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HORMONE DISORDERS AND ITS CORRELATION WITH LIPID PROFILE AND OXIDATIVE STRESS BIOMARKERS IN METABOLIC SYNDROME PATIENTS

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ABSTRACT

Background and aims: Metabolic syndrome" (MS) refers to a group of interrelated metabolic risk factors of developing cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM). The aim of this work is investigation the correlation of melatonin and sprosin hormones with lipid profile and oxidant/antioxidant status in sera of MS patients and to assess the potential of these parameters as diagnostic biomarkers for metabolic syndrome using receiver-operating characteristic (ROC) curve analysis

Methods: This case-control study involved 82 MS patients and 60 controls. The study examined serum levels of glucose, melatonin, asprosin, lipid profile, malondialdehyde (MDA), superoxide dismutase (SOD), glutathione-S-transferase (GST), reduced glutathione (GSH), and total antioxidant activity (AOA).

Results:

The patients group had significantly lower serum levels of melatonin hormone, SOD, GST, GSH, and AOA. While, glucose and asprosin levels increased in MS patients compared to the controls. Similarly, the lipid profile, including cholesterol, very low density lipoprotein (VLDL), and triglyceride (TG), and low density lipoprotein (LDL), were significantly increased. Furthermore, correlation analysis revealed that levels of melatonin were negatively associated with cholesterol and positively associated with TG. Asprosin has negative significant correlation with GSH. Regarding ROC curve analysis, glucose, asprosine, VLDL, TG, and MDA were demonstrated as diagnostic biomarkers for metabolic syndrome.

Conclusions: The study found that melatonin hormone was negatively associated with cholesterol and positively associated with TG, while Asprosin has negative significant correlation with GSH. Additionally, this study proved also that glucose, asprosin, VLDL, TG, and MDA can be used as metabolic syndrome's predictive biomarkers.

KEY WORDS: Metabolic Syndrome, Type 2 Diabetes Mellitus, Melatonin, Asprosin, Reactive Oxygen Species, Oxidative Stress, Antioxidants, Cardiovascular Disease, Free Radicals, Glucose, Lipid Profile, Insulin Resistance

INTRODUCTION

The term "metabolic syndrome" is used to describe a complex condition characterized by a combination of factors that elevate the risk of CVD and insulin resistance (IR). MS is indicated by central obesity, dyslipidemia, hypertension, and abnormal glucose metabolism (Kargar *et al*., 2021). In 1998, the World Health Organization (WHO) introduced the initial formal definition of MS. This definition primarily emphasized the presence of insulin resistance, identified through impaired fasting glucose (IFG) or impaired glucose tolerance (IGT), or the diagnosis of T2DM, which needed to be present at the time of diagnosis. Furthermore, in addition to the aforementioned criteria, the WHO definition also requires the presence of two of the following conditions: hypertension, microalbuminuria, and dyslipidemia characterized by lower levels of high-density lipoprotein (HDL) and higher levels of TG (Alberti, K. G. M. M., & Zimmet, 1998). Over the next 5–10 years, individuals with MS had a five times greater chance of acquiring T2DM and a two times greater chance of developing CVD (Andraweera *et al.*, 2023). There is growing evidence to suggest that oxidative stress, a dominant event in cellular damage and dysfunction, plays an important role in the pathogenesis of MS (Vona *et al*., 2019). Meanwhile, a wide variety of experimental settings pointed out that some specific hormones also have antioxidant properties in the body, for instance, melatonin hormone able to enhance

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the body's antioxidant capacity (Zhang, 2019a). On the other hand, recent research has identified also the vital function of asprosin hormone in this incidence (Hong *et al*., 2021).

Melatonin hormone can increase the body's antioxidant capacity and get rid of oxygen free radicals, thereby lowering oxidative damage, which has been verified both *in vitro* and *in vivo* (Tursinawati *et al.*, 2021). Moreover, the study of Koziróg revealed that melatonin therapy improves blood pressure, the lipid profile, and oxidative stress parameters in patients with MS, thereby, plays a significant role in both the prevention and treatment of metabolic syndrome (Gomes, 2019).

On the other hand, recent researchers have identified also the vital role of asprosin hormone in MS patients. Asprosin hormone regulates hepatic glucose synthesis and insulin sensitivity in response to hunger. Furthermore, studies have also demonstrated that elevated levels of asprosin are linked to metabolic diseases (Hong *et al*., 2021).

The oxidative stress can result from either a systemic increase in reactive oxygen species (ROS) formation or a decrease in antioxidant defense, or both. The contribution of free radicals and oxidative stress in the pathogenesis of MS is widely investigated (Abdelazeem *et al.*, 2021). ROS and free radicals generate the lipid peroxidation process in an organism. Malondialdehyde (MDA) is one of the final products of polyunsaturated fatty acids peroxidation in the cells (Caldiroli *et al.*, 2020).

On the contrary, multiple antioxidants both endogenous and exogenous work together to neutralize ROS in the body. Endogenous antioxidants contain both enzymatic and nonenzymatic components that cooperate to regulate ROS levels (Lönn, 2012). For example, both antioxidant enzymes (SOD and GST) are working as detoxification enzymes for body (Trivedi *et al*., 2014; Balcı *et al*., 2019). Concerning the non-enzymatic antioxidants, it was found that glutathione (GSH) is a one of pivotal anti-oxidant in animal cells and the key cellular redox buffer (Al-zaid and Sayyah, 2023). Indeed, both of these types represent the total antioxidant activity, which act as an antioxidants substance to scavenge free radicals and protect against damage caused by them (Adwas *et al*., 2019).

The metabolic disorders affect some aspects of metabolism, which can result in a range of complications. In MS patients, the alteration in glucose and lipid profile are one of the main causes of metabolic disorder and hence in the diagnosis of this metabolic diseases and assess patient responsiveness to dietary treatments (Al-Harbi *et al*., 2022; Askarpour *et al*., 2023).

The purpose of the current work is to determine the hormone levels, glucose, lipid profile and oxidative stress biomarkers in sera of MS patients in comparison to healthy individuals. Furthermore, to investigate the hormone imbalance as well as study the correlation of hormone levels with glucose, lipid profile and oxidant/antioxidant status in sera of MS patients compared to healthy individuals. In addition to that, to assess the aforementioned parameters as a potential diagnostic biomarkers for metabolic syndrome using ROC curve analysis

2. MATERIALS AND METHODS

2.1. Study design and sampling

This case-control study was conducted at the Chemistry Department, College of Science, University of Duhok, and Azadi Teaching Hospital in the Kurdistan Region of Iraq from November 2021 to June 2022. A total of eighty-two blood samples were collected from individuals diagnosed with MS, following the criteria outlined by the Adult Treatment Panel III (ATP III). The diagnosis was based on the presence of at least three of the following risk factors: fasting plasma glucose ≥ 110 mg/dl (including diabetes), abdominal obesity (waist circumference, WC ≥ 102 cm in men and ≥ 88 cm in women), TG ≥ 150 mg/dl, low HDL levels (< 40 mg/dl in men and < 50 mg/dl in women), and blood pressure ≥ 130/85 mmHg. In this study, individuals who were pregnant, smokers, had liver disease or kidney disease, thyroid disease, or pre-existing heart conditions were excluded from the research. After the exclusion criteria were applied, a total of 82 out of 104 patients and 60 out of 80 controls were enrolled in this study. Blood samples from patients with MS were obtained from the Azadi Teaching Hospital, while blood samples from the control group were collected from the staff at the University of Duhok. After an overnight fasting period of 10-12 hours, blood samples were collected from both groups using a disposable syringe. Approximately 6-8 ml of blood was drawn and collected in clot activator gel tubes

with a capacity of 10 ml. The serum samples were separated by centrifuging them in a Universal 320 centrifuge for 10 minutes at a speed of 4000 rpm. The resulting serum was then utilized for measuring glucose levels and lipid profile parameters, including HDL, TG, and total cholesterol. The remaining serum was subsequently frozen to preserve its integrity for hormone measurements (melatonin and asprosin), enzyme activity assessments (SOD and GST), and the measurement of reduced glutathione, malondialdehyde, and total antioxidant activity

2.2. Ethical Approval

Before the commencement of the study, informed consent was obtained from every participant, and the study was authorized by the Ethics Committee of the Directorate of Health in Duhok, Iraq. For the questionnaire to fit the needs of the study there was a meeting where people were interviewed.

2.3. Measurements

In the present study, the biochemical analysis involved three components: the levels of melatonin and asprosin hormones, which were measured using commercially available kits from Bioassay Technology Laboratory Company in China. The analyses were performed following the manufacturer's instructions utilizing the enzyme-linked immunosorbent assay (ELISA) method (www.bt-laboratory.com). In contrast, the serum glucose and lipid profile levels were determined through a manual enzymatic colorimetric method using a commercial kit (Biolabo SAS in Maizy, France) as follow: serum glucose level was determined by glucose oxidase method; total cholesterol was measured using the cholesterol oxidase-p-aminophenazone method; triglyceride was assessed using the glycerol-phosphate oxidase method; high-density lipoprotein cholesterol was assessed using the HDL-precipitating method; very low-density lipoprotein cholesterol was calculated by dividing TG/5 and finally low-density lipoprotein cholesterol was determined mathematically using Friedewald formula (LDL (mg/dl) = Total cholesterol (mg/dl) − HDL (mg/dl) − TG (mg/dl)/5). The analyses were conducted following the manufacturer's instructions and measured using a spectrophotometer.

In the third set of experiments, the level of antioxidant biomarkers (superoxide dismutase activity, glutathione-S- transferase activity**,** reduced glutathione, total antioxidant activity) and oxidative stress biomarker (MDA) were estimated by using manual methods. Superoxide dismutase activity in the serum was assessed using a modified photochemical NBT (nitroblue tetrazolium) method, which involved the use of potassium cyanide as a peroxidase inhibitor (Winterbourn *et al*., 1975). The activity of glutathione-S- transferase was determined by the method established by Habig *et al*., GST activity was measured spectrophotometrically at 340 nm using the standard substrate (1-chloro-2,4-dinitrobenzene, CDNB) and the co-substrate GSH (Habig, W. H., Pabst, M. J., & Jakoby, 1974). Serum glutathione was determined by a modified procedure utilizing Ellman reagent. The oxidation of glutathione by the sulfhydryl reagent 5,5′-dithio-bis (2-nitrobenzoic acid) (DTNB) (also known as Ellman's reagent), which results in the formation of the yellow derivative 5′-thio-2-nitrobenzoic acid (TNB), it can be measured at a wavelength of 412 nm (Rahman, I., Kode, A., & Biswas, 2006). Total antioxidant activity in serum was measured to predict the total antioxidant potential of the matrix in issue, which is determined by the antioxidants' ability to donate hydrogen to scavenge produced radicals (Miller *et al*., 1995). In this test, the antioxidants in the sample of serum were added to prevent the production of thiobarbituric acid reactive substances (TBARS) from being made. This reaction can be measured with spectrophotometry, and the AOA is the amount that prevents color from developing (Koracevic *et al*., 2001). Finally, the level of serum lipid peroxidation (MDA) was also determined colorimetrically by the method described by (Tukozkan, N., Erdamar, H., & Seven, 2006).

Furthermore, various variables were assessed for the participants, including age groups, duration of T2DM, history of vitamin and mineral intake, presence of diabetes mellitus, hypertension, and dyslipidemia. Additionally, the general characteristics of both MS patients and controls were verified, including body mass index (BMI), waist circumference (WC), and blood pressure.

2.4. Statistical analysis

Data analyses were done using the descriptive statistics. The Chi-Square test was adopted to find out the significance of the differences between the categories of variables. Furthermore, a one-way ANOVA test was used

to find out the differences between sample members according to their personal or demographic characteristics. Moreover, Pearson correlation was used to find out the correlations between the studies variables. Additionally, ROC curve analysis was used to determine the potential biomarkers for the diagnosis of MS disease. A P value of < 0.05 was regarded as statistically significant.

3. RESULTS

3.1. General characteristics of participants

The study includes 142 participants with age between (27-72) years, 82 were metabolic syndrome patients (patient group) and 60 were apparently healthy individuals (control group). The general anthropometric and clinical characteristics of the participants were described in Table 1. As shown in Table 1, significant differences between frequencies (numbers and percentages) between participant groups of age ($P < 0.001$), gender ($P < 0.05$), BMI ($P < 0.01$), history of DM ($P < 0.01$), and WC ($P < 0.001$ and $P < 0.01$) for both males and females, respectively. Besides, blood pressure (systolic and diastolic) ($P < 0.001$ and $P < 0.01$, respectively) was significantly different between the frequencies of MS patients and healthy groups. On the other hand, no significant differences were observed neither in patient group nor in healthy group for frequencies of the history of hypertension, history of dyslipidemia and history of vitamins and minerals taking ($P > 0.05$).

Table(1): -Frequencies of anthropometric and clinical characteristics

Variables	Classes	Groups			Chi-Square		
		Patients	Healthy	Total	Estimate Value	P-Value	Result
Age*	27-50	27 (35%)	50 (65%)	77 (100%)	38.836	0.000	S
	51-60	29 (78%)	8 (22%)	37 (100%)			
	61-72	26 (93%)	2 (7%)	28 (100%)			
Gender***	Male	9 (36%)	16 (64%)	25 (100%)	5.881	0.015	S
	Female	73 (62%)	44 (38%)	117 (100%)			
BMI**	18-25	6 (30%)	14 (70%)	20 (100%)	14.860	0.001	S
	26-30	22 (47%)	25 (53%)	47 (100%)			
	> 30	54 (72%)	21 (28%)	75 (100%)			
History of diabetes mellitus**	Yes	67 (65%)	36 (35%)	103 (100%)	8.195	0.004	S
	No	15(39%)	24 (61%)	39 (100%)			
History of hypertension	Yes	65 (60%)	42 (40%)	107 (100%)	1.099	0.294	N. S
	No	17 (50%)	17 (50%)	34 (100%)			
History of Dyslipidemia	Yes	50 (58%)	37 (42%)	87 (100%)	0.007	0.933	N. S.
	No	32 (58%)	23 (42%)	55 (100%)			
History of vitamins and minerals taking	Yes	13 (52%)	12 (48%)	25 (100%)	0.411	0.522	N. S
	No	69 (59%)	48 (41%)	117 (100%)			
WC	≥ 120 male	6 (50%)	6 (50%)	12 (100%)	16.675	0.000	S

	< 102 male	3 (25%)	9 (75%(	12 (100%)			
	≥ 88 female	72 (66%)	37 (34%)	109 (100%)	10.638	0.001	S
	< 88 female	1 (11%)	8 (89%)	9 (100%)			
Systolic*	≥ 130	55 (80%)	14 (20%)	69 (100%)	26.536	0.000	S
	< 130	27 (37%)	46 (63%)	73 (100%)			
Disystolic**	≥ 85	35 (76%)	11 (24%)	46 (100%)	9.380	0.002	S
	< 85	47 (49%)	49 (51%)	96 (100%)			

Chi-Square Test for n (%), *P value is significant when P < 0.001. **P value is significant when P < 0.01. ***P value is significant when P < 0.05. S, significant, N.S, non-significant.

3.2. Demographic and clinical characteristics of the participants

Table 2 shows the mean ± SD comparison between two groups, the metabolic syndrome patient and the healthy group, of age (55.7 ± 8.1 years, 40.3 ± 9.3 years) and BMI (33.4 ± 6.2 Kg/m^2, 28.8 ± 4.7 Kg/m^2), respectively, where a significant difference (P < 0.001) was observed. Similarly, significant differences (P < 0.05) were observed in gender and history of diabetes mellitus (P < 0.01) between the two groups of participants. On the other hand, no significant difference was observed between the means ± SD of the histories of hypertension, vitamins and minerals, dyslipidemia and WC for MS patients and the healthy group. However, regarding the systolic (138.3 ± 21.1 mmHg, 115.0 ± 16.2 mmHg) and diastolic (81.6 ± 15.4 mmHg, 74.7 ± 12.3 mmHg), a significant difference (P < 0.01) was observed between both groups.

Table(2):- Demographic and clinical characteristics of the participants

Variables	Groups		F test		
	Patients	Healthy	Estimate Value	P-Value	Result
Age*	55.7 ± 8.1	40.3 ± 9.3	45.211	0.000	S
Gender***	1.89 ± 0.315	1.73 ± 0.445	6.048	0.015	S
BMI* (Kg/m^2)	33.4 ± 6.2	28.8 ± 4.7	16.124	0.000	S
History of DM**	1. 8 ± 0.4	1.4 ± 0.5	8.575	0.004	S
History of hypertension	1.2 ± 0.4	1.3 ± 0.5	1.092	0.298	N. S
History of dyslipidemia	1.4 ± 0.5	1.4 ± 0.5	0.007	0.934	N. S
History of vitamins and minerals	1.8 ± 0.4	1.8 ± 0.4	0.406	0.525	N. S
WC (cm)	107.1 ± 10.8	94.6 ± 10.5	0.160	0.689	N. S
Systolic*(mmHg)	138.3 ± 21.1	115.0 ± 16.2	32.175	0.000	S
Diastolic**(mmHg)	81.6 ± 15.4	74.7 ± 12.3	9.902	0.002	S

Mean and standard deviation (One – Way ANOVA), *P value is significant when P < 0.001. **P value is significant when P < 0.01. ***P value is significant when P < 0.05.

3.3. Clinical related biomarkers

The results of all biochemical parameters in serum of MS patients in comparison with healthy groups are summarized in table 3 and figures 1, 2, and 3.

Table(3):- The levels of biochemical parameters in serum of MS patients in comparison with healthy groups

Variables	Groups		F test		
	Patients	Healthy	Estimate Value	P-Value	Result
Glucose* (mg/dl)	173.4 ± 67.7	87.8 ± 16.6	34.648	0.000	S
Melatonin ** (ng/l)	148.2 ± 27.925	181.9 ± 79.337	8.094	0.009	S
Asprosin* (ng/ml)	16.3 ± 0.9	9.9 ± 0.577	90.766	0.000	S
Cholesterol*(mg/dl)	196.4 ± 35.3	158.9 ± 26.1	42.067	0.000	S
LDL ***(mg/dl)	111.4 ± 36.3	88.8 ± 25.6	4.670	0.032	S
VLDL*(mg/dl)	41.5 ± 17.6	17.7 ± 6.3	244.507	0.000	S
Triglyceride*(mg/dl)	207.9 ± 87.1	88.5 ± 31.4	209.983	0.000	S
HDL (mg/dl)	43.4 ± 6.5	52.3 ± 10.4	2.870	0.092	N. S
MDA* (µmol/l)	7.1 ± 0.2	4.5 ± 0.8	118.096	0.000	S
SOD*	0.15 ± 0.03	0.18 ± 0.04	21.728	0.000	S
GST*** (µmol/l)	65.9 ± 32.8	76.9 ± 35.5	6.520	0.012	S
GSH* (µmol/l)	5.4 ± 1.7	10.2 ± 4.2	66.146	0.000	S
AOA* (mmol/l)	1.04 ± 0.46	1.9 ± 0.04	29.707	0.000	S

Mean and standard deviation (One- Way ANOVA). *P value is significant when P < 0.001. **P value is significant when P < 0.01.***P value is significant when p < 0.05.

3.3.1. Hormones and glucose serum levels

The results in Table 3 and Figure 1 showed the mean glucose level, which was (87.8 ± 16.6 mg/dl) in the healthy group when compared to its value in the MS group; that value was doubled to (173.4 ± 67.7 mg/dl) (P < 0.001). While, melatonin hormone mean levels were decreased in the patient group (148.2 ± 27.925 ng/l) compared to the healthy group 181.9 ± 79.337 ng/l. On the other hand, the asprosin hormone mean levels were significantly higher in MS patients (16.3 ± 0.9 ng/ml) compared to healthy group (9.9 ± 0.577 ng/ml).

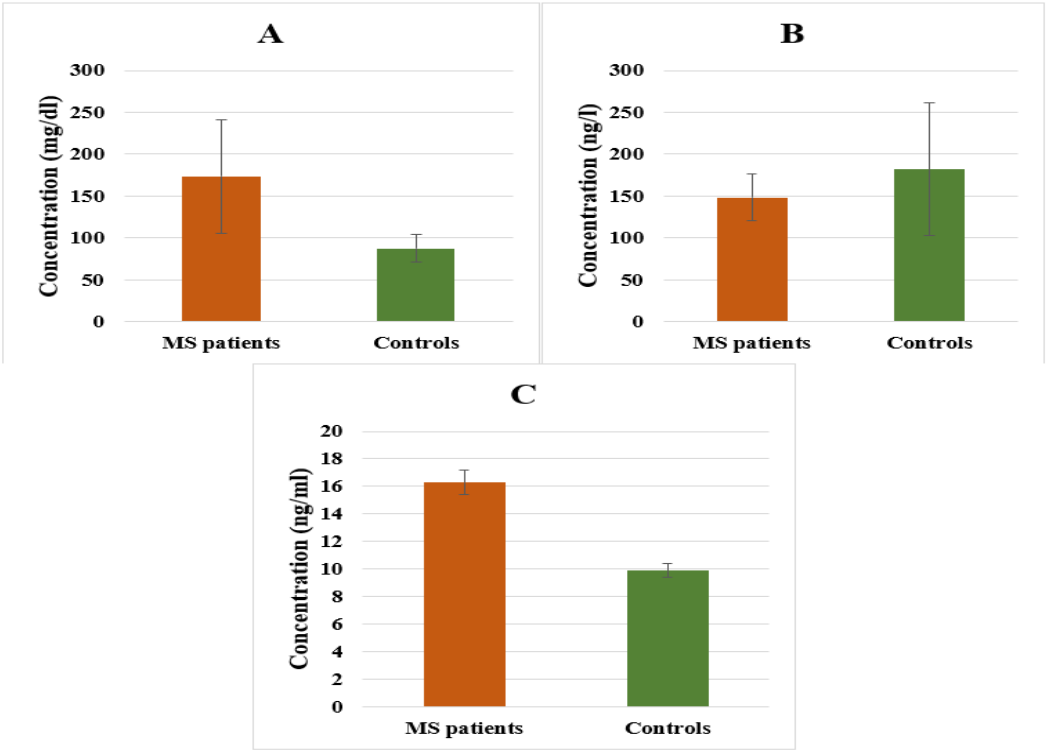

Fig.(1):- Glucose and hormone levels in MS patients in comparison to a healthy group. A: glucose, B: melatonin, C: asprosin.

4.1.1. Serum lipid profile components

The results in Table 3 and Figure 2 showed that serum cholesterol levels in MS patients was higher than in the control group (196.4 ± 35.3 mg/dl, 158.9 ± 26.1 mg/dl), with a significant difference between both groups (P-value < 0.001). Similarly, serum LDL levels in the MS group was significantly higher than in the healthy group, with a mean value of 111.4 ± 36.3 mg/dl and 88.8 ± 25.6 mg/dl, respectively, and a P-value of 0.032. On the same side, serum VLDL concentration was significantly higher in the patient group (41.5 ± 17.6 mg/dl) in comparison to the control group (17.7 ± 6.3 mg/dl) with a (P-value < 0.001). Furthermore, serum TG level in the MS group was also significantly higher than in the healthy group (207.9 mg/dl ± 87.1, 88.5 mg/dl ± 25.6), respectively. However, serum HDL levels were lower in the MS group (43.4 mg/dl ± 6.5) than in the healthy individuals (52.3 ± 10.4 mg/dl), with non-significant differences.

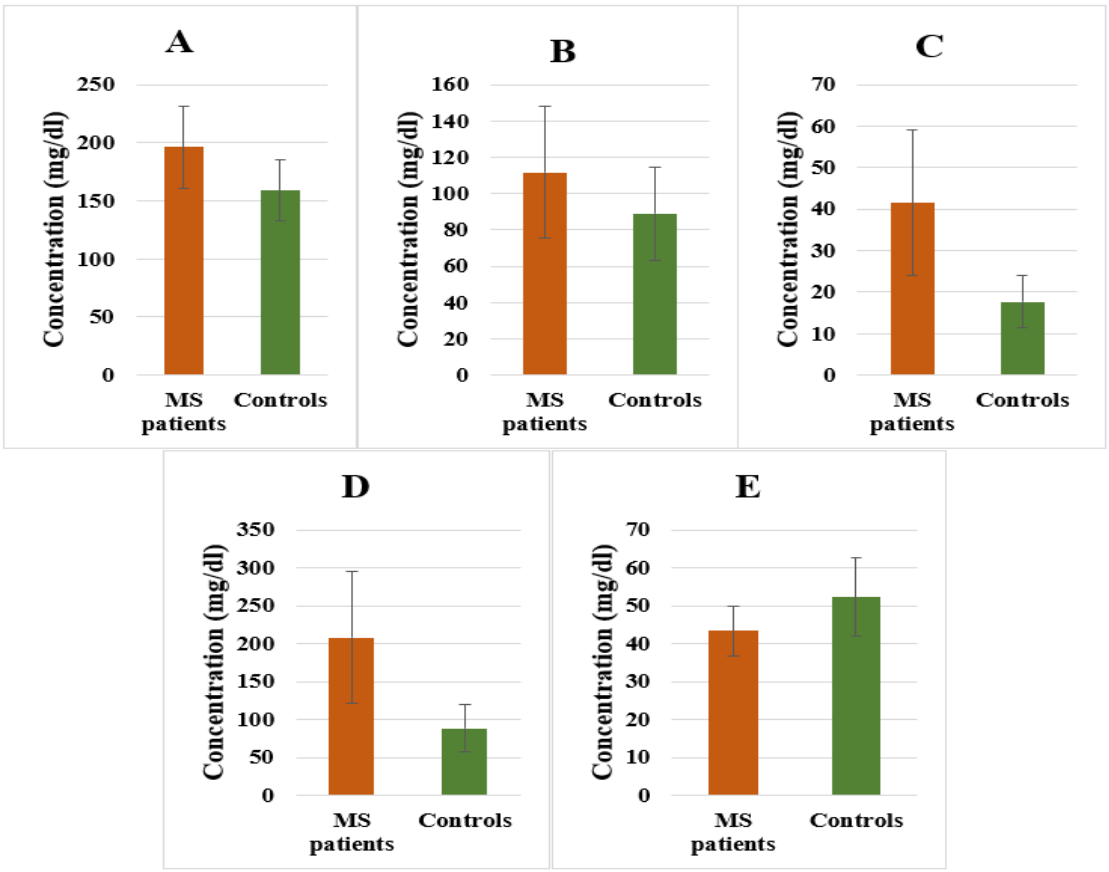

Fig. (2): - Lipid profile levels in MS patients in comparison to the healthy group.
A: cholesterol, B: LDL, C: VLDL, D: TG, E: HDL.

3.3.2. Oxidative stress parameters

The values of oxidative stress parameters were shown in Table 1 and Figure 3 for each participant group. The current study revealed that the pro-oxidant MDA levels were significantly higher in MS patients (7.1 ± 0.2 µmol/l) when compared to the healthy group (4.5 ± 0.8 µmol/l) (P < 0.001). While, serum SOD activity differed significantly in its mean (P < 0.001), its value fell to (0.15 ± 0.03) for the MS group compared to (0.18± 0.04) for the healthy group. Similarly, a significant difference (P < 0.05) was observed in serum GST activity since, it was decreased to (65.9 ± 32.8 µmol/l) in healthy group while, its value in the MS group was (76.9 ± 35.5 µmol/l) (P < 0.05). Furthermore, the study found a significant difference (P < 0.001) in GSH mean levels between the patient and healthy groups, where its levels decreased to (5.4 ± 1.7 µmol/l) in MS patients compared to the healthy group (10.2 ± 4.2 µmol/l). Moreover, serum AOA showed a significant difference (P < 0.001), with a mean value of (1.04 ± 0.46 mmol/l, 1.9 ± 0.04 mmol/l) decreasing in the MS group versus the healthy group.

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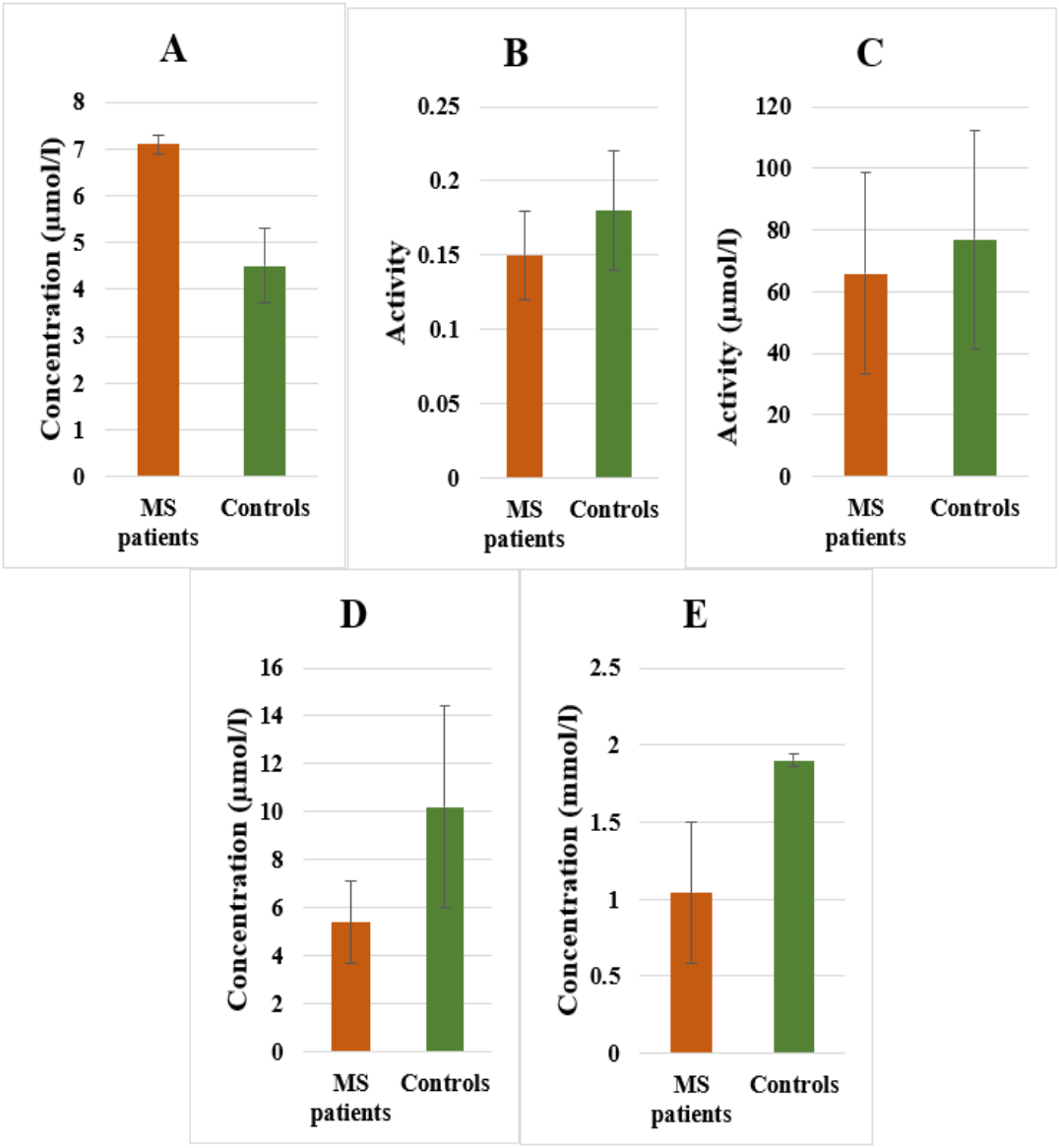

Fig.(3):- Serum levels of oxidative stress parameters in MS patients in comparison to a healthy group. A: MDA, B: SOD, C: GST, D: GSH, E: AOA.

3.4. Correlation of melatonin and asprosin with other biomarkers in MS patients

The present study used the Pearson correlation coefficient (R-value) to compare melatonin and asprosin hormones with other biomarkers, as seen in Table 4. The current study demonstrated that melatonin hormone has no significant correlation with glucose, HDL, LDL, VLDL, MDA, SOD, GST, GSH and AOA. While, it has a significant positive correlation with serum TG ($p < 0.05$) and a significant negative correlation with cholesterol ($p < 0.05$). On the other hand, asprosin showed a significant negative correlation with GSH ($P < 0.05$). While, the correlation of asprosin with other parameters was not significant.

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Table(4):- Correlation of melatonin and asprosin hormones with other parameters.

Variables	Melatonin	P-value	Result	Asprosin	P-value	Result
Glucose	0.131	0.121	N. S	-0.087	0.304	N. S
Cholesterol	-0.213*	0.044	S	0.017	0.843	N. S
HDL	0.009	0.619	N. S	-0.021	0.802	N. S
LDL	0.088	0.296	N. S	0.056	0.506	N. S
VLDL	0.132	0.117	N. S	-0.059	0.489	N. S
TG	0.470*	0.027	S	0.073	0.388	N. S
MDA	0.131	0.121	N. S	-0.087	0.304	N. S
SOD	-0.010	0.902	N. S	-0.068	0.424	N. S
GST	-0.282	0.059	N. S	-0.225	0.136	N. S
GSH	-0.033	0.693	N. S	-0.169*	0.044	S
AOA	0.112	0.186	N. S	0.013	0.881	N. S

* P value is significant when $P < 0.05$.

3.5. ROC curve analysis of serum parameters in patients with MS

The ROC curve analysis was used as useful tool for evaluating the performance of diagnostic tests and more generally for evaluating the accuracy of statistical model. In the current study, ROC was applied for all parameters, including hormones (melatonin and asprosin), glucose, lipid profile, and oxidative stress parameters (MDA, SOD, GST, GSH and AOA). However, only five parameters were successful in this diagnostic analysis (Table 5). Since, asprosin hormone has been considered a potential biomarker for MS disease with an area under the curve (AUC) of 0.967, a standard error (S.E) of 0.016, a 95% confidence interval (CI) of 0.935-0.999, and a cutoff value of (15.27 ng/ml) (Figure 4A). Similarly, glucose can be taken into consideration as a diagnostic biomarker in this disease with an AUC of (1.000), a S.E. of (0.001), a 95% CI of (0.099 to 1.001), and a cutoff value of (108.5 mg/dl) (Figure 4B). Furthermore, VLDL and triglyceride have a strong possibility of being MS biomarkers with AUC values equal to 0.960, S.E. values of (0.021), 95% CIs of (0.918–1.002), and cutoff values of (160 mg/dl and 32 mg/dl, respectively (Figures 4C and D). MDA can also be used as a MS biomarker, with an AUC of (1.000), a S.E of (0.000), a 95% CI of (1.000), and a cutoff value of (6.335 μmol/l) (Figure 4E).

Table (5): -ROC curve analysis of the serum levels of different parameters in patients of MS

Variable	AUC	S. E	95% CI	Result	Cutoff value
Asprosin	0.967	0.016	0.935-0.999	Excellent	15.27 ng/ml
Glucose	1.000	0.001	0.999-1.001	Excellent	108.5 mg/dl
VLDL	0.960	0.021	0.918-1.002	Excellent	32 mg/dl
TG	0.960	0.021	0.918-1.002	Excellent	160 mg/dl
MDA	1.000	0.000	1.000-1.000	Excellent	6.335 μmol/l

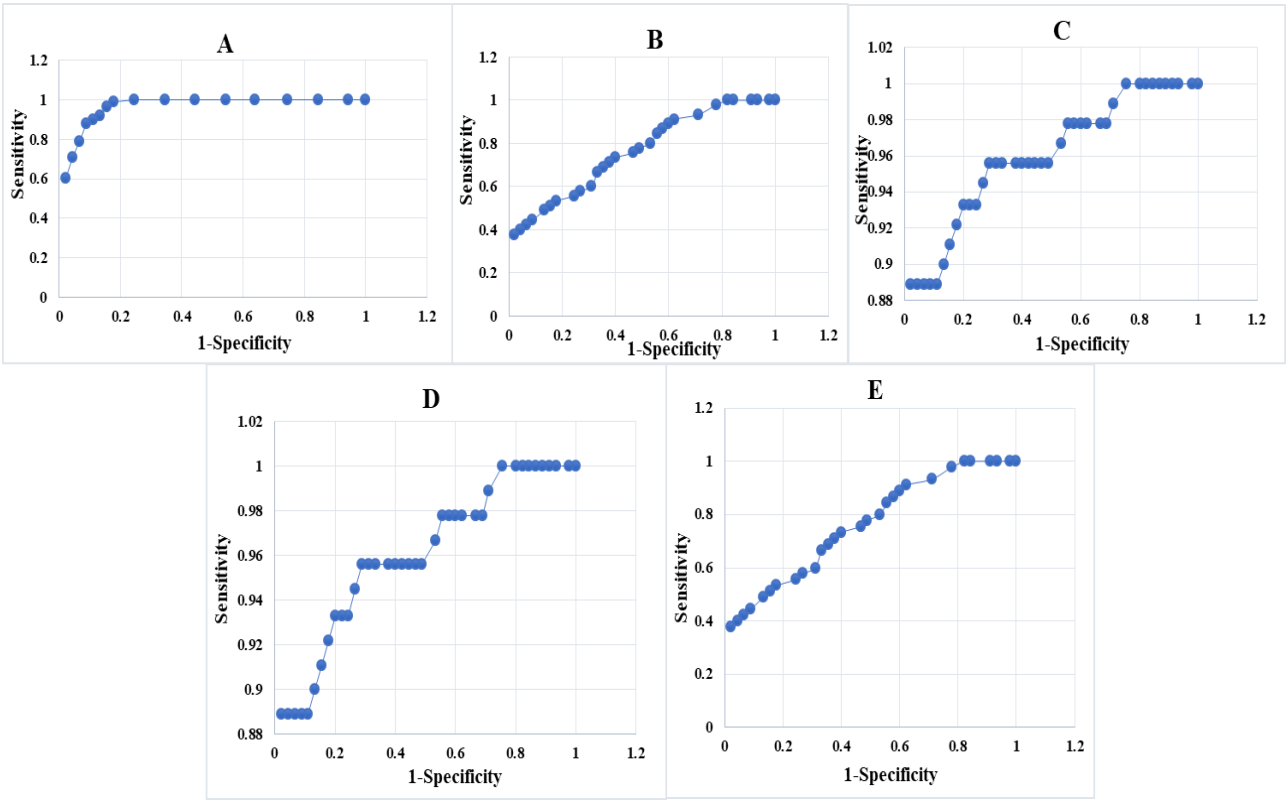

Fig.(4):- ROC curve analysis of asprosin, glucose, VLDL, TG and MDA. A: asprosin, B: glucose, C: VLDL, D: TG, E: MDA.

1. DISCUSSION

Metabolic syndrome is a group of health problems, such as visceral adiposity, insulin resistance, high TG, low HDL levels, and high blood pressure that raises the risk of T2DM and atherosclerotic cardiovascular disease. MS is caused by both genetic and environmental factors that lead to oxidative stress, cellular dysfunction, and systemic inflammation, which are mainly responsible for the pathophysiological pathway (Carresi *et al*., 2020). The current work observed valuable variations in hormone levels as well as sugar, lipid profile and oxidative stress biomarkers. Furthermore, correlation of hormones with other parameters and the evaluation of diagnostic tests efficiency (ROC analysis) were also conducted.

4.1. Hormones level investigation

4.1.1. Melatonin levels in participants

In the current study, morning melatonin levels were considerably lower in cases of metabolic syndrome compared with normal subjects, respectively (148.2 ± 27.925 ng/l vs.181.9 ± 79.337 ng/l, p = 0.009). This hormone decreased with aging, as the mean age in the current study was (55.7 ± 8.1) in MS patients versus (40.3 ± 9.3) in normal subjects. Furthermore, it has been established that the level of melatonin decreases in diseases associated with insulin resistance such as T2DM, which is one of the fundamental criteria in MS patients for the current work (Daniel and Daniel, 2017). Multiple studies showed also that melatonin may also influence diabetes not only by regulating insulin secretion but also by protecting against ROS since pancreatic β-cells are very susceptible to oxidative stress because they possess only low- anti-oxidative capacity (Gürpinar *et al.*, 2012). Furthermore, in Rao *et al.*, study melatonin was showed to decrease oxidative stress in streptozotocin-induced diabetic rats (Rao *et al*., 2002).

4.1.2. Asprosin levels in participants

Asprosin a novel secreted adipokine induced by fasting and targets the liver, promoting hepatic glucose release through the G protein–cyclic adenosine monophosphate–protein kinase A pathway (Romere *et al.*, 2016). The results of the current study showed that asprosin hormone levels were increased in MS patients compared to the healthy group (16.3 ± 0.9 ng/ml, versus 9.9 ± 0.577 ng/ml) (P < 0.001). The association between asprosin with T2DM and IR has been

also investigated in some studies (Wang *et al*., 2018). Asprosin is elevated in patients with insulin resistance, impaired glucose regulation, T2DM and obesity compared with those with the normal glucose regulation group and positively correlated with HOMA-IR (Naiemian *et al*., 2020). Further, hyperlipidemic conditions have been also associated to release of asprosin by pancreatic cells, which triggers pathways that lead to insulin resistance (Alsajri, 2022).

4.1.3. Glucose and lipid profile levels in participants

In the current study the MS patients have high glucose level compared with healthy group, as shown in Table 3 and Figure 1A, there is significant difference $p < 0.001$ in fasting blood sugar levels (173.4 ± 67.7 mg/dl and 87.8 ± 16.6 mg/dl) as previously described by the studies of (Mandal *et al*., 2019; Galicia-Garcia *et al*., 2020), which conducted on T2DM patients. Analysis of serum levels of total cholesterol, LDL and triglyceride between the participant groups showed a statistically significant difference ($p < 0.05$), which included higher levels of these particles in MS patients compared to the healthy group. Similarly, VLDL levels were higher in the patient group than in the healthy group, with a significant difference ($p < 0.001$). While, serum HDL showed a difference between the two groups, which is a lower level for the patient group in comparison to healthy individuals but not significantly ($P = 0.092$). The increased levels of total cholesterol, VLDL, LDL and TG with decreased levels of HDL are markers of dyslipidemia in MS patients. In these patients, free fatty acid (FFA) levels are elevated due to adipose tissue's inability to incorporate it into TG (Ginsberg, H. N., & Huang, 2000). As well as, insulin resistance in MS patients causes reduced retention of free fatty acids by the adipocytes. Both of these abnormalities lead to increased flux of FFA back to the liver. Increased flux of FFA from the periphery to the liver in the insulin resistance state stimulates hepatic TG synthesis, which in turn promotes the assembly and secretion of TG-containing VLDL (Gorter et al., 2004).

4.1.4. Oxidative stress status

One of the defects in MS and its associated diseases is the excess of oxidative stress in the cells (Shrestha *et al*., 2010). In the present study, we have observed that MDA levels, a lipid peroxidation by- product were elevated significantly in MS patients (7.1 ± 0.2 μmol/l) in comparison with healthy subjects (4.5 ± 0.8 μmol/l) ($P < 0.001$). MS patient's serum MDA levels may rise due to increased polyunsaturated fatty acid oxidation in the cellular membrane (Das, 2002). Several studies demonstrated also the association of obesity and diabetes with lipid peroxidation by-products, and the role of aldehydes in impairment of insulin function and signaling was recently pointed out (Pillon, N. J., & Soulage, 2012).

4.1.6. Antioxidant status

The results of the present study suggest that the presence of MS exacerbates oxidative stress in T2DM patients who achieve MS criteria. The serum level of SOD was lower in the MS group than in healthy group (0.15 ± 0.03, 0.18 ± 0.04) ($P < 0.001$). The decrease in SOD activity can be attributable to a rise in endogenous ROS generation, as indicated by an increase in MDA and ROS byproducts. Furthermore, Liu study demonstrated that there is an independent association between decreased SOD activity and MS as well as possible mediating effect of impaired insulin sensitivity and β-cell dysfunction on the relationship between decreased SOD activity and MS (Liu *et al*., 2020).

Similarly, regarding to serum levels of GST, the current study showed that GST levels were lower in the MS patient individuals (65.9 ± 32.8 μmol/l, 76.9 ± 35.5 μmol/l) ($P < 0.05$). The decrease in the GST activity in serum of MS patients may led to an imbalance between its protecting effects and the damaging effects of the free radicals, hence the increased oxidative stress (Roberts *et al.*, 2006).

Besides, non-enzymatic antioxidants were decreased in the patient group compared to the healthy group, such as GSH levels (5.4 ± 1.7 μmol/l, 10.2 ± 4.2 μmol/l). Consequently, levels of GSH decreased as a result of increased oxidative stress in MS patients as demonstrated in the current study. Furthermore, decreased GSH levels may be due to the increased lipid peroxidation in MS patients because GSH serves as a substrate for GST and GP-X during the detoxification of lipid peroxides (Nourazarian, A. R., Kangari, P., & Salmaninejad, 2014). It catalyzes the decomposition of ROS and prevents its accumulation. Regarding plasma total antioxidant activity, in our study, MS patients had significantly lower serum total antioxidant activity level (1.04 ± 0.46 mmol/l) compared with healthy participants (1.9 ± 0.04

mmol/l), as a consequence of metabolic disorders, particularly, dyslipidemia, insulin resistance and oxidative stress. Furthermore, reduced overall antioxidant capacity in metabolic syndrome individuals can be attributed to increased oxidative stress due to endogenous antioxidant system deficiency (Barylski *et al*., 2009).

4.2. Correlation of hormones with other parameters

The correlation of melatonin and asprosin were analyzed with glucose, lipid profile and oxidative stress parameters (MDA, SOD, GST, GSH and AOA).

3.2.1. Correlation of melatonin with other parameters

Depending on the current study, melatonin has a significant positive correlation with TG with a R-value of 0.470 and a P-value of 0.027 in MS patients. In diabetic patients, melatonin is reported to reduce plasma levels of total cholesterol, LDL, and TG and increase HDL (Al-Mahbashy, 2006). Moreover, Tamura study showed that melatonin administration to peri- and postmenopausal women increased their plasma HDL without influencing total cholesterol levels (Tamura *et al.*, 2008). Multiple studies reported that melatonin significantly increases HDL level and decrease TG and LDL level in addition to increased cholesterol catabolism (Koziróg *et al*., 2011).

Our findings are different from those studies because theirs are based on using melatonin as a treatment and then measuring its effects on T2DM, peri- and postmenopausal women, and MS patients, while our study measured the natural morning melatonin levels in MS further than using it as a treatment. Furthermore, previous studies did not establish a link between melatonin and the TG under consideration. However, Zhang *et al*. study demonstrated that there is no significant correlation between melatonin and TG in T2DM patients (J. Zhang *et al*., 2021). Similarly, the Sandyk and Awerbuch study finds that there is no association between melatonin and TG but in patients with multiple sclerosis, which is a different disease from ours (Sandyk, R., & Awerbuch, 1994).

However, these contradictions may be due to the dyslipidemia medication given to MS patients in the current study. Furthermore, the positive correlation between melatonin and TG may be due to differences in the severity of hypertriglyceridemia from patient to patient. Besides, in the current study, morning melatonin was measured, not nocturnal; this can affect the results because, as known, melatonin synthesis is closely linked to the light-dark cycle in humans as well as in other mammals, whether nocturnal or diurnal. Since this "hormone of darkness" is primarily synthesized in the pineal gland during obscurity and its release is inhibited by light (Niles *et al.*, 1999). As a result, its hormonal effects, which directly depend on the circadian and seasonal characteristics of pineal synthesis and secretion, which are dependent on their daily repetition, daily duration of nocturnal signal, and seasonal direction of changing (increasing or decreasing period of synthesis), cannot be deduced and interpreted exclusively, as is usually done for other classic hormones (Cipolla-Neto, J., & Amaral, 2018).

On the other hand, this hormone has a significant negative correlation with cholesterol (R-value = -0.213) ($P < 0.05$). This result agreed with that of Sandyk *et al*., who predicted that there is an inverse correlation between serum cholesterol and melatonin levels (Sandyk, R., & Awerbuch, 1994). Melatonin was shown to reduce serum cholesterol levels in mammalian species (Hoyos *et al.*, 2000). Furthermore, Sack *et al*., reported that in rats pinealectomy produced elevation of blood cholesterol levels suggesting that the pineal gland, via the mediation of melatonin, exerts a cholesterol lowering effect. This observation may explain the increased incidence of hypercholesterolemia in elderly subjects as melatonin secretion declines with advancing age (Altun, A., & Ugur-Altun, 2007). This implies that melatonin is an efficient cholesterol stabilizer regardless of the cause of hypercholesterolemia (diet, metabolic disorders associated with hormonal impairment, side effects of pharmacotherapy, etc.), in contrast to the association of melatonin with hypertriglyceridemia, which has contradictory results as clearly explained before (Karolczak & Watala, 2019).

Regarding to other parameters such as MDA there is no correlation with melatonin. Similarly, in relation to the correlation of melatonin with SOD, the current study showed that there is no association between melatonin and SOD. Considering these findings, it is apparent that melatonin may have a variety of indirect actions to reduce free radical generation and therefore avert oxidative damage (R. Reiter et al., 1999). These findings may explain the reduced level of

melatonin in MS and T2DM patients with reduction of antioxidants. Other parameters, such as glucose, HDL, LDL, VLDL, GST, GSH, and AOA, showed no significant correlation with melatonin.

4.2.2. Correlation of asprosin with other parameters

In the present study, the association of asprosin with other parameters was also investigated. In the current study, the fasting serum asprosin level was not associated with glucose levels both in MS patients and healthy individuals. However, Wang *et al.*, study indicates that the serum asprosin levels were high correlated with glucose metabolism and HbA1c (Wang *et al*., 2018). The conflicting results might be due to the different participants in the present study, as we included previously diagnosed T2DM patients and newly diagnosed T2DM patients. Zhang *et al*., speculated that the dysregulation of asprosin secretion by white adipose tissue might occur in T2DM patients, which leads to pathologically increased asprosin concentrations in those patients (L. Zhang *et al*., 2019). We doubt that with the development of T2DM, this dysregulation might be worse and even affect the response of asprosin to glucose fluctuation, which leads to the impaired relationship between fasting circulating asprosin level and glucose levels in previously diagnosed T2DM patients (L. Zhang *et al*., 2019b).

Asprosin hormone has no significant association with GST in MS. This hormone has significant negative correlation with GSH. This significant association between asprosin and GSH in the current study may be due to the strong association between MS and oxidative stress which lead to decreased antioxidants in those patients. On the other hand, asprosin has no significant relationship with cholesterol, HDL, LDL, VLDL, TG, MDA, SOD and AOA.

4.3. ROC analysis in patients with MS

In the current study, the ROC curve analysis is applied for all parameters, but only serum asprosin, glucose, VLDL, TG, and MDA exhibit as potential biomarkers for MS disease as described in (Table 5). ROC curve is the plotting of (sensitivity) versus (1- specificity) and used to analyze the effectiveness of a diagnostic test. The area under ROC curve is used to define test´s accuracy, the closer to one, the better the test. Regarding cutoffs of analyzed parameters using a ROC curve, they can be determined by summing up specificity and sensitivity. As shown in (Table 5), asprosin with an AUC = 0.967, which is an excellent result and means that this parameter can be used to diagnose MS disease. The cutoff for this biomarker is 15.27 ng/ml, which means that cases with values equal or higher than this value are diagnostic for MS (Figure 4, A). Similarly, glucose and MDA have the same AUC value, which is equal to one, and cutoffs of 108.5 mg/dl and 6.335 µmol/l respectively (Figure 4, B and E, respectively). Besides, both VLDL and TG parameters, which have equal AUC values of 0.960 with different cutoffs of 32 and 160 mg/dl, respectively, can be used to diagnose MS disease (Figure 4, C and D, respectively).

CONCLUSION

In the current study, it was demonstrated that MDA levels increased. While, the levels of antioxidant enzymes such as SOD and GST were decreased. As well, levels of non-enzymatic antioxidants such as GSH and AOA were decreased also. Melatonin hormone is decreased in diseases related to insulin resistance, such as MS. This hormone has a negative association with cholesterol but a positive correlation with triglycerides. Regarding asprosin, there is an increase in its levels in MS patients. This hormone has no correlation with the lipid profile, while it has a negative correlation with GSH. ROC curve analysis demonstrated that asprosine can be considered a biochemical marker to diagnose MS disease. Furthermore, glucose, VLDL, TG and MDA demonstrated to be potential diagnostic biomarkers for MS patients.

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