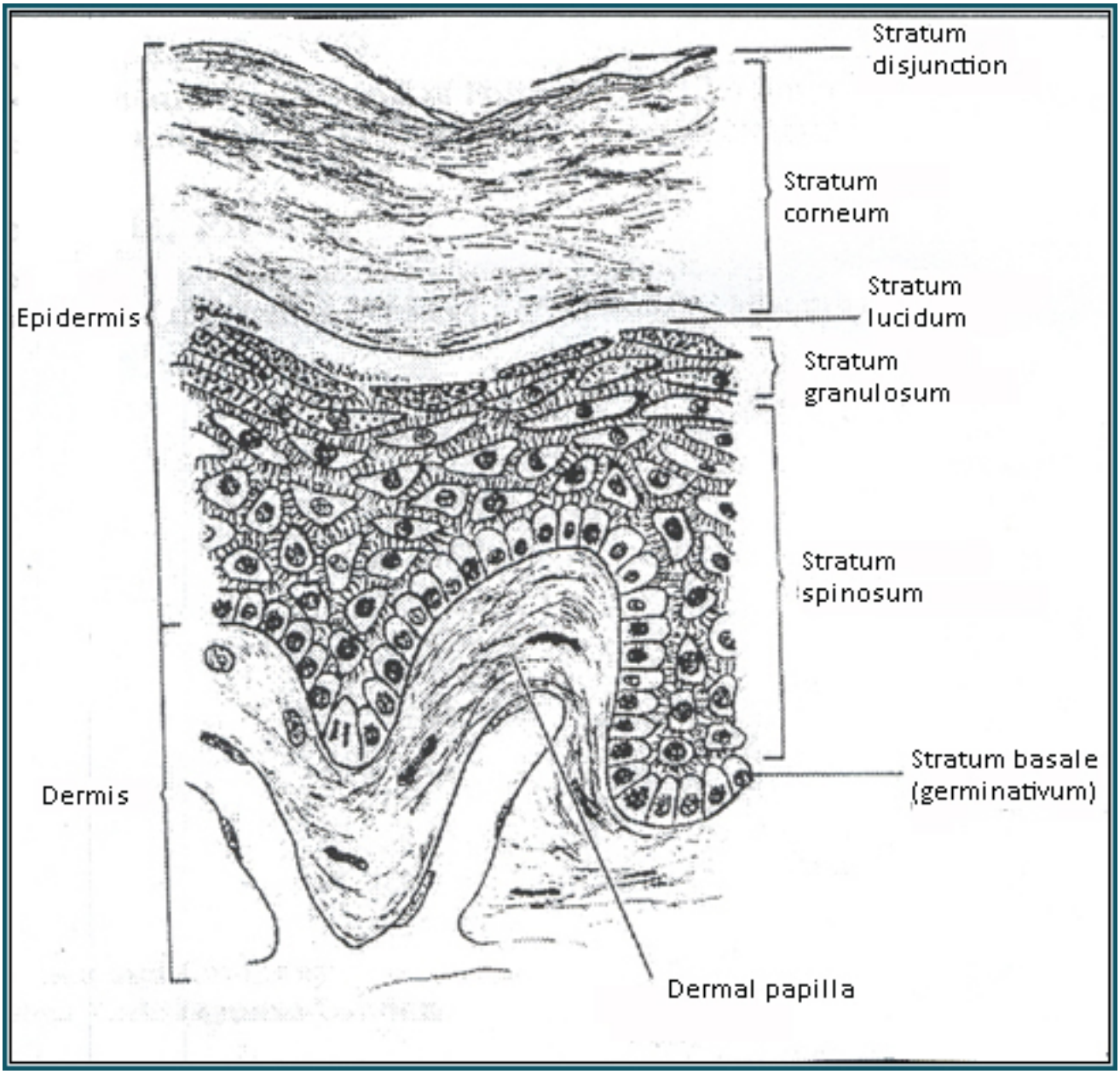
The clinical features of ID lesions in our series (age incidence, female sex predilection, and site of affection and the average duration of the diseases) are comparable to the findings in western societies. The studies performed in these societies reported an average male/female ratio of 1:1 to 1:1.3. The mean age was about 50.4 years. (6) Taken together, these findings suggest common underlying pathogenetic mechanisms in these diseases.

The female sex predilection may indicate that the susceptible genotype is probably characterized by a single inherited dominant allele on the X-chromosome. The disease chronicity, adult onset, female sex predilection and association with other autoimmune diseases suggest the autoimmune nature of ID. Familial lichen planus was reported by others in several studies and this raised the suggestion of genetic predisposition in ID. Several studies examined the role of genetic factors on the development of ID lesions like lichen planus such as HLA-associated antigens.

They showed a role of these antigens in the recruitment of lymphocytes at the site of inflammation.

The diagnosis of lichen planus can be usually made on histologic grounds in more than 90% of cases. However, a number of diseases may simulate the histologic picture of lichen planus and make some difficulties in the diagnosis. These lesions include LP-like keratosis, lichenoid drug eruption, lichenoid lupus erythematosus, chronic graft-versus-host disease, and lichen simplex chronicus. Lichen planus -like keratosis shows focal parakeratosis and adjacent solar lentigines in an otherwise typical histological picture of lichen planus. Lichenoid drug eruptions can be differentiated from lichen planus by the presence of focal parakeratosis with concomitant agranulosis, exocytosis of lymphocytes within the epidermis, and numerous eosinophils in a deeper inflammatory infiltrate. Lichenoid lupus erythematosus differs from lichen planus in the presence of epidermal atrophy in addition to acanthosis, perivascular and periadnexal infiltrate in addition to the superficial band-like infiltrate, dermal mucin deposits, and the presence of a thickened PAS-positive basement membrane. Chronic

Figure 1: The normal skin is composed of three layers: 1) epidermis, 2) dermis, 3) the subcutaneous adipose tissue. Each layer has a complex structure and function. The keratinocytes of the epidermis are arranged in four layers: 1) stratum germinatum, 2) stratum spinosum, 3) stratum granulosum, and 4) stratum corneum



graft-versus-host disease may show epidermal changes similar to lichen planus. However, the inflammatory infiltrate tends to be perivascular and the number of Langerhans' cells is decreased in chronic graft-versus-host disease. Lichen simplex chronicus can be differentiated from lichen planus by the absence of both basal cell destruction and the band-like infiltrate. (35)

The prognosis for lichen planus is good, as most cases regress within 18 months. However, some cases may recur. Hypertrophic lesions may leave residual hyperpigmentation. Alopecia is often permanent. Malignant transformation of cutaneous LP occurs in less than 1% of cases. (36) Squamous cell carcinoma may arise occasionally in long-standing lesions of lichen planus situated on mucous membranes or the vermillion border . (12) Ulcerative lesions of oral lichen planus, particularly in men, have a higher risk for malignant transformation. (37)

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Figure 2: Lichen planus, violaceous flat-topped scaly papules and plaques

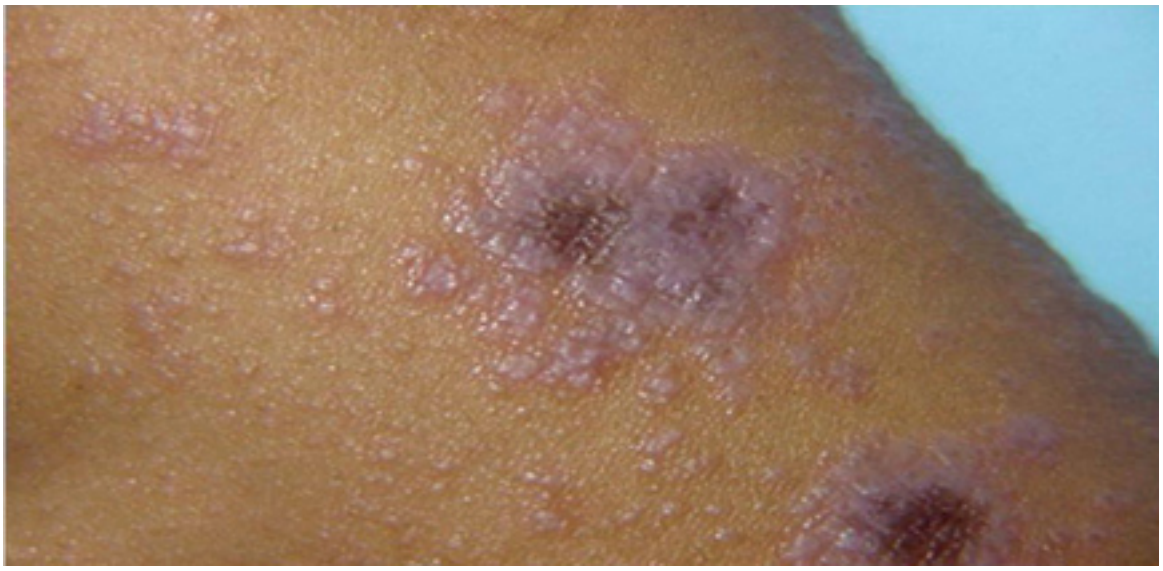
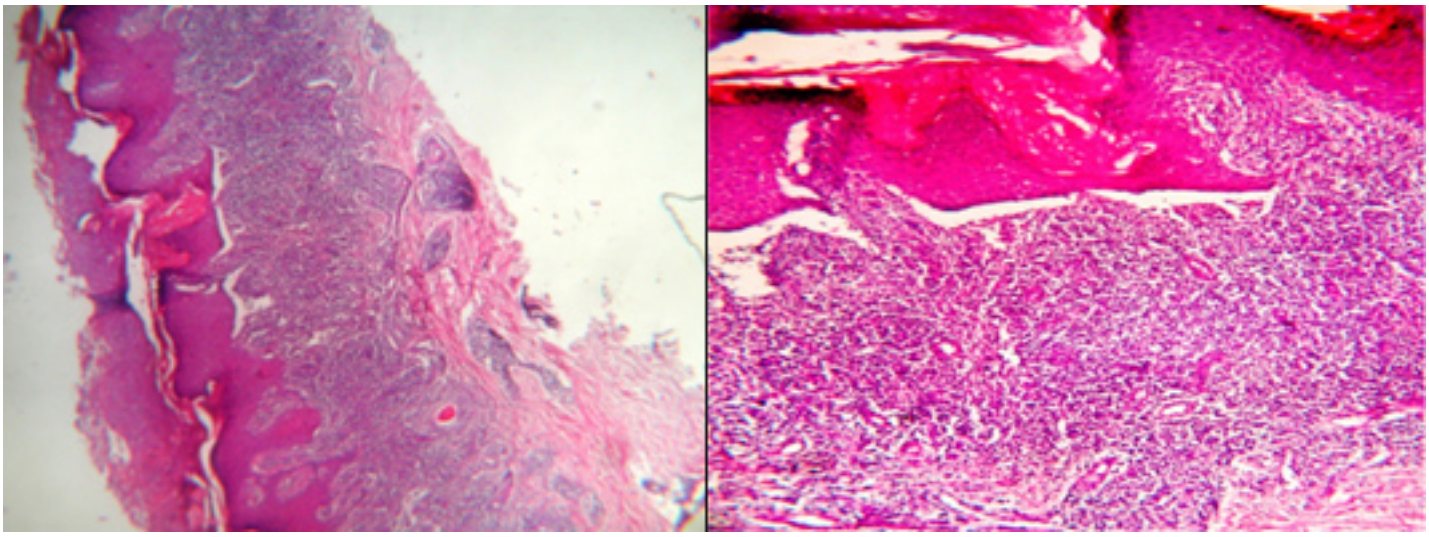


Figure 3 : Histological features of lichen planus. dense dermal inflammatory infiltrate. Occasional inflammatory cells are seen abutting on the basal cell keratinocytes together with apoptotic keratinocytes



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Seroprevalence of Herpes Simplex Virus Type 2 (HSV-2) in Pregnant Women and its Relation to Some Blood Cells and IL-2 in Kirkuk, Iraq

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ABSTRACT

Background: The HSV-2, is a widespread viral pathogen. It has been described as an important etiological agent in uterus and during the intrapartum period in pregnant women.

Objectives: Estimate the prevalence of HSV-2 antibodies among pregnant women in Kirkuk city.
Patients and Methods: A cross sectional study (M.Sc. Thesis) was conducted in Kirkuk city and included 176 pregnant women, and 134 non-pregnant married women (control group) who attended at Azadi Teaching Hospital and Al Ta'akhi Health Care Center from the 20th of November 2012 to the 23rd of April 2013.

Results: The study revealed that the 62.48 % of pregnant women were infected with HSV-2. The highest rate of IgM antibodies was found in 50% of pregnant women aged 18-23; this was also true for both IgM and IgG antibodies together that were found in 41.17% of women. The relation of seropositive HSV-2 antibodies with the total white blood cells (W.B.Cs) count showed a non-significant result with the probability (P) value >0.05. This was also true for the relation with absolute lymphocyte count (ALC), while its relation with absolute eosinophil count (AEC) showed a significant result, P <0.05. In regards to the relation of HSV-2 antibodies with serum interleukin-2 (IL-2), the result was non-significant. The relation with abortion number was significant. There was significant relation of abortion with gestational time of pregnancy in seropositive pregnant women.

Conclusion: The seroprevalence of HSV-2 was relatively high in pregnant women in Kirkuk city. Primary and re-infection of latency occurred at the highest rate in age group 18-23 years old. Primary HSV-2 infection increases the AEC and IL-2 during pregnancy. The highest rate of abortion occurred during the first trimester of pregnancy in women with HSV-2.

Key words: HSV-2, Iraq.

Introduction

The HSV-2, is a widespread viral pathogen. It has been described as an important etiological agent in uterus and during the intrapartum period in pregnant women. The HSV-2 infection has been found to be a sexually transmitted disease affecting most commonly, individuals who are in their adolescence or young adulthood[1,2].

The HSV-2 belongs to the Herpesviridae family. The virion particle is spherical 150-200 nanometer (nm) in diameter [3,4], with four structural elements; an electron opaque core, a protein capsid, surrounding the virus core comprising 162 capsomeres, an amorphous tegument surrounding the capsid, and an outer envelope with spikes on its surface. The core is composed of linear dsDNA [5,6].

The primary route of acquisition of HSV-2 infections is through genital sexual contact with an infected partner who is shedding the virus symptomatically or asymptomatically [7]. The HSV-2 infection is more common in women than men[8].

Neonatal HSV-2 infection is acquired from the mother during vaginal delivery [9]. The chances of the baby becoming infected increase if there is an outbreak at the time she delivers the infant[10]. The risk of transmission of HSV-2 during primary infection in the third trimester of pregnancy to the infant is estimated to be 30%-50%[11]. Intrauterine and postnatal transmissions are rare[12]. The HSV-2 virus is also associated with a higher rate of miscarriages than normal[13]. Latency of the virus is in sacral nerve ganglia[14]. The HSV-2 can reactivate upon stress[15].

Direct detection of viral DNA by liquid or in situ hybridization, and after, by the polymerase chain reaction (PCR), are considerably more sensitive[16]. Enzyme-linked immunosorbent assay (ELISA) can be used to detect immunoglobulin M and G (IgM and IgG respectively) in serum[17].

Acyclovir is selectively effective against HSV-2. Other drugs effective in treating HSV-2 infection include famciclovir and topical Penciclovir[16].

Objectives: Estimate the prevalence of HSV-2 antibodies among pregnant women in Kirkuk city, and its relation to some blood cells and IL-2.

Materials and Methods

Study Population:

A cross sectional study [M.Sc. thesis] conducted in Kirkuk city included 176 pregnant women, and 134 non-pregnant married women (control group) attending Azadi Teaching Hospital and Al Ta'akhi Health Care Center from the 20th of November 2012 to the 23rd of April 2013 and aged (18-40) years old. A blood sample of 7.5 ml was drawn from each patient and separated into two parts; one part 5 ml was with no Ethylene Diamine Tetraacetic Acid (EDTA) anticoagulant used for detection of anti-HSV-2 IgM, IgG antibodies, and serum IL-2 using Enzyme Linked-Immunosorbant assay (ELISA) technique, and the other part 2.5 ml was with EDTA for detection of blood cells using specialized fully automated hematological analyzer machine (CELL-DYN RUBY).

Detection of anti-HSV-2 IgM and IgG antibodies

Enzyme Immunoassay for Detection of IgM antibodies to HSV-2 in Human serum From BioCheck, Inc 323 Vintage Park Dr. Foster City, CA 94404.

Purified HSV-2 antigen is coated on the surface of microwells. Diluted patient serum is added to the wells, and the HSV-2 IgM-specific antibody, if present, binds to the antigen. All unbound materials are washed away. Horse radish peroxidase (HRP-conjugate) is added, which binds to the antibody-antigen complex. Excess HRP-Conjugate is washed off and a solution of Tetramethyl Benzidine (TMB) reagent is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of HSV-2 IgM-specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and control.

Samples: Serum (stored at -20 °C).

Enzyme Immunoassay for Detection of IgG Antibodies to HSV-2 in Human Serum. From BioCheck, Inc 323 Vintage Park Dr. Foster City, CA 94404.

Purified HSV-2 antigen is coated on the surface of microwells. Diluted patient serum is added to the wells, and the HSV-2 IgG-specific antibody, if present, binds to the antigen. All unbound materials are washed away. HRP-conjugate is added, which binds to the antibody-antigen complex. Excess HRP-conjugate is washed off and a solution of TMB reagent is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of HSV-2 IgG-specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

Samples: Serum (stored at -20 °C).

Detection of Human IL-2 in Human Serum

Enzyme Immunoassay for Detection of Human IL-2 in Human Serum. From Biolegend Inc. Pacific Heights Blvd. San diego, CA 92121

Human IL-2 EIA Kit is a sandwich enzyme immunoassay (EIA) with a 96-well strip plate that is pre-coated with a capture antibody. This kit is specifically designed for the accurate quantization of human IL-2 from cell culture supernatant, serum, plasma, and other biological fluids. This kit is analytically validated with ready-to-use reagents.

Samples: Serum (stored at -20 °C).

Detection of Blood Cells

The CELL-DYN Ruby uses flow cytometric techniques to analyze the RBC/PLA, WBC, and nuclear optical count (NOC) populations. Flow cytometry is a process in which individual cells or other biological particles in a single file produced by a fluid stream are passed through a beam of light. A sensor or sensors measure, by the loss or scattering of light, the physical or chemical characteristics of the cells or particles.

Samples: EDTA anticoagulant treated vein's whole blood.

Statistical Analysis

Computerized statistical analysis was performed using Mintab version 11 statistic program. Comparison was carried out using; Chi-square (X2), and probability (P value). The P value < 0.05 was considered statistically significant, and for results where its P value was less than 0.01 was considered highly significant, while for those which its P value was greater than 0.05 was considered non-significant statistically.

Results

Detection of anti-HSV-2 IgM and IgG antibodies

The current findings revealed that anti-HSV-2-IgM was found in 7.95 % of pregnant women, anti-HSV-2-IgG in 35.22 % and both IgM and IgG at the same time in 19.31 %, while 37.3 % of them had neither IgM nor IgG against HSV-2. Regarding the control group, the rate of IgM, IgG, and both IgM and IgG (at the same time) was 11.19%, 22.38 % and 8.2 % respectively. However 58.2 % were negative for both IgM and IgG. The result was highly significant (Table 1).

The highest rate (50 %) of HSV-2- IgM antibodies was found in pregnant women aged 18-23 years, while the highest rate (33.87 %) of HSV-2- IgG antibodies was found in those aged 24-29 years. Also the highest rate (41.17 %) of HSV-2- IgM &IgG together was found in the age group 18-23 years. The result was non-significant (Table 2).

Detection of Blood Cells

Detection of total W.B.Cs

The highest rate (35.71%) of increased W.B.Cs counts was seen with seropositive HSV-2-IgM antibodies. The result was non-significant (Table 3).

Detection of ALC

The highest rate (29.41 %) of increased to ALC was found with HSV-2 (IgM & IgG) antibodies. The result was non-significant (Table 4)

Table 1: Summary of the HSV-2 Antibodies Seroprevalence in Pregnant Women and Control Group 1 (Non-Pregnant Married Women)

Seroprevalence of HSV-2 Antibodies	Seroprevalence of HSV-2 Antibodies			
	Pregnant Women		Control Group[1]	
	No.	%	No.	%
IgM (+) and IgG (-)	14	7.95	15	11.19
IgM (+) and IgG (+)	34	19.31	11	8.20
IgM (-) and IgG (+)	62	35.22	30	22.38
IgM (-) and IgG (-)	66	37.5	78	58.20
Total	176	100	134	100
X^2=18.571	P= 0.0002	P < 0.01	Highly significant (Hs)	

Table 2: Relation of Seropositive HSV-2 Antibodies to Age Groups of pregnant Women

Age Groups (Years)	Seropositive HSV-2 Antibodies					
	HSV-2- IgM		HSV-2 - IgG		HSV-2 (IgM & IgG)	
	No.	%	No.	%	No.	%
18-23	7	50	18	29.03	14	41.17
24-29	3	21.42	21	33.87	10	29.41
30-35	3	21.42	16	25.80	7	20.59
36-40	1	7.14	7	11.29	3	8.82
Total	**14**	**100**	**62**	**100**	**34**	**100**
X^2 = 3.029	P = 0.992	P > 0.05	Non significant(Ns)			

Table 3: Relation of Seropositive HSV-2 Antibodies with the Total W.B.C.s Counts

Total W.B.C.s Count	Seropositive HSV-2 Antibodies					
	HSV-2- IgM		HSV-2- IgG		HSV(IgM & IgG)	
	No.	%	No.	%	No.	%
Normal *	8	57.14	52	83.87	22	64.71
Increased**	5	35.71	8	12.90	11	32.35
Decreased***	1	7.14	2	3.23	1	2.94
Total	**14**	**100**	**62**	**100**	**34**	**100**
X^2 = 7.508	P = 0.512	P>0.05	Ns			

Table 4: Relation of Seropositive HSV-2 Antibodies with Peripheral ALC

Peripheral ALC	Seropositive HSV-2 Antibodies					
	HSV-2- IgM		HSV-2- IgG		HSV-2 (IgM & IgG)	
	No.	%	No.	%	No.	%
Normal*	8	57.14	46	74.19	23	67.65
Increased**	4	28.57	12	19.35	10	29.41
Decreased***	2	14.29	4	6.45	1	2.94
Total	**14**	**100**	**62**	**100**	**34**	**100**
$X^2 = 3.625$		P = 0.471		P>0.05		Ns

Table 5: Relation of Seropositive HSV-2 Antibodies with Peripheral AEC

Peripheral AEC	Seropositive HSV-2 Antibodies					
	HSV-2- IgM		HSV-2- IgG		HSV-2(IgM & IgG)	
	No.	%	No.	%	No.	%
Normal *	8	57.15	57	91.94	32	94.12
Increased**	4	28.58	5	8.07	1	2.95
Decreased***	2	14.29	0	0	1	2.95
Total	**14**	**100**	**62**	**100**	**34**	**100**
$X^2 = 17.650$		P = 0.014		P <0.05		Significant

Table 6: Relation of Seropositive HSV-2 Antibodies with Serum IL-2 Levels

IL-2 Levels	Seropositive HSV-2 Antibodies					
	HSV-2- IgM		HSV-2- IgG		HSV-2 (IgM & IgG)	
	No.	%	No.	%	No.	%
Normal *	2	18.19	6	23.08	3	23.08
Increased**	9	81.82	20	76.93	10	76.93
Total	**11**	**100**	**26**	**100**	**13**	**100**
$X^2 = 0.120$		P= 0.942		P>0.05		NS

Table 7: Relation of Seropositive HSV-2 Antibodies in Pregnancy with History of Abortion and Frequency of Abortion

Total Number of HSV-2 Antibodies Seropositive Pregnant Women	History of Abortion							
	No abortion		One abortion		Two abortions		Three abortions or more	
	No.	%	No.	%	No.	%	No.	%
110	83	75.45	19	17.27	4	3.64	4	3.64
			27 (24.55 %)					
Abortion	$X^2 = 9.444$		P= 0.031		P<0.5		Significant	
Abortion freq.	$X^2 = 5.840$		P= 0.082		P>0.05		Ns	

Table 8: Relation of Abortions with Gestational Time in Pregnant Women with Seropositive HSV-2 Antibodies

Total Number of Aborted Pregnant Women	Gestational Time of Pregnancy					
	1st trimester		2nd trimester		3rd trimester	
	No.	%	No.	%	No.	%
27	21	77.78	5	18.52	1	3.70
$X^2 = 8.424$	P = 0.026		P<0.05		S	

Detection of AEC

The highest rate (28.58%) of increased AEC was found with HSV-2-IgM antibodies. The result was significant (Table 5).

Detection of serum IL-2

The highest rate of increased IL-2 level was found in all types of the HSV-2 antibodies and as following: 81.82 %, 76.93 %, and 76.93 % for HSV-2-IgM, HSV-2-IgG, and HSV-2 (IgM & IgG) respectively. The result was non-significant (Table 6).

Relation of Anti-HSV-2 Antibodies to History of Abortion, and Frequency of Abortion

The total rate of abortion was (24.55 %) out of a total 110 seropositive pregnant women. The rate of abortion number was 17.27 % for one abortion and 3.64 % for each of two and three abortions or more. The results were significant for abortion, and non-significant for frequency of abortion (Table 7).

Relation of Abortion with Gestational Time of Pregnancy in Pregnant Women with Seropositive HSV-2 Antibodies

The highest rate (77.78 %) of abortion was found in the 1st trimester, while the lowest rate was found in the 3rd trimester. The result was significant (Table 8).

Discussion

The HSV-2 is the leading cause of genital ulcer disease worldwide. The virus can be transmitted to neonates[18]. Maternal-fetal transmission of HSV-2, which is frequently asymptomatic, can cause severe and permanent neurological damage to the neonate[19]. The prenatal form of the infection in newborns can be observed when, a neonate passes through the infected birth canal. Contamination of the neonate with this condition may cause meningitis with a serious complication[20]. Furthermore and according to our information, no such study of similarity has been published regarding pregnant women with HSV-2 infection in Iraq.

In the present study, the HSV-2 infection was relatively common among pregnant women. ELISA method was used as a serological method for detection of seropositive HSV-2 antibodies, and then the results were classified according to seropositive HSV-2 antibodies type to: HSV-2-IgM represented the acute state of (primary) infection, HSV-2-IgG represented the past (chronic) infection, and both HSV-2-IgM & HSV-2-IgG at the same time represented the re-infection or reactivation of latent infection[21].

The rate of seropositive anti-HSV-2-IgM antibodies obtained by the current study was similar to that obtained from other Iraqi cities like Baghdad (8.1 %) and Waset province (7.7 %), but slightly lower than that recorded in Mosul (10 %)[22,23,24]. In Turkey, it was 8.2% which is close to our

findings too, but in another study in Turkey, it was 11.2 % which is slightly higher than that recorded by the current findings[25]. These variations in results may be attributed to the fact that different ELISA kits used in the other studies from different companies may be with different reagents qualities and properties. Other factors which may also be attributed to the differences are steps, and techniques used by the investigators. While our finding was higher than that recorded in Saudi Arabia 0.5 %[26], this may be due to the lack of a nationwide screening program in our country to control the infection, which maybe Saudi Arabia has. Results obtained by the current study were largely lower than that reported in northern India (33.5 %), [27], in which most people from this area are known to have a very low living standard, this high rate may be an indication that the HSV-2 infection may be endemic in this area.

Regarding the anti-HSV-2-IgG antibodies rates; the present study revealed that, the rate of anti-HSV-2-IgG antibodies was 35.22 % of the pregnant women. This result was similar to those reported in Waset province (31.3 %), Tanzania (33 %) and Sweden (34 %)[23][28,29]. While the rate was lower than that recorded in Turkey (63.1 %), Iran (43.75 %), and Uganda (86 %)[25][29,30]. This may be related to different cultural factors and different socioeconomic factors too. In addition it may be associated with co-infection of HSV-2 with other viruses infections that enhance the transmission and increase the prevalence of HSV-2, especially HIV which has the same route of transmission and is present at high rates in these areas and may be endemic. The current findings were higher than that recorded In Japan (7 %), Italy (7.6 %), USA (22 %), and Germany (18 %)[28][31,32]. This may be attributed to the fact that these countries are considered as developed countries, and may have good nationwide surveillance programs to control the infection. The HSV-2 infection has a high prevalence rate in pregnant women in developing countries, especially those with a high rate of HIV prevalence[33].

The rate of both anti-HSV-2 (IgM & IgG at the same time) antibodies in pregnant women in the present study was 19.31 %. This was higher than that recorded in India (2.9 %)[34,35]. This may be due to the fact that in India, a safety program may have been developed for pregnant women to protect them from HSV-2 infection by following some special criteria like examining those mothers who got primary infection in the past and were at great risk of reactivation during pregnancy. Primary infection with HSV-2 acquired by women during pregnancy accounts for a half of the morbidity and mortality from HSV-2 among neonates, and the other half results from reactivation of old infection. (24) Since there are physiological changes during pregnancy that might affect the hormone levels; hormones like progesterone for instance may increase the susceptibility and decrease the immune response to genital herpes infection[36].

In the current study the highest rate (50 %) of seropositive anti-HSV-2-IgM antibodies was found in pregnant women aged 18-23 years. This was also true for the seropositive anti-HSV-2 (IgM &IgG) antibodies which were 41.17 % in pregnant women aged 18-23 years (as shown in Table 2). Age is one of the determinant factors associated with the prevalence of HSV-2[37]. Ashley, et al, [38], said that the acquisition of primary infection of HSV-2 increases in earlier ages, less than the third decade of life. This also agrees with Sen, et al [27]. The reason may be due to the fact that most pregnancies occur at this age. In addition to that, this age group may have more contact with infected persons.

Data obtained by the current work revealed that the re-infection and reactivation had also occurred at a highest rate in age group 18-23 years. This may be associated with some factors like stress, hormonal changes, especially most of these women have married recently, so once they got married and pregnant a lot of physiological changes may be happening in their bodies which make them more vulnerable to the infection. The highest rate of anti-HSV-2-IgG antibodies was 33.87 %, which was lower than that recorded in Colombia (64.3 %), and in Thailand (36.8 %)[39]. This may be due to socio-demographic reasons, and most women in these areas might have been infected with the virus at younger ages. Although the age was the determinant factor influencing HSV-2 seroprevalence, the results were non-significant ($P > 0.05$), in correlation with age groups which was disagreed with by Smith, et al [40]. This is may be due to low differences in demographic distribution of the virus in our society compared to the other countries.

Regarding the total W.B.Cs count; the current study agrees with Lakhan, et al, [41] who found normal W.B.Cs count in HSV-2 positive patients. On the other hand, Navaneethan, et al, [42] recorded a higher rate of decreased W.B.Cs in seropositive HSV-2 pregnant women. These differences may be due to the fact that pregnant women are particularly susceptible as immunological changes during pregnancy suppress T-cell mediated immunity promoting disseminated infection like HSV-2 hepatitis.

The current study showed that the highest rate of normal ALC was found with all types of the anti-HSV-2 antibodies, while the highest rate of increased ALC was found with seropositive anti-HSV-2 (IgM & IgG together) antibodies. The HSV-2 is considered one of the infectious agents that lead to lymphocytosis and increase in the peripheral ALC[43,44]. Although lymphocytes increased during HSV-2 infections in pregnant women, the result was non-significant ($P > 0.05$) (as shown in Table 4). This may be due to the differences in kinetic responses of the lymphocyte cells in these pregnant women. In addition, these pregnant women may have other viral infections or hematological conditions that affect the response of the lymphocytes. These findings agree with Koelle, et al, [45] who recorded non-significant ($P > 0.05$) results regarding the relation of HSV-2 infection with lymphocytes count.

The present study showed that the highest rate of normal AEC was found with all types of the anti-HSV-2 antibodies, while the highest rate of increased AEC was found in pregnant women who were seropositive for anti-HSV-2-IgM antibodies. The result was significant ($P < 0.05$) (as shown in Table 5).

The HSV-2 infection is associated with eosinophilia[46]. This may be attributed to the fact that HSV-2 causes severe itching when infecting immune-compromised individuals such as pregnant women leading to increase in the eosinophils count. This finding agrees with Tarkkanen, et al[47], who recorded high eosinophil count in relation with seropositive anti-HSV-2 antibodies. Eosinophilia may be associated with HSV-2 infections because of its effects on the skin causing rashes, on the eyes, on the genitalia and so on[48].

The IL-2 is a cytokine secreted by Th1 cells[49]. Although the current study showed that the highest rate of increased IL-2 was found with all types of the anti-HSV-2 antibodies, there were non-significant ($P > 0.05$) differences in the results in regards to the relation of IL-2 levels with the seropositive anti-HSV-2 antibodies in the pregnant women (as shown in Table 6). This agrees with Rushbrook, et al[50], who said that there was no relation between IL-2 levels and HSV-2 severity. In other studies using whole HSV antigen, adults who had a better IFN- response during genital HSV infection had a longer interval to recurrence, and recurrences have been associated with decreased IL-2 production induced by HSV antigen[51]. These differences in the results may be due to the differences in the ability of HSV-2 to switch the Th2 cells to Th1, the latter which are responsible for secreting of IL-2, in pregnant women. Besides, these women may have had other infections that led to the changes in IL-2 secretion, and as a result these infections led to the differences in the results that have been noted above.

The acquisition of genital herpes during pregnancy has been associated with spontaneous abortion, prematurity, and congenital and neonatal herpes[52]. The present study showed that the highest rate of seropositive anti-HSV-2 antibodies was found in pregnant women who have had no history of abortion, followed by those who had a history of one abortion and showed significant ($P < 0.05$) results in regards to the history of abortion with seropositive anti-HSV-2 antibodies (as shown in Table 7). This agrees with Kim, et al [53], who recorded significant difference in regards to HSV-2 infections with a history of abortion.

Regarding the frequency and recurrent abortion due to HSV-2 infection in pregnant women; the current study showed non-significant ($P > 0.05$) difference in number of abortions among single, twice, and three times or more of abortion frequency (as shown in Table 7). This agrees with Jasim, et al[23] who observed a non-significant relation in regards to abortion frequency and seropositive anti-HSV-2 antibodies. These findings point to that acute infection or reactivation of latent infection of HSV-2 that may occur as a result of immune suppression or certain physiological changes in the body during pregnancy.

Furthermore to have a safe pregnancy there has to be a switch from Th1 to Th2 and not the other way around, and this is due to the fact that Th1 cytokines are considered to be detrimental to pregnancy, via direct embryo toxic activity, or via damage to the placental trophoblast, or possibly by activating cells that are deleterious to the conceptus, whereas Th-2 cytokines may directly or indirectly contribute to the success of pregnancy by down regulating potential Th-1 reactivity[54].

The present study showed that the highest rate (77.78 %) of abortion was found in the first trimester of pregnancy, and the result was significant ($P < 0.05$) (as shown in Table 8). This agrees with Borhani, et al [55] who said; the danger of intrauterine HSV transmission is highest during the first trimester of gestation and it can lead to abortion, stillbirth and congenital anomalies. The differences in results may be due to some maternal infections, such as CMV, especially during the early gestation, which can result in fetal loss or malformations because the ability of the fetus to resist infectious organisms is limited and the fetal immune system is unable to prevent the dissemination of infectious organisms to various tissues. The fetus and/or neonate are infected predominantly by viral and also by bacterial and protozoal pathogens. Infections with various pathogens cause miscarriage or may lead to congenital anomalies in the fetus while others are associated with neonatal infectious morbidity[56].

Conclusions

The seroprevalence of HSV-2 was relatively high in pregnant women in Kirkuk city. Primary and re-infection of latency occurred at highest rate in age group 18-23 years old. Primary HSV-2 infection increases the AEC and IL-2 during pregnancy. The highest rate of abortion occurred during the first trimester of pregnancy in women with HSV-2.

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Predictive value of pain intensity in the clinical severity of painful crises in children and adolescents with sickle cell diseases

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ABSTRACT

Objectives: Painful crisis is a significant problem for patients with sickle cell diseases (SCD). We tried to understand whether or not there is an association between severity of pain and complication rate in hospitalized children and adolescents with SCD in the present study.

Methods: All hospitalized SCD patients with painful crisis between September 2012 and September 2013 were included into the study. The intensity of pain was assessed at the first visit. Pain scores were obtained using the Faces Pain scale and Verbal Descriptor Scale. Severity of pain was divided into three groups as mild, moderate, and severe according to the scales.

Results: Seventy-nine patients under the age of 18 years-old with SCD and 146 episodes of painful crisis were evaluated. Forty-five (57%) patients were women and mean age was 11.5 years. The white blood cell counts, aspartate aminotransferase and C-reactive protein (CRP) were significantly higher while erythrocytes, hemoglobin, hematocrit and albumin levels were significantly lower in the severe pain episodes group ($p<0.05$ for all). The number of patients transfused was significantly high in the severe pain episodes group than the other two groups ($p=0.006$, $p=0.001$). Most of severe pain episodes group had complicated vaso-occlusive crisis (acute chest syndrome 41.6 %, Hepatic sequestration crisis 6.7%), ($p<0.05$).

Conclusion: There may be an direct relationship between prevalence of complicated vaso-occlusive crisis and pain intensity of SCD. Patients with sickle cell anemia should be classified according to their pain scores during hospitalization, and patients with high pain scores should be closely monitored for complications.

Key words: Sickle cell diseases, Vaso-occlusive crisis, Severity of pain, Pain score

Introduction

Sickle cell disease (SCD) is inherited as an autosomal recessive disorder. It is now well established that SCD results from a single change of one amino acid, valine, instead of glutamic acid at the sixth position of the hemoglobin beta chain. The prevalence in Turkey as a whole is 0.3-0.6%, although this rises to 3%-44% in some parts of the Çukurova region.(1)

SCD is characterized by chronic hemolytic anemia, dactylitis, and acute episodic clinical events known as "crises." Vaso-occlusive (painful) crises (VOC) are the most common and start in infancy and early childhood. Other crises are acute chest syndrome, central nervous system crisis, sequestration crisis and aplastic crisis. The factors that precipitate or modulate the occurrence of sickle cell crisis are not fully understood, but infections, hypoxia, dehydration, acidosis, stress and cold are believed to play some role. Frequent episodes of crisis, infections and organ damage reduce the quality of life of patients with SCD. A high rate of VOC is an index of clinical severity that correlates with early death.(1,2) VOC is also the most prevalent complication of SCD. Pain is the insignia of SCD. Acute VOC is a common medical emergency in patients with SCD, necessitating hospitalization. Tissue damage due to vaso-occlusion releases numerous inflammatory mediators that initiate the transmission of painful stimuli and the perception of pain. Sickle cell vaso-occlusion, which may involve both the micro- and macrovasculature, is the most important pathophysiological event in SCD and explains most of its clinical manifestation.(3)

The decision to admit a patient with SCD requires multi-modal evaluation of severity of anemia, presence of infection, priapism, acute chest syndrome, acute stroke or another life-threatening complication.(4) The pain severity ratings Visual Analog Scale (VAS), Numeric Rating Scale (NRS), Verbal Descriptor Scale (VDS) and Faces Pain Scale (FPS) are used. The Verbal Descriptor Scale (VDS) is based on the patient selecting the most appropriate word to describe his/her condition. Pain is classified as mild, moderate or severe on a simple pain scale.(5-7) VAS and NRS are used with patients with SCD.(8) Jones et al.(6) converted pain-intensity scores associated with the Bieri FPS, NRS and VDS into four levels (none, mild, moderate, and severe) to analyze the effectiveness of a pain intervention.

Painful crisis is a significant problem for children with SCD, and there has been little progress to date in its management. There are insufficient studies concerning pain severity scores and prevalence of VOC complications in patients with severely painful crises, and also the management of these patients. The purpose of this study was to estimate the effect of initial pain severity ratings on progress, complications and management of hospitalized pediatric patients with SCD.

Methodology

This study comprised a retrospective chart review of a single-center series of 79 patients with SCD. The sample studied included all pediatric patients hospitalized at Antakya State Hospital, Turkey, between September 2012 and September 2013, with SCD with painful crisis. Data collected included demographic characteristics, clinical, hematological and biochemical data, and Verbal Descriptor Scale and Face Pain Scale scores. Data were obtained from patients' medical charts. The following clinical variables were recorded: age, gender, length of hospital stay (days), duration of pain (days), fever (axillary temperature equal to or greater than 38.0 ^{o}C), transfusion, exchange transfusion, type of pain crisis, factors triggering the painful crisis and intensity of pain.

Baseline values for hematological parameters, and liver and renal functions were recorded. Blood samples were collected in EDTA containing tubes for measurement of leukocyte (WBC), erythrocyte (RBC), hemoglobin (HGB), hematocrit (HCT) and platelet (PLT) levels. A complete blood count (CBC) was carried out on an automated hematology analyzer (Sysmex XT- 2000i, USA). Biochemical parameters (glucose, blood urea nitrogen (BUN), creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, direct bilirubin, protein and albumin) were assessed in blood samples. All biochemical investigation was performed on a Modular Analytics P800 analyzer (Roche Diagnostics, Indianapolis, IN) using spectrophotometric methods. Concentrations of serum C-reactive protein (CRP) in 146 samples were measured by nephelometry using a BN II Nephelometer (Siemens). Serum CRP values were considered normal between 0 and 5 mg/dl.

Intensity of pain was assessed at the first visit, before any analgesia was administered. Pain scores were obtained using the Faces Pain Scale (FPS) and Verbal Descriptor Scale (VDS). Pain severity based on the Verbal Descriptor Scale was based on the patient selecting the most appropriate word for his/her condition. Pain was classified as mild, moderate or severe on the basis of FACES Pain Scale and VSD pain scores (VDS: no pain; slight and mild pain = mild pain; moderate pain; severe pain, very severe pain, and most intense pain possible = severe pain. Bieri FPS: face 1 = no pain, faces 2-3 = mild pain, faces 4-5 = moderate pain, faces 6 -7 = severe pain).(5-7) For statistical analysis, patients were divided into three groups: one group with mild pain, one group with moderate pain and one group with severe pain. Patients were compared according to severity of pain in terms of demographic, clinical, hematological and biochemical parameters.

Statistical analysis

Statistical analyses were performed on SPSS software, version 15. Data are expressed as arithmetic mean ± standard deviation (SD) for quantitative data and as percentages (%) for qualitative data. Values are presented as mean (minimum-maximum). The Mann-Whitney U test or Chi-square test was used for comparisons between groups, as appropriate. Categorical variables were assessed using the Pearson's chi-square test. A p value of <0.05 was considered significant.

Results

One hundred and forty-six episodes of painful crisis in 79 patients were evaluated. Forty-five (57%) patients were girls and 34 (43%) boys. Mean age was 11.5 years. Mean number of painful crises per year per patient was 1.8. Patients'

demographic characteristics are summarized in Table 1. Painful episodes most often involved vaso-occlusive crisis (n=91, 62.3%), followed by acute chest syndrome (n=35, 24%), splenic sequestration crisis (n=15, 10.3%) and hepatic sequestration crisis (n=4, 2.7%). Only one patient experienced central nervous system crisis (0.7%). Mean hospital length of stay for the 146 painful crisis episodes was 5.5±3.5 days, and mean duration of pain was 4.2±1.9 days.

The mild pain group experienced 15 (11%) episodes, the moderate pain episodes group 71 (48%) and the severe pain group 60 (41%). Age and gender distribution were similar between the groups. Mean length of hospital stay was significantly higher in the severe pain group than in the mild and moderate pain groups (p=0.001, p=0.001). Mean duration of pain was significantly longer in the severe pain group than in the other two groups (p=0.003, p=0.028). Fever was highest in the severe pain group ($p<0.05$). The number of patients transfused was also significantly higher in the severe pain group than in the other two groups (p=0.006, p=0.001). Eight patients were treated with exchange transfusion, six of whom were in the severe pain group ($p>0.05$). Most of the moderate pain group experienced VOC (77%), while most of the severe pain group had acute chest syndrome (41.6%) ($p<0.05$). Hepatic sequestration crisis occurred in four patients (6.7%) in the severe pain group. There were also significant differences between this group and the moderate pain group (p=0.027). Stress was the most common trigger of painful episodes in the mild pain group, and infection in the severe pain group (p=0.001, 0.002). Details of comparative values are given in Table 2.

Hematological and biochemical values were similar in the mild and moderate pain groups. Sickle cell anemia patients with severe pain episodes had significantly higher WBC and CRP levels compared with the other two groups (p=0.014, p=0.003, p=0.025, p=0.004, respectively). Erythrocyte count was significantly lower in the severe pain group than in the mild pain group (p=0.008). Sickle cell anemia patients with severe pain episodes had significantly lower HGB and HCT levels compared with the moderate pain group (p=0.002, p= 0.002). Aspartate aminotransferase levels were significantly higher in the severe pain group (p= 0.022) and albumin levels significantly lower (p=0.001) than in the moderate pain group. Normal range creatinine was higher in the severe pain group compared with the moderate pain group (p=0.04). The hematological and biochemical values of the groups are presented in Table 3 (page 32).

Discussion

Patients with SCD with pain crises were hospitalized in this study. Patients were divided into three groups on the basis of pain assessment scales. Length of hospitalization and duration of pain, WBC, AST and CRP were significantly higher in the SCD group with severe painful episodes, while RBC, hemoglobin, hematocrit and albumin values were significantly lower. More complicated VOC was also observed in this group.

Patients with SCD suffer from acute, painful vaso-occlusion crises, infections and life-threatening acute chest syndrome. Vaso-occlusive crises are the most common causes of acute morbidity and medical emergency in sickle cell anemia patients requiring hospitalization.(9-11) Lionnet F et al.(12) reported prevalences of hospitalized painful VOC, acute chest syndrome and priapism of 36%, 20% and 20%, respectively. In our study frequencies of hospitalized painful VOC, acute chest syndrome, splenic sequestration crisis, hepatic sequestration crisis and central nervous system crisis were 62.3%, 24%, 10.3%, 2.7% and 0.7%, respectively. No priapism was observed in any patient. No patients died and all were discharged after treatment. Thirty-five painful crisis episodes exhibited acute chest syndrome in this study. Acute chest syndrome was recorded based on the current criteria: new infiltrate visible at chest X-ray associated with one or more symptoms, such as fever, cough, tachypnea, breathing difficulties or new-onset hypoxia.(12) Blood exchange was performed in 2 of the patients when no response to medical therapy was achieved. Twenty-five of the 35 patients with acute chest syndrome were in the severe pain group. VOC with complications (acute chest syndrome, splenic sequestration crisis, hepatic sequestration crisis and central nervous system crisis) was more prevalent in the group with severely painful crises. The prevalence of complications rose with severity of pain. Nine of the 15 patients with splenic sequestration crises, all of the 4 patients with hepatic sequestration crises and the one patient with central nervous system crisis were in the severe pain group. Hemorrhagic stroke was determined in our patients with central nervous system crisis. Primary hemorrhagic stroke is an uncommon complication of SCD, with reported mortality rates of 24% to 65%. Most reported cases are in adults, and little is known about the occurrence in children. The incidence of hemorrhagic stroke is greatly increased in patients with sickle cell anemia (HbSS) compared with the general population and affects children and young adults to a disproportionate extent.(13) All 4 episodes with hepatic sequestration were in the severe pain group. Acute hepatic sequestration is a rarely recognized complication of VOC. Patients with right upper quadrant hepatic syndrome generally report right upper quadrant pain and fever. Clinical examination is significant for jaundice and hepatic enlargement.(14) Right upper quadrant pain and fever, significant jaundice and hepatic enlargement were present in all our cases. Blood exchange transfusion was performed in all hepatic sequestration attacks when no response was obtained to medical therapy. Acute splenic sequestration crisis results from the rapid sequestration of red blood cells in the spleen. Splenic sequestration occurs in 10%-30% of children with SCD, most commonly between the ages of 6 months and 3 years, and may follow a febrile illness.(15,16) Similarly in this study, splenic sequestration crisis was determined at a level of 10.3%.

Infection is a major cause of morbidity and mortality in these patients. Patients with SCD have impaired immunity and are thus predisposed to infections which frequently precipitate VOC. Infection was also determined as the factor precipitating 82.2% of painful crisis episodes in this study. Many inflammatory markers of acute phase reaction are elevated in SCD patients. Routine laboratory tests including total leukocyte count and C-reactive protein are sensitive for infection.(9,11) SCD is considered an inflammatory

Table 1. Patients' demographic characteristics.

	(n; means/range) ms/range)
Number of patients	79
Age (years)	11.5±4.57 (1-18)
Gender (male/female)	34/45
The number of painful SCD crises	146
Number of painful crises per patient	1.8±1.13 (1-6)

Data are arithmetical means ± SD. SCD; Sickle cell disease

Table 2: Patients' clinical characteristics

Parameter	Mild pain group (n=15)	Moderate pain group (n=71)	Severe pain group (n=60)
Length of hospital stay (days)	3.40±2.61	4.56±2.44	7.08±3.99[a,b*]
Duration of pain (days)	2.6±1.12	4.14±1.12[a]	4.88±1.82 [a,b*]
Number of patients with fever (n,%)	8 (53%)	30 (42%)	50 (83%)[a,b]
Number of patients transfused (n,%)	3 (20%)	15 (21%)	36 (60%) [a,b]
Exchange transfusion (n,%)	0 (0%)	2 (2.8%)	6 (10%)
Vaso-occlusive crisis (n,%)	15 (100%)	55 (77%) [a]	21 (35%)[a,b]
Acute chest syndrome (n,%)	0 (0%)	10 (14%)	25 (41.6%)[a]
Splenic sequestration crisis (n,%)	0 (0%)	6 (9%)	9 (15%)
Central nervous system crisis (n,%)	0 (0%)	0 (0%)	1(1.7%)
Stress (n,%)	4 (27%)	7 (10%)	3 (5%)[a]
Effort or fatigue (n,%)	0 (0%)	8 (11.2%)	2 (3%)
Infections (n,%)	11 (73%)	54 (76%)	55 (92%)[b]
Cold (n,%)	0 (0%)	1 (1.4%)	0 (0%)
Dehydration (n,%)	0 (0%)	1 (1.4%)	0 (0%)

Data are arithmetical means ± SD (standard deviation). a Statistically significant at $p < 0.05$ compared to the mild pain group. b Statistically significant at $p < 0.05$ compared to the moderate pain group. *p-values were calculated by Mann-Whitney U test. Other p-values were calculated by Pearson's chi-square test.

Table 3: Hematological and biochemical parameters of patients

Parameter	Mild pain group (n=15)	Moderate pain group (n=71)	Severe pain group (n=60)
White blood cell count (/mm³)	14000±4500	15400±6500	20737±10735[a,b]
Erythrocytes count (x 10⁶ /mm³)	3.39±0.67	3.15±0.77	2.74±0.84[a]
Hemoglobin (gr /dl)	8.37±1.50	8.88±1.75	7.67±2.05[b]
Hematocrit (%)	24.48±4.05	25.40±4.60	22.03±5.43[b]
Platelet count (x 10³/L)	360±192	402±232	348±217
Glucose (mg/dl)	103.54±15.37	99.83±18.06	104.16±22.24
BUN (mg/dl)	9.01±3.21	8.20±2.78	8.79±3.02
Creatinine (mg/dl)	0.31±0.08	0.33±0.14	0.29±0.11[b]
AST (U/L)	45.67±18.52	43.24±30.91	67.95±117.82[b]
ALT (U/L)	19.40±11.07	24.45±19.58	38.18±116.46
Total bilirubin (mg/dl)	2.67±2.32	2.63±1.86	3.66±3.72
Direct bilirubin (mg/dl)	0.57±0.11	0.76±0.68	1.29±2.62
Protein (g/dl)	7.50±0.87	7.58±0.56	7.26±0.71
Albumin (g/dl)	4.65±0.25	4.74±0.34	4.49±0.41[b]
CRP (mg/dl)	30.33±34.94	46.52±55.01	80.07±63.65[a,b]

Data are arithmetical means ± SD (standard deviation). a Statistically significant at $p < 0.05$ compared to the mild pain group. b Statistically significant at $p < 0.05$ compared to the moderate pain group. BUN; blood urea nitrogen, AST; Aspartate aminotransferase, ALT; Alanine aminotransferase , CRP; C-reactive protein. p-values were calculated by Mann-Whitney U test.

condition due to abnormally high leukocyte counts and increased levels of WBCs during and after VOC. Clinical studies show that leukocytosis is a risk factor for major sickle cell-related complications such as stroke, acute chest syndrome and early death.(17-19) This study demonstrates that patients with SCD have significantly high WBC levels in severely painful episodes. As a marker of inflammation, CRP has been used to predict prognosis and relapse in patients with some chronic diseases, as well as morbidity in others.(10) Akohoue et al.(10) reported higher serum CRP levels in patients with SCD than in healthy controls. In the present study, CRP levels were higher in SCD patients with painful crisis episodes. CRP levels were highest in patients with severe crises. Previous studies have shown that serum CRP levels are markedly increased in patients with SCD with VOC and that sequential measurements of CRP are useful in predicting the subsequent development of severe painful crisis in patients hospitalized for VOC.(20,21) Previous studies have also shown that CRP levels correlate well with VOC with fever in patients with SCD.(22)

Liver abnormality results in AST and ALT release, making this a useful test for detecting liver damage. Hemolysis also raises AST, ALT and bilirubin levels in SCD.(23) One recently published study reported elevated bilirubin, total protein random glucose AST and ALT levels in SCD.(23) Ojuawo et al.(24) reported significantly higher ALT, alkaline phosphatase and bilirubin levels during crisis than at

recovery, especially in young patients. However, total protein and albumin levels between crisis and at recovery were not significantly different. In this study, AST levels were significantly high and albumin significantly low in the severe pain crisis group. Koh et al.(25) reported that increased hospital stay was associated with lower albumin and hemoglobin/hematocrit levels. In agreement with Koh et al.(25) we determined low albumin, hemoglobin and hematocrit values in the severe pain SCD group, that with the longest hospitalization. This shows the presence of greater hemolysis and hepatic damage in this group.

In conclusion, SCD continues to represent a major public health problem in Turkey, and especially in our region. Patients are mostly admitted to the emergency department with VOC. This analysis of 146 painful crisis episodes suggests that VDS and FPS should be used in determining the severity of painful episodes in patients with SCD, and that patients with high pain intensity scores should be monitored closely in terms of complicated VOC.

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Effects of Omega-3 on lipid profile and haematological parameters in hyperlipidemic rats

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ABSTRACT

Background: There is good evidence that omega-3 fatty acids found in fish oil can help to prevent and treat atherosclerosis by preventing the development of plaque and blood clots. Omega-3 can also help prevent heart disease, lower blood pressure, and reduce the level of triglycerides in the blood. The present study was designed to evaluate and compare the effects of different doses of omega-3, gemfibrozil and atorvastatin on lipid profile and haematological parameters in hyperlipidemic rats.

Methods: Forty eight rats were divided into two groups. The first groups included 18 rats' they were subdivided into three subgroups each of 6 rats. The first subgroup served as a control. The second and third subgroups received omega-3 (15 mg/kg) and (30 mg/kg) orally (PO) daily respectively. The second group included 30 rats and received atherogenic diet throughout the treatment period and served as hyperlipidemic rats. The hyperlipidemic model rats were subdivided into five subgroups of six rats each. The first subgroup served as a positive control. The second and third subgroups received omega-3 (15 mg/kg) and (30 mg/kg) PO daily respectively. The fourth and fifth subgroups received gemfibrozil (3.5 mg/kg) PO daily and atorvastatin (2 mg/kg) PO daily respectively. At the end of treatment period of all these groups, the rats were subjected to various biochemical and hematological tests.

Results: After four weeks of therapy, (30mg/kg) of omega-3 showed a significant reduction in the level of triglyceride (TG), total cholesterol (TC) and low density lipoprotein (LDL-C) in control rats, whereas (15mg/kg) omega-3 could only reduce the level of TC and LDL-C significantly. Four weeks of daily administration of both doses of omega-3 produced significant reduction in serum (TC, TG and LDL-C) of hyperlipidemic rats. However neither (15mg/kg) of omega-3 nor omega-3 (30mg/kg) could increase the level of high density lipoprotein HDL-C in the treated and non-treated hyperlipidemic rats.

Both doses of omega-3 produced a significant increase in the level of HB, RBC and MCH in normal rats. The same doses of omega-3 showed a significant increase in the levels of hemoglobin (HB), red blood cell (RBC), hematocrit (HTC) and mean corpuscular hemoglobin (MCH) in hyperlipidemic rats after 4 weeks of therapy.

Following four weeks treatment with both gemfibrozile and atorvastatin there was a significant reduction in serum (TC, TG and LDL-C) and a significant rise in serum HDL-C in hyperlipidemic rats.

Conclusion: Omega-3 was effective in controlling lipid profile especially serum (TC, TG and LDL-C). No significant differences were found between the effects of both doses omega-3 and gemfibrozile or atorvastatin on TC, TG, and LDL-C of hyperlipidemic rats. In contrast to omega-3, gemfibrozile and atorvastatin induced a significant raise in the level of HDL-C. Omega-3 was effective in increasing the levels of HB, RBC, HTC and MCH in hyperlipidemic rats.

Key words: Omega 3, Gemfibrozile, Atorvastatin, Lipid profiles, hyperlipidemic rats

Introduction

Hyperlipidemia is a lipid abnormality with genetic or familial origins (primary hyperlipidemia). Hyperlipidemia could also be caused by endocrine, hepatic or renal diseases (secondary hyperlipidemia). Primary hyperlipidemia includes familial or polygenic hypercholesterolemia, familial combined hyperlipidemia, familial hypertriglyceridemia, and dysbetalipoproteinemia (1).

Concomitant elevation of circulating levels of triglyceride-rich VLDL and cholesterol-rich LDL is recognized as being associated with an increased risk of premature coronary artery disease (2).

There is good evidence that omega-3 fatty acids (namely EPA and DHA) found in fish oil can help prevent and treat atherosclerosis by preventing the development of plaque and blood clots. Omega-3 can also help prevent heart disease, lower blood pressure, and reduce the level of triglycerides (fats) in the blood. One preliminary study found that people with high cholesterol who took fish oil and red yeast rice lowered cholesterol levels about as much as people who took simvastatin. People with heart disease or those who need to lower triglycerides may need to take fish oil supplements (3) and are characteristic of subjects who exhibit a lipid phenotype typical of combined hyperlipidemia (4).

Atherosclerosis is a disease of large and medium-sized muscular arteries characterized by inflammation and dysfunction of the lining of the involved blood vessels and the buildup of cholesterol and lipids. This results in the formation of a plaque, obstruction of blood flow and diminished oxygen supply to target organs (5).

This dysfunction may arise due to many factors like vessel injury and collagen exposure, metabolite deposition in the vessel wall (increase in lipid, cholesterol), or change in vascular reactivity due to change in the rate or force with which blood flows (6, 7).

The present study was designed to evaluate and compare the effects of different doses of omega-3, gemfibrozil and atorvastatin on lipid profile and haematological parameters in hyperlipidemic rats.

Materials and Methods

Animals

A total of 48 rats of both sexes were used in the present study. Their weight ranged from (170- 250 grams) and they were aged 60 days, the rats were obtained from Mousil and Abu ghreb. Once received they were kept in the animal house in the College of Medicine under controlled conditions of a 12 hour light / 12 hour dark cycle in a room temperature of 25 C°.

The rats were divided into two groups. The first groups included 18 rats which received standard diet throughout the experimental period and were subdivided into three subgroups each of 6 rats. The first subgroup served as a control. The second subgroup received a daily single dose of omega-3 (15mg/kg) orally (PO). The third subgroup received a daily double dose of omega-3 (30mg/kg) PO.

The second group included 30 rats and received an atherogenic diet (79% standard diet + 21% Butter fat) throughout the treatment period and served as hyperlipidemic rats. The hyperlipidemic model rats were subdivided into four subgroups, each group having six rats. The first subgroup served as a positive control. The second subgroup received daily single dose of omega-3 (15mg/kg) PO. The third subgroup received a daily double dose of omega-3 (30mg/kg) PO. The fourth subgroup received a daily single dose of gemfibrozil (3.5mg/kg) PO, and the fifth subgroup received a daily single dose of atorvastatin (2mg/kg) PO.

At the end of the treatment period, the animals were subjected to various biochemical parameters (biochemical and hematological parameters). The animals were deprived of food overnight, anesthetized using light chloroform and sacrificed by cervical decapitation. Blood samples were collected from the rats for determination of serum total cholesterol, triglycerides, high density lipoprotein-C and low density lipoprotein-C, besides some of hematological parameters (HB, RBC, HTC, and MCH).

Statistical analysis

All data are expressed as means± standard error means (M±SEM) and statistical analysis was carried out using statistically available software (SPSS Version 11.5). Data analysis was made using one-way analysis of variance (ANOVA). The comparison among groups was done using Duncan test. $P<0.05$ was considered as statistical significance.

Results

Effects of omega-3 on lipid profiles

Daily administration of omega-3 (30mg/kg) induced a significant reduction in the level of TG in normal rats. The level of triglyceride of normal rats also decreased by (15mg/kg) of omega-3 but the result turned out to be statistically non-significant (Table 1 - next page).

Both doses of omega-3 (15mg/kg) and (30mg/kg) have the same significant efficacy in reducing the level of both TC and LDL-C of the normal rats, whereas they have no significant effects on the level of HDL-C of normal rats as shown in Table 1.

Effects of omega-3 on lipid profiles of hyperlipidemic rats

There was a marked increase in the level of serum triglyceride and TC and LDL-C in the animals treated with atherogenic diet compared to the control group indicating the induction of hyperlipidemia as shown in Table 2 (next page).

Different letters indicate the significance of the result ($P<0.05$).

Table 1: Effect of different doses of omega-3 (15mg/kg) and (30mg/kg) on the lipid profile of normal rats (n=18)

Parameters	Control	Omega-3 (15mg/kg)	Omega-3 (30mg/kg)
TG mg/100ml	67.25±14.45	44.08±2.55	27.26±2.349 *
TC mg/100ml	58.31±9.70	23.88±1.55 *	24.19±3.78 *
HDL-C mg/100mL	32.96±6.002	24.33±1.38	25.21±2.39
LDL-C mg/100mL	6.51±1.71	2.31±0.25 *	2.76±0.65 *

* (P<0.05) when compared to control group

Table 2: Effect of different doses of omega-3, gemfibrozile and atorvastatin on the lipid profile of hyperlipidemic rats (n=36)

Parameter	Control	Hyperlipidemic rats model	Omega -3 15mg/kg	Omega -3 30mg/kg	Gemfibrozile 3.5mg/kg	Atorvastatin 2 mg/kg
TG mg/100ml	57.25±17.45 a	174.83±53.86 b	33.78±2.34 a	28.83±3.55 a	65.24±9.63 a	65.93±9.31 a
Cholesterol mg/100ml	58.31±9.70 a	104.33±23.91 b	41.006±5.81 a	35.79±3.85 a	68.29±6.04 a	46.51±5.38 a
HDL-C mg/100ML	32.96±6.002 a	32.66±5.77 a	35.08±3.02 a	28.81±2.42 a	70.08±5.96 c	48.02±4.45 b
LDL-C mg/100ML	6.51±1.71 a	21.50±12.62 b	4.4±0.73 a	4.5±0.54 a	11.80±1.95 a	5.81±0.51 a

Different letters indicate the significance of the result (P<0.05).

Table 3: Effect of different doses of omega-3 on the haematological parameters of normal rats

Parameters	Control	Omega-3(15mg/kg)	Omega-3(mg/kg)
HB g/dl	13.1000±0.7895 a	15.7333±0.29515 b	15.6833±0.25221 b
RBC 10^12/ µl	6.5650±0.38125 a	7.2250±0.07873 b	7.4967±0.10834 b
HTC %	37.3333±2.54659 a	41.2000±0.86323 a	41.0167±0.96243 a
MCH pg/L	19.9833±0.44827 a	21.3417±0.19080 b	20.8333±0.21705 b

Table 4: Effects of different doses of omega-3 on the haematological profiles of hyperlipidemic rats

Parameter	Control	Hyperlipidemic rats model	Omega -3 (15mg/kg)	Omega-3(30mg/kg)
HB g/dl	13.1000±0.7895 a	10.9667±0.82610 a	15.1333±0.67856 b	15.2833±0.40118 b
RBC10^12/ µl	6.5650±0.38125 ab	5.9800±0.51046 a	7.1683±0.26196 ab	7.5583±0.18718 b
HTC%	37.3333±2.54659 a	32.4833±2.82683 a	41.0333±2.05340 b	41.5500±1.28705 b
MCH pg/L	19.9833±0.44827 b	18.5000±0.54894 a	21.1000±0.45092 b	20.2167±0.15366 b

Compared to the hyperlipidemic rat model both doses of omega-3 (15mg/kg) and (30mg/kg) produced significant reduction in the level of TG. Moreover the same doses of omega-3 could decrease the level of TC and LDL-C hyperlipidemic rats significantly. Compared to the control group no significant changes appeared in the level of HDL in the treated and non-treated hyperlipidemic rats (Table 2).

Effects of omega-3 on some haematological parameters

Both doses of omega-3 significantly increased the level of HB, RBC and MCH of control rats, while the same doses of omega 3 induced a non-significant rise in the level of HTC as shown in Table 3.

Rats fed with atherogenic diet for thirty days displayed non-significant reduction in the levels of HB, RBC and HTC, whereas it significantly reduced MCH compared to the control group. Both doses of omega-3 (15mg/kg) and (30mg/kg) significantly increased the level of HB, RBC and HTC compared with both normal and hyperlipidemic groups as shown in Table 4.

Compared to the control group both doses of omega-3 showed no significant changes in the level of MCH. However there was a significant difference between the effects of omega-3 with that of hyperlipidemic rats as shown in Table 4.

Effects of gemfibrozil on lipid profiles of hyperlipidemic rats:

Compared to the hyperlipidemic rat model gemfibrozil produced significant reduction in the level of TG, TC and LDL-C (Table 2).

No significant differences were found between the effects of both doses omega-3 and gemfibrozil on TG, total cholesterol and LDL of hyperlipidemic rats. However gemfibrozil unlike omega-3 significantly increased the level of HDL-C as shown in Table 2.

Effects of atorvastatin on lipid profiles of hyperlipidemic rats:

Compared to the hyperlipidemic rat model atorvastatin produced significant reduction in the level of TG, total cholesterol and LDL (Table 2).

No significant differences were found between the effects of both doses omega-3 and atorvastatin on TG, total cholesterol and LDL of hyperlipidemic rats however atorvastatin could significantly increase the level of HDL as shown in Table 2.

Discussion

According to the lipid hypothesis, abnormally high cholesterol levels (hypercholesterolemia), or more correctly, higher concentrations of LDL-C and lower concentrations of functional HDL-C are strongly associated with cardiovascular disease because these promote atheroma development in arteries (atherosclerosis). This disease process leads to myocardial infarction (heart attack), stroke and peripheral vascular disease. Since higher blood concentrations of LDL-C, especially the smaller and denser LDL particles, contribute to this process, they are often termed "bad cholesterol" because they have been linked to atheroma formation, while high concentrations of functional HDL-C, which can remove cholesterol from cells and atheroma, offers protection (8).

Concomitant elevation of circulating levels of triglyceride-rich VLDL and cholesterol-rich LDL is recognized as being associated with an increased risk of premature coronary artery disease (2) and is characteristic of subjects who exhibit a lipid phenotype typical of combined hyperlipidemia (4).

In the present study, serum triglycerides were significantly reduced in hyperlipidemic and normal rats treated with omega-3 at the dose of (15mg/kg) for a single dose and (30mg/kg) for a double dose after 4 weeks of treatment. This result is in agreement with another study by Harris et al (1983) who found that omega-3 significantly reduced serum triglycerides in hypertriglyceridemic patients by 25 % to 35 % after 12 weeks of therapy (9). Similar findings were reported by Sanders and Hochland (1983), Negakawa et al. (1983) and Zucker et al. (1988) (10, 11, 12) who found that fish oil

(< 20 g/d) induced a marked decrease in triglyceride concentration in hyperlipidemic patients.

This antitriglyceridemic effect of omega-3 on hyperlipidemic rats is in consensus with Simopoulos (1991) and Thomas et al. (2000) who observed that triglyceride concentration was reduced considerably by omega-3 in patients with hypertriglyceridemia (13, 14).

The mechanism responsible for the triglyceride-lowering effect of omega-3 is poorly defined. In theory it could be related to decreased VLDL-C production (presumably secondary to decreased availability of hepatic free cholesterol for particle assembly), increased clearance of VLDL-C through the LDL receptor (or other lipoprotein receptors), increased delipidation of VLDL particles via LPL, or a combination of the above mechanisms (15).

In this study, the level of total cholesterol was significantly reduced in hyperlipidemic rats treated with both doses of omega-3 after 4 weeks of treatment. This finding is in agreement with Kobatake et al. (1984) who observed that omega-3 significantly reduced serum total cholesterol after 20 days of therapy in hyperlipidemic subjects (16). Whereas Harris (1997) found that a large dose of omega-3 (4 g per day) has no significant effect on the level of total cholesterol in hyperlipidemic subjects after 2 weeks of treatment so this difference might be due to the short term treatment with omega-3 (17).

In the present study, hyperlipidemic rats treated with omega-3 at the doses of (15mg/kg) and (30mg/kg) showed no significant increase in the level of HDL after 4 weeks of treatment. This result is incompatible with another study by (Mori 2000) who found that HDL-C concentration was increased significantly in hyperlipidemic subjects (18). Furthermore Harris (1997) concluded that 4 g per day of omega-3 increased HDL-C cholesterol levels by 1 to 3 percent after 4 weeks of treatment. This effect of omega-3 could be due to the fact that omega-3 significantly reduced total cholesterol in hyperlipidemic and normal rats (19).

LDL-C was significantly reduced in hyperlipidemic rats treated with both doses of omega-3 after 4 weeks of treatment. This is incompatible with the observation of Mori (2000) who observed that there was usually no significant changes in LDL-cholesterol concentration associated with omega-3 administration in hyperlipidemic subjects (18). On the contrary, especially with high doses of omega-3 FAs used in the treatment of hypertriglyceridemia, LDL levels may rise by 10 %, this effect being even more pronounced in patients with extreme TG elevations at baseline (17. 19).

In another investigation Sanders and Hochland (1983) reported that there were modest decreases in LDL concentration for the normal subjects who received (< 20 g/d) of fish oil after 4 weeks of treatment (10), similar findings were reported by Negakawa et al (1983) and Zucker et al (1988) (11, 12).

In the present study, HB and RBC were noticeably increased in hyperlipidemic rats treated with omega-3 at the dose of (15mg/kg) and (30mg/kg). These results are compatible with another study by Abbas et al (2009) who found that administration of omega-3 was associated with an increase in the levels of HB and RBC in sucrose treated rats (20).

In this study, HTC was significantly increased in hyperlipidemic rats treated with omega-3. This result is incompatible with another study by Ghaderpanahi et al (2010) who found that administration of 1g of fish oil in elderly subjects has no significant effects on the level of HTC (21).

In this research, MCH was increased significantly in hyperlipidemic rats treated with omega-3 (15mg/kg) and (30mg/kg). This result was replicated in another study by Nwabueze et al (2011) who found that (MCH) was significantly ($P<0.05$) higher in Heterobranchus bidorsalis fish fed on feeds containing 2000mg and 1000mg omega-3 than in control fish (22).

Gemfibrozil treatment produced a significant reduction in the serum triglyceride of hyperlipidemic rats similar to that of omega-3. This result was quite similar to that reported by Keijiro Saku et al (1985) who found that gemfibrozil significantly reduced serum triglycerides by 46 % after 12 weeks of therapy in hyperlipidemic patients (23).

Moreover it is accordance with the result of Irish and Thompson (1996) who detected that gemfibrozil lowered serum triglycerides by 44% after 6 weeks of therapy in hyperlipidemic patients (2).

The result of this study showed that total cholesterol was significantly reduced in hyperlipidemic rats treated with gemfibrozil after 4 weeks of treatment. This result is in agreement with another study by Keijiro Saku et al (1985) who found that gemfibrozil significantly reduced total cholesterol by 47% after 12 weeks of therapy in hyperlipidemic patients (23).

In this study, HDL was significantly increased in hyperlipidemic rats treated with gemfibrozil after 4 weeks of treatment. This result is in consensus with studies by Irish and Thompson (1996) who concluded that gemfibrozil significantly increased HDL by 36% and 31% respectively after 12 weeks of therapy in hyperlipidemic patients (2, 24).

In the present study serum LDL was reduced in hyperlipidemic rats treated with gemfibrozil after 4 weeks of treatment. This result is in agreement with another study by Manninen et al (1982) who found that gemfibrozil significantly reduced LDL by 23% after 12 weeks of therapy in hyperlipidemic patients (25), whereas Irish and Thompson (1996) reported that gemfibrozil has no significant effect on the level of LDL in hyperlipidemic patients even after 12 weeks of treatment. Therefore this result is incompatible with the finding of this study (24).

In this study, serum TG was significantly reduced in hyperlipidemic rats treated with atorvastatin after 4 weeks of treatment. This result is in agreement with studies of Athyros et al (2002) and Branchi et al (1999) who found that atorvastatin 20 mg daily dose significantly reduced TG by 31%, 20 % respectively in hyperlipidemic patients after 2 months of therapy (26, 27). In accordance with reports of Stein et al (1998) the effect of atorvastatin on serum TG was largely dependent on the baseline serum triglyceride level and, in patients with low serum triglyceride; there was little if any hypotriglyceridemic response (28). The relationship of the hypotriglyceridemic activity to the baseline serum triglyceride level may explain why some authors found only small effects of statins on serum triglycerides, whereas others reported greater lowering effects.

The differences in the hypotriglyceridemic response among the studies are likely to be due to differences in the patient populations. It is generally accepted that HMG CoA reductase does not play a direct role in the repletion of TG levels. Atorvastatin administration, however, produces marked reduction in TG levels in hyperlipidemic patients (29).

In the present study, atorvastatin as gemfibrozil and omega-3 significantly reduced the level of total cholesterol in hyperlipidemic rats after 4 weeks of treatment. These results are in agreement with results of studies conducted by Nawrocki et al (1995) and Marian et al (2006) who found that atorvastatin reduced plasma cholesterol up to 45% in patients with primary hypercholesterolemia (30, 31).

In this research, LDL-C was significantly reduced in hyperlipidemic rats treated with atorvastatin after 4 weeks of treatment. This is in accordance with the observations of Hing-Chung et al (2006) who found that atorvastatin 20 mg daily for 12 weeks of treatment significantly decreased LDL-C in comparison with 10 and 40 mg of atorvastatin in hyperlipidemic patients (32).

This reduction of serum cholesterol could be due to inhibition of HMG-CoA reductase which catalyzes the conversion of HMG-CoA to mevalonate which decreases the cholesterol synthesis (33, 34).

In the present study, HDL-C was significantly increased in hyperlipidemic rats treated with atorvastatin after 4 weeks of treatment. This result was quite similar to that reported by Jeevan et al, (2008) who found that atorvastatin significantly increased HDL-C for 12 weeks of treatment (35).

The mechanism underlying the increase in HDL-C levels observed during statin therapy is poorly understood. Available evidence suggested that increase in HDL-C with statin therapy results from a combination of increased expression of apoA-I and reduced HDL remodeling as a consequence of lowering triglyceride levels (35, 36).

There is also evidence that increases in HDL-C during statin therapy may be related to the decrease in the activity of cholesteryl ester transfer protein, likely due to depletion of levels of very low-density lipoprotein and LDL particles (37).

No significant differences were found between the effects of both doses of omega-3 with gemfibrozil and atorvastatin on TG, total cholesterol and LDL-C of hyperlipidemic rats, however gemfibrozil and atorvastatin unlike omega-3, significantly increased the level of HDL-C.

Conclusion

1- Omega-3 was efficient in reducing serum TC, TG and LDL-C. However it was not effective in significantly altering serum HDL-C in hyperlipidemic rats.

2- Omega-3 was effective in increasing the levels of HB, RBC, HTC, and MCH in hyperlipidemic rats.

3- No significant differences were found between the effects of both doses of omega-3 and gemfibrozile or atorvastatin on TG, TC and LDL-C of hyperlipidemic rats. In contrast to omega-3, gemfibrozile and atorvastatin induced a significant rise in the level of HDL-C.

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Prevention of Otologic disorders in Nigeria: The Case of Primary School Children in Rivers State, South South of Nigeria

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ABSTRACT

Introduction: Common ear diseases in children are preventable. In developing countries like Nigeria, most health care programmes concentrate on secondary school pupils to the disadvantage of those in primary schools. This has delayed reduction of preventable ear diseases. Ear diseases among children can lead to disabilities especially permanent hearing loss. The question is to what extent are otologic disorders prevented among primary school children? The researchers carried out school health programmes to identify common ear diseases in primary school children so as to contribute a quota in prevention of otologic diseases among them.

Materials and method: This was a cross-sectional descriptive study. Random sample of 1,200 pupils from primary 1-6 between 5 to 13 years were selected for study from 13 primary schools. Only 802 pupils whose parents gave consent eventually participated in this study. Administered semi-structured questionnaire was used to obtain information on participants from parents. Also, otoscopic examination of respondents was carried out by the researchers after being trained in use of otoscope by a specialist. Complete physical examination of the 802 children was done. Cerumen was recorded if no part of tympanic membrane was visible.

Thereafter, cerumen, other debris and foreign bodies were removed in such children. External canals and tympanic membranes were inspected for likely abnormalities. Data analyses were done using SPSS version 17.0 statistical software. Results were presented on tables using frequency and percentages.

Results: Findings showed that out of 802 children studied, only 279(34.8%) were found normal.
The common Otologic diseases found among the respondents were impacted cerumen 319(39.7%), chronic suppurative otitis media 95 (11.8%), debris 55 (6.9%), Otitis media with effusion 28 (3.5%), and acute otitis media 10 (1.3%).

Conclusion: Based on the proportion of children identified with otologic problems, there is need for periodic and well coordinated school health programmes.

Key words: Otologic disorders, school children, otitis media, hyperemic.

Introduction

The importance of prevention of ear diseases as an organ of hearing cannot be overemphasized. Disorders of the ear among children in developing countries have been widely reported as a major public health problem [1-6]. Delayed speech, cognitive, emotional, social and academic developments have been reported as some of the direct effects of hearing impairment in children [7].Studies have shown that a good proportion of children with ear diseases have difficulties in learning and as a result, their academic performances are negatively affected [8,9]. It is a well known fact that with early medical intervention, disability from diseases of the ear can be prevented. However, most times children with ear problems are not diagnosed early and as a result, the diseases progress to profound deafness.

Ear disorders in children can arise from congenital problems such as Crouzons's disease, Down's syndrome, Achondroplasia or Marfan's disease and others [10-12]. Ear disorders in children can also result from acquired causes like otitis media with effusion (OME), otitis externa, trauma leading to perforation of the tympanic membrane or impacted cerumen [13,14]. There have been various views regarding the effect of removing impacted cerumen (wax). Some researchers argue that removing impacted cerumen (wax) prevents hearing loss [15,16], while others emphasize that there will be persistent hearing loss even after the wax has been removed [17].

Studies have recommended a high index of suspicion in the diagnosis of ear diseases among children. This is necessary because most studies are of the view that Otitis media constitute the main ear disorder in children living in developing countries including Nigeria [18-20]. Some authors further argue that most ear disorders present with or without symptoms and that only a thorough history and physical examination of children with ear problems would guarantee effective diagnosis, treatment and prevention of disability. Such authors recommended that physicians should conduct thorough examination of the head and neck area of a child for a possible predisposing factors to developing ear diseases when a history of high grade fever, pain in the ear, ear discharge, pre or post auricular swelling, hearing impairment, deep seated headache, dizziness, tinnitus and vertigo is reported [21-25]. WHO (2000) recommended that periodic screening for hearing impairments in schools will ensure early diagnosis and treatment for children with ear diseases especially those in developing countries. This is necessary because some ear diseases are gradual in onset, painless, without signs and symptoms and at times invisible (26). Therefore, without routine screening of school children especially in places where there is no working national guidelines, the likelihood of detecting children with ear disorders could go unnoticed until later in the child's age when prevention becomes difficult [27-29]. In developed countries, where neonates were screened for diseases of the ear, studies have reported a high proportion of neonates with hearing impairment [30]. Prevention of ear diseases in children requires follow-up for the purposes of repeat audiology test and for intervention after definitive diagnoses (31,32).

The benefits of screening school children for disabilities especially hearing loss should not be underestimated. In developing countries, like Nigeria, children with disabilities are totally dependent on others and as such, have little or no contribution towards the economic development of the country where they reside. The need to ensure that youths live satisfying and fulfilled lives devoid of disabilities and dependence motivated the researchers to conduct this study. This research enabled the researchers to contribute a quota to the recent call for the prevention of disabilities among school children in Nigeria. In this study, the researchers emphasized the need to strengthen routine ear screening from neonatal period to school period, periodic physical examinations and health education in schools. The study will help to complement the limited studies on childhood ear diseases in Nigeria.

Materials and Methods

This cross-sectional descriptive study was carried out to identify common otologic disorders among primary school children in Rivers State. The sample was made up 1,200 pupils in primary 1-6 within the ages of 5 to 13 years randomly selected from 13 primary schools. This study was conducted in Port-Harcourt City (PHC). Port-Harcourt was chosen for the study because it is the capital of Rivers State and the major oil-producing State in Nigeria. It attracts lots of investors and tourists from all parts of the country and beyond. Moreover, Port-Harcourt has the largest number of highly populated primary schools among the 23 local governments in the State and therefore afforded enough sample for the study. The thirteen (13) schools selected for study were based on the fact that their populations were made up of both boys and girls (co-educational). The sample size was calculated at a 95% confidence level for a 13.9% proportion of hearing loss among primary school children in Lagos, Nigeria [20]. To get the sample for study, simple random sampling by balloting was used to select 1200 pupils from classes 1-6. Only 802 pupils whose parents gave consent eventually participated in this study. Semi-structured self administered questionnaire was used to obtain information on the participants from the parents. Also questionnaire was used to explore the social status of the parents of the respondents. The social status of the respondents' parents was graded into five (1-5) categories based on their monthly income. In addition, otoscopic examination of the respondents was carried out by the researchers after being trained on the use of otoscope by a specialist. Also, complete physical examination on the 802 children was done and cerumen was recorded if no part of the tympanic membrane was visible. Thereafter, cerumen, other debris and foreign bodies were removed in such children. The external canals and tympanic membranes were later inspected for likely abnormalities. Children were classified as having abnormal ear drums or having otitis media if the drums were perforated, hyperemic, retracted or showed evidence of scarring with or without fluids. Data analyses were done using SPSS version 17.0 [21] statistical software. Results were presented in tables using frequency and percentages.

Limitations to the study

The population studied was school children therefore, the observed prevalence of ear diseases w ould not reflect the actual picture in the general population because institutional based study may not give a clear picture of the situation outside the school.. Also children not in school as well as those whose parents refused to consent to the study might have epidemiological characteristics different from those who took part in the study. The cross-sectional descriptive design of the study could not allow causality to be determined.

Results

The mean age of the respondents was 8.6 years ± 2.3 years with median 8.0 years. About 405(50.5%) males and 397(49.5%) females were studied. The age of the respondents was evenly distributed. About 281 (35.0%) pupils were between the age of 5-7 years and 253 (31.6%) were between 11-13 years. See Table 1 for more details As contained in Table 2, the social class of the parents of the study group was classified into 5 categories using their monthly income. Those in category 1 were on monthly income of USD 2890, category 2, USD 2312, category 3 on USD 1692, category 4 on USD 867 and category 5 were on USD 289. The social status of 48(6.0%) of the respondents , could not be ascertained because enough information was not provided by their parents/guardians. Study showed that a good proportion of the respondents 268 (33.4%) came from social class category 3.

The social class of the parents of the respondents who had more otologic disorders than others was noted. From the result of the study, respondents from social status category 5 had the highest incidence of otologic disorders. This is statistically significant P<0.001 see Table 3 (next page) for more details. The types of otologic diseases seen among the respondents were noted. From the findings, impacted cerumen 319(39.7%), Chronic suppurative otitis media 95(11.8%), debris 55(6.9%), Otitis media with effusion 28(3.5%), and acute otitis media 10(1.3%) were noted. See Table 4 for more details.

Discussion

From the result of this study, several otologic diseases were identified among the respondents after the otologic examination. The most common otologic diseases was impacted cerumen. The fact that impacted cerumen was the most common otologic disease among the population studied showed the extent to which the tympanic membranes of the respondents were occluded as well as the extent they are exposed to hearing loss. Impacted cerumen occluding the tympanic membrane has been widely reported by several researchers as the major cause of hearing loss in children

Table 1: Age and Sex Distribution of Study Population

Age group (years)	Males (%)	Females (%)	Total number (%)
5-7	144(51 .2)	137(48.8)	281(35.0)
8-10	133(49.6)	135(50.4)	268(33.4)
11-13	128(50.6)	125(49.4)	253(31.6)
Total	405(50.5)	397(49.5)	802(100.0)

Table 2: Social Class of the Study Population

Social class	Total (%)
I	74 (9.2)
2	222 (27.7)
3	268 (33.4)
4	167 (20.8)
5	23 (2.9)
Unknown	48 (6.0)
Total	802 (100.0)

Table 3: Social status of the parents of respondents and Otologic disorders

Social class	Normal Ear (%)	Ear disorder (%)	Total (%)	X^2/P
1	54(73.0)	20(27.0)	74(100.0)	X^2=159.4
2	127(57.2)	95(42.8)	222(100.0)	P<0.001
3	68(25.4)	200(74.6)	268(100.0)	
4	23(13.8)	144(86.2)	167(100.0)	
5	3(13.0)	20(87.0)	23(100.0)	
Unknown	4(8.3)	44(91.7)	48(100.0)	
Total	279(34.8)	523(65.2)	802(100.0)	

Table 4: Respondents and types of Otologic diseases noted

Types of Otologic diseases	Frequency	Percentage
Impacted Cerumen	319	39.7
Chronic suppurative Otitis media	95	11.8
Debris	55	6.9
Otitis media with effusion	28	3.5
Mucoid discharge	12	1.5
Acute Otitis media	10	1.3
Foreign body	4	0.5
Normal	279	34.8
Total	802	100

[12, 26]. This finding is similar to studies conducted in other parts of Nigeria [20, 23], Turkey [24] and Swaziland [25] where impacted cerumen was identified as the main otologic disease among the population studied. Usually impacted cerumen in children is asymptomatic and can easily be missed by parents and caregivers. This could possibly lead to hearing impairment in the affected children. Identifying foreign bodies as the least otologic disease among the respondents (0.5%) is comparable to that identified by some other studies [26, 27]. It is not surprising that foreign bodies constitute the least otologic disease among the group studied. This is because foreign bodies usually present some discomforting symptoms that will demand immediate attention for their removal.

Chronic suppurative otitis media (CSOM) has been implicated as the major risk factor for ear diseases in children [28]. It has been seen as the major health challenge in several parts of the world [22, 28, 29]. The finding in this study reveals a CSOM prevalence of 11.8%. This is low compared to a study conducted by Akinpelu etal [30] in which a prevalence as high as 33.9% was seen among the population studied. This high prevalence noted in the previous studies could be attributed to the wider age group interval (6months to 18years) that participated in the study as against the present study which used (5 years to 13 years).

Social status was an important factor in the occurrence of otologic diseases among the group studied. The lower the respondents' social status the more otologic diseases they presented with. In this study, chronic suppurative otitis media was high among social status categories 4 and 5 which had monthly income of USD 867 and USD 289 respectively. Chronic suppurative otitis media has been linked to poor hygiene, nutrition, and housing conditions and these poor conditions encourage viral and bacterial infections [29]. This finding is comparable to previous studies which found higher prevalence of chronic suppurative otitis media among African-Americans than Caucasians [31]. This difference in the prevalence was attributed to poor standard of living among African-Americans. Most individuals in the low socio-economic class avoid living in places where they will be expected to pay high house rent and as a result, they tend to prefer living in suburbs where house rents are low but the environmental conditions are poor. The fact that the respondents from the lowest social status categories 4 and 5, whose monthly income was poor presented with more otologic problems than others, showed that low social status constitutes a major risk factor for otologic diseases among school children. The prevalence of otologic diseases of 65.2% among school children as identified in this study, is lower than that reported by other researchers in a study conducted in Nepal where a prevalence of 75.7% was noted [22]. The observed difference in the two studies conducted could be influenced by the socio-demographic variations in the two populations studied.

In ranking the prevalence of acute otitis media (AOM) among the respondents, AOM with 1.3% was the sixth otologic disorder. This ranking is comparable to a study conducted in Nepal [22] in which AOM with 1.4% prevalence was ranked as the 4th otologic disorder seen among the group studied. In another study [32] the prevalence of AOM was 28% and ranked higher than the two studies above. The difference in this ranking might be in the population studied. The sample studied here comprised children with febrile illnesses. AOM is an acute illness that commonly presents with other febrile illnesses in children [8]. Most often, in developing countries where malaria is endemic, children who present with feverish conditions with no other symptoms suggestive of any ear disease are treated for malaria, thereby making clinicians miss AOM, except if the clinician has a high index of suspicion for ear disorders and this often will lead to further delay in making the diagnosis. The fact that some ear diseases are asymptomatic and could be missed or given late diagnosis when prevention may be unattainable, periodic physical examination as well as health education is needed in primary schools.

Since cerumen impaction which was associated with low social status and poor environmental condition was the commonest otologic problem seen in this study , the need to prevent it by routine examination, aural toileting or simple cleaning with cotton buds to check reaccumulation is necessary. Parents, care givers, and teachers should be health educated on how to identify symptoms for ear diseases in children. During health programmes in school, clinicians should examine every child's ear for a possible ear disease.

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