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RESEARCH ARTICLE

Lipid peroxidation and antioxidant status in overt and subclinical hypothyroidism

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Background: Oxidative stress, caused by an imbalance in reactive oxygen species produced during normal cell metabolism and/or deficiency of scavenger antioxidant defense, has been implicated in the pathology of several human diseases, including endocrine diseases of thyroid gland.

Aim of the work: The aim of this study is to evaluate the serum levels of malondialdehyde (MDA), a product of lipid peroxidation, and the antioxidant enzymatic activities of superoxide dismutase (SOD) and catalase (CAT) as well as the total antioxidant capacity (TAC) in overt and subclinical hypothyroidism.

Subjects and methods: Forty eight newly diagnosed hypothyroid patients, 24 patients of them have subclinical hypothyroidism (SHT) while the other 24 patients have overt hypothyroidism (OHT), as well as 24 healthy euthyroid control subjects were enrolled in this cross sectional study. Laboratory investigations including lipid profile, thyroid functions as well as levels of serum MDA, SOD, catalase and TAC were estimated.

Results: Both OHT and SHT patients had significantly higher serum TSH, total cholesterol (TC), triglycerides (TGs), LDL-C, catalase, SOD and MDA levels as well as significantly lower serum HDL-C and TAC levels when compared to euthyroid control subjects.

The correlation study revealed that serum TSH levels were significantly positively correlated with TC, LDL-C and TGs serum levels and significantly negatively correlated with HDL-C levels. Moreover, serum MDA, CAT and SOD levels showed significantly positive correlation with TSH, TC, LDL-C and TGs serum levels and a significant negative correlation with free T4, free T3 and HDL-C serum levels. Furthermore, TAC levels showed a significant positive correlation with free T4, free T3 and HDL-C serum levels and a negative correlation with TSH, TC, TGs and LDL-C serum levels.

Conclusions: The present study demonstrated that patients with both OHT and SHT showed increased lipid peroxidation and oxidative stress which was evidenced by the increased levels of MDA, a lipid peroxidation product, and the induction of SOD and CAT antioxidant enzymatic activities. Increased MDA and other free radical production could overload the antioxidant system resulting in consumption and severe depletion of antioxidants with concomitant reduction in total antioxidant capacity.

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Introduction:-

Hypothyroidism is a clinical entity resulting from the deficiency of thyroid hormones. It is mainly of two types (i) overt hypothyroidism (OHT) which is characterized biochemically by an increase in serum thyroid stimulating hormone (TSH) concentration and a decrease in serum triiodothyronine (T3) and thyroxine (T4) concentrations below

normal levels(ii) subclinical hypothyroidism (SHT) which is a common thyroid disorder characterized by mild elevation in TSH while T3 and T4 are within normal levels [1].

A variety of free radicals such as reactive oxygen species (ROS) are generated during cellular metabolism and their levels are kept in equilibrium with the antioxidant defense systems that scavenge and minimize their formation. Oxidative stress (OXS) is a condition in which the equilibrium between free radical production and their scavenging by antioxidants becomes perturbed favoring increased production of free radicals and/or diminished antioxidants. This leads to accumulation of free radicals or their products within the cells and damage of important biomolecules, such as DNA, proteins and lipids. OXS has been implicated in the pathology of several human diseases, including endocrine diseases of thyroid gland [2, 3]. Thyroid hormones regulate mitochondrial respiration and oxidative metabolism, and thus play an important role in free radical production [4]. In addition, thyroid hormones also regulate the synthesis and degradation of the antioxidant proteins, vitamins and enzymes [4, 5]. Therefore, variations in the thyroid hormone level may modulate in vivo cellular OXS [6].

Lipid peroxidation is a free radical or ROS-mediated oxidation of cell membrane lipids which results in the formation of toxic and reactive aldehyde metabolites called lipid peroxidation products such as malondialdehyde (MDA) and thiobarbituric acid reactive substances (TBARSs) [7]. Free radical-scavenging enzymes, such as superoxide dismutase (SOD) and catalase (CAT), are important intracellular antioxidant enzymes present in aerobic cells and forms the first line of cellular defense against oxidative injury, SOD catalyzes the dismutation of the superoxide anion radicals into hydrogen peroxide (H_2O_2) which is then deactivated to water (H_2O) by catalase or glutathione peroxidase (GPx) [8,9]. Serum total antioxidant capacity (TAC) represents a measurement of the combined activities of all antioxidants including enzymatic and non-enzymatic antioxidants in a biological sample and not just the antioxidant capacity of a single compound which may be more informative about the overall antioxidant status [10].

Research interest concerning OXS in hypothyroidism has been in focus in recent years. The available data concerning OXS and antioxidant capacity in hypothyroidism are controversial. Some authors suggested that tissues may be protected from oxidant damage because of the hypometabolic state and the slow rate of free-radical generation caused by the lowered thyroid hormone levels in hypothyroidism [5], whereas; other studies showed that oxidative stress was increased in both overt and subclinical hypothyroid disorders [3].

Therefore, the aim of this study was to evaluate the serum levels of malondialdehyde, a product of lipid peroxidation, and the antioxidant enzymatic activities of superoxide dismutase and catalase as well as the total antioxidant capacity in overt and subclinical hypothyroidism.

Subjects and methods:-

Forty eight newly diagnosed hypothyroid patients, 24 patients of them have subclinical hypothyroidism (SHT) while the other 24 patients have overt hypothyroidism (OHT), as well as 24 healthy euthyroid control subjects were enrolled in this cross sectional study. All patients were recruited from outpatient clinics of Endocrinology Unit of the Internal Medicine Department, Faculty of Medicine, Zagazig University, Egypt between November 2013 and October 2014. All enrolled subjects gave an informed consent for participation in this study and the study protocol was approved by the research ethical committee of Faculty of Medicine, Zagazig University, Egypt.

Hypothyroidism was classified to SHT or OHT according to American Association of Clinical Endocrinologists and The American Thyroid Association guidelines reported in 2012 (i.e. SHT is diagnosed by a serum TSH above the upper reference limit in combination with a normal free thyroxine (T4), while OHT is diagnosed by an elevated TSH, usually above 10 mIU/L, in combination with a subnormal free T4) [1]. Exclusion criteria to the study were patients taking lipid-lowering drugs, antioxidant vitamin supplements, acetylsalicylic acid, antihistamines, antihypertensives, hormone replacement therapy, smokers, alcoholics, diabetes mellitus, pregnant, and acute, chronic, or malignant diseases.

All enrolled personnel were submitted to history taking including past history of thyroid diseases and/or thyroidectomy as well as full clinical assessment including thorough clinical examination and blood pressure measurements. Body mass index (BMI) (kg/m^2) was calculated by dividing weight (kg) by height in squared meters (m^2). Laboratory tests were done in the Medical Biochemistry Department, Zagazig University, Egypt.

Blood sample collection:-

A 10-mL blood sample was taken from all studied personnel under complete aseptic conditions after 12-14 hours overnight fast. The blood sample was divided into 2 portions: 6 mL of whole blood was collected in sterile EDTA-containing tubes for assessment of plasma catalase activity and preparation of erythrocyte lysate for estimation of superoxide dismutase. The other part (4 mL) was centrifuged at 4000 rpm for 10 minutes and aliquots of serum samples were stored at -80°C until analysis. Serum samples were used for determination of lipid profile, thyroid function tests, MDA and TAC.

Biochemical measurements:-

Total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), triglycerides (TGs), high density lipoprotein cholesterol (HDL-C), albumin, total bilirubin, alanine aminotransferase (ALT) aspartate aminotransferase (AST), blood urea and serum creatinine were measured by routine enzymatic methods. Fasting blood glucose (FBG) was measured by a glucose oxidase procedure.

Malondialdehyde (MDA) Assay:-

Measurement of MDA level was done by a commercial chemical colorimetric assay kit according to manufacturer's protocol (Biodiagnostic, Egypt). MDA in the sample reacts with thiobarbituric acid reagent under acidic conditions at 95°C for 30 min to generate a pink colored product determined at 534 nm.

Catalase activity (CAT) assay:-

CAT activity was measured using a calorimetrically enzymatic assay kit at 510 nm (Biodiagnostic, Egypt). In this assay, catalase reacts with a known quantity of hydrogen peroxide and the reaction is stopped after 1 min with catalase inhibitor. In the presence of peroxidase, the remaining hydrogen peroxide reacts with 3,5-dichloro-2-hydroxybenzene sulfonic acid and 4-aminophenazone to form a chromophore with a color intensity inversely proportional to the amount of catalase in the sample.

Determination of SOD activity:-

SOD activities were measured according to manufacturer's protocol (Biodiagnostic, Egypt). This assay relies on the ability of the enzyme to inhibit the phenazinemetosulphate-mediated reduction of nitrobluetetrazolium dye. The change of absorbance is measured at 560 nm calorimetrically.

Total antioxidant capacity (TAC) assay:-

Serum TAC was measured using a commercial kit following the manufacturer's protocol (Biodiagnostic, Egypt) on the basis of the oxidation reduction colorimetric assay at a wavelength of 532 nm. The method is based on that the antioxidants in the sample eliminate a certain amount of the provided hydrogen peroxide. The remaining H₂O₂ is determined colorimetrically by an enzymatic reaction converting 3, 5-dichloro-2-hydroxyl benzenesulfonate to a colored product.

Thyroid profile assay:-

Thyroid profile was assessed by estimation of serum TSH, T₃, and fT₄ that were measured by chemiluminescent immunometric assay by COBAS E 411 Roche Hitachi Apparatus according to manufacturer's protocol (Roche Elecsys reagent kits, Roche Diagnostica, USA). Detection limits for TSH was 0.005–100 µIU/mL, fT₄ was 0.3-100 pmol/L, and T₃ was 0.4-50 pmol/L.

Statistical analysis:-

Continuous variables were checked for normality by using Shapiro-Wilk test. Continuous variables were expressed as the mean±SD, and the categorical variables were expressed as a number (percentage). Kruskal-Wallis H test was used to compare between more than two groups of independent non-normally distributed variables while one way ANOVA test was used for normally distributed variables. Percent of categorical variables were compared using the Chi-square (χ^2) test. Spearman's rank correlation coefficient was calculated to assess the relationship between various non-normally distributed variables while Pearson's correlation coefficient was calculated for normally distributed variables. All tests were two sided with $p < 0.05$ was considered statistically significant (S), $p < 0.01$ was considered highly statistically significant (HS), and $p > 0.05$ was considered non statistically significant (NS). All data were analyzed using SPSS 22.0 for windows (SPSS Inc., Chicago, IL, USA) & MedCalc 13 for windows (MedCalc Software bvba, Ostend, Belgium).

Results:-

The clinical characteristics and biochemical data of patients with SHT and OHT as well as euthyroid control subjects and comparison between the three groups were summarized in **Table 1**.

There was no significant difference in the mean values of age, male/female ratio, DBP, albumin, total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea and creatinine among the study groups.

The mean values of SBP, BMI and FBG levels were significantly elevated in OHT patients compared with SHT patients and euthyroid control subjects. Overt hypothyroid patients had significantly higher serum TSH, total cholesterol, triglycerides, LDL-C, catalase, SOD and MDA levels as well as significantly lower serum free T3, free T4, HDL-C and TAC levels when compared to subclinical hypothyroid patients and euthyroid control subjects.

Moreover, SHT patients had significantly higher serum TSH, TC, TGs, LDL-C, CAT, SOD and MDA levels as well as significantly lower serum TAC and HDL-C levels when compared to euthyroid control subjects. However, there was no significant difference between both groups in the mean values of serum free T3 and free T4 levels.

Correlations between thyroid hormones, lipid profile and oxidant/antioxidants profile in all studied subjects (N=72) were summarized in **Table 2 and 3**. The correlation study revealed that serum TSH level was significantly positively correlated with TC (Fig. 1A), LDL-C (Fig. 1B) and TGs serum levels and significantly negatively correlated with HDL-C levels. While, free T3 and free T4 serum levels showed a significant negative correlation with TC, TGs and LDL-C serum levels and a significant positive correlation with HDL-C levels. Moreover, MDA (Fig. 2), catalase and SOD levels showed significantly positive correlation with TSH (Fig. 2A), TC (Fig. 2C), LDL-C (Fig. 2D) and TGs serum levels and a significant negative correlation with free T4 (Fig. 2B), free T3 and HDL-C serum levels. Furthermore, TAC levels showed a significant positive correlation with free T4 (Fig. 3B), free T3 and HDL-C serum levels and a significant negative correlation with TSH (Fig. 3A), TC, TGs and LDL-C serum levels.

Table (1): Demographic, Clinical and biochemical characteristics of the studied groups

Variables	Euthyroid Control (n=24)	Subclinical hypothyroidism (n=24)	Overt hypothyroidism (n=24)	Test	P
Age (years)	41.33 ± 8.10	43.54 ± 5.05	42.62 ± 6.51	0.663	0.522
Sex					
Male	7 (29.2%)	8 (33.3%)	9 (37.5%)	0.375§	0.829
Female	17 (70.8%)	16 (66.7%)	15 (62.5%)		
BMI (Kg/m ²)	26.54 ± 2.58	26.75 ± 2.69	29.50 ± 3.18*#	8.143•	0.001
Systolic BP (mmHg)	110.62 ± 11.91	116.66 ± 11*	124.79 ± 7.86*#	11.199•	<0.001
Diastolic BP (mmHg)	74.79 ± 7.29	75.41 ± 10.20	80.20 ± 9.38	4.979‡	0.083
FBG (mg/dl)	81.91 ± 6.31	82.91 ± 6.27	86.79 ± 5.51*	7.442‡	0.024
Blood urea (mg/dl)	30.95 ± 6.18	29.29 ± 6.60	27.12 ± 2.96	2.932•	0.060
Serum creatinine (mg/dl)	0.84 ± 0.22	0.77 ± 0.19	0.78 ± 0.23	1.210‡	0.546
Serum ALT (mg/dl)	24.20 ± 6.94	20.79 ± 3.91	20.83 ± 5.45	3.853‡	0.146
Serum AST (mg/dl)	25.33 ± 6.43	23.91 ± 4.33	22.62 ± 4.48	2.378‡	0.305
Total bilirubin (mg/dl)	0.64 ± 0.23	0.67 ± 0.19	0.63 ± 0.26	0.837‡	0.658
Albumin (g/dl)	4.09 ± 0.42	4.03 ± 0.51	3.99 ± 0.53	1.128‡	0.569
Total cholesterol (mg/dl)	169.73 ± 11.37	204.75 ± 14.96*	223.83 ± 19.46*#	74.587•	<0.001
Triglycerides (mg/dl)	130.33 ± 8.65	162.66 ± 16.24*	183.16 ± 15.09*#	90.172•	<0.001
LDL-C (mg/dl)	91.00 ± 13.13	122.91 ± 16.27*	142.83 ± 23.11*#	50.663•	<0.001
HDL-C (mg/dl)	52.83 ± 5.29	49.29 ± 5.55*	43.58 ± 6.87*#	14.770•	<0.001
TSH (uIU/ml)	1.63 ± 0.63	11.55 ± 1.01*	56.40 ± 6.58*#	1368.861•	<0.001
Free T3 (pmol/L)	4.75 ± 1.11	4.41 ± 0.79	1.15 ± 0.46*#	136.673•	<0.001
Free T4 (pmol/L)	16.79 ± 2.40	16.17 ± 2.21	5.70 ± 1.15*#	232.301•	<0.001
MDA (nmol/ml)	0.82 ± 0.06	2.47 ± 0.30*	2.89 ± 0.16*#	685.379•	<0.001
Catalase (U/L)	12.30 ± 1.27	15.55 ± 1.15*	18.35 ± 2.25*#	81.852•	<0.001
SOD (U/L)	8.09 ± 1.02	9.67 ± 0.97*	10.69 ± 1.15*#	36.879•	<0.001
TAC (nmol/ml)	1.19 ± 0.19	0.86 ± 0.19*	0.64 ± 0.08*#	69.124•	<0.001

•One Way ANOVA test; ‡Kruskal-Wallis H test; § Chi-square test; p< 0.05 is significant.

* p<0.05 compared with the euthyroid control group, and # p<0.05 compared with the subclinical hypothyroid group

BMI: body mass index; BP: blood pressure; FBG: fasting blood glucose; ALT: alanine aminotransferase; AST: aspartate aminotransferase; LDL-C: low density lipoprotein-cholesterol; HDL-C: high density lipoprotein-cholesterol; TSH: thyroid stimulating hormone; T3: triiodothyronine; T4: thyroxine; MDA: Malondialdehyde; SOD: Superoxide dismutase; TAC: total antioxidant capacity.

Table (2): Correlation between thyroid function tests, lipid profile and oxidant/antioxidants profile

Variables	TSH (uIU/ml)		Free T3 (pmol/L)		Free T4 (pmol/L)	
	r	p	r	p	r	p
Total cholesterol (mg/dl)	+0.950	<0.001	-0.775	<0.001	-0.770	<0.001
Triglycerides (mg/dl)	+0.951	<0.001	-0.796	<0.001	-0.794	<0.001
LDL-C (mg/dl)	+0.916	<0.001	-0.760	<0.001	-0.748	<0.001
HDL-C (mg/dl)	-0.699	<0.001	+0.674	<0.001	+0.650	<0.001
Malondialdehyde (nmol/ml)	+0.954	<0.001	-0.795	<0.001	-0.783	<0.001
Catalase (U/L)	+0.959	<0.001	-0.781	<0.001	-0.778	<0.001
Superoxide dismutase (U/L)	+0.819	<0.001	-0.683	<0.001	-0.674	<0.001
TAC (nmol/ml)	-0.958	<0.001	+0.830	<0.001	+0.846	<0.001

r: correlation coefficient; p<0.05 is significant.

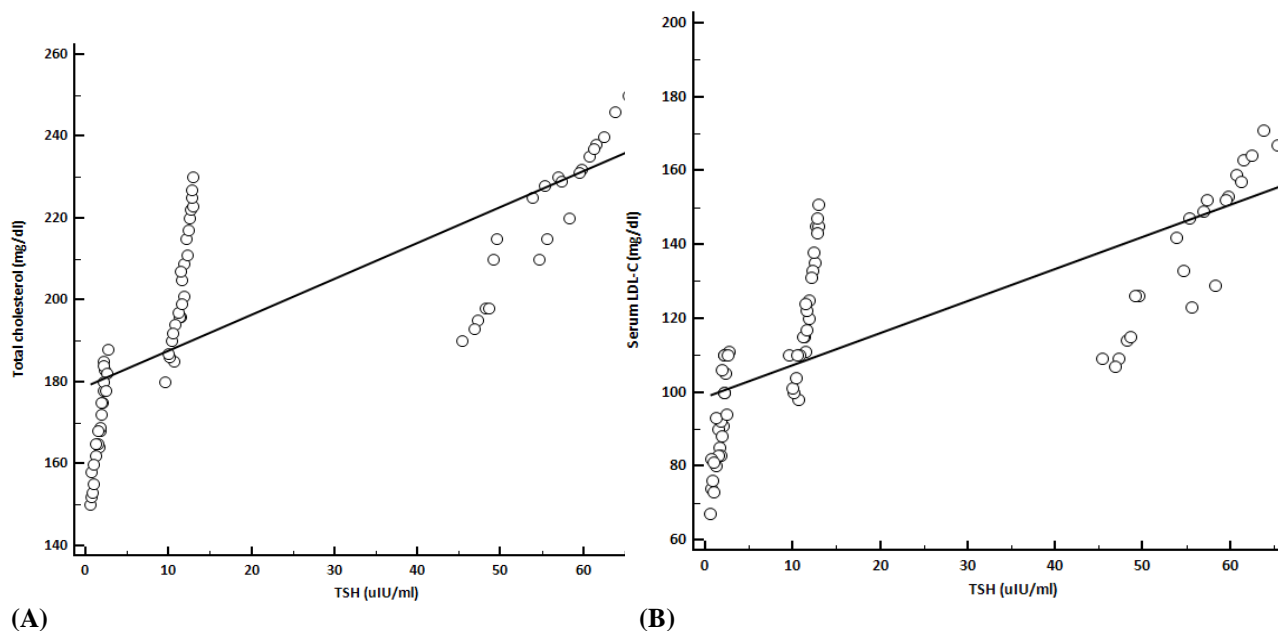
TSH: thyroid stimulating hormone; T3: triiodothyronine; T4: thyroxine; LDL-C: low density lipoprotein-cholesterol; HDL-C: high density lipoprotein-cholesterol; TAC: total antioxidant capacity.

Table (3): Correlation between lipid profile and oxidant/antioxidants profile

Variables	Total cholesterol (mg/dl)		Triglycerides (mg/dl)		LDL-C (mg/dl)		HDL-C (mg/dl)	
	r	p	r	p	r	p	r	p
Malondialdehyde (nmol/ml)	+0.933	<0.001	+0.953	<0.001	+0.905	<0.001	-0.712	<0.001
Catalase (U/L)	+0.958	<0.001	+0.957	<0.001	+0.944	<0.001	-0.786	<0.001
Superoxide dismutase (U/L)	+0.841	<0.001	+0.821	<0.001	+0.838	<0.001	-0.681	<0.001
TAC (nmol/ml)	-0.963	<0.001	-0.959	<0.001	-0.946	<0.001	+0.764	<0.001

r: correlation coefficient; p<0.05 is significant.

LDL-C: low density lipoprotein-cholesterol; HDL-C: high density lipoprotein-cholesterol; TAC: total antioxidant capacity.



(A)

(B)

Fig. (1): Scatter plot with regression line show correlation between TSH (uIU/ml) and selected study parameters in all studied subjects (N=72) (A) TSH (uIU/ml) & total cholesterol (mg/dl); (B) TSH (uIU/ml) & serum LDL-C (mg/dl).

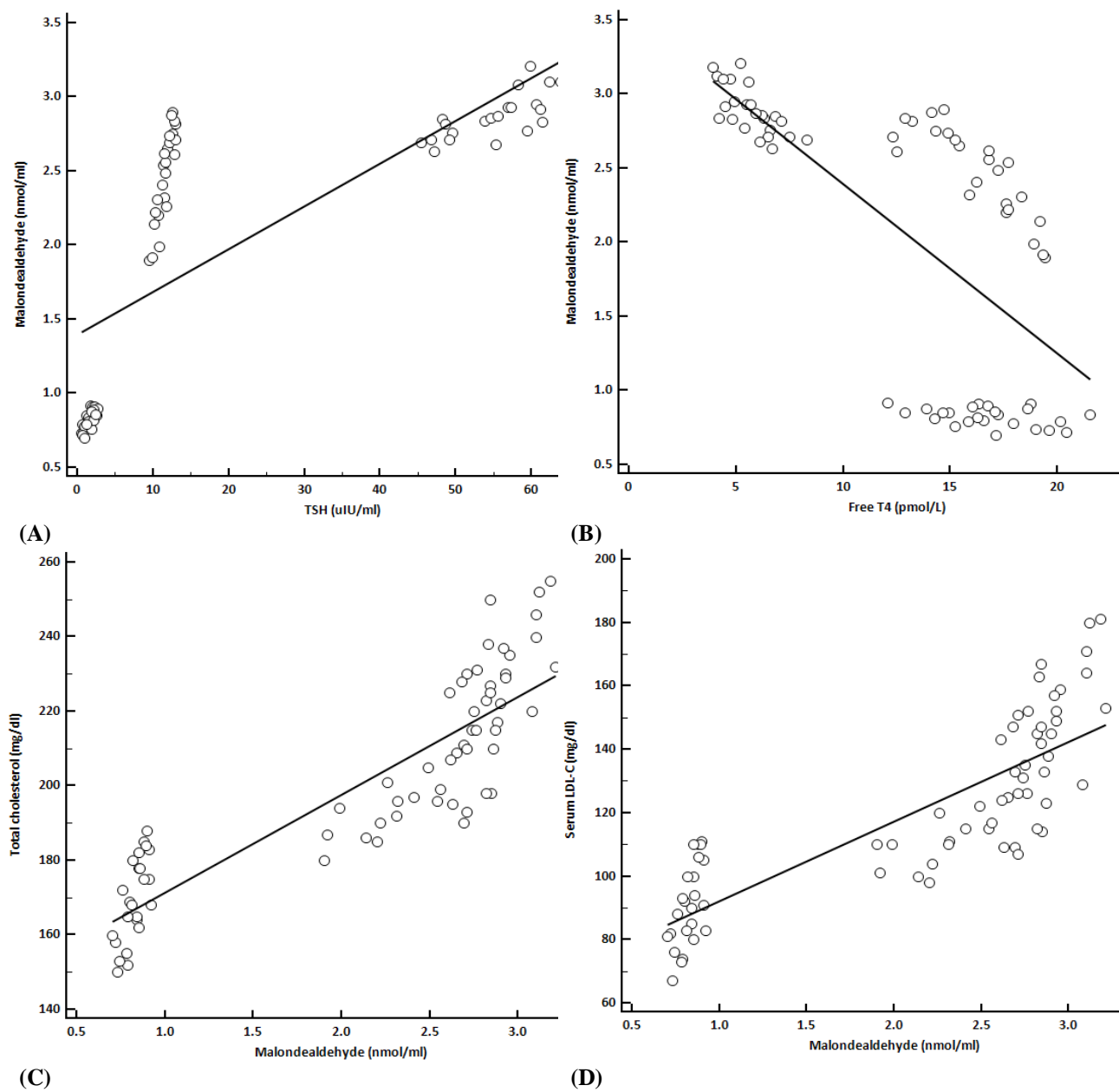


Fig. (2): Scatter plot with regression line show correlation between malondialdehyde (nmol/ml) and selected study parameters in all studied subjects (N=72) (A) TSH (uIU/ml)& malondialdehyde (nmol/ml); (B) Free T4 (pmol/L)& malondialdehyde (nmol/ml); (C) Malondialdehyde (nmol/ml) & total cholesterol (mg/dl); (D) Malondialdehyde (nmol/ml) & serum LDL-C (mg/dl).

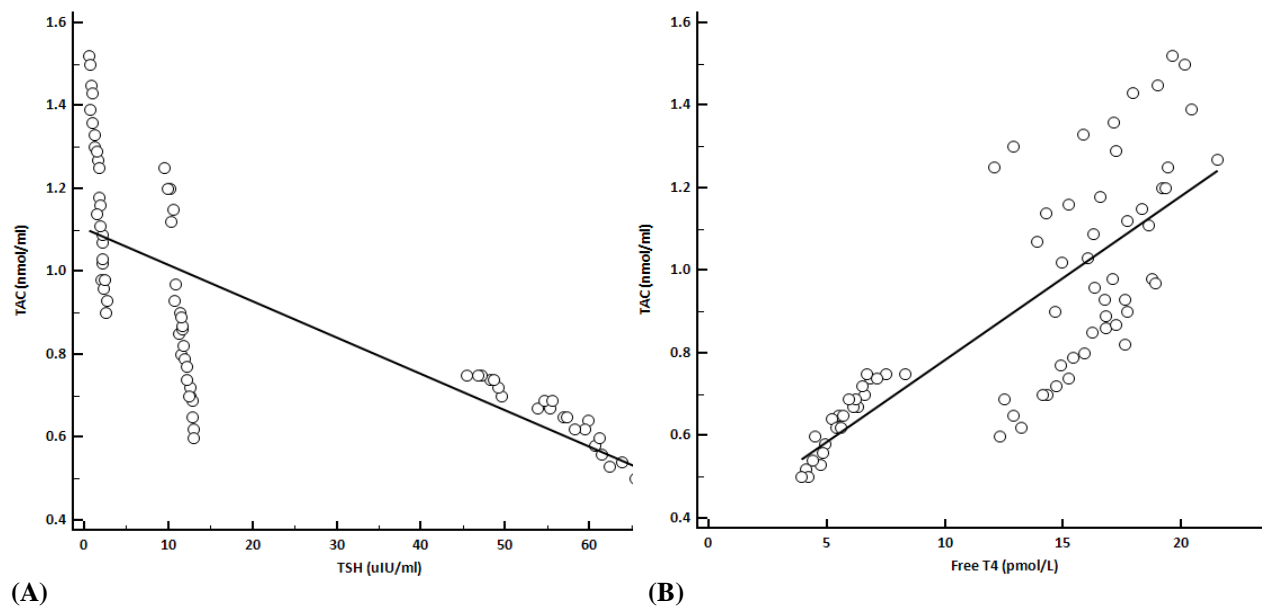


Fig. (3): Scatter plot with regression line show correlation between TAC (nmol/ml) and selected study parameters in all studied subjects (N=72) (A) TSH (uIU/ml) & TAC (nmol/ml); (B) Free T4 (pmol/L) & TAC (nmol/ml).

Discussion:-

Oxidative stress, caused by an imbalance in reactive oxygen species (ROS) produced during normal cell metabolism and/or deficiency of scavenger antioxidant defense, has been implicated in the pathology of several human diseases, including endocrine diseases of thyroid gland [2,3]. The available data concerning OXS and antioxidant capacity in hypothyroidism are controversial. Some authors suggested that tissues may be protected from oxidant damage because of the hypometabolic state in hypothyroidism [5], whereas; other studies found that oxidative stress is increased in both OHT and SHT disorders [3]. Therefore, in this study we aimed to evaluate the serum levels of MAD and the antioxidant enzymatic activities of SOD and CAT as well as the TAC in overt and subclinical hypothyroidism.

The results of the present study revealed that both OHT and SHT had significantly higher serum TC, TG and LDL-C levels as well as significantly lower serum HDL-C levels compared with euthyroid control subjects. Correlation between thyroid hormones and lipid profile revealed that serum TSH level was significantly positively correlated with TC, TGs and LDL-C serum levels and significantly negatively correlated with HDL-C levels. Furthermore, free T3 and free T4 serum levels showed a significant negative correlation with TC, TGs and LDL-C serum levels and a significant positive correlation with HDL-C levels. In this respect, our results showed that patients with both SHT and OHT had hypercholesterolemia, hypertriglyceridemia, elevated LDL-C and reduced HDL-C levels thus displaying a more atherogenic lipid profile when compared with healthy individuals.

In accordance with our results, several studies have reported increased levels of TC and LDL-C in OHT [11-13] and SHT [14-18]. In addition, some studies have shown that dyslipidemia may also be accompanied by elevated TG levels in OHT [11-13] as well as in SHT [18, 19]. Data regarding HDL-C are scarce reporting higher HDL-C [11], similar HDL-C [3, 20] or lower HDL-C [19, 21] levels in OHT and SHT compared to euthyroid subjects. Furthermore, some studies demonstrated that serum TSH was positively correlated with TC, TGs, and LDL-C, but no associations were observed between serum TSH and HDL-C [15, 22]. Indeed, even within the normal range of TSH values, a linear increase in total cholesterol, LDL-C and triglycerides and a linear decrease in HDL-C levels have been observed with increasing TSH [23]. However, other studies showed no significant differences in serum TC, LDL-C, HDL-C, or TG levels between SHT patients and the euthyroid controls [22, 24, 25].

Elevations in total cholesterol and LDL-C may occur in hypothyroidism due to several changes in the synthesis, metabolism, and mobilization of lipids. Thyroid hormones especially T3 induces 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase which is the first step in cholesterol biosynthesis [26]. They also upregulate LDL-C receptors that mediate uptake of cholesterol-rich LDL-C via directly binding to specific thyroid hormone

responsive elements [27]. Therefore, hypercholesterolemia in both OHT and SHT is due to decreased fractional clearance of cholesterol from the plasma and reduced LDL-C uptake by liver and other tissues as a result of reduced number of LDL-C receptors [27]. Likewise, hypertriglyceridemia in both OHT and SHT is due to decreased activity of lipoprotein lipase, which is also stimulated by T3 [28]. Lipoprotein lipase lowers TG levels through hydrolysis of triglycerides in TG-rich lipoproteins (i.e. Very low density lipoproteins (VLDL) and chylomicrons) into fatty acids and glycerol [17].

Catalase and SOD are important intracellular free radical-scavenging antioxidant enzymes present in aerobic cells, and form the first line of cellular defense against oxidative injury [20, 29].

In the present study, both OHT and SHT patients had significantly higher CAT and SOD levels compared to the control subjects. In addition, CAT and SOD levels showed significantly positive correlation with TC, TGs, LDL-C and TSH serum levels and a significant negative correlation with HDL-C, free T3 and free T4 serum levels. Previous studies have reported controversial findings on antioxidant enzymes CAT and SOD in OHT and SHT. In agreement with our findings some studies demonstrated an increase in CAT activity in OHT [20, 30], as well as in SHT patients [29]. Also, our results were in accordance with several studies which demonstrated a significant increase in SOD activity in OHT patients [20, 30, 31, 32]. In addition, some recent studies showed that CAT levels were correlated with TSH, TC, and LDL-C. However, after controlling TC levels, the association between CAT and TSH was not significant suggesting that oxidative stress is likely to be potentially related to the hypercholesterolemia which occurs secondary to thyroid dysfunction and not directly to thyroid hormone levels in both OHT [20] and SHT [29]. However, there are contrasting reports showing significantly decreased SOD activity in OHT [33, 34, 35] and in SHT [34] patients, or no change in SOD activity in OHT [4] and in SHT [29] patients. Also, some studies, contradictory to our findings, have demonstrated significantly decreased CAT activity in OHT [32, 35] or no change in CAT activity in OHT [36] and SHT [34] patients compared to controls.

Lipid peroxidation is caused by ROS-mediated oxidation of cell membrane lipids. Decomposition of peroxidized lipid yields a wide variety of end-products, including malondialdehyde (MDA) and thiobarbituric acid reactive substances (TBARSs) [7]. In this study, MDA levels were significantly elevated in both OHT and SHT patients compared to the control subjects indicating increased lipid peroxidation. Our finding of high MDA level in OHT group compared to SHT group indicates that oxidative damage increases with increased severity of hypothyroidism. Moreover, serum MDA levels showed significantly positive correlation with TC, TGs, LDL and TSH serum levels and a significant negative correlation with HDL, free T3 and free T4 serum levels. Our results are consistent with several studies which demonstrated that MDA levels were significantly elevated in OHT [3, 21, 34, 36–39] as well as in SHT [3, 21, 34, 37, 40] patients compared with euthyroid controls which may be an indirect suggestion of increased oxidative stress in these pathological states. In addition, some of the previous studies found a significant correlation between TSH and MDA which was lost after nullifying the effects of each of the coronary lipid risk factors among hypothyroid subjects and suggested that the increase in MDA was not associated with thyroid hormones but was related to elevated plasma lipids [38, 39].

In agreement with our results, some studies reported increased concentrations of TBARSs, another marker of lipid peroxidation, in the circulation of OHT [20] and in SHT [29] patients. Moreover, in the previous studies, TBARSs were correlated with TSH, TC, and LDL-C; however, after controlling TC levels, the association between TSH and TBARSs was not significant suggesting that increased lipid peroxidation could be attributed to lipid levels in hypothyroid status. In contradictory to our results, some studies showed that there is no significant alteration of plasma TBARSs in OHT [41] or SHT patients [41, 42] compared to euthyroid controls. Thus, lipid oxidation in OHT and SHT may not have been directly caused by low thyroid function, but was enhanced by the presence of elevated plasma cholesterol and LDL-C secondary to hypothyroidism. Prolonged availability of oxidation substrates, mainly LDL-C, in the plasma increases their susceptibility to oxidative modification [43]. Oxidized LDL-C impairs endothelial function leading to atherosclerosis, the first major cardiovascular disease event. It is plausible that thyroid hormones impacted first on lipids causing hyperlipidemia, and excess lipids acted as substrates for T3. Consequently, accelerated consumption of oxygen by T3 occurred, resulting in enhanced generation of ROS, higher consumption of cellular antioxidants and inactivation of antioxidant enzymes [44, 45]. Aerobic organisms have developed complex antioxidant systems that can counteract ROS and free radicals. This system can prevent and/or reduce ROS and free radical-induced oxidative damage to tissues and cells and is composed of different antioxidant substances, including enzymatic, non-enzymatic antioxidants, an array of small molecules and many unknown antioxidant substances [20, 46].

In recent years, interest has grown in studying the role played by oxidative stress in hypothyroidism by investigating one or more of the antioxidant markers including SOD, CAT [4,20,29,34,35], GPx, glutathione-S-transferase (GST), glutathione [34,35], vitamin A (β -carotene), vitamin E (α -tocopherol), Vitamin C (ascorbic acid)[4,35]. However, the measurement of different antioxidant molecules separately was not only impractical and time-consuming but also held no clinical significance because the effects of antioxidants can be additive, moreover, the dynamic distribution of different antioxidants in various biological samples and their potential interactions make it difficult to measure each antioxidant separately, and such measurements are also unlikely to represent the overall antioxidant substances in the body. Therefore, it is essential to measure the levels of overall antioxidants, including those known and unknown antioxidants to assess the antioxidant status in vivo [46].

In the present study, we measured serum total antioxidant capacity (TAC) that represents a measurement of the combined activities of all antioxidants including enzymatic and non-enzymatic antioxidants in a biological sample and not just the antioxidant capacity of a single compound, which may be more informative about the overall antioxidant status [10]. In the present study, both OHT and SHT patients had significantly lower serum TAC levels compared to the control subjects. TAC levels showed a significant positive correlation with HDL, free T3 and free T4 serum levels and a negative correlation with TC, TGs and LDL and TSH serum levels. In consistency with our results, some studies found that TAC was decreased in OHT as well as SHT patients compared to control [3,21]. Our findings are -however- contradictory to a previous study that demonstrated no changes in TAC in SHT patients when compared to euthyroid control [40].

Although the pathophysiological consequences of the decreased levels of antioxidant capacity are not yet elucidated, this biochemical change is thought to be a physiological adaptation and response to hypothyroidism. Thyroid hormones are involved in combating against the toxicity of oxidative stress in animals and in human as they regulate the synthesis and degradation of the antioxidant proteins, vitamins and enzymes [5, 35]. Another explanation for decreased TAC in OHT and SHT is the persistent hypercholesterolemia that enhances lipid peroxidation with increased MDA and other free radical production; this could overload the antioxidant system resulting in consumption and severe depletion of antioxidants with concomitant reduction in total antioxidant capacity [40].

In this study, oxidative stress was found to be increased in both overt and subclinical hypothyroid patients as indicated by the elevated lipid peroxidation product, malondialdehyde and the oxidant imbalance due to LDL-C oxidation caused the cells to respond by increasing antioxidant enzymes SOD and catalase activities to counter ROS generation. The mechanism of increased OXS in hypothyroidism is likely to be multifactorial. It was described that, despite being a hypometabolic state, increased lipid peroxide levels can occur in hypothyroidism due to slower clearance rate [38]. Hyperlipidemia, decreased cholesterol clearance, deficient antioxidant system, effects of thyroid hormones on regulation of antioxidant enzymes and excess TSH are the other proposed mechanisms associated with increased OXS in hypothyroidism [37].

In overt hypothyroidism, administration of substitution therapy with L-thyroxine is usually needed to correct dyslipidemia [27]. In addition, some studies demonstrated that after achieving euthyroid state there was a significant decrease in MDA levels and improvement of oxidative stress in the patients with hypothyroidism [4, 39]. Also, hypercholesterolemic patients with SHT especially those with prominent thyroid dysfunction (TSH levels >10 mIU/L), may be treated with thyroxine substitution therapy, since the restoration of euthyroidism can effectively improve lipid levels, relieve certain symptoms, and may also prevent progression to overt hypothyroidism [27].

Conclusion:-

The present study demonstrated that patients with both OHT and SHT showed an increased lipid peroxidation and oxidative stress which was evidenced by the increased levels of MDA, a lipid peroxidation product, and the induction of SOD and catalase antioxidant enzymatic activities. Increased MDA and other free radical production could overload the antioxidant system resulting in consumption and severe depletion of antioxidants with concomitant reduction in the total antioxidant capacity.

Recommendations:-

We suggest that monitoring of thyroid hormones and lipids should be considered in the management of hypothyroidism to determine the appropriate dosage of L-thyroxine substitution therapy. Also, lipid lowering drugs

may be useful in the reduction of oxidative stress in hypothyroidism. In addition to routine monitoring of hypothyroid patients for their TSH levels, measurement of MDA and TAC might be considered for evaluation of oxidative injury. Hypercholesterolemic patients with SHT, especially those with prominent thyroid dysfunction (TSH levels >10 mIU/L), may be treated with thyroxin substitution therapy and/ or lipid lowering drugs, since the restoration of euthyroidism can effectively improve lipid levels and reduce oxidative stress. Furthermore, supplementation of antioxidants (antioxidant vitamins, copper, and zinc) in doses sufficient to combat oxidative stress may be a useful adjunct and should be considered in the management of hypothyroid patients. Further larger clinical studies are required to better understand the role of oxidative stress on the physiopathology of hypothyroidism.

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