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Evaluation of Melatonin Hormone and Nitric oxide Levels in Non-alcoholic Fatty Liver Patients in Relation to Obesity and Oxidative Stress

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Abstract:

Objective: To assess the levels of melatonin hormone and oxidative stress markers(TBARS, MDA and GSH) in NAFLD patients and obese subjects and compared with healthy group.

Material and Methods: A total of (63) volunteers inserted in study, divided to three groups :group A :(21) obese patients with NAFLD, group B: (21) obese subjects and group C: (21) healthy subjects. Melatonin hormone, TBARS, MDA and GSH were measured.

Results: The current study recorded that there was a significant decrease in melatonin hormone in both group A & B when compare to control ($p > 0.05$). The mean of GSH also significantly decrease in NAFLD and obese patients when compared to healthy subject ($p > 0.05$). Both TBARS and NO were significantly rise in group A among to other groups($p>0.05$), while MDA not show and difference between studied groups

Conclusion: Melatonin hormone effected by both NAFLD and obesity.

Key words: Melatonin, TBARS, MDA, GSH, obesity

INTRODUCTION:

Non-alcoholic fatty liver disease (NAFLD) is one of the most famouscauses of chronic liver impairment. It is distinguished by asymptomatic increase in serum enzymatic liver levels but not used excessive alcohol intake or suffered from other chronic liver diseases. [1]. NAFLD is most common in obese patientswith type 2 diabetes mellitus and insulin resistance [2]. Recent estimations suggest that about 20%–25% of the populations are obese. The percentage of NAFLD in the obese subjects can be about 95% depending on the degree of the obesity. As the prevalence of obesity increases, the prevalence of NAFLD will increase, making it the most common cause of chronic liver disease [3].

NAFLD encompasses a range of clinic-pathologic exist, all of which involve fat accumulation in the liver parenchyma. Nonalcoholic steatohepatitis (NASH) is a subtype of NAFLD association with hepatocyte necrosis with or without fibrosis; it develop to chronic liver disease and cirrhosis [4]. In NAFLD patients appear that fat diffusion inside liver tissue and secreted hepatic steatosis and take place in the absence of alcohol intake. NAFLD is a serious cause of concern because of its direct association with diabetes, obesity and hyperlipidemia [5]. Simple steatosis is shown around the liver and then leads to the proliferation of fat cells into hepatocytes causing nonalcoholic steatohepatitis (NASH), which develops into cirrhosis of the liver [6]. Obesity is a major risk factor lead to the development of NAFLD [7]. Fat-rich food is the (first hit) causing NAFLD and inflammation is the (second hit) leading to convert NAFLD to NASH [8]. Currently, there are no direct treatments for liver in the case of NAFLD but all therapies targeting to reducethe risk of NAFLD [9].

Melatonin is a hormone produced by the pineal gland. The secretion of melatonin is controlled by light intensity [10]. Melatonin play an important role inmultiple biological functions and acts as an anti-oxidant [11]. It has act as anti-inflammatory molecules [12], regulates heart rate [13] and support the human immunity [14]. It also has anti-cancer properties [13]. Recent study suggested that melatonin also regulated the lipid metabolism [16], decrease insulin resistance [17], control glucose levels [18], and decrease body weight [19]. This study was designed to evaluate the effect of NAFLDon melatoninlevels and oxidative stress markers.

MATERIAL AND METHODS:

The study was conducted at the Center of Liver and Gastrointestinal Diseases Baghdad Teaching Hospital. The participants included 63 volunteers divided into three groups: the first group (21) volunteers, obese patients (BMI > 30) with NAFLD, and the second group (21), obese volunteers (BMI > 30) without other disease. Third (21) volunteers are control group.

Blood samples were collected from fasting patients in the morning (8:00 am - 10:00 am). The time of sample taken was affected on our results. A total of (5 ml) was draw from venous and transfer into the tubes and left for (15 min.) and then placed in the centrifuge for (10 min) and then separated the serum and stored in frozen degree (-20) until the day of the analysis.

Measurements:

Serum melatonin hormone and oxidative stress marker (glutathione (GSH), Thiobarbituric acid reactive substances (TBARS), Malondialdehyde (MDA)) and nitric oxide (NO) were measured.

Melatonin was measured quantitatively by using ELISA kit (CUSABIO, China) . GSH levels were measured according to Julins (1994) method (20). TBARS and MDA levels were determined spectrophotometrically according to Alam Zeb (2016) methods (21).

RESULTS:

The current study shows that there were a significant difference in both ALT and AST in group A when compared to other studied groups. Alkaline phosphatase show no difference among groups.

Table (1)

	No	ALT	AST	ALP
Group A	21	47.14±22.52 a	49.65±15.39a	95.1±22.41 a
Group B	21	28.61±7.90 b	32.54±8.66 b	96.19±24.64a
Group C	21	27.80±6.35 b	34.32±7.41b	96.71±25.29a
P value	63	0.002	0.004	0.093
LSD		3.4136	11.226	0.447

Table (2)

	No	Melatonine	GSH	TBARS	MDA	NO
Group A	21	12.24±5.45b	65.75±17.59a	7.61±0.58a	5.53±0.94 a	122.76±59.78a
Group B	21	12.61±4.90b	73.34±18.53b	6.90±0.64b	5.99±1.04a	95.42±38.67b
Group C	21	17.80±6.15a	80.62±18.41c	5.51±0.90c	5.91±0.86a	79.76±21.57b
P value	63	0.002	0.04	0.003	0.25	0.001
LSD		3.4136	11.226	0.447	0.5901	26.541

The current study recorded that there was a significant decrease in melatonin hormone in both group A & B when compare to control ($p > 0.05$). The mean of GSH also significantly decrease in NAFLD and obese patients when compared to healthy subject ($p > 0.05$). Both TBARS and NO were significantly rise in group A among to other groups($p>0.05$), while MDA not show and difference between studied groups, table (2).

DISCUSSION:

Several studies have evaluated that melatonin treatment decreased body weight in animal and human [22,23]. A recent study recorded that melatonin has action on inflammatory process, fat degardation, and bioenergic reaction in animals[24]. Current study found that melatonin significantly decreased body weight in pateints when compared to control group.Melatonin act as controlling factor in glucose metabolism.Two types of melatonin receptors calledMT1 and MT2,which called G-protein-coupled receptors[25]. Eachreceptors are located in the islet of Langerhans and are participated in the controlling of glucagon production from α-cells and insulin releasing from β-cells [25]. Other study suggested thatstimulating of MT1 or MT2 act as an inhibitor for second messenger cAMP or cGMPaccompanied by decrease insulin production [26, 27].

This study had manylimitations. Firstly, it is not recorded that the food intake for volunteers , which was one of the limitations of the study.

Decreased in body weight could lead to a **decline** of adiposity and fat accumulation, which lead to decrease in hepatic steatosis. A decrement of steatosis, in turn, down-controlled inflammatory markers. Recent study suggest that melatonin treatment was cause weight reduction, which could be elucidated the melatonin action on body mass, or other **advantageous** actions. However, melatonin as therapeuticagent in NAFLD patients as a result of both anti-inflammation and anti-obesity effects[28].

Oxidative stress defined as inequality between defense factors which include superoxide dismutase (SOD) reduced glutathione (GSH), and reactive oxygen species

(ROS) include superoxide. Reduction of antioxidants molecules as glutathione, , β-carotene, or vitamin C and E or rise increasing of ROS in the liver may occur in nonalcoholic steatohepatitis (NASH).The contribution of serum stress parameters in liver cirrhosis is unclearfor hepatocellular carcinoma.Oxidative stress markers (serum levels of 8-hydroxy-2-deoxyguanosine 8-OHdG or 4-hydroxy-2-nonenal 4HNE), have been recorded to be elevated and antioxidants (superoxide dismutase) decline in NASH patients; however, no significant difference in serum 8-OHdG levels between NASH and FL was reported [29,30]

Nitric oxide (NO), which has short half-life free radical elaborate in different biological functions, consist of vascular balance, neurotransmission and host protection, is result from L-arginine by the action of nitric oxide synthase (NOS) enzyme. There are three isoforms of NOS (neuronal, endothelial, and inducible NOS) have been known. However, when there is increase in the NO production which lead to inducethe inflammation-associated tissue damage, which reported a major role in an inducibleNOS-mediated NO secretion .Garcia-Monzon and colleges suggest that the pathogenesis of NASH, NO has a cytotoxicity effect by effected on superoxide anions to produce peroxynitrite, a strong oxidant that activate nitration of tyrosine to produced nitrotyrosine[31]. The NOS was found to be significantly elevated in the advance form of NASH; NO has been suggested to have a major role in the process of fibrosis in NASH patient[32].

CONCLUSION:

This study concluded that melatonin level decrease in NAFLD patient and Obese subject when compared to healthy subjects. All oxidative stress markers effected be both obesity and NAFLD disease except MDA.

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