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Research International Journal of Endocrinology and Diabetes

Research Article

Paraoxinase-1 Activity in Patients with Subclinical Hypothyroidism: A link to Atherosclerosis

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Received: 20 September 2020; **Accepted:** 30 October 2020; **Published:** 05 November 2020

Citation of this article: El-Kafrawy N, El-Barbary H, El-Kerch A, Ashraf M (2020) Paraoxinase-1 Activity in Patients with Subclinical Hypothyroidism: A link to Atherosclerosis. Re Int J of End and Diabe. 1(1): 026-036. DOI: 10.37179/rijed.000007.

ABSTRACT

Objective: To investigate paraoxinase-1 (PON-1) activity in subclinical hypothyroidism (SCH) patients in comparison with acute coronary syndrome (ACS) patients and to determine the prevalence of SCH among patients with ACS.

Background: Paraoxonase1 (PON1) is an antioxidant enzyme that plays an important role in lipoprotein metabolism; it is an indicator of the risk of atherosclerosis and coronary artery disease development. Thyroid hormones are associated with both the oxidant-antioxidant balance and lipoprotein metabolism; SCH and its correlation with the atherosclerotic cardiovascular diseases is a subject of debate.

Subjects and Methods: This study included 95 subjects from Menoufia University Hospital after exclusion of all known cardiovascular risk factors, classified into :Group I (control group) (n=15); Group II (ACS subjects) (n=46), Group III (SCH subject) (n=20) and Group IV (subjects with both ACS and SCH) (n=14).Thorough history taking; complete physical examination, thyroid function tests, lipid profile, PON-1 activity, electrocardiogram (ECG) and echocardiographic examination of the heart were done.

Results: PON-1 activity was highly significant lower in group II and IV than group III than control; there are a significant negative correlation between PON-1 activity and thyroid stimulating hormone and a highly significant positive correlation with FT4.In addition the severity of dyslipidemia and the prevalence of diastolic dysfunction in group III were comparable to that in group II and the prevalence of SCH among ACS patients is 23.3%.

Conclusion: This study suggests that SCH is an independent risk factor for ACS, and the prevalence of SCH among patient with ACS was 23.3%.

Keywords: Atherosclerosis, Paraoxinase-1, Subclinical Hypothyroidism.

Introduction

Subclinical hypothyroidism is a common biochemical finding in the general population [1]. SCH is typically an asymptomatic condition, biochemically defined by a raised serum thyroid-stimulating hormone (TSH) concentration combined with a normal level of free thyroxine (FT4) [2]. *The prevalence ranges from 4% to 15%, it is present more often in females than males, and lower in blacks than in whites and more in elderly* [3]. Dependent on the level of serum TSH, SCH can be mild (TSH = 4.5–9 mU/L) or severe (TSH ≥10 mU/L) [4]. Subclinical hypothyroidism may act directly on the heart, impairing both systolic and diastolic functions or indirectly by altering peripheral vascular resistance and serum lipid and coagulation profiles [5]. Atherosclerosis is an inflammatory disease, regarding its pathogenesis and according to "response to retention hypothesis," the whole sequence of events is found to be initiated by the retention of modified LDL [6]. High-density lipoprotein has a protective role against atherosclerosis by its involvement in reverse cholesterol transport. Recent evidence, however, suggests HDL also prevents atherosclerosis by inhibiting the oxidative modification of LDL particle, which is done by some of the enzymes of HDL like Paraoxinase 1 (PON-1) [6]. Recently, dysfunctional, or pro-inflammatory HDL was indicated as a pro-atherogenic factor. The protective function of HDL against atherogenesis could be partly explained by its main constituent, PON1 [7].

Paraoxonase-1 is a high-density lipoprotein associated -enzyme capable of hydrolyzing lipid peroxides thus, PON-1 plays a preventing role in atherosclerosis by protecting against lipid peroxidation, modulates the susceptibility of LDL to atherogenic modifications and even plays an anti-inflammatory role [8]. Since reduced PON1 concentration and/or activity might have a deleterious effect on the protective function of HDL [7] PON1 has been the focus of intensive research and has been reported as an independent risk factor for CVD [9]. Paraoxinase-1 activity has been suggested to be inversely related to oxidative stress [10], Thyroid hormones have a strong impact on oxidative stress and the antioxidant system [11], in subclinical hypothyroidism, lower PON-1 activity indicated increased oxidative damage [12]. In some studies, it was shown that elevated serum level of TSH has an inverse effect on the levels of serum lipoproteins, due to these lipid abnormalities, subclinical hypothyroidism may be considered as a risk factor for atherosclerosis [12].

Subjects and Methods

Study Population

This study included 95 subjects aged from 19-59 years old from outpatient clinics and cardiovascular care unit (CCU) at Menoufia University Hospitals from March2014 to December 2016 after approval of ethical committee of faculty of medicine Menoufia University and all participants provided informed consent. They were classified into four groups: Group I (controls group) (n=15), Group II (subjects with ACS) (n=46) Group III (subjects with SCH) (n=20) Group IV (subjects with both ACS and SCH) (n=14).

Inclusion Criteria: Patients included in the study were those with either Subclinical hypothyroidism diagnosed in outpatient clinic by thyroid profile or with acute coronary syndrome diagnosed in cardiovascular care unit (CCU) by clinical presentation, ECG, and cardiac enzymes.

Exclusion Criteria: Obese subjects (BMI ≥ 25 kg/m2), Smokers, subjects with diabetes mellitus or pre-diabetes, subjects on anti-thyroid medications, and patients with chronic renal failure and chronic liver disease all were excluded from the study.

Evaluation of Patients: All subjects underwent full history taking and clinical examination with emphasis on thyroid gland examination and anthropometric measurements including body mass index (BMI) and waist circumference.

Laboratory

Specimen Collection and storage:

Serum: Blood collected by venipuncture, allowed to coagulate at room temperature for 10-20 min, then, centrifuge at the speed of 2000-3000 r.p.m. for 20 min to collect supernatant. Specimens held for a longer frozen only once at -40°C prior to assay.

Routine Investigations: Were done for all subjects in the form of complete blood count (CBC), Liver function tests (serum albumin, prothrombin concentration, total and direct bilirubin, ALT and AST), Kidney function tests (serum urea and creatinine), fasting blood glucose , 2h-post-prandial blood glucose and lipid profile (TC, TG, HDL and LDL).

Specific Investigations

Thyroid function tests (TSH, FT3, FT4)

Principle of TSH: DS-EIA-TSH is a one-step immunoassay, based on the principle of the sandwich method. This test uses monoclonal antibody of high affinity and specificity (enzyme conjugated and immobilized) directed against a distinct antigenic determinant on the intact TSH molecule, and normal expected values of TSH: 0.37-5.1 μIU/ml.

Principle of free T4 and free T3: DS-EIA-T4 (or DS-EIA-T3 for T3 Kits) is a one-step immunoassay to determine the presence of free T4 (or T3) in human serum using competitive microplate enzyme immunoassay, and Normal expected values of free T3: 0.8-2 ng/ml and of free T4: 4.5-13.8 μg/dl.

Determination of PON-1 Concentration: A commercially available kit (PON-1 ELISA Kit, BYEK2123) (Chongqing Biopsies Co., Ltd): was used for four groups according to the method described by Bajaj et al. [13].

Echocardiographic Examinations

All echocardiographic examinations were made by one investigator in blinded fashion; the examinations were made with two-dimension, M-mode, and pulse Doppler echocardiography(Hewlett Packard Inc., USA) equipped with a 2.5 MHz transducer, together with the lead II ECG.The following parameters were measured :left ventricular end-diastolic diameter, left ventricular end-systolic diameters, posterior wall thickness, inter-ventricular septal thickness, fractional shortening, left ventricular ejection fraction ,left ventricular mass index, diastolic trans-mitral peak velocity (E and A wave), E/A ratio, deceleration time of mitral E wave.

Statistical Analysis

The statistical package for the social sciences (SPSS, version 20; SPSS Inc., Chicago, Illinois, USA) software computer program was used for analysis of our data. Data was expressed into two phases:

I – Descriptive

Mean value and Standard Deviation [SD]: for quantitative data and Frequency and percentage for qualitative date.

II- Analytic

T test for comparison of two independent quantitative variables normally distributed, U test (Mann Whitney test) for comparison of more than two independent quantitative variables not normally distributed, K test (Kruskal Wallis) for comparison of two independent quantitative variables not normally distributed, X2 (Chi 2) for comparison between two or more independent qualitative variables normally distributed, Fisher exact test for comparison between two independent qualitative variables with one cell < 5, Pearson's Correlation Coefficient [r] for comparison between two dependents quantitative normally distributed variable. The significance level (*P*-value) was expressed as follows: *P* value ≥ 0.05 is insignificant; *P* value < 0.05 is significant and *P* value < 0.001 is highly significant. The area under the receiver operating characteristic curve (ROC) was used to assess the discriminative ability of PON-1 activity in SCH in comparison to that in ACS.

Results

This study included 95 subjects from outpatient clinics and cardiovascular care unit (CCU) Menoufia University Hospitals from March 2014 to December 2016. The mean ages of the studied groups (Table 1) were: 34.7±9.7 years in control group, 48.8±4.8years ingroup II, 34.3±11.2 years in group III and 38.7±3.1 years in group IV and these results showed that the mean of age was highly significant higher in group II than other groups (p- value <0.001) and there was no significant difference between group I, III and IV regarding age (P- value> 0.05). Regarding gender (Table 1), control group included 9 males (60%) and 6 females (40%) and group II included 31 males (67.4%) and 15 females (32.6%), and these results showed that acute coronary syndrome was more prevalent in males when studied in these groups with euthyroidism (I, II). Group III included 7 males (35%) and 13 females (65%) and group IV included 5 males (35.7 %) and 9 females (64.3%) and these results showed that SCH are highly prevalent in females when studied in these groups with SCH (III, IV).

All subjects with BMI ≥ 25 were excluded from the present study, the mean of BMI (Table 2) was (20.8±1.4) kg/ m^2 in group I, (22.8±1.8) kg/ m^2in group II, (21±2.4) kg/ m^2 in group III and (22.8±1.6) kg/ m^2 in group IV and these results showed that there was no significant difference between studied groups regarding BMI (P- value > 0.05). All subjects with WC ≥ 102 or 88 in male or female respectively were excluded from the study, the mean of WC was (89.7±8.2) cm in group I, (90.9±8.3) cm in group II, (84.7±7.5) cm in group III and (87.6±10.4) cm in group IV and these results showed that there was no significant difference between studied groups regarding WC (P-value > 0.05). Regarding fasting blood glucose (FBG) level, all subjects with diabetes mellitus or impaired fasting glucose were excluded from the study, the mean of FBG (Table 3) was (80.9±6.8) mg/dl in group I and (80.5±7.1) mg/dl in group II, (81.8±7.1) mg/dl in group III and (78.3±6.1) mg/dl in group IV and these results showed that there was no significant difference between studied groups regarding FBS (P-value > 0.05). Regarding 2h-pp, the mean (Table 3) was (95.5±3.5) mg/dl in group I , (100.4±7.2) mg/dl in group II , (97.3±4.2) mg/dl in group III and (96.2±6.7) mg/dl in group IV and these results showed that there was no significant difference between studied groups regarding 2h PP blood glucose level(P- value > 0.05).

The mean of TC (Table 4) was (165.4±25.9) mg/dl in group I, (229.8±30) mg/dl in group II, (232.1±38.5) mg/dl in group III and (285.7±22.3) mg/dl in group IV. And these results showed that the mean of TC was: Highly significant higher in all groups than group I (P- value < 0.001), highly significant higher in group IV than group II and group III (P-value< 0.001) and there was no significant difference between group II and group III regarding TC (P- value > 0.05). The mean of TG (Table 4) was (129.9±10.1) mg/dl in group I, (174.8±28.6) mg/dl in group II, (169.1±19.8) mg/dl in group III and (228.7±47.8) mg/dl in group IV and these results showed that the mean of TG was: highly significant higher in all groups than group I (P- value < 0.001), highly significant higher in group IV than group II and group III (P- value < 0.001) while there was no significant difference between group II and group III regarding TG (P- value > 0.05).

The mean of HDL (Table 4) was (55.6±3.3) mg/dl in group I,

Table 1: Distribution of the studied patients regarding their demographic data.

Demographic data		Group I (n= 15)		Group II (n= 46)		Group III (n= 20)		Group IV (n= 14)		X^2	P value	LSD
		NO.	%	NO.	%	NO.	%	NO.	%			
Gender	Male	9	60	31	67.4	7	35	5	35.7	8.3	< 0.05	
	female	6	40	15	32.6	13	65	9	64.3			
Age in years (Mean ±SD)		36.7±9.7		48.8±4.8		34.3±11.2		38.7±3.1		24.2*	<0.001	P1: <0.001 P2: 0.347 P3:0.944 P4:<0.001 P5:<0.001 P6:0.087

P-value >0.05 means non-significant, < 0.05 means significant p-value<0.001 means highly significant
SD: standard deviation No. : means number. *Anova (f) test.
P1: between group I&II, P2: between group I&III, P3: between group I & IV
P4: between group II &III, P5: between group II &IV, P6: between group III & IV
Group I: patients with normal TFTs and without ACS
Group II: patients with normal TFTs and with ACS
Group III: patients with SCH and without ACS
Group IV: patients with SCH and ACS

Table 2: Comparison between studied groups regarding anthropometric measurement:

Anthropometric measurements	Group I (n= 15) X ± SD	Group II (n= 46) X ± SD	Group III (n= 20) X ± SD	Group IV (n= 14) X ± SD	f-test	P value	LSD
BMI (kg/ m²)	20.8±1.4	20.8±1.8	21±2.4	22.8±1.6	5.2	>0.05	P1:0.875 P2:0.995 P3:0.944 P4:0.324 P5:0.226 P6:0.937
WC (cm)	89.7±8.2	90.9±8.3	84.7±7.5	87.6±10.4	2.6	0.057	P1:0.093 P2:0.628 P3:0.522 P4:0.108 P5:0.0.329 P6:0.212

P-value > means non-significant, < 0.05 means significant p-value<0.001 means highly significant
SD: standard deviation, n: means number, BMI= body mass index, WC= waist circumference, cm: centimeter.
P1: between group I&II, P2: between group I&III, P3: between group I & IV
P4: between group II &III, P5: between group II &IV, P6: between group III & IV
Group I: patients with normal TFTs and without ACS
Group II: patients with normal TFTs and with ACS
Group III: patients with SCH and without ACS
Group IV: patients with SCH and ACS

Table 3: Comparison between studied groups regarding blood glucose level.

	Group I (n= 15) X ± SD	Group II (n= 46) X ± SD	Group III (n= 20) X ± SD	Group IV (n= 14) X ± SD	f-test	P value	LSD
FBG (mg/dl)	80.9±6.8	80.5±7.1	81.8±7.1	78.3±6.1	0.729	0.537	P1:0.694 P2:0.867 P3:0.318 P4:0.492 P5:0.148 P6:0.292
2h PP (mg/dl)	95.5±3.5	100.4±7.2	97.3±4.2	96.2±6.7	3.5	0.068	P1:0.373 P2:0.08 P3:0.745 P4:0.068 P5:0.598 P6:0.069

FBG= fasting blood glucose, 2h PP= 2 hours post prandial blood glucose level
P-value > 0.05 means non-significant, SD: standard deviation, n: means number,
P1: between group I&II, P2: between group I&III, P3: between group I & IV
P4: between group II &III, P5: between group II &IV, P6: between group III & IV
Group I: patients with normal TFTs and without ACS
Group II: patients with normal TFTs and with ACS

(34.1±5.7) mg/dl in group II, (43.4±5.7) mg/dl in group III and (30.6±2.4) mg/dl in group IV and these results showed that the mean of HDL was: highly significant lower in all groups than group I (P- value < 0.001), highly significant lower in group III and group IV than group II (P- value < 0.001) and significantly lower in group IV than group III (P- value < 0.05). The mean of LDL (Table 4) was (86.1±12.4) mg/dl in group I, (130.2±18.5) mg/dl in group II, (132.2±17.5) mg/dl in group III and (170.4±22.1) mg/dl in group IV and these results showed that the mean of LDL was: highly significant higher in all groups than group I (P- value < 0.001), highly significant higher in group IV than both group II and III (P- value < 0.001) while there was no significant difference between group II and group III regarding LDL (P- value > 0.05).

The mean of TSH (Table 5) was (1.9±0.5) in group I, (2.1±0.7) in group II, (7.7±1.6) in group III and (7.8±0.6) in group IV and these results showed that the mean of TSH was: Highly significant higher in group III and group IV than group I (P- value < 0.001), highly significant higher in group III and group IV than group II (P- value < 0.001) and there was no significant difference between group I and group II or between group III and group IV regarding TSH (P- value > 0.05). The mean of FT3 (Table 5) was (1.3±0.4) in group I, (1.4±0.4) in group II, (1.4±0.3) in group III and (1.3±0.3) in group IV and these results showed that there was no significant difference between studied groups regarding FT3 (P- value > 0.05).

The mean of FT4 (Table 5) was (9.8±2) in group I, (9.7±2.3) in group II, (6.3±2.1) in group III and (7.9±2.6) in group IV and these

Table 4: Comparison between studied groups regarding lipid profile.

	Group I (n= 15) X ± SD	Group II (n= 46) X ± SD	Group III (n= 20) X ± SD	Group IV (n= 14) X ± SD	f test	P value	LSD
TC (mg/dl)	165.4±25.9	229.8±30	232.1±38.5	285.7±22.3	38.02	<0.001	P1:<0.001 P2:<0.001 P3:<0.001 P4:0.774 P5:<0.001 P6:<0.001
TG (mg/dl)	129.9±10.1	174.8±28.6	169.1±19.8	228.7±47.8	28.7	<0.001	P1:<0.001 P2:<0.001 P3:<0.001 P4:0.462 P5:<0.001 P6:<0.001
HDL (mg/dl)	55.6±3.3	34.1±5.7	43.4±5.7	30.6±2.4	91	<0.001	P1:<0.001 P2:<0.001 P3:<0.001 P4:<0.001 P5: 0.025 P6:<0.001
LDL (mg/dl)	86.1±12.4	130.2±18.5	132.2±17.5	170.4±22.1	52.9	<0.001	P1:<0.001 P2:<0.001 P3:<0.001 P4:0.683 P5:<0.001 P6:<0.001

p-value<0.001 means highly significant SD: standard deviation n : means number.
TG: Triglycerides, HDL-c: high density lipoprotein, LDL-c: low density lipoprotein.
P1: between group I&II, P2: between group I&III, P3: between group I & IV
P4: between group II &III, P5: between group II &IV, P6: between group III & IV
Group I: patients with normal TFTs and without ACS
Group II: patients with normal TFTs and with ACS
Group III: patients with SCH and without ACS
Group IV: patients with SCH and with ACS

Table 5: Comparison between studied groups regarding thyroid profile.

Thyroid function tests	Group I (n= 15) X ± SD	Group II (n= 46) X ± SD	Group III (n= 20) X ± SD	Group IV (n= 14) X ± SD	f test	P value	LSD
TSH (µIU/ml)	1.9±0.5	2.1±0.7	7.7±1.6	7.8±0.6	270.3	<0.001	P1: 0.576 P2:<0.001 P3:<0.001 P4:<0.001 P5:<0.001 P6: 0.624
FT3 (ng/ml)	1.3±0.4	1.4±0.3	1.4±0.4	1.3±0.3	0.411	0.746	P1:0.831 P2:0.409 P3:0.969 P4:0.52 P5:0.802 P6:0.395
FT4 (µg/dl)	9.8±2	9.7±2.3	6.3±2.1	7.9±2.6	16.9	<0.001	P1: 0.611 P2:<0.001 P3:0.029 P4:<0.001 P5: 0.036 P6: 0.063

P-value>0.05 means non-significant, p-value<0.05 means significant, p-value<0.001 means highly significant
SD: standard deviation, n: means number. TSH: thyroid stimulating hormone FT3:free T3 FT4: free T4
P1: between group I &II, P2: between group I & III, P3: between group I & IV
P4: between group II&III, P5: between group II&IV, P6: between group III & IV
Group I: patients with normal TFTs and without ACS
Group II: patients with normal TFTs and with ACS
Group III: patients with SCH and without ACS
Group IV: patients with SCH and with ACS

results showed that the mean of FT4 was: Highly significant lower in group III than group I and group II (P < 0.001), significantly lower in group IV than both group I and II (P < 0.05) while there was no significant difference between group I and group II and between group III and group IV regarding FT4 (P > 0.05).

The mean of PON-1 activity (Table 6) was (65.1±7.6) U/L in group I, (12.05±4.8) U/L in group II, (18.7±5.2) U/L in group III and (11.4±4.5) U/L in group IV and these results showed that the mean of PON-1 activity was: Highly significant lower in all groups than group I (P < 0.001), highly significant lower in group II and group IV than group III (P< 0.001) while there was no significant difference between group II and group IV regarding PON-1 activity (P > 0.05). The mean of LVEF (Table 7) was (56.1±3.1) % in group I, (53.1±2) % in group II, (53.6±5) % in group III and (50.3±2.8) % in group IV and these results showed that there was no significant difference between studied groups regarding LVEF (P- value > 0.05). Prevalence of diastolic dysfunction (Table 7) was significantly higher in group IV than both group II and III than group I (p-value <0.001) and there was no significant difference between group II and group III regarding diastolic dysfunction (50% in both groups).

Pearson correlation efficient between PON-1 activity and both of demographic and laboratory data of studied groups showed that (Table 8): There was negative and significant correlation between PON-1 and age (p= 0.003, r= - 0.297).There was negative but non-significant correlation between PON-1 and BMI and WC (p- value > 0.05). There was negative but non-significant correlation between PON-1 and FBS and 2h PP blood glucose level (p- value > 0.05). There was negative and highly significant correlation between PON-1 and TC, TG and LDL (p < 0.001, r = - 0.638, - 0.46, and – 0.65 respectively) while there was positive and highly significant correlation between PON-1 and HDL (p < 0.001, r = 0.77). There was negative and significant correlation between PON-1 and TSH (p< 0.05, r = - 0.247). There was positive and highly significant correlation between PON-1 and FT4 (p < 0.001, r = 0.34).

Among patients with acute coronary syndrome (Group III, n=60), there was 14 patients with subclinical hypothyroidism (23.3%), 44 patients with normal thyroid function (73.3%) and 2 patients with euthyroid sick syndrome (3.3 %). So, the prevalence of SCH among ACS patients was 23.3% (Table 9). ROC curve is used to assess the efficiency of PON-1 activity in predicting the cardiovascular risk in studied groups (Table 10), AUC=0.873 at cut off point 18.15, and

Table 6: Comparison between studied groups regarding paraoxinase-1 activity.

	Group I (n= 15) X ± SD	Group II (n= 46) X ± SD	Group III (n= 20) X ± SD	Group IV (n= 14) X ± SD	f test	P value	LSD
PON-1 (U/L)	65.1±7.6	12.05±4.8	18.7±5.2	11.4±4.5	391.8	<0.001	P1:<0.001 P2:<0.001 P3:<0.001 P4:<0.001 P5: 0.693 P6:<0.001

P-value>0.05 means non-significant, p-value<0.001 means highly significant
SD: standard deviation, n: means number.
P1: between group I &II, P2: between group I & III, P3: between group I & IV
P4: between group II&III, P5: between group II&IV, P6: between group III & IV
Group I: patients with normal TFTs and without ACS
Group II: patients with normal TFTs and with ACS
Group III: patients with SCH and without ACS
Group IV: patients with SCH and with ACS

Table 7: Comparison between studied groups regarding echocardiographic findings.

	Group I (n= 15) X ± SD		Group II (n= 46) X ± SD		Group III (n= 20) X ± SD		Group IV (n= 14) X ± SD		f test	P value	LSD
LVEF (%)	56.1±3.1		53.1±8		53.6±5		52.3±6.8		0.972	0.409	P1:0.282 P2:0.133 P3:0.127 P4:0.764 P5:0.561 P6:0.689
DD	N	%	N	%	N	%	N	%	19.2	<0.001	
Present	0	0	23	50	10	50	11	78.6			
Absent	15	100	23	50	10	50	3	21.4			

P-value>0.05 means non-significant, p-value<0.001 means highly significant
SD: standard deviation, n: means number. LVEF= left ventricular ejection fraction DD= diastolic ysfunction
P1: between group I &II, P2: between group I & III, P3: between group I & IV
P4: between group II& III, P5: between group II&IV, P6: between group III & IV
Group I: patients with normal TFTs and without ACS
Group II: patients with normal TFTs and with ACS
Group III: patients with SCH and without ACS
Group IV: patients with SCH and with ACS

sensitivity and specificity are (85.7% and 65% respectively). ROC curve also applied to group II and III separately to compare sensitivity and specificity of PON-1 activity in both groups (Table 11, 12), the AUC was (1 and 0.89 in group II and III respectively) at cut off point (39 and 34 in group II and III respectively) , in group II , sensitivity is 100% , specificity is 100% while in group III sensitivity is 89%, specificity is 80.3 with PPV and NPV (82.3% and 90% respectively).

Table 8: Pearson Correlation Coefficient between PON-1 and age, anthropometric measurements, and laboratory data.

		PON-1	
		r	p - value
Age	(years)	-0.297	0.003*
BMI	(kg/m^2)	-0.017	0.869
WC	(cm)	-0.016	0.876
FBS	(mg/dl)	-0.05	0.626
2h PP	(mg/dl)	-0.174	0.09
TC	(mg/dl)	-0.638	<0.001*
TG	(mg/dl)	-0.46	<0.001*
HDL	(mg/dl)	0.77	<0.001*
LDL	(mg/dl)	-0.65	<0.001*
ALT	(U/L)	-0.03	0.775
AST	(U/L)	-0.33	0.001*
Albumin	(gm/dl)	0.04	0.678
PC	(%)	-0.011	0.919
Bilirubin	(mg/dl)	0.025	0.809
TSH	(µIU/ml)	-0.247	0.016*
FT3	(ng/ml)	-0.015	0.887
FT4	(µg/dl)	0.34	<0.001*

Table 9: Prevalence of subclinical hypothyroidism (SCH) among patient with acute coronary syndrome (ACS).

	ACS (n=60)	
	No.	%
Euthyroid	**44**	**73.3**
SCH	**14**	**23.3**
Euthyroid sick syndrome	**2**	**3.30%**
Overt hypothyroidism	**0**	**0%**
Hyperthyroidism	**0**	**0%**

Discussion

Human and animal studies support the hypothesis that oxidative modification of LDL plays a crucial role in the pathogenesis of atherosclerosis. PON-1 may protect against cardiovascular disease because it is capable of hydrolyzing oxidized LDL cholesterol. It has been found that decreased serum PON-1 activity is associated with more severe atherosclerosis [14]. The influence of subclinical hypothyroidism on atherosclerosis is still not very well understood. There is substantial evidence that subclinical hypothyroidism alters several traditional risk factors such as increased circulating levels of low-density lipoprotein (LDL) cholesterol, direct effects on vascular endothelium, and altered coagulability. However, we do not yet have a good understanding of the mechanism of risk factors for cardiovascular diseases [15]. The mean age was averaged (36.7±9.7) years in group I (subjects with normal TFTs without ACS), (48.8±4.8) years in group II (subjects with normal TFTs with ACS), (34.3±11.2) years in group III (subjects with SCH without ACS) and (38.7±3.1) years in group IV (subjects with SCH with ACS) (Table 1) and these results showed that the mean of age was highly significant higher in group II than group I, III and IV (p- value <0.001) .

Regarding gender (Table 1) there were 9 males (60%) and 6 females (40%) in group I , 31 males (67.4%) and 15 females (32.6%) in group II and these results showed that acute coronary syndrome was more prevalent in males, group III included 7 males (35%) and 13 females (65%) and group IV included 5 males (35.7%) and 9 females (64.3%) and these results showed that subclinical hypothyroidism was more prevalent in females. These results agree with that of *Prasad et al.* (regarding gender) as in his study SCH patients comprises 55% women and 36.7% men, which shows that SCH is more common in women than in men [16]. The present results agree with that of *Singh and Sarkar,* (regarding gender) who found that within subclinical hypothyroid group (n=56) 30% were males whereas 70% were females, they concluded that the prevalence of thyroid disease is higher in women and increasing with advancing age [17].This finding was consistent with that of ***Saxena et al.,*** who noted the predominance of females in cases of hypothyroidism with M : F ratio 1: 6.5 [18]. The higher prevalence of thyroid disease in women suggests that estrogen might be involved in the pathophysiology of thyroid dysfunction.

Table 10: sensitivity and specificity of PON1 in prediction of ACS in studied groups.

	AUC	p-value	cut off	sensitivity	specificity	PPV	NPV	accuracy
PON-1	0.873	<0.001	18.15	85.7%	65%	63.1%	86.7%	73.5%

PON-1= paraoxinase-1, AUC= area under curve, PPV= positive predictive value, NPV= negative predictive value

Table 11: Validity of PON1 in prediction of CVD in patients with ACS (group II).

	AUC	p value	cut off	sensitivity	specificity	PPV	NPPV	accuracy
PON-1	1	<0.001	39	100%	100%	100%	100%	100%

PON-1= paraoxinase-1, AUC= area under curve, PPV= positive predictive value, NPV= negative predictive value.

Table 12: Validity of PON-1 in prediction of CVD in patients with SCH (group III).

	AUC	p value	cut off	sensitivity	specificity	PPV	NPPV	accuracy
PON-1	0.89	<0.001	34	89%	80.3%	82.3%	90%	88.5%

PON-1= paraoxinase-1, AUC= area under curve, PPV= positive predictive value, NPV= negative predictive value.

Estradiol has an antagonistic effect on the hormones T3 and T4. The reason being estradiol competes with T3 and T4 for binding sites on the receptor proteins [17]. All these studies were done on general population to study the prevalence of SCH or on patients with SCH on comparison with controls, only few studies were carried on patients with ACS, the results of these studies do not agree with our results.

For example, *Helmy et al.,* who studied the prevalence of subclinical hypothyroidism among patients with ACS according to age and gender, showed no statistically significant difference in the prevalence of SCH between both males and females. It was 4.5% in male's vs .5.9% in females [19]. Regarding to lipid profile, we found that the mean of TC is highly significant higher in all groups than group I (P- value < 0.001), highly significant higher in group IV than group II and group III (P- value < 0.001) and there was no significant difference between group II and group III regarding TC (P- value > 0.05). These results show that the mean of TG is highly significant higher in all groups than group I (P- value < 0.001), highly significant higher in group IV than group II and group III (P- value < 0.001) and there was no significant difference between group II and group III regarding TG (P- value > 0.05). The mean of HDL is highly significant lower in all groups than group I (P- value < 0.001), highly significant lower in group III and group IV than group II (P- value < 0.001) and significantly lower in group IV than group III (P- value < 0.05). The mean of LDL is highly significant higher in all groups than group I (P- value < 0.001), highly significant higher in group IV than group II and group III (P- value < 0.001) and there is no significant difference between group II and group III regarding LDL (P- value > 0.05).

So, to summarize our results regarding to lipid profile, we found that TC, TG and LDL were significantly higher in subjects with both ACS and SCH (Group IV) than subjects who have either ACS (Group II) or SCH (Group III) than control (Group I). Regarding HDL it was decreased in SCH subjects with or without ACS i.e. (Group III and IV) more than group II (ACS), in addition it shows more reduction in group IV than group III (i.e. Group IV < III < II < I). Also, we observed that there was no significant deference between Group II and Group III regarding dyslipidemia which means that dyslipidemia in subjects with SCH is comparable to that in subjects with ACS. Up to our knowledge no previous study assessed the lipid profile in subjects with SCH (alone and in association with ACS) in comparison with subjects with ACS.

The present results agree with that of Patel *et al.,* who found that in SCH, TC and LDL levels were increased while HDL level was decreased when compared to control [20]. These results are consistent with that of both *Prasadet al.* and *Sadariyaet al.* studies that show increase in the values of all lipid parameters except HDL as compared to that of the euthyroid [16, 21]. Contrary to the present observations, National Health and Nutrition Examination Survey III (n=8586) reported no significant differences in lipid parameters in SCH subjects as compared to euthyroid individuals when adjusted for confounding variables [22].

The present study confirms that in patients with SCH, levels of lipid parameters are increased except HDL, which is decreased, these lipid parameters are known to be atherogenic in nature. If thyroid replacement therapy decreases the levels of atherogenic lipids, thyroid replacement therapy may be advisable in SCH and it may further avoid the harmful effects of the atherogenic lipids [20]. The possible explanation for dyslipidemia here could be decreased LDL and IDL catabolism. This might either be due to reduction in cell surface receptor for LDLor due to their decreased activity. This would increase TC and LDL concentration in blood [16]. LDL receptors contain the SREBP-2 gene which is regulated by T3. Hence, LDL receptors could decrease when the SREBP-2 gene expression reduces [23]. Moreover, decreased activity of hepatic lipase (also stimulated by T3) results in increase in the level of TG-rich lipoproteins, resulting in high concentration of VLDL and TG in blood (16). Recently, the anti-atherogenic role of HDL-C has been challenged by studies showing that genetically elevated HDL-cholesterol does not offer protection against CVD [24].The doubts concerning the protective role of HDL-C have been supported by *in vitro* studies which indicate that the HDL-C from patients with CVD does not have a protective action, but does stimulate inflammation and free radical synthesis [25]. This data suggests that HDL-C in some circumstances becomes pro-atherogenic and dysfunctional [26]. Studies have indicated that PON1 is strongly associated with the HDL-C fraction, and this interaction is stabilized by the apolipoprotein A-I. PON1 is essential for the antioxidative HDL-C protection of LDL-C [27]. So in this work we have studied PON 1activity in patients with SCH in comparison not only with controls but also with patients having ACS to strengthen and ensure the validity of PON 1 as a marker of inflammation, oxidative stress and hence atherosclerosis .

In this study the mean of PON-1is highly significant lower in all groups than group I (P < 0.001), highly significant lower in group II and group IV than group III (P < 0.001) and there was no significant difference between group II and group IV regarding PON-1(P > 0.05). To summarize, PON-1 in II and IV was lower than group III than group I, which means that SCH cause significant reduction of PON-1 activity, but this reduction is still lower than that occur in ACS. Up to our knowledge no previous study assessed the PON-1 activity in subjects with SCH (alone and in association with ACS) in comparison with ACS, all studies compare PON-1 in SCH and control or overt hypothyroidism.

The present results agree with that of *Singh and Sarkar,* who found a significant decrease in PON-1 levels in SCH (P≤0.01) and in overt hypothyroidism cases (P≤0.001) than control , in this study L-thyroxine therapy significantly increased (p<0.001) serum PON-1 activity [17]. Also, the results of this study agree with the study of *Cebeci et al.,* which enrolled 25 cases with SCH and 20 healthy controls. The patient group and the control group were compared in terms of the activity of paraoxonase 1 and the oxidative stress index, and they concluded that the activity of paraoxonase was significantly low and oxidative stress was significantly high [13].In the study performed by *Başkol et al.*, PON 1 activity was found to be lower in the pre-treatment SCH patients compared to control group and post-treatment cases of subclinical hypothyroidism. The PON 1 activity in post-treatment SCH group showed a significant increase but remained significantly lower compared to control group [28].

These results are inconsistent with the study of Coria *et al.*, in which PON-1 activity did not show differences between SCH, overt hypothyroidism and euthyroid woman [29].The present study disagree with that of *Millionis et al.,* where PON 1 activity was found to be similar among the patients with subclinical hypothyroidism and control group [30]. Evidence regarding the association of SCH and the risk of CV disease appear conflicting in epidemiological studies

[30]. The Cardiovascular Health Study, a community-based study (n = 3233), showed no increased incidence of fatal or non-fatal CV events in individuals with SCH (n = 496) [31]. Another population study (n = 2730) showed an increased risk for heart failure but not for any atherosclerotic events and mortality associated with SCH (n = 338) [32]. In contrast, a meta-analysis of 15 studies showed an increased prevalence and incidence of coronary heart disease (CHD) in patients with SCH compared with euthyroid individuals; nevertheless, this was true only among subjects younger than 65 years [33]. In another community-based study, CHD was significantly more prevalent among patients with SCH (n = 119) than euthyroid individuals (n = 1906) even after adjustment for conventional CV risk factors [34]. In this the left ventricular systolic function was normal as evidenced by the normal ejection fraction. But regarding diastolic function it was impaired and the prevalence of diastolic dysfunction was higher in subjects with SCH with or without ACS (group III and IV) than control (group I).The present results also showed that the prevalence of diastolic dysfunction in subject with isolated SCH (group III) was comparable to that of subjects with ACS (group II). Up to our knowledge no study assessed the cardiac function and echocardiographic findings in subjects with SCH (alone and in association with ACS) in comparison with ACS, All studies compare it in SCH and control and in some studies assess improvement of these findings and parameters after treatment.

Our results agree with a recent one which concluded that the left ventricular systolic function was normal, but 17% of the patients showed grade 1 diastolic dysfunction as evidenced by prolonged deceleration time, isovolumic relaxation time and reduced E/A ratio, and there was significant improvement in the diastolic parameters after thyroxine replacement. Significant correlation is found between TSH level and diastolic dysfunction [35].The present study also agree with that of ***Nag et al.,*** which conclude that there was significant left ventricular diastolic dysfunction as measured by increased mitral peak A velocity ($P < 0.001$), decreased E/A ratio ($P < 0.001$), prolonged isovolumetric relaxation time ($P = 0.035$). While left ventricular systolic function was similar in both groups with no significant change [36].

The results of the present work are consistent with that of *Karki et al.,* who concluded that the diastolic dysfunction was found in 37.5% (n=15/40). They also found that with replacement therapy, 13 patients reverted to the normal whereas one having grade 2 diastolic dysfunction reverted to grade 1. One patient who had grade 1 diastolic dysfunction (impaired relaxation) did not improve [37]. The present results disagree with that of ***Nakova et al.,*** who concluded that Subclinical hypothyroidism was associated with a statistically significant reduction in systolic function of the left ventricle [38].

In Pearson analysis of our study there was negative and significant correlation between PON-1 and age *($p < 0.05$, $r = -0.297$).* There was negative and highly significant correlation between PON-1 and TC, TG and LDL *($p < 0.001$, $r = -0.638$, -0.46,* -0.65 respectively*).* While there was positive and highly significant correlation between PON-1 and HDL *($p < 0.001$, $r = 0.77$).*There was negative and significant correlation between PON-1 and TSH ($p < 0.05$, r = -0.247).Also here was positive and highly significant correlation between PON-1 and FT4 ($p < 0.001$, r = 0.34). Among patients with acute coronary syndrome (Group II, IV) (n=60), there were 44 patients with normal thyroid function (73.3%), 14 patients with subclinical hypothyroidism (23.3%), and 2 patients with euthyroid sick syndrome (3.3 %). *thus, the prevalence of SCH among ACS patients was 23.3%.*

This result is consistent with that of Zhang *et al.*, from the Mayo Clinic, who reported that among 2,430 patients treated with PCI prevalence of subclinical hypothyroidism was quite high (28.2%) [39]. The present results agree with that of *Khalil et al.,* who found that the prevalence of thyroid dysfunctions in 196 patients with acute coronary syndrome were 23% from which the most prevalent thyroid dysfunction is Euthyroid Sick syndrome (ESS) (68.9%) (which disputes with our findings) followed by Subclinical Hypothyroidism (24.5%) then Subclinical Hyperthyroidism (6.6%) [40].the results of this work disagree with that of, *Helmy et al.,* who showed that among 300 ACS patient there were 243 (81 %) euthyroid, 21 (7 %) with subclinical hyperthyroidism , 15 (5 %) with overt hyperthyroidism, 15 (5 %) with SCH and 6 (2 %) with over hypothyroidism (39). To analyze the diagnostic accuracy of PON-1 activity in predicting ACS in studied groups, the ROC curve was analyzed (Table 10). Area under curve (AUC) was 0.87, at cut off point of 18.15 U/L and sensitivity = 85.7% and specificity = 65% with positive predictive value (PPV) = 63.1% and negative predictive value (NPV) = 86.7%. The validity of PON-1 in prediction of ACS was compared in group II and group III (Table 11,12), the AUC was (1 and 0.89 in group II and III respectively) at cut off point (39 and 34 in group II and III respectively) , in group II , sensitivity is 100% , specificity is 100% while in group III sensitivity is 89% , specificity is 80.3 with PPV and NPV (82.3% and 90% respectively).

Conclusion

The level of reduction of PON-1 in subjects with SCH is comparable to that in subject with ACS; it is concluded that SCH could be considered as an independent risk factor for ACS. There is a significant negative correlation between PON-1 and TSH and a highly significant positive correlation between PON-1 and FT4, suggesting that the risk of cardiovascular disease and ACS increase with the progression (severity) of SCH. It is found that the prevalence of subclinical hypothyroidism is high (23.3 %) among young non-obese, non-diabetic and non-smoker patients with ACS.

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