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THE MOST GRACIOUS,
THE MOST MERCIFUL

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The inaugural issue of JUMJ first appeared September 1, 2014 with Prof. Dr. Saleh A. Al-Damegh, Founding Dean of Unaizah College of Medicine and Applied Medical Sciences and General Supervisor for Unaizah Colleges New Campus at Qassim University, Qassim University, Qassim, Saudi Arabia, as the Founding Editor-In-Chief.

The journal was initially name as Aljouf University Medical Journal (AUMJ). As the university name changed into Jouf University, the journal's name was also modified into Jouf University Medical Journal (JUMJ) starting from the 3rd issue of the year 2018.

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JUMJ full text articles and their serial code Digital Object Identifier (DOI) address number (according to the International DOI Foundation) are accessible online through searching Journals for Aljouf University Medical Journal at Al-Manhal Platform (https://platform.almanhal.com/Search/Result?q=&sf_21_0_3=Aljouf+University+Medical+Journal&opsf_21_0=1&f_title_type_exact_loc_en=Article&opf_title_type_exact_loc_en=2). JUMJ is indexed by the three major players in the world of academic library discovery services: EBSCO Discovery Services (EDS), OCLC (Worldcat Discovery) and ProQuest (SUMMON). The DOI of AUMJ is 10.12816.

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3. Expanding, increasing the depth, and spreading of internationally competent and updated medical knowledge among the JUMJ target audiences for the benefit of advancing the medical service to the local and international communities.
4. While insuring integrity and declaration of any conflict of interest, JUMJ is adopting an unbiased, fast, and comprehensively constructive one-month peer review cycle from date of submission to notification of final acceptance.

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Original Article

Prevalence and Risk Factors of Depression among Patients with Type 2 Diabetes in Qassim, Saudi Arabia: A Cross-Sectional Study

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Abstract

Background: Diabetes mellitus type 2 (T2DM) is one of the most prevalent health problems affecting the general public, and depression is the most common psychological disorder encountered among diabetic patients.

Objectives: To assess the prevalence of depression and to identify its associated sociodemographic risk factors among diabetic patients at Qassim, Saudi Arabia.

Patients and Methods: An analytical cross-sectional study was conducted at the Diabetes Clinic at National Guard Center for Primary Health Care, Qassim. It involved 186 Saudi, of both genders, with an age range of 26 - 85 years. People with T2DM who completed the Zung's Self-Rating Depression Scale were enrolled. Data analysis was performed using SPSS V.24.0 (SPSS, Chicago, USA).

Results: Overall, 40.9% of the patients experienced depression, however; those with moderate to severe depression were 15.6%. Factors that showed significant relationship with depression were gender ($p = 0.001$), marital status ($p = 0.012$), education ($p = 0.001$) and occupation ($p = 0.001$), however there was a weak positive correlation between Hemoglobin A_{1C} level and depression score ($r = 0.291$ and $P = 0.001$).

Conclusions: The prevalence of depression is high among T2DM patients from Qassim, Saudi Arabia. This should alert clinicians to identify and treat depression as part of multidisciplinary diabetes care. Larger community-based studies are needed to identify the magnitude of the problems and to tackle the related risk factors to ameliorate the problem.

Key words: Depression, Risk factors, Type 2 diabetes mellitus, HbA_{1C} %.

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Introduction

Diabetes mellitus type 2 (T2DM) is a chronic noncommunicable disease that poses a severe impact on society and governments such as; decreased life expectancy, high health care costs and reduced quality of life are the most threatening complications of T2DM. By 2035, six hundred million people will experience diabetes mellitus [(1)]. Diabetes mellitus is increasing in dreadful manner in Saudi Arabia; affecting one in every five Saudis[(2-4)].

T2DM is associated with dangerous short term consequences such as decreased blood sugar, in addition to long term consequences such as nephropathies, neuropathies, retinopathies and cardiovascular morbidities. Diabetes is correlated with many psychological problems [(5,6)], and many studies reported higher prevalence of psychiatric diseases among diabetic patients, (16 - 50%)[(7-9)], compared to general population (6 - 40%)[(10,11)]. Depression is the most common psychiatric complication among diabetic patients[(12,13)], and it occurs as a

direct effect of neurological changes accompanying diabetes[14]. The presence of diabetes and depression together lead to increase in disabilities and decrease in patient's quality of life[15]. The available literature reveals that one third of diabetic patients complain of clinically manifested depression, and moreover, they were more likely to have depression twice as nondiabetics[16]. Internet addiction, obesity, reduced physical activity, diabetes consequences and bad glycemic control are correlated with depression in diabetic patients[17-21]. Many dangerous consequences are related to depression in diabetic patients such as increased diabetic complications[18], advanced morbidities[22], hyperglycaemia[17,18], reduced quality of life[23-26] and premature death[27].

The present study aimed to assess the prevalence and risk factors of depression among patients with T2DM in Qassim, Saudi Arabia.

Patients and Methods

Study design and recruitment criteria:

The present study was done using analytical cross-sectional design. The sampling technique was systematic random sampling. Participants with T2DM who were registered at the Diabetic Clinic in the National Guard Center for Primary Health Care, Qassim, Saudi Arabia (NGHA-QPHC) and who met the diagnostic criteria by the American Diabetes Association (ADA) 2017[28] were chosen by a systematic random sampling technique using medical records' serial numbers in the registration list at the Clinic, where we choose every fifth patient attending the clinic. Attending diabetic patients who were hospital-diagnosed 6 months before the study - utilizing clinical guidelines and laboratory information, were included in the present study. The study was conducted between March and May 2018. Patients who had diabetes type 1, psychiatric illnesses, night shifts working in the previous 3 months, diabetic neuropathy, sleep disturbances, age less than 18 years, endocrinal diseases, pregnancy and lactation were excluded from the present study.

Sample size determination was done considering prevalence of depression to be 25%[27], confidence level of 95%, 90% power of the study and design effect of 2.5. After adding 20% as a non-response rate, the calculated sample size was 150. Two hundred and thirteen eligible patients with T2DM aging 25 - 92 years were invited to take part in the present study. Among 213 patients, 5 left because of workload, 12 refused to participate in the study and 10 didn't fill the questionnaire completely. The response rate was 87.3%. Overall, 186 people with T2DM participated in the study. All participants gave informed verbal consent. Ethical clearance was taken from Qassim Quality and Continuous Medical Education Committees. The procedures followed were in accordance with the standards of International Ethical Guidelines for Health-related Research Involving Humans - Geneva 2016[29].

Evaluation of symptoms of depression:

Depressive symptoms were assessed using the Zung's Self-Rating Depression Scale[30]. The Scale was designed by Zung to assess the level of depression for patients diagnosed with depressive disorder. The Scale is a short self-administered survey to quantify the depressed status of a patient. All the symptoms were reported by patients. This scale comprise 20 questions measured on a scale of 1 = rarely, 2 = some of the time, 3 = very often/often, and, 4 = almost/always. Then the total score of the 20 questions was multiplied by 1.25, with the integer score as a standard score. The score ranges from 25 - 100 and is grouped into 4 different categories; 25 - 49 = Normal range (no psychopathology), 50 - 59 = Minimal to mildly depression, 60 - 69 = Moderate to marked depression, and, ≥70 = Severe to extreme depression.

Validation of Zung's Self-Rating Depression Scale:

Many different studies have used the Zung's depression scale for assessment of depression[31-33]. This tool was validated and shown to be efficient in many countries using different languages [33,34] The Arabic translation of the English Zung's Self-Rating Depression Scale was validated in 2005[31].

Assessment of glycemic control:

Based on the American Diabetes Association (ADA) 2017 Guidelines[28], a hemoglobin A_{1c} (HbA_{1c}) level <7.0% (54 mmol/mol) was considered to be a good glycemic control and a HbA_{1c} level ≥7.0% was defined as a poor glycemic control.

Assessment of other variables:

Other variables such as age, marital status, sex, level of education, employment status, net household monthly income, duration of diabetes since diagnosis and insulin usage were assessed using a standardized data collection questionnaire.

Statistical analysis:

SPSS V.24.0 (SPSS, Chicago, USA) was used for statistical analysis. Mean ± SD was calculated for continuous variables while, absolute values and percentages were recorded for categorical variables. Differences in categorical variables were assessed using Chi-square and Fisher's Exact test. Pearson correlation test was applied to test the relationship between quantitative variables. The minimum statistical significance level for all analyses was $p < 0.05$ at a confidence level of 95%.

Results

Socio-demographic and clinical features of participants are shown in Table 1. Most of our respondents were >40 years of age (89.8%). Males were majority (55.9%) and most of them were married (91.9%). Less than half of participants were illiterates (47.8%), unemployed/housewife (44.1%) were or were retired (41.4%). The majority (85.5%) has a total net income per month of <7500 Saudi Arabian Riyal (SAR).

One third of participants (32.3%) had controlled diabetes (HbA_{1C} <7%) and (40.9%) of type 2 DM included in the study had depression (Figure 1).

Table 1: Sociodemographic and clinical features of the investigated type 2 diabetic patients (n = 186), Qassim, Saudi Arabia. Data shown are frequencies; n (%).

Characteristic		n (%)
Age, Years	18 - 40 >40	19 (10.2) 167 (89.8)
Gender	Male Female	104 (55.9) 82 (44.1)
Marital status	Married Divorced/Widowed	171 (91.9) 15 (8.1)
Education level	Illiterate Primary Preparatory Secondary University	89 (47.8) 41 (22) 20 (10.8) 25 (13.4) 11 (5.9)
Occupation	Employed Businessman Unemployed/Housewife Retired	24 (12.9) 3 (1.6) 82 (44.1) 77 (41.4)
Income, SAR	<7500 7500-15000 >15000	159 (85.5) 25 (13.4) 2 (1.1)
Duration of diabetes, Years	<10 10-20 >20	96 (51.6) 68 (36.6) 22 (11.8)
HbA_{1C} level	<7% (controlled) 7.1-9% (poorly controlled) >9% (very poorly controlled)	60 (0.3) 83 (44.6) 43 (23.1)
Insulin use	Yes No	76 (40.9) 110 (59.1)

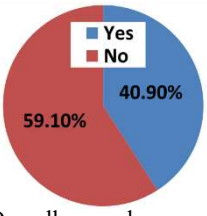

Figure 1: Overall prevalence of depression among type 2 diabetic patients from Qassim, Saudi Arabia. Depression = Yes. No depression = No. Data shown are percentages (total n = 186).

Regarding the degree of depression as assessed by Zung's Self-Rating Depression Scale, 25.3%, 12.4% and 3.2% of the participants had minimal, moderate and severe depression, respectively - as demonstrated in Figure 2.

Table 2 shows a statistically significant relationship between depression on one hand and gender, marital status, education and occupation on the other hand. Depression was observed more common among females (59.8%), divorced/widowed patients (73.3%), illiterate patients (55.1 %) and patients who were businessman (66.7%).

However, no statistically significant associations were found between depression and other variables such as age, monthly income, duration of diabetes, HbA_{1C} level and insulin use. A weak positive correlation was found between HbA_{1C} level and depression score as demonstrated in Figure 3.

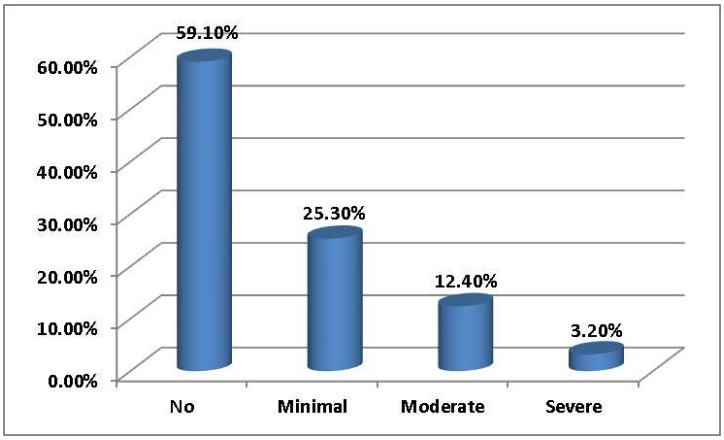

Figure 2: Distribution of the various degrees of depression among type 2 diabetic patients from Qassim, Saudi Arabia. Data shown are percentages (total n = 186).

Discussion

The present study showed that the prevalence of depression among diabetic patients attending the Diabetic Clinic of the National Guard Center for Primary Health Care, Qassim, Saudi Arabia is 40.9% which is comparable to previous studies[35-38]. Management of psychiatric diseases and their risk factors among diabetic patients depends mainly on identifying properly their spectrum among diabetic patients. Reported studies revealed that diabetic patients are more likely to have depression - two times higher than nondiabetic patients[12,13]. Therefore, periodic assessment of psychological status of diabetic patients is recommended by the International Diabetes Federation Global Guideline[39]. The assessment of psychological status of diabetic patients should be done on regular basis[40-41].

The prevalence of depression among diabetic patients was 40.9% in the present study, however, other studies have shown different figures, such as, 49.6%[42], 57.9%[43], 61.8%[44] and 12.5%[45]. The marked discrepancy in the prevalence among these studies may be related to the differences in methodology used, cultural, religion, genetic and environmental background, case definition, method of data collection and sampling technique. A large scale multicenteric study using validated instrument will be more

appropriate to measure depression among diabetic patients accurately. The prevalence of depression among diabetic patients in this study was consistent with other international studies among diabetic patients such as, 40.3% in Malaysia[46], 42% in UK[47] and 37%[48] in Ireland.

In this study, depression was shown higher among females and divorced/widowed individuals. Similarly, Hawamdeh and his colleagues found that depression among diabetic patients was significantly higher among women in Arab countries[49]. In agreement with the present study, a study has revealed that unmarried patients were three times more depressed than married patients[42]. The present study has revealed that depression is lower among those who are retired if compared with unemployed/housewife patients, which is not in consistent with other studies[50]. This may be because Saudi patients don't have any worry about the costs of treatment after retirement as the government is ensuring strong free-of-charge healthcare services for them. Educational level also affects the development of depression. It was shown that illiterates were more vulnerable to depression in the present study and this was consistent with other studies[51,52], whereas, others investigators stated non-significant association[53].

The present study showed that 44.6% of patients had poor glycemic control and 23.1% had very poor glycemic control. However; there was a weak positive correlation between HbA_{1C} level and depression score that is inconsistent with other studies[54, 55].

Table 2: Determinants of depression among the investigated type 2 diabetic patients, Qassim, Saudi Arabia. Data shown are frequencies; n (%) and p values.

Characteristic		**Depression (n = 76)**	**No Depression (n = 110)**	**p**
		n (%)	**n (%)**	
Age, Years	18-40 >40	7 (36.8) 69 (41.3)	12 (63.2) 98 (58.7)	0.707
Gender	Male Female	27 (26) 49 (59.8)	77 (74) 33 (40.2)	0.001
Marital status	Married Divorced/Widowed	65 (38) 11 (73.3)	106 (62) 4 (26.7)	0.012
Education level	Illiterate Primary Preparatory Secondary University	49 (55.1) 13 (31.7) 2 (10) 9 (36) 3 (27.3)	40 (44.9) 28 (68.3) 18 (90) 16 (64) 8 (72.7)	0.001
Occupation	Employed Businessman Unemployed/Housewife Retired	7 (29.2) 2 (66.7) 48 (58.5) 19 (24.7)	17 (70.8) 1 (33.3) 34 (41.5) 58 (75.3)	0.001
Income, SAR	<7500 7500-15000 >15000	65 (40.9) 10 (40) 1 (50)	94 (59.1) 15 (60) 1 (50)	0.962
Duration of Diabetes, Years	<10 10-20 >20	43 (44.8) 23 (33.8) 10 (45.5)	53 (55.2) 45 (66.2) 12 (54.5)	0.333
HbA_{1C} level, %	<7% (controlled) 7.1 - 9% (poorly controlled) >9% (very poorly controlled)	19 (31.7) 34 (41) 23 (53.5)	41 (68.3) 49 (59) 20 (46.5)	0.085
Insulin Use	Yes No	25 (39.7) 51 (41.5)	38 (60.3) 72 (58.5)	0.815

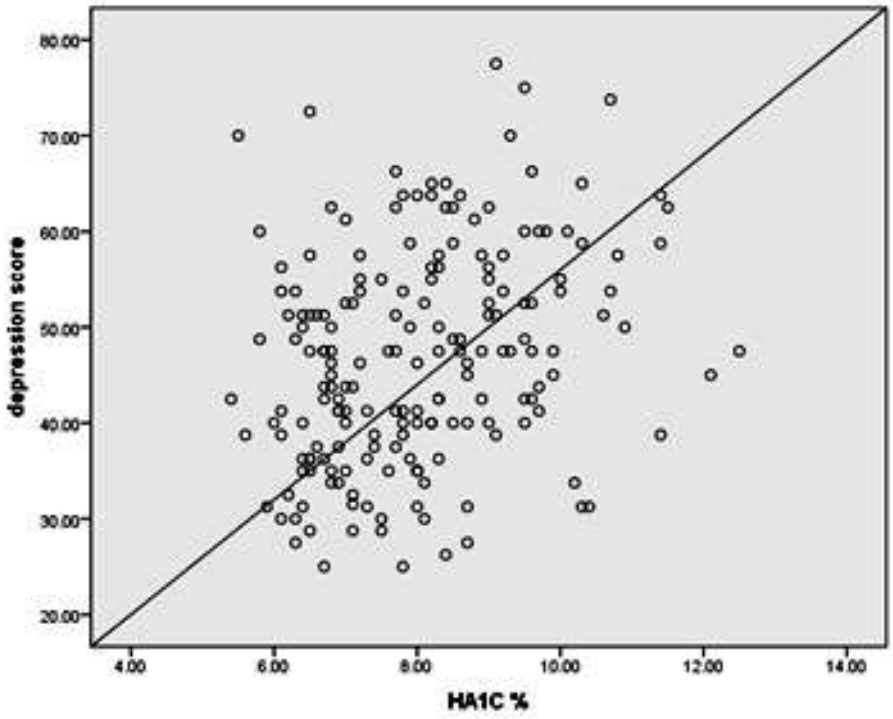

Figure 3: Correlation between HA_{1C} and depression score among type 2 diabetic patients from Qassim, Saudi Arabia ($r = 0.291$ and $p = 0.001$).

Conclusion

Our study revealed a high prevalence of depression among patients with T2DM from Qassim, Saudi Arabia. Females, divorced/widowed patients, illiterates and businessmen were more likely to suffer from depression than others. Depressive symptoms were associated with poor glycemic control. Prevention necessitates better glycemic control, appropriate screening, increasing social support, and health education & promotion.

Limitations of the Study

First, as it is a single center-based study, it will not provide reliable data about the general estimated prevalence. Also, being a cross-sectional study, it did not assess the temporal sequence between depression and other risk factors. Thirdly, misclassification was liable to occur in the present study, as self-report questionnaires were used. Fourth, other risk factors such as dietary intake which influence the occurrence of depression among diabetic patients weren't studied in the present study.

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Conflict of Interests

The authors declared no conflict of interests.

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Original Article

Evaluation of Neutrophils: Lymphocytes Ratio among Children with Falciparum Malaria in a Sudanese Endemic Area

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Abstract

Background: Severe Falciparum malaria causes high mortality and morbidity among children, especially in tropical regions. Neutrophils to lymphocytes ratio (NLR) is a prognostic indicator of inflammatory diseases, cancers, cardiovascular events, and many types of infectious diseases - including parasitic ones, e.g., Falciparum malaria.

Objectives: We aimed to evaluate NLR among Sudanese children, infected with Falciparum malaria, attending Wad Medani Pediatric Hospital, Gezira State, Sudan, to assess its relationship to the intensity of parasitemia and disease severity.

Methodology: This case-control study included 300 Sudanese children. They were composed of 100 severe Falciparum malaria (aging 8.63 ± 3.40 years; 39% female), 100 uncomplicated Falciparum malaria (aging 8.83 ± 4.20 years; 55% female) and 100 normal age-matching healthy controls (aging 10.08 ± 3.58 years; 50% female). Total and differential white blood cell counts were measured using by Sysmex XP300N Autoanalyzer, and NLR was calculated (absolute neutrophils count/absolute lymphocytes count). The data were analyzed using SPSS software (V 20.0) and Statdisk software (V 13.0).

Results: Leukocytosis (34%) and increased NLR (30%) were observed in Falciparum malaria cases. The mean of NLR in Falciparum malaria patients was significantly higher than controls (3.47 ± 3.51 and 2.87 ± 3.01, respectively; $p < 0.001$). However, NLR did not show significant difference comparing patients stratified for disease severity (severe vs. mild malaria; $p = 0.735$). Severe Falciparum malaria and absolute neutropenia were significantly correlated ($p = 0.006$ and $r = 0.194$; OR = 3.17).

Conclusion: The study concluded that leukocytosis, increased NLR, absolute neutropenia and absolute lymphocytopenia were most common WBCs changes observed in Falciparum malaria. Significant association was observed between development of severe falciparum malaria and absolute neutropenia. To improve management of severe malaria, absolute neutrophil count may be recommended, along with others parameters, as predictive indicator for the disease severity.

Key words: Neutrophils to lymphocytes ratio, Leukocytosis, Lymphocytopenia, Falciparum malaria, Neutrophilia, Sudanese children.

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Introduction

Falciparum malaria is one of the common worldwide infections that lead to high mortality and morbidity - especially in tropical regions and among children. World Health Organization (WHO) estimated that 99.7% of malaria cases are

due to *Plasmodium falciparum*. Because of it a number of 285,000 children die before their fifth birthdays in Africa[1]. Falciparum malaria cases among Sudanese patients is estimated to be 87.6%[2].

Falciparum malaria is associated with several hematological changes that affect the whole CBC profile; RBCs, WBCs and platelets[3]. Lymphocytosis, lymphopenia and fluctuation in neutrophil count were frequently reported[4], with a relationship with disease severity and complications[5]. Neutrophils to lymphocytes ratio (NLR) is a simple indicator of inflammatory conditions. It is considered as a strong prognostic and predictor factors for infectious diseases[6]. Previous study reported higher prevalence of lymphopenia in malaria cases as compared to healthy controls. However, association between NLR and malaria was controversial; insignificant association[7] and significant correlation with parasite load were reported[8].

The diagnosis/prognosis of malaria poses a challenge particularly in developing countries such as Sudan, where there is a need for simple, rapid, cost-effective, and sensitive tests. Variations in the different hematological parameters have been studied to help identify patients at risk for severe or complicated malaria. Data considering changes in NLR and its correlation with disease severity and intensity of parasitemia in Sudan are scarce. Therefore, the present study reported on assessment of changes in hematological profile, particularly NLR, and their prognostic role in Falciparum malaria in Sudanese children.

Patients and Methods

Participants and setting

This case-control and facility-based study was conducted at Wad Medani Pediatric Hospital, Gezira State, Sudan during period between March to December 2017. The study enrolled 300 Sudanese children (age range of 1.5 - 18 years; mean ± SD was 9.85 ± 4.05 years).

The samples were collected using simple randomized method from Falciparum malaria patients attending the Outpatient Clinics. The sample size was 300 samples based on the epidemiology of malaria in Sudan reported by National Ministry of Health[9]. The required sample size was determined according to following formula: $N = z^2pq/d^2$, where, Z = 1.96, P = 0.75, q = 0.25, d = 0.5. $N = 1.96 \times 1.96 \times 0.75 \times 0.25/0.5 \times 0.5$. $N = 288 \simeq 300$.

Peripheral blood samples were collected from 100 subjects diagnosed as severe Falciparum malaria by blood film and WHO criteria[10], 100 subjects diagnosed as uncomplicated Falciparum malaria by blood film or immune-chromato-graphic test (ICT), and 100 age-matching normal healthy controls. The uncomplicated malaria to stages (a cold stage, a hot stage, and a sweating stage) with presence of one of severe malaria features or without presence of severe malaria features. The presence of 2 or more of the clinical features (coma, respiratory distress/pulmonary edema, bleeding, shock, intravascular hemolysis, jaundice, or convulsions) or laboratory features (severe anemia, hypoglycemia, lactic/metabolic acidosis, renal failure, and hyperparasitemia) classifies the patient as suffering from severe malaria[11]. Severe Falciparum malaria also is associated with cerebral malaria, hemoglobinuria, hypotension, hematological changes (as thrombocytopenia), hepatic dysfunction and cardiovascular collapse shock[10].

We included sick children with Falciparum malaria aging 1 month to 18 years old, from both genders and residing in Gezira State who were admitted to Wad Medani Pediatric Teaching Hospital. We excluded sick children with mixed malaria or Vivax malaria, those aging ≥18 years old, those residing outside Gezira State, those suffering from recent infection, malignancy, and thrombosis, and those on anticoagulant and anti-inflammatory medication. The study design was approved by the Ethics Committees of the Ministry of Health, Gezira State, Sudan. Informed consent was obtained from guardian of each participant.

Sampling and investigations

A 2.5 mL venous blood specimen was collected by clean venipuncture in K_3EDTA containers. Thin and thick films were immediately made. Parasitemia staging and parasite intensity (%) were determined directly from thick and thin

blood films. Total and differential white blood cell counts were measured using a hematology Autoanalyzer (Sysmex XP300N®, Kobe, Japan). NLR was calculated by dividing absolute neutrophil count by the absolute lymphocytes count.

Leukocytopenia was defined as a leukocyte count of $<3.5 \times 10^9$/L and leukocytosis was defined as a leukocyte count $>8.5 \times 10^9$/L. Absolute neutropenia was defined as absolute neutrophil count of $<2.1 \times 10^9$/L and neutrophilia was defined as absolute neutrophil count of $>7.0 \times 10^9$/L. Absolute lymphocytopenia was defined as absolute lymphocyte count of $<1.1 \times 10^9$/L and lymphocytosis was defined as absolute lymphocyte count $>3.0 \times 10^9$/L[12]. The Lower limit of neutrophil: lymphocyte ratio was defined as NLR <0.78 and the upper limit was defined as NLR >3.53[6].

Statistical analysis

Comparison of WBCs parameters between study groups and between severe and uncomplicated Falciparum malaria were analyzed using t-test. Comparison of WBCs parameters among different grades of malaria parasitemia was analyzed using One Away ANOVA. Correlation among WBCs parameters, malaria severity and intensity of parasitemia were analyzed using Pearson's correlation test. Relationship between neutrophil count and the severity of Falciparum malaria infection was analyzed using Chi-square test. The data were analyzed using IBM® SPSS Software (V 20.0) and Statdisk Software (V 13.0).

Results

The 300 Sudanese children participated in this study were allocated into complicated Falciparum malaria (n = 100), uncomplicated Falciparum malaria (n = 100) and healthy controls (n = 100). Their demographic characteristics are shown in Table 1. The hyperparasitemia represented 72% in severe malaria, and, 18% in uncomplicated malaria. The average intensities of parasitemia for severe and uncomplicated malaria were 0.88 ± 0.42 and 0.39 ± 0.30, respectively (Table 1).

Table 1: Demographic characteristics of the investigated children with Falciparum malaria (total n = 200) sub-grouped according to the severity of the disease. Data shown are frequencies; n (%) and mean ± SD.

Factors		Severe (n = 100)	Uncomplicated (n = 100)	All patients
Age (years)		8.63 ± 3.40	8.83 ± 4.20	8.73 ± 3.81
Age group (years)	<5 years	19 (19 %)	24 (24 %)	43 (21.5 %)
	6 – 10 years	47 (47 %)	41 (41 %)	88 (44 %)
	11 – 15 years	33 (33 %)	29 (29 %)	62 (31 %)
	>15 years	1 (1 %)	6 (6 %)	7 (3.5 %)
Gender	Male	61 (61 %)	45 (45 %)	106 (53 %)
	Female	39 (39 %)	55 (55 %)	94 (47 %)
Parasitemia	+	4 (4%)	43 (43 %)	47 (23.5 %)
	++	3 (3 %)?	25 (25 %)	28 (14 %)
	+++	21 (21 %)	14 (14 %)	33 (16.5 %)
	++++	72 (72 %)	18 (18 %)	90 (45 %)
Intensity of parasitemia (%)		0.88 ± 0.42	0.39 ± 0.30	0.64 ± 0.44

As show in Table 2, the mean of total WBCs, absolute neutrophil count, absolute lymphocyte count and NLR in Falciparum malaria cases were 7.14 ± 3.24 × 10^9/L, 4.52 ± 2.85 × 10^9/L, 1.78 ± 1.03 × 10^9/L & 3.47 ± 3.51, respectively.

Table 2: The investigated WBCs parameters in Falciparum malaria infected children (total n = 200) stratified according to the disease severity. Data shown are mean ± SD.

Parameter	Severe (n = 100)	Uncomplicated (n = 100)	All patients
Total WBCs × 10^9/L	6.74 ± 3.49	7.54 ± 2.92	7.14 ± 3.24
Absolute neutrophil count × 10^9/L	4.23 ± 3.21	4.81 ± 2.42	4.52 ± 2.85
Absolute lymphocytes count × 10^9/L	1.71 ± 1.00	1.84 ± 1.07	1.78 ± 1.03
Neutrophil/Lymphocytes Ratio	3.39 ± 3.91	3.56 ± 3.09	3.47 ± 3.51

High NLR, leukocytosis, absolute neutrophilia & absolute lymphocytopenia were observed in 3%, 2%, 16.5% & 19%, respectively in malaria cases (Table 3).

Table 3: Variation in WBCs parameters investigated in Falciparum malaria infection children (total n = 200). Data shown are frequencies; n (%).

Parameters	Low	Normal	High
Total WBCs × 10^9/L	13 (6.5%)	129 (64.5%)	58 (29%)
Absolute neutrophil count× 10^9/L	29 (14.5%)	138 (69%)	33 (16.5%)
Absolute lymphocytes count× 10^9/L	38 (19%)	148 (7 %)	14 (7%)
Neutrophil/Lymphocytes Ratio	14 (7%)	118 (59%)	68 (34%)

NLR, absolute neutrophil count and absolute lymphocyte count in malaria cases were 3.47 ± 3.51, 4.52 ± 2.85 × 10^9/L, and 1.78 ± 1.03 × 10^9/L vs. 2.87 ± 3.01, 3.65 ± 1.38 × 10^9/L, & 2.29 ± 0.69 × 10^9/L in controls, respectively. This gave highly significant differences for the three parameters comparing patients & controls (P <0.001, 0.004 & 0.001, respectively). However, difference was insignificant in total WBCs between the two groups (P = 0.330) (Table 4).

Table 4: Comparison of WBCs parameters between children with Falciparum malaria (n = 200) and healthy controls (n = 100). Data shown are mean ± SD and p value.

Parameters	Patients	Controls	P
Total WBCs × 10^9/L	7.14 ± 3.24	6.79 ± 1.98	0.330
Absolute neutrophil count× 10^9/L	4.52 ± 2.85	3.65 ± 1.38	0.004
Absolute lymphocytes count× 10^9/L	1.78 ± 1.03	2.29 ± 0.69	0.000
Neutrophil/Lymphocytes Ratio	3.47 ± 3.51	2.87 ± 3.01	0.000

There were no significant differences in NLR, total WBCs, absolute neutrophil count and absolute lymphocyte count comparing severe malaria vs. uncomplicated malaria (P = 0.734, 0.080, 0.145 and 0.382, respectively) (Table 5).

Table 5: Comparison of the investigated WBCs parameters between children with uncomplicated vs. severe Falciparum malaria. Data shown are mean ± SD and p value.

Parameters	Severe (n = 100)	Uncomplicated (n = 100)	P
Total WBCs × 10^9/L	7.54 ± 2.92	6.74 ± 3.49	0.080
Absolute neutrophil count× 10^9/L	4.81 ± 2.42	4.23 ± 3.21	0.145
Absolute lymphocytes count× 10^9/L	1.84 ± 1.07	1.71 ± 1.00	0.382
Neutrophil/Lymphocytes Ratio	3.56 ± 3.09	3.39 ± 3.91	0.734

NLR in malaria cases stratified according to intensity of the parasitemia (namely, +, +++, +++ and ++++) were 2.53 ± 2.62, 3.25 ± 2.44, 4.82 ± 4.99 and 3.52 ± 3.38, respectively. This revealed significant difference among them (P = 0.032). There was significant difference in absolute lymphocytes count in relation to intensity of parasitemia (P = 0.050). No significant differences in total WBCs & absolute neutrophil count in relation to intensity of parasitemia (P = 0.729 & 0.414, respectively) (Table 6).

Table 6: Relationship between the investigated WBCs parameters and the intensity of parasitemia in children infected with Falciparum malaria (namely, +, +++, +++ and ++++). Data shown are mean ± SD and p value.

Parameters	Intensity of Parasitemia				P
	+ (n = 47)	++ (n = 28)	+++ (n = 35)	++++ (n = 90)	
Total WBCs × 10^9/L	7.09 ± 2.66	6.99 ± 3.39	7.70 ± 3.64	6.99 ± 3.33	0.729
Absolute neutrophil count× 10^9/L	4.11 ± 2.17	4.49 ± 2.64	5.18 ± 3.30	4.49 ± 3.03	0.414
Absolute lymphocytes count× 10^9/L	2.14 ± 1.02	1.59 ± 0.77	1.64 ± 1.24	1.70 ± 0.99	0.050
Neutrophil/Lymphocytes Ratio	2.53 ± 2.62	3.25 ± 2.44	4.82 ± 4.99	3.52 ± 3.38	0.032

The absolute lymphocyte count had significant negative correlation with malaria parasitemia (P <0.017/r = -0.130) despite having no significant correlation with parasite intensity (P = 0.270). Total WBCs, absolute neutrophil count and NLR had no significant correlation with malaria parasitemia (P = 0.454, 0.972 and 0.152, respectively) and no significant correlation with intensity of parasitemia (P = 0.167, 0.173 and 0.808, respectively) (Table 7).

Table 7: Correlation between the investigated WBCs parameters and intensity of the parasitemia of children with Falciparum malaria. Data shown are r/p values.

Parameters	Total WBCs	Neutrophil count	Lymphocytes count	NLR
Parasitemia	-0.040/0.454	-0.002/0.972	-0.130/0.017	0.077/0.152
Parasitic intensity	-0.067/0.167	-0.066/0.173	-0.055/0.270	-0.012/0.808

22% of the severe malaria cases had absolute neutropenia and 13% had absolute neutrophilia. 7% of the uncomplicated malaria cases had absolute neutropenia and 20% had absolute neutrophilia. This revealed statistically difference comparing absolute neutrophil count frequency and malaria severity (P <0.008) (Table 8). There were no significant differences between WBCs count, NLR and absolute lymphocyte count among different grade of malaria severity.

Table 8: Relationship between neutrophil count and the severity of Falciparum malaria in malaria infection in children (n = 200). Data shown are n and p value.

Parameters	Uncomplicated (n = 100)	Severe (n = 100)	All Patients	P
Absolute neutropenia	7	22	29	0.008
Normal neutrophil	73	65	138	
Absolute neutrophilia	13	20	33	

Discussion

Falciparum malaria constitutes a major public health problem in Sudan; accounting 87.6%. Furthermore, 75% of the population is at risk of developing the disease. The children are 3 times more likely to get malaria than adults. The treatment outcome of malaria depends on appropriate early diagnosis and appropriate management with the recommended therapy to reduce deaths attributed to severe malaria. The neutrophils and lymphocytes play vital role in induction of immune responses to Falciparum malaria and their ratio (NLR) have been proven as predictors of malaria infection and its clinical severity in studies elsewhere[8].

High NLR, leukocytosis, absolute neutrophilia and absolute lymphocytopenia were observed in 34%, 29%, 16.5% and 19% of our pediatric Falciparum malaria in malaria patients, respectively. With the except of absolute lymphocyte count, the WBCs parameters (NLR, total WBCs and absolute neutrophil count) were significantly increased in Falciparum malaria in malaria as compared to healthy controls. The increasing in total WBCs in malaria may be due to an increased release of leukocytes at the initial stage of infection to fight against malaria infection. This is related to the effective immune response to malaria in malaria endemic areas. Garba et al reported higher total WBCs in Falciparum malaria in malaria than controls[13]. In contrast, Kini and Chandra that showed that total WBCs was decreased in Falciparum malaria in malaria than controls[14]. Controversy could be due to timing of analyzing the CBC relevant to the infection cycle. The increase in absolute neutrophil count in Falciparum malaria in malaria could be due to early release of neutrophil from the bone marrow in response to the infection. Other studies reported similar increases in neutrophil count in Falciparum malaria in malaria than controls[14,15].

We observed that 19% of our patients had lymphopenia. Akhtar et al reported that 17% of their malaria patients had lymphopenia[4]. Furthermore, Chaudry et al reported that 16.7% of their malaria

patients had lymphopenia[16]. Prasad et al observed that 11.1% of their malaria patients had lymphopenia[5]. Chronic stimulation of lymphocytes by environmental microorganisms may increase apoptosis of lymphocytes that is also seen in healthy donors from endemic areas. Moreover, increased sequestration of activated lymphocytes during malaria and their depletion are known to be activated by plasmodium infection[8,17]. Increases in neutrophil count is usually considered as a sign of systemic inflammation[3,18].

NLR was high in our Falciparum malaria in malaria cases controls. This finding agree with studies reported from Ghana[3], Thailand[19], and Germany[18]. The accompanied increase in neutrophil count is usually considered as a sign of systemic inflammation and decrease in lymphocytes[3, 18]. The mild increase in NLR in uncomplicated malaria made the difference insignificant compared with cases with severe Falciparum malaria i. This finding is consistent with Louis Dias and Sumanth[20] and Berens-Riha et al[18]. The increases in WBCs parameters investigated in our study in uncomplicated falciparum malaria were insignificantly different from levels in severe cases. van Wolfswinkel et al and Frimpong et al observed higher WBCs parameters in severe Falciparum malaria[8, 21].

Interestingly, NLR had no significant correlation with intensity of malaria parasitemia, despite its significant increase with the disease. The absolute lymphocyte count had significant negative correlation with intensity of malaria parasitemia and having significant difference comparing controls and patients. There was no significant correlation between malaria parasitemia vs. each of total WBCs and absolute neutrophil. Antwi-Baffour et al reported significant negative correlations between malaria parasitemia and absolute lymphocyte count[3]. van Wolfswinkel et al reported significant correlations between parasitemia vs. total WBCs and NLR, with no significant correlations between parasitemia vs. absolute lymphocyte and neutrophil counts[8]. Kotepui et al, also reported a correlation between NRL with malaria parasitemia[19].

Leukocytopenia in the present study represented 13% of Falciparum cases (76.9% in severe malaria compared to 23.1% in uncomplicated malaria). Similar results were stated by a previous study with a rate of 11.5% of leukocytopenia[22]. Decreased NLR was observed in 71.4% in severe malaria compared to 28.6% in uncomplicated malaria. Among 68 patients with increased NLR, 52 patients (76.5%) had hyperparasitemia. From 58 patients with leukocytosis, 37 patients (63.8%) had hyperparasitemia. However, NLR, total WBCs, and absolute neutrophil and absolute lymphocyte counts had no significant correlation with intensity of malaria parasitemia. Disagreeably, van Wolfswinkel et al reported significant correlations between NLR and parasite count[8].

Neutropenia and neutrophilia were observed in 75.6% and 61.1% in severe malaria compared to 38.8% in uncomplicated malaria. Among 29 patients with absolute neutropenia, 22 patients (75.6%) had severe malaria and 19 patients (65.5%) had hyperparasitemia. Among 33 patients with absolute neutrophilia, 23 (69.7%) had hyperparasitemia. 20% of patients with uncomplicated falciparum malaria had absolute neutrophilia. Prasad et al reported predominance of neutrophilia in cases positive for simple plasmodium malaria[5]. When associated with severe malaria and hyperparasitemia, this finding indicates failure of production, alteration of distribution or abnormality of neutrophil lifespan - in a manner analogous to the mechanism of malaria-induced anemia and thrombocytopenia. This can explain the increasing in average NLR in uncomplicated as compared with severe Falciparum malaria. Lymphocytopenia and lymphocytosis were observed in 76.3% and 57.1% in hyperparasitemia. Lymphocytopenia with P. falciparum has been reported in several previous studies[4,16, 23-27].

The age had significant negative correlation with total WBCs, absolute neutrophil count and absolute lymphocyte count, while it insignificantly correlated with NLR. Similar results were observed in a previous study[28]. A gradual decline

in WBCs count in association with advancement of age as a normal feature in most populations[3].

Conclusion

High NLR, leukocytosis, absolute neutrophilia and absolute lymphocytopenia were major changes associated with Falciparum malaria among children in Sudan. Furthermore, absolute lymphocyte count had significant negative correlation with malaria parasitemia. Severe Falciparum malaria was associated with absolute neutropenia. The high NLR in Falciparum malaria may qualify to be the simple prognostic recommended hematological marker to assess the disease severity and to improve the management of complicated malaria among high risk patients.

Limitations of the Study

We wished to include the other prognostic indices, effect of disease duration and effect of type/duration of treatment, but they all require longitudinal studies that were out of our reach.

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Conflict of Interests

The authors declared no conflict of interests.

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Original Article

Thrombomodulin Gene Ala455Val Polymorphism and Risk of Coronary Heart Disease

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Abstract

Background: Coronary heart disease (CHD) remains a major cause of death worldwide. Thrombomodulin (TM) Ala455Val polymorphisms had been correlated with the risk of CHD. There is no published data considered the association between TM Ala455Val with CHD in Sudan.

Objective: This study aimed to detect the association of TM C1418T gene polymorphism among Sudanese patients with CHD.

Patients and Methods: This is a case-control study conducted from October to December 2018, Sudan Heart Center, Khartoum, Sudan. A total of 80 participants were enrolled in this study. The cases group comprised of 40 patients; 21 males and 19 females diagnosed with CHD; their mean age was 50 years. 40 normal healthy volunteer matching in age and gender were used as the control group. Whole genomic DNA from peripheral blood was extracted using the salting-out method. TM C1418T polymorphism was detected using allele-specific polymerase chain reaction. The sociodemographic and clinical data were collected using direct interviewer questioner. Data were analyzed using SPSS version 20.

Result: The frequency; n (%), of TM genotypes in our patients were CC = 8 (20.0%), C/T = 25 (62.5%) and TT = 7 (17.5%). In control the frequencies were CC = 17 (42.5%), C/T = 21 (52.5%) and TT = 2 (5.0%).

Conclusion: in the instigated sample of Sudanese CHD patients, TM C1418T polymorphism looked like a significant risk marker.

Key words: Thrombomodulin, Thrombomodulin C1418T SNP, Gene polymorphism, Coronary Heart Disease, Sudan.

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Introduction

The narrowing or blockage of the coronary arteries due to the gradual build-up of fatty material and plaque inside the wall of arteries, as result of atherosclerosis, is the cause of Coronary Artery/Heart Disease (CAD/CHD). The latter is the most common type of heart disease[(1)]. Several traditional coronary risk factors that are invloved in CAD include diabetes mellitus, obesity, hypertension, smoking, alcohol intake, and family history. The contribution of polymorphic variants of genes as risk factors for CAD is gaining popularity among researchers and clinicians[(2)]. Thrombomodulin (TM) is an important vaso-protective and thrombo-resistant molecule. It is expressed as a transmembrane glycoprotein on the surface of endothelial cells[(3)]. Mechanism of its thrombo-resistant role is through binding and modulating thrombin conformation. Such cofomational change turns thrombin from being a potent prothrombotic into an antithrombotic enzyme. Next step is the cleavage and activation of protein C by TM-bound thrombin. The active protein C inactivates, by proteolytic degradation,

the coagulation factors VIIIa and Va[4], thereby reducing coagulation reactions.

inflammatory mediators reduce the basal level of TM expression on the surface of unstimulated endothelial cells[5]. Therefore, reduction in TM levels is probably causative in the vascular damage and thrombosis seen in inflammation and septicemia[6]. In the coding and the promoter regions of the intron-less TM gene alrge number of SNP and mutations were reported[7-11]. Although the effect of the reported TM single nucleotide polymorphism (SNP) on the level of its protein expression and activity is not well-established, it was reported that a dimorphism that results in cytosine transition to thymidine resulting in a missense alanine (A) to valine (V) substitution at amino acid position 455 (A455V), may be associated with myocardial infarction[12]. However, the implication of TM gene SNP in CAD is currently controvisial, where a number of reports stated a positive association[13], others negated negated such relationship[9,14].

Nationally in Sudan, there are no previous studies that tackled this point. We planned this study to investigate the possible association between Ala455Val TM polymorphism and CHD among healthy and Sudanese CHD patients.

Patients and Methods

Participants and Setting

This is a case-control study that was conducted during December 2018 at Sudan Heart Center, Khartoum, Sudan. A total of 80 participants were enrolled in this study. Forty cases with hospital-diagnosed CHD were comprised of 21 males and 19 females with a mean age of 50 years. Forty normal healthy volunteers matching in age and gender were used as the control group. Under full aseptic technique, 5 mL of peripheral blood was collected on ethylenediamine tetra-acetic acid as an anticoagulant. DNA was extracted using the salting out method. Patients with an unclear diagnosis were excluded from this study. The sociodemographic and clinical data were collected using direct interviewer questioner. The present study was approved by Alneelain University Ethical Board. The informed consent was taken from all subjects before samples were collected.

Genotyping of TM C1418T polymorphism:

Allele-specific polymerase chain reaction (PCR) was for detection of TM SNP. A stretch of 384 bp of TM gene encompassing 1418 site was amplified using the following established allele-specific primers: 1) Forward primer for TM 1418 allele C-Ala is "5'-GGGCCCGACTCGGCCCTTGC-3', 2) Forward primer for TM 1418 allele T-Val is "5'-GGGCCCGACTCGGCCCTTGT-3', and, 3) The reverse primer is the same for both TM 1418 alleles is 5'-GGGCCCGACTCGGCCCTTGT-3'"[15]. PCR reaction was carried out using Techne (TC-412) thermal cycler in a total volume of 20 µL. The reaction mix contained 0.5 µL from each primer, 5 µL of genomic DNA, 3 µL of "5X FIREPoL" ready-to-load master mix that contained 7.5 mM $MgCl_2$ (Solis Biodyne, Tartu, Estonia) and 11 µL distilled water. The intial step of PCR was the denaturation at 95 °C for 2 minutes. This was followed by 40 cycles each was composed of: 1) denaturation at 95 °C for 30 seconds, 2) annealing temperatures ranged between 67.5 - 68.5 °C for 30 seconds, and 3) extension at 72 °C for 40 sec that was followed by a final extension at 72 °C for 10 minutes. After amplification, 100-volt/60-A electrophoresis of 5 µL of the PCR reaction mixed with the loading solution was applied to resolved the products on 2% agarose gel containing ethidium bromide (0.05 µg/mL) in 1X TBE running buffer. The separated bands were visualized by a gel documentation system against 1 µL DNA ladder of 100 bp as molecular weight marker.

Statistical analysis

Data was analyzed using statistical package for social sciences (SPSS) version 20. The correlation between variables was tested using Chi-square. Regression test analysis used to find the association between CHD and polymorphism. The Hardy-Weinberg equation was used to detect the allele frequency.

Results

Demographics and risk factors

Based on gender, our patients were 21 (52.5%) males and 19 (47%) females. Most of them had age >50 years; n = 25 (26%). The current study found that the patient's age was statistically significantly associated with CHD (p = 0.001). In regards to the risk factors, the present study reported that 27 patients (67.5%) had a family history, 25 patients (62.5%) were hypertensive, 15 patients (37.5%) had diabetes, 20 patients (50%) were obese, & 25 patients (62.5%) were smokers.

Molecular Findings

The frequency of TM genotypes in our patient were CC = 8 (20.0%), CT = 25 (62.5%), and TT = 7 (17.5%), while in controls were CC = 17 (42.5%), CT = 21 (52.5%) and TT = 2 (5.0%). There was a statistically significant difference between the two groups (p = 0.04). The allele frequency of T genotype in CHD patient using Hardy-Weinberg equilibrium was higher than controls (p = 0.015 and OR = 2.15), as showed in Table 1.

A statistically significant association between the frequency distribution of CT/TT and TT/CC and CHD was evedent (p = 0.026 and p = 0.023 with an OR of 7.438 and 2.957, respectively), as shown in Table 2.

Table 1: Thrombomodulin (TM) C1418T genotype polymorphism in Sudanese coronary heart disease (CHD) patients and healthy controls. Data shown are frequencies; n (%) and p values. CT = Heterozygous genotype, CC = Homozygous common genotype, TT = Homozygous rare genotype. $X^2 = 5.452$. OR = 2.15 (CI lower = 1.12, CI Upper = 4.91).

TM Genotype/Allele		CHD Cases	Controls	Total	p
Genotype	CC	8 (20.0)	17 (42.5)	25	
	CT	25 (62.5)	21 (52.5)	46	0.040
	TT	7 (17.5)	2 (5.0)	9	
Allele	C	41 (50.6)	55 (68.8)	95	0.015
	T	39 (49.4)	25 (31.3)	64	

Table 2: Comparison of thrombomodulin C1418T genotypes in Sudanese coronary heart disease (CHD) patients and healthy control subjects. Data shown are frequencies (comma was removed after frequencies) (,added instead of semicolon after frequencies) n (%), and OR (95% CI), and p values.

Genotype	CHD Cases	Controls	OR (95% CI; Lower - Upper)	p
CT	25 (55.3)	21 (75.8)	2.53 (0.911, 7.024)	0.059
CC	8 (44.7)	17 (24.2)		
Total	33	38		
TT	7 (46)	2 (10.5)	7.438 (1.252, 44.193)	0.023
CC	8 (53.3)	17 (89.5)		
Total	15	19		
CT/TT	32 (80)	23 (57.5)	2.957 (1.091, 8.009)	0.026
CC	8 (20)	17 (42.5)		
Total	40	40		

Discussion

The endothelial cell surface TM glycoprotein is vaso-protection through binding and converting the procoagulant thrombin into an anticoagulant[3,15]. The current study revealed that the C1418T genotypes of TM among Sudanese patients with CHD vary from that in normal healthy control participants (p = 0.041). Assuming that both cases and controls were in Hardy-Weinberg equilibrium, the frequency of CT and TT genotypes was higher in our patients in comparison with normal controls. This finding was in agreement with the results of a study done in Pakistan, by Mughal et al. They investigated 182 CAD patients

and reported a significant correlation between TM C1418T polymorphism and CAD. They also proposed that TM polymorphism a risk marker for CAD in the population of Karachi[16].

Our observations are in the same line with a study of Chao et al, who studied 345 patients in Nigeria. The TM C1418T gene polymorphism was significantly associated with increased risk to CAD[14]. The finding of this study supported Dogra et al (2013) who showed that TM C1418T (Ala455Val) genotype was a risk marker for CAD patients(17). Our results also are inconsistent with that of Norlund et al, who investigated 97 patients and 159 healthy controls. They revealed that TM gene C/T dimorphism might be etiologically involved in the pathophysiology of myocardial infarction (MI) and that Ala455Val missense replacement may affect the function of TM, and consequently the activation of protein C anticoagulant pathway[18]. A meta-analysis study, published in 2014, reviewed a total of 14 case-control studies that included 5493 cases and 8297 controls. Similar to our results, it showed a significant association between TM Ala455Val polymorphism and risk of CAD (OR = 1.14; 95% CI, 1.05 – 1.24; I2 = 0%)[19].

On the contrary, a cross-sectional study that was conducted in the Netherlands on 51 subjects to investigate the association between TM-455 genotype and venous thromboembolic diseases did not find any significant difference between cases and controls[8]. A Swedish study enrolled 91 patients with CAD and 159 normal healthy volunteers to assess the relationship between TM-455 genotype frequency. They noticed a significantly higher frequency for A allele in CAD patients[12]. Moreover, our observations did not match with several studies reporting no association between TM C1418T polymorphism and CAD[8-10,14,20]. These differences may be attributed to differences in the ethnic background of different human populations. TM is the endothelial cell cofactor for protein C activation the failure of which may cause thrombotic disorders. This is why defect in TM gene were accountable for heriditory thrombophilia[21].

Conclusion

According to our results, the C1418T polymorphism of TM gene appeared as a significant risk marker in Sudanese CHD patients. We recommend that this polymorphism should be added to the list of risk factors in CHD.

Limitations of the Study

The major limitation of the present study is the small sample size, considering the short study period.

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This study was self-funded.

Conflict of Interests

The authors declared no conflict of interests.

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Original Article

Metabolic and Histopathological Changes Correlating with High-Fat Diet-Induced Non-Alcoholic Fatty Pancreatic Disease in Mice: The Role of Cotreatment with Olive Oil

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Abstract

Background: Consumption of high energy diets is not only associated with obesity characterized by insulin resistance and dyslipidemia, but is also associated with accumulation of fat in various organs of the body including pancreas.

Objectives: To evaluate serum lipogram and glycemic control indices, and, tissue content of pro-inflammatory IL-1β and TNF-α and the histopathological changes in pancreas of Swiss albino mice fed with high-fat diet (HFD) to induce nonalcoholic fatty pancreatic disease (NAFPD), and, the ability of co-treatment with olive oil to mitigate and/or reverse these changes.

Animals and Methods: Adult male mice were divided into four groups, 10 mice each; reference control group, olive oil control group, HFD-fed group, and, HFD-fed olive oil co-treated group. All mice were fed with the respective diets for a period of 16 weeks. After overnight fast, mice were sacrificed under light diethyl ether anesthesia to enable collection of blood and the pancreas was removed, weighed and used for histopathology and measurement of IL-1β and TNF-α. Serum was analyzed for total cholesterol, HDL-C, LDL-C, triglycerides, glucose and insulin levels. Insulin resistance (IR) was calculated. One-way analysis of variance (ANOVA) using SPSS program was used to statistically compare between groups for significance of differences.

Results: HFD feeding induced NAFPD, caused marked elevation in fasting serum total and LDL-cholesterol, glucose, insulin levels and IR, and, reduced HDL-C. Pancreatic tissue contents of IL-1β and TNF-α were elevated in HFD-fed mice. Pancreatic tissue sections from HFD-fed mice showed ectopic fat deposition in the intra-lobular as well as interlobular space along with lipid vesicles in the acinar cells. Olive oil cotreatment caused marked improvement in serum lipid and glycemic control indices, and, pancreatic tissue cytokines content and fat deposits.

Conclusion: HFD leads to NAFPD, deranged serum glucose and insulin and lipid profile, increased pancreatic tissue content of pro-inflammatory cytokines and lipid deposition. Cotreatment with olive oil ameliorated these changes. Incorporating olive oil as part of a healthy diet could protect against NAFPD and insulin resistance.

Keywords: Nonalcoholic fatty pancreatic disease, Lipid profile, Pro-inflammatory cytokines, Olive oil, Insulin resistance, Lipid deposit.

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Introduction

Increased intake of high calorie foods rich in carbohydrates and fats and decreased physical activity have been attributed as the fundamental causes of obesity. Obesity is a major risk factor for developing diabetes, cardiovascular diseases, musculoskeletal diseases and some cancers[1]. Moreover, obesity culminates in fatty infiltration of various organs and tissues of human body, including nonalcoholic fatty liver disease that progresses into steatohepatitis,

fibrosis and cancer[2,3]. Moreover, continuous consumption of high energy diets increase the levels of oxidative stress and causes microcirculatory changes which predisposes to chronic pancreatic injury and nonalcoholic fatty pancreatic disease (NAFPD)[4]. NAFPD is characterized by the fatty infiltration of the pancreas with bigger size, increased free fatty acids and triglyceride contents. The increased production of pro-inflammatory cytokines in NAFPD together with increased amounts of toxic fats may act as key factors in the pathogenesis for steato-pancreatitis and pancreatic cancer[5].

A plethora of experimental studies have demonstrated the detrimental effects of high-fat diets (HFD) on the pancreatic tissue which include the alterations in the exocrine and endocrine structure and functions of the pancreas[6-8]. Experimentally, pancreatic steatosis leads to various structural and functional abnormalities of the islet cells resulting in hyperglycemia[9,10]. Exocrine changes include the fat accumulation in pancreatic acinar cells followed by fibrosis of the pancreas[11]. Chronic HFD induces peroxidation injury of the pancreatic cells, and increases the synthesis of collagen by activating the stellate cells of pancreas[12]. Some studies have found the high prevalence of NAFPD among pancreatic cancer patients further emphasizing the deleterious effects of high fat diets on the pancreas[13,14].

Olive oil, is well-recognized component of the healthy Mediterranean diet, with antioxidant[15], anti-inflammatory[16], hypolipidemic, hepato-protective[17] and other favorable metabolic properties[18]. Along with a large amount of the monounsaturated oleic acid[19], olive oil is also rich in phenolic antioxidant compounds such as tyrosol, hydroxytyrosol, and oleuropein[20]. The role of olive oil in the prevention and treatment of the chronic diseases relates to its distinct fatty acid composition and high content of antioxidant phenolic compounds[21].

The current study was undertaken to evaluate the histopathological changes in pancreatic tissue and its content of the pro-inflammatory IL-1β and TNF-α, and, changes in blood lipid profile, glucose, insulin and insulin resistance in mice fed with HFD. Furthermore, the ability of co-treatment with olive oil to mitigate and/or reverse these changes was also evaluated.

Animals and Methods

Setting and Design

This study was performed in the Department of Pathology, College of Medicine, Jouf University, Sakaka, Saudi Arabia from 1st of August, 2016 to 31st of January, 2017. Adult male Swiss albino mice (20-25 g in weight) were used in this study. They were obtained from the animal house, College of Pharmacy, Jouf University. All the mice selected were given a three-day period for getting acclimatized ad libitum access to food (Standard laboratory rodent pellet diet; Grain Silos & Flour Mills Organization, Riyadh, Saudi Arabia)) and tap water in a natural light-dark cycle. The mice were divided into four groups of 10 mice each: 1) reference control group was given basal diet, 2) olive oil control group was fed with basal diet plus 1.3 mL/kg b.w olive oil (given orally/daily), 3) HFD-fed group was given HFD, and 4) Cotreatment group received HFD plus olive oil (1.3 mL/kg b.w orally/daily). HFD was prepared every two days by mixing 8% coconut oil and 1.5% cholesterol to the standard rodent pellet[22]. The extra virgin olive oil used in the present experiment was the product of the local Aljouf Agricultural Development Company, Aljouf (Aljouf olive oil). All animals were fed with the respective diets for a period of 16 weeks and after an overnight fast of 12 hours, the mice were sacrificed in the morning. All the protocols of this research were approved by the Local Committee of Bioethics, Jouf University and watched the EU Directive 2010/63/EU on the Protection of Animals used for Scientific Purposes.

Sampling and Investigations

Animals were scarified under light diethyl ether anesthesia and whole blood aspirated from the heart was centrifuged at 15000 rpm for 5 min to isolate sera that were stored at -80 °C till used. Glucose,

insulin levels and the lipogram (total cholesterol, HDL-C, LDL-C and triglycerides) were measured using commercial kits (Cat# K603-100, K613-100, K613-100, K622-100, K686-100, and K4271-100; Biovision, California, USA) on Autoanalyzer Beckman Coulter Synchron LX20 (Beckman Coulter Inc., USA). The homeostasis model assessment for insulin resistance (HOMA-IR) index, as modified for HFD-fed mice, was used to calculate insulin resistance (IR); HOMA-IR index = [fasting glucose; mmol/L X fasting insulin; mU/mL] / 14.1)[23].

Pancreatic tissue was removed from all mice and weighed. A slice of pancreatic tissue from each mouse was immediately fixed in 10% formalin. The tissue samples were processed for histopathology following H&E standard techniques[24]. Micrograph images were recorded by the Digital micro-imaging device Leica DMD108 (Morrisville, USA). Rest of the pancreatic tissue from each mouse was frozen at -80 °C for the measurement of IL-1β and TNF-α by quantitative sandwich ELISA kits (Cat# K4796 and A1093-200 Biovision, California, USA). At use, 100 mg of pancreatic tissue was homogenized in 50 mM potassium phosphate buffer (pH 7.4) and the homogenate was centrifuged at 3500g for 30 minutes to get rid of debris. Total protein content of the supernatant was measured by Bio-Rad Protein Assays to normalize the cytokine contents to tissue protein content (pg or ng/mg protein).

Data analysis

Statistical Package for Social Sciences (SPSS) version 18.0 was used for data entry and analysis. We presented our data means ± standard deviation (SD). The multiple comparisons by using one-way analysis of variance (ANOVA) was utilized to test for significance of differences in the parameters studied in the experiment groups. A p value of <0.05 was considered as statistically significant.

Results

Table 1 shows the changes in pancreatic tissue levels of IL-1β and TNF-α, and fasting serum insulin, glucose and lipogram of the four groups of mice, along with their calculated IR, wet weight of the pancreas and animal body weights. The HFD-fed mice showed significant elevation in tissue IL-1β and TNF-α, and fasting serum insulin, glucose, IR, wet weight of the pancreas and lipid profile (triglyceride, total and LDL cholesterol) but a decrease in HDL-C levels compared to reference and olive oil controls. Cotreatment with olive oil almost completely normalized most of these parameters to control levels.

Pancreatic tissue IL-1β content was significantly higher in the HFD-fed mice compared to each of the control groups ($p <0.001$) and the olive oil-cotreated mice ($p <0.009$). While the two controls were insignificantly different, they both had lower IL-1β than the co-treated mice ($p <0.001$). Pancreatic tissue TNF-α content was significantly higher in the HFD-fed mice compared to each of the control groups ($p <0.001$) and the cotreated mice ($p <0.005$). The two controls were insignificantly different, and, were insignificantly different from the cotreated mice, too.

Serum insulin level was significantly higher in HFD-fed mice ($p <0.001$) compared to each of the other three groups that were insignificantly different. Serum glucose showed significantly higher levels in HFD-fed mice compared to each of the other three groups ($p <0.001$). Although cotreatment significantly lowered glucose level, it was still significantly higher than the controls ($p <0.001$). This was reflected as a very high IR in the HFD-fed mice vs. no resistance in controls ($p <0.001$) and vs. very mild resistance in the cotreated mice ($p <0.001$). The latter was insignificantly different from controls.

The 4 groups showed nonsignificant difference comparing their serum triglyceride levels. Serum total cholesterol level was higher in HFD-fed mice than each of reference control, olive oil control and cotreatment mice ($p <0.04$, $p = 0.02$ and $p = 0.008$, respectively). The latter 3 groups were insignificantly different. Serum HDL-C showed lower levels in HFD-fed mice compared to each of the reference control, olive oil control and cotreated mice ($p = 0.06$, $p = 0.007$ and p

<0.001, respectively). The latter 3 groups were insignificantly different. Serum LDL-C level was significantly higher in HFD-fed mice than each of reference control, olive oil control and cotreated mice (p <0.001, p = 0.03 and p = 0.007, respectively). The latter 3 groups were insignificantly different.

The wet weight of the pancreas/per mice was significantly higher in HFD-fed mice compared to each of reference control, olive oil control and cotreated mice (p =0.015, p = 0.001 and p <0.001, respectively). The latter 3 groups were insignificantly different. Body weight was higher in the HFD-fed mice compared to each of reference and olive oil controls (p = 0.0018 and p = 0.008, respectively), but insignificantly different from cotreated mice although the latter was insignificantly different from controls, too.

Table 1: Changes in body and pancreas weight and pancreatic tissue and serum parameter measurements in mice with non-alcoholic fatty pancreatic disease induced by feeding high-fat diet (HFD) vs. controls (reference and olive oil) and those cotreated with olive oil (OO-HFD-cotreatment) - after 16 weeks of experiment. Data presented as mean ± SD. For p value of comparison, see the text. n = 10 mice per group.

Biochemical parameters	Reference control	Olive oil control	HFD	OO-HFD-cotreated
IL-1β, pg/mg protein	11.3 ± 1.89	11.9 ± 2.21	25.3 ± 3.54	21.3 ± 2.57
TNF-α, pg/mg protein	2.61 ± 0.35	2.59 ± 0.31	3.48 ± 0.47	2.85 ± 0.40
Serum insulin, µIU/mL	1.96 ± 0.28	2.21 ± 0.33	11.79 ± 2.34	3.21 ± 0.74
Serum glucose, mg/dL	85.3 ± 11.21	92.4 ± 11.87	181.9 ± 19.36	121.7 ± 13.35
Insulin Resistance Index	0.71 ± 0.12	0.87 ± 0.19	9.12 ± 1.74	1.66 ± 0.36
Serum triglycerides, mg/dL	121.2 ± 21.43	122.87 ± 22.05	126.8 ± 22.31	123.17 ± 19.8
Serum t. cholesterol, mg/dL	172 ± 24.19	169 ± 22.96	203 ± 29.71	165 ± 21.56
Serum HDL-C, mg/dL	33.17 ± 8.32	36.67 ± 9.13	22.58 ± 6.12	41.9 ± 11.91
Serum LDL-C, mg/dL	73.34 ±10.84	80.58 ± 11.29	95.41 ± 12.71	77.52 ± 11.32
Pancreas weight, mg	137.53 ± 17.3	131.24 ± 13.01	160.52± 19.29	120.81±14.21
Body weight, gm/animal	31.94 ± 4.03	33.16 ± 4.37	40.85 ± 6.32	36.51 ± 5.09

Histological examination of pancreatic sections from different groups stained with H& E is presented in Figures 1-4. It revealed that HFD-fed mice showed ectopic fat deposition in the intra-lobular as well as interlobular space, and, lipid vesicles in the acinar cells. Mice co-treated with olive oil showing reduction in the fat deposits in all three compartments. Healthy reference and olive oil controls showed intact pancreatic architecture and cells

Discussion

Obesity is one of the important nutritional disorders in most of the developed countries and is becoming increasingly significant in the developing countries also[25]. Obesity due to consumption of energy-rich diets with high content of carbohydrate and/or fat elicits oxidative stress by stimulating the production of free radicals via multiple biochemical mechanisms[26,27]. Systemic oxidative stress and inflammation appear to play a key role in the pathogenesis of obesity-related comorbidities. The latter include insulin resistance, fatty liver, atherosclerosis, type 2 diabetes, and cancer[28]. Experimental studies have shown that HFD increases the levels of triglycerides and cholesterol in the blood[4,8,29]. In the present study, the body weight of HFD-fed mice was significantly elevated along with increases in serum triglyceride and cholesterol levels. In comparison, olive oil cotreated mice showed considerable reduction in serum triglycerides and cholesterol levels; signifying the protective effects of olive oil against dyslipidemia. Similar findings have been observed by Rincon-Cervera et al regarding effects of extra virgin olive oil on HFD-fed mice[29].

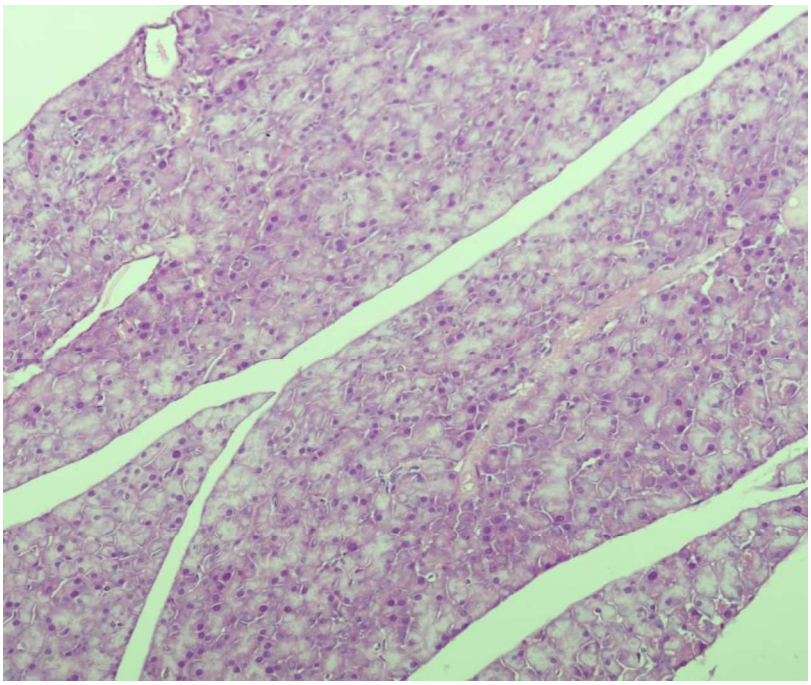

Figure 1: Representative histopathological sections of reference control mice pancreas stained by H&E showing normal pancreatic architecture.

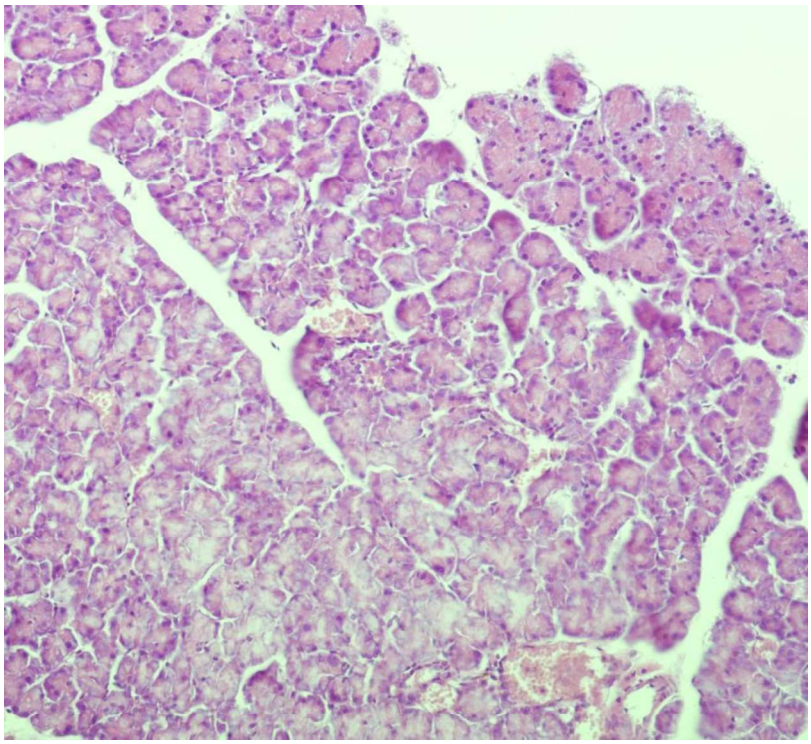

Figure 2: Representative histopathological sections of mice pancreas stained by H&E of olive oil control showing normal pancreatic architecture.

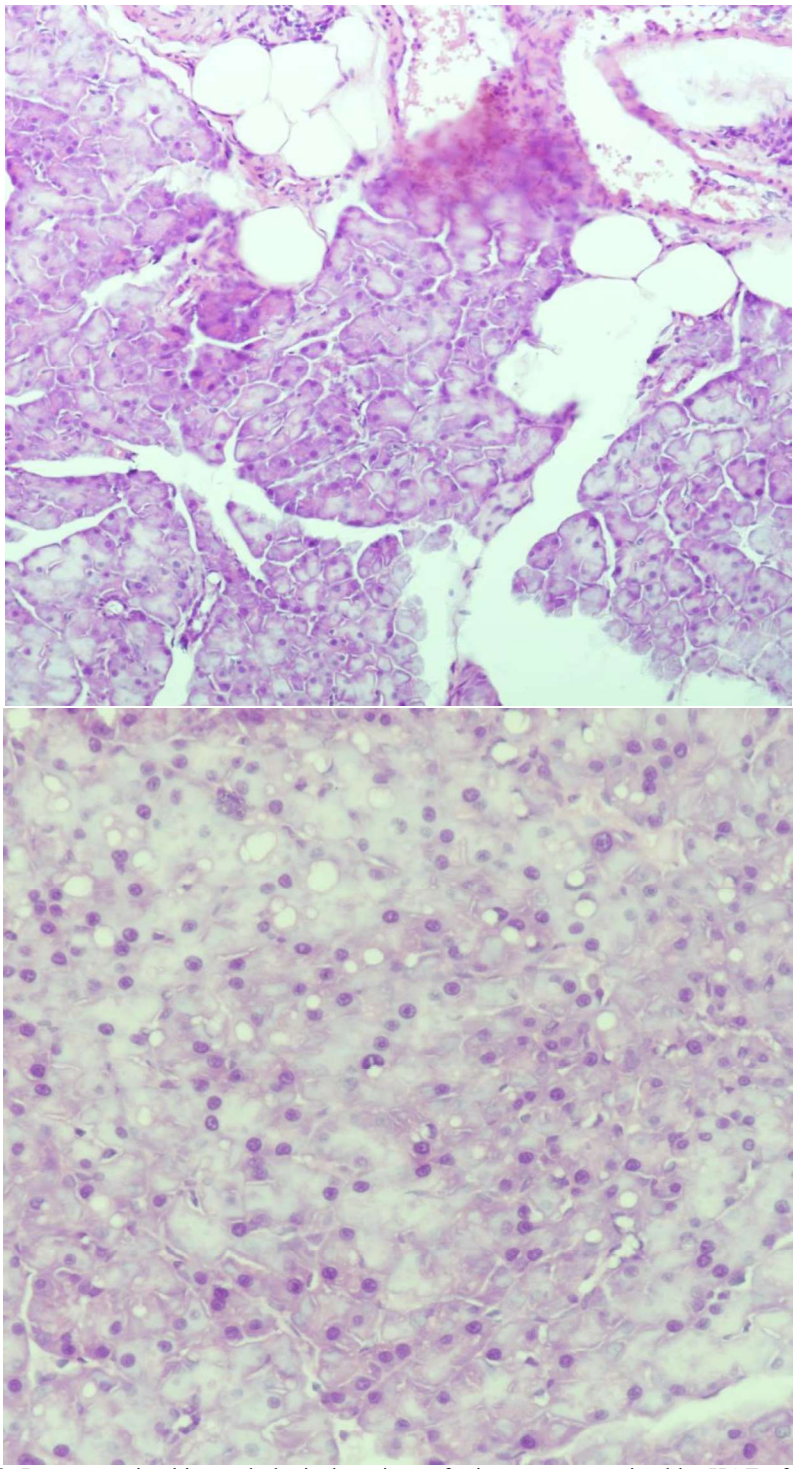

Figure 3: Representative histopathological sections of mice pancreas stained by H&E of high fat diet-fed mice showing ectopic fat deposition in the intra-lobular as well as interlobular space (Upper image), and, lipid vesicles in the acinar cells (lower image).

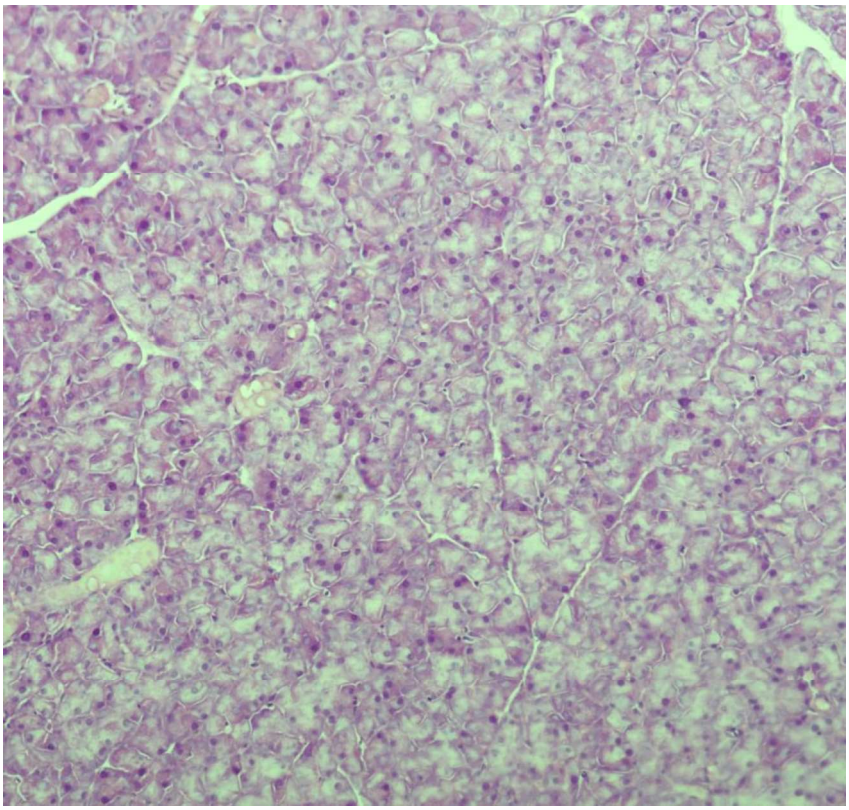

Figure 4: Representative histopathological sections of mice pancreas stained by H&E of high fat diet-fed mice co-treated with olive oil showing reduction in the fat deposits.

In the present study HFD-fed mice exhibited significantly increased levels of fasting serum insulin and glucose levels and IR index, when compared to the other groups; demonstrating the metabolic derangement induced by HFD. In contrast, the cotreated group showed significant improvement in serum insulin and glucose levels and IR index which implies the supportive ability of olive oil to intermediary metabolism and insulin sensitivity. Similar results were observed by Rincon-Cervera et al[29]. HFD induces alteration in the adipokines leading to increased secretion of leptin and decrease in adiponectin levels correlating with infiltration of the monocytes and macrophages into the fat tissue[30]. Increased amounts of altered fat stimulate the production of cytokines such as IL-1β and TNF-α[5]. In the present study, the pancreatic tissue levels of TNF-α and IL-1β were significantly elevated in HFD-fed group when compared to other groups. Similarly, Mathur et al found a significant elevation of IL-1β and TNF-α in obese mice when compared to lean mice[5]. Olive oil significantly decreased the levels of pancreatic IL-1β and TNF-α when compared to HFD-fed group that indicates the anti-inflammatory properties of the olive oil.

The wet weight of the pancreas was significantly elevated in HFD mice as compared to the controls and cotreated mice. Similarly, Mathur et al observed increased pancreatic weight in obese mice[5]. The wet weight of the pancreas in olive oil co-treated mice was significantly lower when compared to the obese mice. The body weight was significantly higher in HFD-fed mice compared to the controls but insignificantly different from cotreated mice. Increase in the body weights of mice fed with HFD has been observed in various other studies also[4,5,11].

Ogilvie postulated the entity of pancreatic fat infiltration[31]. The obesity-induced fat infiltration of the pancreas is called as nonalcoholic fatty pancreatic disease. The progression of the latter may result in an inflammatory state called Non-alcoholic steato-pancreatitis[5]. Histologic observation showed pancreatic ectopic fat accumulation in the inter-lobular space and adipose infiltration in pancreas in the mice fed with HFD. Similar observations were reported by Fraulob et al in their study on mouse model of metabolic syndrome[32]. Chowdary et al found that the high-fat intake induces hyperlipidemia and leads to pancreatic endocrine and exocrine alterations[7]. Also, Zhang et al found that hyperlipidemia induces increased pancreatic free fatty acid, lipid peroxidation and collagen synthesis by activated pancreatic stellate cells[33].

Conclusion

The present results showed that HFD causes derangement of lipid profile, pro-inflammatory cytokines, insulin resistance, and accumulation of fat in the pancreas leading to nonalcoholic fatty pancreatic disease. Olive oil co-treatment ameliorated all of these HFD harmful effects by improving insulin sensitivity, lipid profile, fat infiltration in the pancreas and levels of pro-inflammatory cytokines. Therefore, olive oil, as part of a normal healthy diet, can combat the obesity-related nonalcoholic fatty pancreatic disease and insulin resistance.

Limitations of the Study

Further ultrastructural and molecular studies could be extremely helpful in further elucidating the changes in the pancreas at the ultrastructural and molecular levels. Such studies could not be undertaken due to funding restraints as this study was self-funded.

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Conflict of Interests

The author declared no conflict of interests.

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Introduction

Provide adequate background that highlights the importance and gap information of your research point in relation to previous studies but avoiding a detailed literature survey. State the hypothesis or rationale and objectives of the work and a brief description of how you planned to approach them.

Materials or Patients and Methods

Provide sufficient detail to allow the work to be reproduced, with details of supplier and catalogue number when appropriate. Methods already published should be indicated by a reference: only relevant modifications should be described.

Patients and Normal Subjects

If human participants were used in the experiment please make a statement to the effect that this study has been approved by your Institution Ethics Review Board for human studies (the number of the approval should be stated in the methods section and JUMJ may ask for submission of the original ethical approval with the manuscript), and, that patients or their custodians have signed an informed consent that also states right of withdrawal without any consequences. Sample sized should be appropriately calculated. The manuscript should describe how the size of the experiment was planned. If a sample size calculation was performed this should be reported in detail, including the expected

difference between groups, the expected variance, the planned analysis method, the desired statistical power and the sample size thus calculated. For parametric data, variance should be reported as 95% confidence limits or standard deviations rather than as the standard error of the mean. Normal participants and patients criteria, inclusion and exclusion criteria should be stated. Name and address where the work was done and when it was done (time period, from …. to …..) should be clearly stated, too.

Experimental animals

When animals were used in the experiments, a local Institutional Ethics Review Board for animal studies should review and approve the experiment and that all animal procedures were in accordance with the standards set forth in guidelines for the care and use of experimental animals by Committee for Purpose of Supervision of Experiments on Animals (CPCSEA) and according to National Institute of Health (NIH) protocol. The precise species, strain, sub-strain and source of animals used should be stated. Where applicable (for instance in studies with genetically modified animals) the generation should also be given, as well as the details of the wild-type control group (for instance littermate, back cross etc.). The manuscript should describe the method by which animals were allocated (randomized) to experimental groups, particularly for comparisons between groups of genetically modified animals (transgenic, knockout etc.), the method of allocation to for instance sham operation or focal ischemia should be described.

Experimental

Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described. Where and when the study was conducted should be stated.

Results

Results should be clear and concise. Data should be presented in an appropriately organized tables, figures and/or artworks. The statistical analysis used should be suitable for the objectives of the study and type of data analyzed. Prospective authors are highly advised to consult a biostatician.

Footnotes

Footnotes should be used sparingly. For table footnotes, indicate each footnote in a table with a superscript lowercase letter or add them into the title.

Graphical abstract

A Graphical abstract is optional and should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership online. Authors must provide images that clearly represent the work described in the article. Please provide an image with a minimum of 531 × 1328 pixels (h × w) or proportionally more. The image should be readable at a size of 5 × 13 cm using a regular screen resolution of 96 dpi. It is preferable to be inserted at its normal place to the relevant text or otherwise be submitted as a separate TIFF, EPS, PDF or MS Office files.

Discussion

This should explore the significance, interpretation and reasoning of the results of the work vs. other studies. Do not repeat describing the results in this section. A combined Results and Discussion section is acceptable. Avoid extensive citations and discussion of published literature. In the same time, avoid speculations without a supporting literature. Avoid discussion based on "Data not Shown" or "Personal Communications".

Limitations and Future Prospective

The authors may wish to pinpoint the limitations of the study and their reason and foresee the next step to go from their study. This may be presented in a short Limitations and Future Prospective section standing alone or as a separate paragraph in the Discussion or Results/Discussion section.

Conclusions

The main conclusions of the study may be presented in a short Conclusions section standing alone or as a separate paragraph at the end of the Discussion or Results/Discussion section. Conclusions should not be biased and should be based on the data, presented and discussed inside the manuscript only.

Gain of Knowledge

Following the conclusion section, it is mandatory for manuscripts submitted for final publication in JUMJ to have a Gain of Knowledge section that is consisted of 2 - 5 bullet points (maximum 90 characters, including spaces, per bullet point) that convey the core findings of the article.

Acknowledgements and Funding

Collate acknowledgements in a separate section at the end of the article before the references. List individuals or organizations that provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.). Whoever would

be acknowledged should be informed and a verification for that could be requested by JUMJ Editor. If funded, the source of funding should be mentioned.

Appendices

If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly, for tables and figures: Table A.1; Fig. A.1, etc.

CASE REPORT WRITING TEMPLATE

Title. Include the words "case report" in the title. Describe the phenomenon of greatest interest (e.g., symptom, diagnosis, diagnostic test, intervention, and outcome).

Abstract. Summarize the following information if relevant: 1) Rationale for this case report, 2) Presenting concerns (e.g., chief complaints or symptoms, diagnoses), 3) Interventions (e.g., diagnostic, preventive, prognostic, therapeutic exchange), 4) Outcomes, and 5) Main lesson(s) from this case report.

Key Words. Provide 3 - 8 key words that will help potential readers search for and find this case report.

Introduction. Briefly summarize the background and context of this case report.

Presenting Concerns. Describe the patient characteristics (e.g., relevant demographics - age, gender, ethnicity, occupation) and their presenting concern(s) with relevant details of related past interventions.

Clinical Findings. Describe: 1) the medical, family, and psychosocial history including lifestyle and genetic information; 2) pertinent co-morbidities and relevant interventions (e.g., self-care, other therapies); and 3) the physical examination (PE) focused on the pertinent findings including results from testing.

Timeline. Create a timeline that includes specific dates and times (table, figure, or graphic).

Diagnostic Focus and Assessment. Provide an assessment of the; 1) diagnostic methods (e.g., PE, laboratory testing, imaging, questionnaires, referral), 2) diagnostic challenges (e.g., financial, patient availability, cultural), 3) diagnostic reasoning including other diagnoses considered, and, 4) prognostic characteristics (e.g., staging) where applicable.

Therapeutic Focus and Assessment. Describe: 1) the type(s) of intervention (e.g., preventive, pharmacologic, surgical, lifestyle, self-care) and 2) the administration and intensity of the intervention (e.g., dosage, strength, duration, frequency.

Follow-up and Outcomes. Describe the clinical course of this case including all follow-up visits as well as 1) intervention modification, interruption, or discontinuation, and the reasons; 2) adherence to the intervention and how this was assessed; and 3) adverse effects or unanticipated events. In addition, describe: 1) patient-reported outcomes, 2) clinician-assessed and -reported outcomes, and 3) important positive and negative test results.

Discussion. Please describe: 1) the strengths and limitations of this case report including case management, 2) the literature relevant to this case report (the scientific and clinical context), 3) the rationale for your conclusions (e.g., potential causal links and generalizability), and 4) the main findings of this case report: What are the take-away messages?

Patient Perspective. The patient should share his or her experience or perspective of the care in a narrative that accompanies the case report whenever appropriate.

Informed Consent. Did the patient or their custodian give the author of this case report informed consent? Provide if requested .

Case Report Submission Requirements: 1) Competing interests, are there any competing interests? 2) Ethics Approval, Did an ethics committee or institutional review board review give approval? If yes, please provide if requested, 3) De-Identification, has all patient's related data been de-identified?

RANDOMIZED CLINICAL TRIALS WRITING TEMPLATE

In this particular type of original study, individuals are randomly allocated to receive or not receive a preventive, therapeutic, or diagnostic intervention and then followed up to determine the effect of the intervention. All randomized clinical trials should include a flow diagram and authors should provide a completed randomized trial checklist (see CONSORT Flow Diagram and Checklist; http://www.consort-statement.org) and a trial protocol.

Authors of randomized controlled trials are encouraged to submit trial protocols along with their manuscripts.

All clinical trials must be registered (before recruitment of the first participant) at an appropriate online public that must be independent of for-profit interest, e.g.:

- http://www.clinicaltrials.gov;
- http://www.anzctr.org.au;

- http://www.umin.ac.jp/ctr;
- http://isrctn.org;
- http://www.trialregister.nl/trialreg/index.asp).

Each manuscript should clearly state an objective or hypothesis; the design and methods (including the study setting and dates, patients or participants with inclusion and exclusion criteria, or data sources, and how these were selected for the study); the essential features of any interventions; the main outcome measures; the main results of the study; a comment section placing the results in context with the published literature and addressing study limitations; and the conclusions.

Data included in research reports must be original. A structured abstract not exceeding 300 words is required. Clinical trials are limited to 2700 words (not including abstract, tables, figures, and references), 40 references, and no more than 5 tables and figures.

REVIEW, MINIREVIEW AND META-ANALYSIS PAPERS

These papers will not have empirical data acquired by the authors but will include historical perspectives, analysis and discussion of papers published and data acquired in a specific area.

Systematic reviews and meta-analyses are a particular type of original articles that perform systematic, critical assessment of literature and data sources pertaining to clinical topics, emphasizing factors such as cause, diagnosis, prognosis, therapy, or prevention. All articles or data sources should be searched for and selected systematically for inclusion and critically evaluated, and the search and selection process should be described in detail in the manuscript. The specific type of study or analysis, population, intervention, exposure, and tests or outcomes should be described for each article or data source. A structured abstract of less than 300 words is required. The text is limited to 3500 words (not including abstract, tables, figures, and references); about 4 tables (a flow diagram that depicts search and selection processes as well as evidence tables should be included) - and no reference limit.

Minireview is a brief historical perspective, or summaries of developments in fast-moving areas covered within the scope of the journal. They must be based on published articles; they are not outlets for unpublished data. They may address any subject within the scope of the journal. The goal of the minireview is to provide a concise very up-to-date summary of a particular field in a manner understandable to all readers.

SHORT COMMUNICATION AND SHORT RESEARCH ARTICLE

Short Communications are urgent communications of important preliminary results that are very original, of high interest and likely to have a significant impact on the subject area of the journal. A Short Communication needs only to demonstrate a 'proof of principle'. Authors are encouraged to submit an Original Research Paper to the journal following their Short Communication. There is no strict page limit for a Short Communication; however, a length of 2500-3500 words, plus 2-3 figures and/or tables, and 15-20 key references is advisable. Short Research Article may be smaller single-result findings as a brief summary that include enough information, particularly in the methods and results sections, that a reader could understand what was done.

POLICY PAPER

The purpose of the policy paper is to provide a comprehensive and persuasive argument justifying the policy recommendations presented in the paper, and therefore to act as a decision-making tool and a call to action for the target audience.

COMMENTARIES/OPINION ARTICLES

An opinion-based article on a topical issue of broad interest, which is intended to engender discussion.

STUDY PROTOCOLS AND PRE-PROTOCOLS

JUMJ welcomes publishing protocols for any study design, including observational studies and systematic reviews. All protocols for randomized clinical trials must be registered and follow the CONSORT guidelines; ethical approval for the study must have been already granted. Study pre-protocols (i.e., discussing provisional study designs) may also be submitted and will be clearly labeled as such when published. Study protocols for pilot and feasibility studies may also be considered.

METHOD ARTICLES

These articles describe a new experimental or computational method, test or procedure, and should have been well tested. This includes new study methods, substantive modifications to existing methods or innovative applications of existing methods to new models or scientific questions. We also welcome new technical tools that facilitate the design or performance of experiments or operations and

data analysis such as software and laboratory and surgical devices, or of new technologies to assist medical diagnosis and treatment such as drug delivery devices.

Maximum length of submissions

Full length original research articles should not exceed 10000 words (maximum 60 references), and up to 6 tables and/or figures.

Short communications comprising up to 1800 words of text, maximum 15 references, and two illustrative items (Tables and/or Figures).

Letters and Case Reports (provide novel insight into disease mechanisms, diagnostic and management applications). *Clinical Laboratory Notes* (technical evaluation or important insight into analytical methodology), or *Letters to the Editor* (focused on a specific article that has appeared in JUMJ within 4 weeks of print issue date of article). For all 3 types of letters listed above, the text should not exceed 600 words, with no abstract, a maximum of 1 table or figure and up to 5 references.

Review Articles, Surveys, Essays, and Special Reports may exceed the word and reference limit for Full-length articles as per the comprehensive nature of these articles. However, both of these articles (Reviews and Special Reports) will still require an abstract (unstructured, 350 word maximum).

Editorials, Meeting summary, Commentaries, Book review and Opinion pieces will not require an abstract and will be limited to 2000 words and up to 20 references. A book review is a brief critical and unbiased evaluation of a current book determined to be of interest to the journal audience. Publication of a submitted book review is at the discretion of the editor.

Artwork

General points

Make sure you use uniform lettering and sizing of your original artwork. Preferred fonts: Arial (or Helvetica), Times New Roman (or Times), Symbol, Courier. Number the illustrations according to their sequence in the text. Use a logical naming convention for your artwork files. Indicate per figure if it is a single, 1.5 or 2-column fitting image. For Word submissions only, you may still provide figures and their captions, and tables within a single file at the revision stage.

Formats

Regardless of the application used, when your electronic artwork is finalized, please 'save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below). Please do not supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); the resolution is too low, supply files that are too low in resolution, and, submit graphics that are disproportionately large for the content.

- EPS (or PDF): Vector drawings. Embed the font or save the text as 'graphics'.
- TIFF (or JPG): Color or grayscale photographs (halftones): always use a minimum of 300 dpi.
- TIFF (or JPG): Bitmapped line drawings: use a minimum of 1000 dpi.
- TIFF (or JPG): Combinations bitmapped line/half-tone (color or grayscale): a minimum of 500 dpi is required.

Color artwork

Please make sure that artwork files are in an acceptable format (TIFF (or JPEG), EPS (or PDF), or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures the Journal will ensure that these figures will appear in color on the Web regardless of whether or not these illustrations are reproduced in color in the printed version. Because of technical complications which can arise by converting color figures to 'gray scale' please submit in addition usable black and white versions of all the color illustrations.

Figure captions

Ensure that each illustration has a caption (Legend). A caption should comprise a brief title below the figure that describes its content and not to be general. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used in the legend. Figure caption should stand for itself (self-explanatory) without the need for consulting the text.

Tables

Number tables consecutively in accordance with their appearance in the text. Place footnotes to tables below the table body and indicate them with superscript lowercase letters within the table. If necessary, such footnotes could be placed at the end of the table title. Avoid vertical rules. Be sparing in the use of tables and ensure that the data presented in tables do not duplicate results described elsewhere in the article (Figures or text). The table caption (Title) should be brief but describes its content and not to be general. Explain all symbols and abbreviations used in the table in the footnote. Table title should stand for itself (self-explanatory) without the need for consulting the text. The table structure should be scientifically organized

(columns and rows) and its message should be easily comprehendible.

The Editor-in-Chief, on accepting a manuscript, may recommend that additional tables and/or graphs containing important backup data, too extensive to be published in the article, may be published as supplementary material. In that event, an appropriate statement will be added to the text. However, the author should submit such material for consideration with the manuscript.

References

References cited should be relevant, up-to-date and adequately cover the field without ignoring any supportive or conflicting publications. Please ensure that every reference cited in the text is also present in the reference list (and vice versa). If present, unpublished results and personal communications may be mentioned in the text and not in the reference list. Citation of a reference as 'in press' implies that the item has been accepted for publication and shows up on PubMed literature search or a copy of the title page of the relevant article must be submitted. DOI of the references - whenever applicable should be presented. Authors are encouraged to cite primary literature rather than review articles in order to give credit to those who have done the original work.

Reference management software

This journal has standard templates available in key reference management packages EndNote (http://www.endnote.com/support/enstyles.asp) and Reference Manager (http://refman.com/support/rmstyles.asp). Using plug-ins to word processing packages, authors only need to select the appropriate journal template when preparing their article and the list of references and citations to these will be formatted according to the journal style, which is described below.

Reference formatting

There are no strict requirements on reference formatting at submission but should be consistent, complete and up-to-date. Where applicable, author(s) name(s), chapter title/article title, journal title/book title, year of publication, volume number-issue number/book chapter and the pagination must be present. For the book reference, the edition number, editors (if they are not the authors), publisher and its main address (City and Country) should be added as described below in the example. The reference style used by the journal should be applied to the accepted article at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct. Use peer-reviewed references only except for national and international organizational reporting and registers. If you do wish to format the references yourself, they should be arranged according to the following examples:

Reference style

Indicate references by number(s) in curved brackets as a bolded superscript at the end of the cited text(s) before the full stop, e.g., ……. shorter hospital stay and lower cost[(20)]. The actual authors can be referred to, but the reference number(s) must always be given. Number the references in the list in the order in which they appear in the text. The authors list should not be shortened, all authors' names should be mentioned up to 10 authors and end longer list by et al. For further details you are referred to 'Uniform Requirements for Manuscripts submitted to Biomedical Journals' (J Am Med Assoc 1997; 277: 927-34) (see also http://www.nlm.nih.gov/bsd/uniform_requirements.html).

Examples:

Reference to a journal publication: Format your journal publications according to the following examples depending on whether; 1) It is already published with specific page numbers, 2 and 3) It is already published with article ID number and pages from 1 to …, 4) It is published put ahead of print, or, it is accepted for publication.

1. Van der Geer J, Hanraads JAJ, Lupton RA. The art of writing a scientific article. J. Sci. Commun., 2010;163(1):51-9.
2. Leta S, Dao TH, Mesele F, Alemayehu G. Visceral Leishmaniasis in Ethiopia: An Evolving Disease. PLoS Negl Trop Dis., 2014; 8(9):e3131;1-7.
3. Arjmand MH, Ahmad Shah F, Saleh Moghadam M, Tara F, Jalili A, Mosavi Bazaz M, Hamidi Alamdari D. Prooxidant-antioxidant balance in umbilical cord blood of infants with meconium stained of amniotic fluid. Biochem Res Int., 2013;2013:ID270545;1-4.
4. Teferra RA, Grant BJ, Mindel JW, Siddiqi TA, Iftikhar IH, Ajaz F, Aliling JP, Khan MS, Hoffmann SP, Magalang UJ. Cost minimization using an artificial neural network sleep apnea prediction tool for sleep studies. Ann Am Thorac Soc., 2018 Jul 28 (ahead of print).
5. Alduraywish AA, Almani AZ, Alanazi AD-A, Alruwaili FS, Alolaywi AN, Almaeen AH, El-Metwally TH. Vitamin D insufficiency among healthy participants

and type 2 diabetic patients from the northern Al-Jouf region of Saudi Arabia: Correlation with the prognostic indices of the disease. International Medical J, 2019 (Accepted for publication; http://www.seronjihou.co.jp/imj/).

Reference to a book: Strunk Jr W, White EB (Editors). The elements of style, 4th Edition, Longman, New York; 2000, pp. 210-9.

Reference to a chapter in an edited book: Mettam GR, Adams LB. How to prepare an electronic version of your article. In: Jones BS, Smith RZ (Editors), Introduction to the electronic age, 1st Edition, E-Publishing Inc., New York, 2009, Chapter 2: pp. 281-304.

Reference to a homepage: It is acceptable to refer to an Organizational Guidelines, Reports, Forms, Data sheets, Questionnaires, etc. It should follow the following format. World Health Organization. Non-communicable Diseases (NCD) Country Profile, 2014 (http://www.who.int/globalcoordinationmechanism/publications/ncds-country-profiles-eng.pdf; last accessed March 1, 2017).

Journal abbreviations source

Journal names should be abbreviated according to the List of Title Word Abbreviations: http://www.issn.org/services/online-services/access-to-the-ltwa/.

Abbreviations and units

Standard abbreviations as listed in the Council of Biology Editors Style Manual may be used without definition. Use non-standard abbreviations sparingly, preceding their first use in the text with the corresponding full designation. Use units in conformity with the standard International System (SI) of units.

Video data

The journal accepts video material and animation sequences to support and enhance your scientific research. Authors who have video or animation files that they wish to submit with their article are strongly encouraged to include links to these within the body of the article. This can be done in the same way as a figure or table by referring to the video or animation content and noting in the body text where it should be placed. All submitted files should be properly labeled so that they directly relate to the video file's content. To ensure that your video or animation material is directly usable, please provide the files in one of our recommended file formats with a preferred maximum size of 50 MB. Video and animation files supplied will be published online in the electronic version of your article. Since video and animation cannot be embedded in the print version of the journal, please provide text for both the electronic and the print version for the portions of the article that refer to this content.

Audio Slides

JUMJ encourages authors to create an Audio Slides presentation with their published article as supplementary material. This gives authors the opportunity to summarize their research in their own words and to help readers understand what the paper is about. Authors of this journal will automatically receive an invitation e-mail to create an Audio Slides presentation after acceptance of their paper.

Supplementary data

JUMJ accepts electronic supplementary material to support and enhance your scientific research. Supplementary files offer the author additional possibilities to publish supporting applications, high-resolution images, background datasets, sound clips and more. Supplementary files supplied will be published online alongside the electronic version of your article. In order to ensure that your submitted material is directly usable, please provide the data in one of our recommended file formats. Authors should submit the material in electronic format together with the article and supply a concise and descriptive caption for each file.

Supplementary material captions

Each supplementary material file should have a short caption which will be placed at the bottom of the article, where it can assist the reader and also be used by search engines.

THE COPYRIGHTS

Submission checklist

The following list will be useful during the final checking of an article prior to sending it to the journal for review. Please consult this Guide for Authors for further details of any item.

To avoid unnecessary errors, you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details for all authors:

- E-mail address.
- Full postal address.
- Telephone.

All necessary files have been uploaded, and contain:

- Keywords.
- All figures and their captions.
- All tables (including title, description, footnotes).

Further considerations:

- Manuscript has been 'spell-checked' and 'grammar-checked'.
- All references mentioned in the Reference list are cited in the text, and vice versa.
- Permission has been obtained for use of copyrighted material from other sources (including the Web).
- Color figures are clearly marked as being intended for color reproduction on and in print, or to be reproduced in color electronically and in black-and-white in print.

PEER REVIEW PROCESS

High quality manuscripts are peer-reviewed by minimum of two peers of the same field along with a biostatistician in the case the study requires. Pre-reviewing advice and help will be provided by the Editor-In-Chief on first submission for initial improvements to meeting the minimum criteria of peer-reviewing. The journal follows strict double-blind fold constructive review policy to ensure neutral evaluation. During this review process identity of both the authors and reviewers are kept hidden to ensure unbiased evaluation. A cycle of one-month reviewing process is the target of the journal from submission to final acceptance. For meeting this goal, the Editor-In-Chief is expecting strict compliance from author hastening corrections and replying editorial requests. Continuous post-publication open peer reviewing is highly encouraged through submitting comments to the Editor on any of the published article that will show up with author reply in the subsequent issue to the journal.

The reviewers' comments are sent to authors once received. With the help of the reviewers' comments, FINAL decision (accepted or accepted with minor revision or accepted with major revision or rejected) will be sent to the corresponding author. Reviewers are asked if they would like to review a revised version of the manuscript. The editorial office may request a re-review regardless of a reviewer's response in order to ensure a thorough and fair evaluation.

In order to maintain this journal's mission of one-month publication cycle, authors are encouraged to submit the revised manuscript within one week of receipt of reviewer's comment (in case of minor corrections). However, revised manuscript submission should not go beyond 2 weeks (only for the cases of major revision which involves additional experiment, analysis etc.). Along with corrected manuscript authors will be requested to submit filled a point-by-point answers to the reviewers' comments and any rebuttal to any point raised. The Editor-In-Chief of the journal will have exclusive power to take final decision for acceptance or rejection during any dispute.

Under special circumstance, if the review process takes more time, author(s) will be informed accordingly. The editorial board or referees may re-review manuscripts that are accepted pending revision. Manuscripts with latest and significant findings will be handled with the highest priority so that it could be published within a very short time. The journal is determined to promote integrity in research publication. In case of any suspected misconduct, the journal management reserves the right to re-review any manuscript at any stages before final publication.

Our massage to JUMJ potential reviewers says "Although the Manuscript General Evaluation Form is attached, we like to instigate a policy of constructive reviewing and to do our best to make the submitted manuscripts publishable - provided that it is genuine and contain no major frauds of republication, duplicate use of self-data or plagiarism of intellectual properties of the others. Please, make your changes and insert your corrections, comments and suggestions directly into the manuscript text but in a different color. Please also make sure that the author(s) presented an inclusive and updated list of genuine references, applied proper statistics and extracted justified conclusions".

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The Manuscript Assigned Number and Title: ……..

Although the following manuscript general evaluation form is sent to reviewers, JUMJ asks the reviewer for further meticulous one-

word-at-a-time revision, please. Please insert corrections, changes, suggestions, questions, comments and points of deficiency directly into the manuscript text but in a different color. Also, please do not worry much for the formatting.

The Manuscript Evaluation Score: Please, score the manuscript from 0 to 4 (highest) for each of the following items, and sum the total score. Please, also check if the statistics of the results require revision.

Items \ Scores	0	1	2	3	4	Item Score
The Study is a Priority Problem						
Originality						
Significance of the Work						
Research Design						
Quality and Clarity of the English Writing						
Standard and Reproducible Methodology						
Results Presentation and Appropriate Statistics						
Relevant Discussion and Justified Conclusions						
Tables/ Illustrations/ Figures						
References: Inclusive and Updated						
Total score						
Requires Statistical Revision (Mark Please)				Yes		No

Justification for Decision & Feedback for the Author (REQUIRED): JUMJ recommends the reviewer to introduce such justifications and feedback into the text at the appropriate places within the manuscript (Specific and Comprehensive Revision).

Note to the Editor (WELCOMED): JUMJ welcomes reviewer writing a note to the Editor including conflict of interest that will not be disclosed to the Author(s).

The Decision: Should JUMJ publish the manuscript? Please, check the appropriate box.

Yes, without any revision:	Accepted	
Yes, with minor revision and alterations:	Accepted with revision	
Yes, with major revision and alterations:	Re-Evaluation after substantial revision	
No, this manuscript should be denied publication:	Denied	

AFTER ACCEPTANCE

Online proof correction

Corresponding authors will receive an e-mail with annotation for correction of MS format proofs and resend the corrected version. All instructions for proofing will be given in the e-mail we send to authors. We will do everything possible to get your article published quickly and accurately - please upload all of your corrections within 48 hours. It is important to ensure that all corrections are sent back to us in one communication. Please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is solely your responsibility.

Offprints

The corresponding author, at no cost, will be provided with a PDF file of the article via email (the PDF file is a watermarked version of the published article and includes a cover sheet with the journal cover image and a disclaimer outlining the terms and conditions of use). For an extra charge, paper offprints can be ordered at any time via the offprint order form, which is sent once the article is accepted for publication.

Policy Regarding Corrections & Retractions

JUMJ differentiates the post-publication amendments into: Comments, Addenda, Corrections, and Retractions (or Partial Retractions).

Comments and Replies: An interested reader may approach the Editorial Office in cases of questionable results or experimental design and methods of a published article. Upon request, the Editorial Office will ask the reader to formulate his/her argument in a one-page evidence-supported comment on the article. In

case the comment is valid after revision, the Editorial Office will approach the authors of the article in question and invite them to prepare a Reply. Reply will be reviewed, and if accepted, will be compiled with the comment for publication in the current issue of JUMJ, with page numbers added - with a hyperlink to the Comments and Replies appearing on the original publication. If the reader's complaints are substantiated without a valid refute from the authors, the authors in collaboration with the Editorial Office may consequently publish a Correction or decide to retract the paper entirely.

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