Evaluation of some markers of subclinical atherosclerosis in Egyptian young adult males with abdominal obesity

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ABSTRACT

Young adults with abdominal obesity are liable to have subclinical atherosclerosis that may contribute to an increased risk of cardiovascular disease later in life. This study aims to evaluate subclinical atherosclerosis and its possible correlation with some inflammatory and biochemical markers in Egyptian young adult males with abdominal obesity. The study includes 50 young adult males (age range: 19–29 years) divided into two groups. Group 1 comprises 20 non-obese subjects (controls). Group 2 comprises 30 apparently healthy obese subjects. Carotid intima media thickness (carotid-IMT) was estimated using B-mode ultrasonography of the common carotid arteries, and abdominal ultrasonography was performed to assess the presence of a fatty liver. Laboratory investigations included fasting levels of serum glucose, triglycerides (TG), cholesterol (total [TC], high-density [HDL-cholesterol] and low-density [LDL-cholesterol] lipoprotein fractions), high-sensitivity C-reactive protein (hs-CRP), neopterin, lipoprotein-a (Lp[a]), gamma glutamyl transferase (GGT), aspartate and alanine aminotransferases (AST, ALT), plasma plasminogen and fibrinogen. Results showed that carotid IMT, serum hs-CRP, neopterin, Lp(a), fibrinogen, plasminogen, TC, TG, LDL-cholesterol and liver enzymes were significantly elevated ($P<0.001$) in the obese group compared to controls. All obese subjects showed evidence of fatty liver. A significant positive correlation was found between carotid-IMT and body mass index, waist circumference, waist/hip ratio, cholesterol, triglycerides, neopterin, hs-CRP, AST, ALT and GGT. Elevated serum levels of inflammatory biomarkers and increased ALT, AST and GGT, and non-alcoholic fatty liver disease biomarkers may be useful predictors of subclinical atherosclerosis.

KEY WORDS: Atherosclerosis.
C-reactive protein.
Liver diseases.
Neopterin.
Obesity.

Introduction

The prevalence of obesity among young adults is increasing dramatically worldwide.[1] Visceral obesity that begins in childhood and continues to adulthood may be associated with increased risk of atherosclerosis.[2] Patients, especially overweight and obese subjects, with subclinical atherosclerosis have a two-fold increased risk of developing overt cardiovascular disease.[3]

Obesity represents a state of chronic low-level inflammation as many inflammatory markers found in obese individuals originate from adipose tissue.[4,5] Inflammation plays a key role in the initiation and progression of atherosclerosis and atheromatous plaque disruption.[6,7]

Neopterin (NP), a pteridine derivative, is a catalytic product of guanosine triphosphate (GTP). It is a marker of inflammation and immune system activation that is produced by activated monocytes and macrophages in response to interferon-γ.[8] Neopterin induces pro-atherothrombotic lesions in arteries as it modulates the intracellular redox state by stimulating inducible nitric oxide synthase (iNOS), a part of the inflammatory response that impairs endothelial function.[6]

C-reactive protein (CRP) is a positive acute-phase reactant and a marker of inflammation. With the advent of high-sensitivity assays, high-sensitivity CRP (hs-CRP) is important in predicting future cardiovascular events in initially healthy individuals.[9–11] Although, hs-CRP screening in children and adults is performed, less attention has been given to young adults.[12]

Fibrinogen, a 340 kDa glycoprotein synthesised by the liver, is involved in platelet aggregation and coagulation. It is an acute-phase protein produced in response to inflammation. A relationship has been established between its elevation and the risk of cardiovascular disease.[13]

Lipoprotein-a (Lp[a]) is a low-density lipoprotein (LDL)-like particle containing a molecule of apo(a) that has homology with plasminogen, thus it competes with plasminogen for its high-affinity binding sites on endothelium, platelets and macrophages.[14,15] It can interfere with fibrinolytic processes by acting as a procoagulant, as it localises fibrinogen in the arterial wall. This supports the hypothesis that Lp(a) is thrombogenic.[16] Rajtari *et al*.[17] reported that fibrinogen, Lp(a) and plasminogen are related

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to visceral fat and could be considered as haemostatic risk factors for cardiovascular disease.

High-resolution B-mode ultrasonography provides a non-invasive method for quantifying arterial wall thickness.[9] Increased carotid intima media (carotid-IMT) thickness is an established marker for subclinical atherosclerosis.[18]

Clinical studies have reported a strong association between elevated alanine aminotransferase (ALT) and gamma glutamyl transferase (GGT) – as markers of non-alcoholic fatty liver disease (NAFLD) – and coronary heart disease. However, information on the persistence of increased levels of liver enzymes and their effect on cardiovascular risk in young adults is limited.[19–21]

This study aims to evaluate subclinical atherosclerosis and its possible correlation with some inflammatory and biochemical markers in Egyptian young adult males with abdominal obesity.

Materials and methods

The study was approved by the ethical committee of the Medical Research Institute (MRI), Alexandria University. Written informed consent was received from the study participants.

Fifty young adult males (age range: 19–29 years) of similar socioeconomic status were selected from workers at MRI and included in the study. The participants were divided into two groups. Group 1 comprised 20 healthy non-obese subjects (body mass index [BMI]: <24.9 kg/m^2, waist circumference [WC] ≤94 cm and waist:hip ratio [WHR] ≤0.8) (controls). Group 2 comprised 30 apparently healthy obese subjects (BMI <30 kg/m^2, WC >94 cm and WHR >1).

All subjects were non-smokers, not alcoholics and were free from anaemia, acute infection (including hepatic viral infection), cardiac, pulmonary, renal, endocrine and collagen diseases and malignancy. They were not receiving any medication.

All study subjects received the following:

- clinical history
- clinical examination including blood pressure measurement and resting electrocardiogram
- anthropometric measurements including BMI, WC and WHR[22,23]
- abdominal ultrasonography to assess the presence of fatty liver (in conjunction with high ALT and/or AST levels and the absence of viral hepatitis, autoimmune hepatitis and metabolic liver diseases)[24,25]
- carotid-IMT measurement.[26]

Blood (7 mL) was taken from each subject after overnight fast. A 5 mL sample was collected in a plain tube, centrifuged and the separated serum divided into two aliquots. One was used for fasting serum glucose (FSG), total cholesterol , high-density lipoprotein (HDL-cholesterol), low-density lipoprotein (LDL-cholesterol), triglycerides (TG), creatinine, hs-CRP and ALT, AST and GGT.[27] These were measured using a Konelab chemistry analyser (Thermo Electron. Vantaa, Finland). The AST:ALT ratio was calculated. Serum LDL-cholesterol level was estimated using the equation of Friedwald *et al*.[28] The other aliquot was frozen at –80°C for serum neopterin measurement using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (RE 59321; IBL, Hamburg, Germany; assay sensitivity: 0.7 nmol/L, intra-assay coefficient of variation [CV]: 3.6%),[29] and also for the determination of serum Lp(a) using an ELISA kit (Diamedex Is-Ap-tek Lp[a]; assay sensitivity: 3.1 mg/dL, intra-assay CV: 2.2%).[30]

The other 2 mL blood was citrated and centrifuged. The plasma was used to measure plasminogen (assay sensitivity: 6 mg/dL, intra-assay CV: 1.5%)[31] and fibrinogen using an immunochemical reaction (Dade Behring, Marburg; assay sensitivity: 180 mg/dL, intra-assay CV: 2.7%).[32]

Data analysis was performed using SPSS version 11.5. The Kolmogorov-Smirnov test of normality revealed non-normally distributed variables (except in the case of age, WC, cholesterol, neopterin and plasminogen). Non-normally distributed variables were reported as median (minimum and maximum range), while normally distributed variables were reported as mean (standard deviation). Comparison between obese subjects and controls was performed using the Mann-Whitney test for all variables except the normally distributed data using Student's *t*-test. Correlation between carotid-IMT and all parameters was performed using Pearson's correlation. All tests were two-sided and the level of significance was set at $P \leq 0.05$.

Results

Table 1 shows the clinical data obtained for the two groups. Systolic and diastolic blood pressure, BMI, WC and WHR were significantly higher in the obese group (Group 2) compared to the control group (Group 1) ($P<0.001$). All the obese subjects had fatty liver diagnosed by abdominal ultrasonography with significant increase of right hepatic lobe diameter at mid-clavicular line ($P<0.001$) compared to controls.

Table 2 shows the results of laboratory parameters. Serum levels of total cholesterol, TG, LDL-cholesterol and liver enzymes (AST, ALT and GGT) were significantly higher ($P<0.001$), while HDL-cholesterol and AST:ALT ratio were significantly lower ($P<0.001$) in the obese group compared to the controls.

Table 3 shows the results of carotid-IMT, serum neoptrin, hs-CRP, plasma Lp(a), fibrinogen and plasminogen testing. Significant increase was found in carotid-IMT, serum neopterin, hs-CRP and plasma fibrinogen levels in the obese subjects compared to the controls ($P<0.001$). With plasma Lp(a) and plasminogen, a significant increase was seen in the obese subjects but values were within the normal reference range.

Table 4 shows correlations between carotid-IMT and the studied variables in the obese group (Group 2).

Discussion

In the present work, a significant increase in carotid-IMT was found in young obese males compared to controls. The increased anthropometric measures of obesity (BMI, WC and WHR) and dyslipidaemia significantly positively correlated with carotid-IMT, indicating that young subjects with abdominal obesity have subclinical atherosclerosis and may be at risk of future cardiovascular events. Juonala *et al*.[33] reported that dyslipidaemia and obesity from childhood

Table 1. Clinical data of control and obese subjects.

		Control group (n=20)	Obese group (n=30)	Test of significance	P value
Age (years)	Mean±SD	25.65±2.18	27.3±2.93	1.75	0.095
Systolic blood pressure (mmHg)	Median (minimum–maximum)	117.5 (90–130)	130 (120–140)	81	<0.001
Diastolic blood pressure (mmHg)	Median (minimum–maximum)	75 (65–80)	80 (70–85)	98	<0.001
BMI (kg/m^2)	Median (minimum–maximum)	25.5 (22–29)	35.98 (30.3–41.2)	0.00	<0.001
WC (cm)	Mean±SD	82.15±5.95	114.5±8.11	15.27	<0.001
WHR	Median (minimum–maximum)	0.80 (0.7–0.85)	1.04 (0.96–1.12)	0.00	<0.001
Obesity duration (years)	Mean±SD	–	10.70±5.75	–	–
Fatty liver	–	+	–	–	
Right hepatic lobe diameter (cm)	Median (minimum–maximum)	13 (12.8–13.5)	17.7 (14–20)	0.00	<0.001

BMI: body mass index, WC: waist circumference, WHR: waist:hip ratio.
Statistically significant: $P \leq 0.05$.

have detrimental effects on the vasculature and contribute to atherosclerosis risk in adulthood.

Subclinical inflammation is implicated in the initiation and/or progression of atherosclerosis in obese subjects.[34,35] Kazaki *et al*.[8] reported that neopterin may provide more comprehensive information about cardiovascular risk than hs-CRP measurement, particularly when macrophage activation is implicated. In the present study, significant elevation of the two inflammatory markers (hs-CRP and neopterin) was found in the obese subjects, which supports the relationship between obesity and low-grade inflammation. Moreover, the significant positive correlations of hs-CRP and neopterin with carotid-IMT confirms the role of the inflammatory process in promoting subclinical atherosclerosis.

The elevated hs-CRP could be considered a procoagulant as it is reported to bind to LDL-cholesterol and very low density lipoproteins (VLDL), which in turn activates the complement system, stimulates tissue-factor production by macrophages and initiates the coagulation process. C-reactive protein also mediates the uptake of LDL-cholesterol into macrophages, converting them to foam cells.[36]

The association between neopterin and carotid-IMT could be explained by the fact that activated macrophages can stimulate smooth muscle cells, expressing nitric oxide synthase (NOS) with production of large, potentially toxic amounts of nitric oxide, interstitial forms of collagen and the proliferation of the extracellular matrix.[37,38]

In the present work, obese subjects showed significantly higher plasma fibrinogen levels compared to the control group, and it also significantly positively correlated with carotid-IMT, indicating its role in subclinical atherosclerosis in this group. These findings are in accordance with those of Paramo *et al*.,[39] who suggested that fibrinogen can be an independent marker of subclinical atherosclerosis in asymptomatic subjects.

Fibrinogen production in the liver is regulated by cytokines and is greatly enhanced in response to different inflammatory responses. Therefore, it is possible that elevated fibrinogen levels may reflect the chronic inflammatory state that characterises atherosclerosis. In addition, fibrinogen may promote vascular disease by increasing blood viscosity, promoting fibrin formation and enhancing platelet-platelet interaction.[40] Levels of Lp(a) and plasminogen were significantly increased in obese subjects but their values were within the normal reference ranges and were not associated with carotid-IMT.

In the present study, all the obese subjects showed fatty

Table 2. Laboratory parameters in control and obese subjects.

		Control group (n=20)	Obese group (n=30)	Test of significance	P value
Fasting serum glucose (mmol/L)	Median (minimum–maximum)	5 (3.74–5.5)	5.44 (4.24–6)	205	0.057
Total cholesterol (mmol/L)	Mean±SD	3.67±0.53	5.76±0.89	10.285	<0.001
Triglycerides (mmol/L)	Median (minimum–maximum)	0.86 (0.41–1.45)	1.83 (0.89–2.42)	42	<0.001
LDL-cholesterol (mmol/L)	Median (minimum–maximum)	2.34 (1.56–3.64)	4.22 (2.52–5.54)	21	<0.001
HDL-cholesterol (mmol/L)	Median (minimum–maximum)	1.09 (0.86–1.85)	0.74 (0.49–1.2)	93	<0.001
GGT (U/L)	Median (minimum–maximum)	29.5 (20–38)	47 (24–133)	84	<0.001
AST (U/L)	Median (minimum–maximum)	20.0 (9–31)	24 (16–88)	142	0.002
ALT(U/L)	Median (minimum–maximum)	17.5 (10–31)	39 (30–216)	67.5	<0.001
AST:ALT ratio	Median (minimum–maximum)	0.57 (0.41–0.97)	0.98 (0.3–0.99)	100	<0.001
Creatinine (mmol/L)	Median (minimum–maximum)	0.07 (0.05–0.10)	0.07 (0.06–0.1)	287	0.791

Statistically significant: $P \leq 0.05$.

Table 3. Results of carotid-IMT, serum neopterin, hs-CRP, Lp(a) and plasma fibrinogen and plasminogen in control and obese subjects.

		Control group (n=20)	Obese group (n=30)	Test of significance	P value
Carotid-IMT (mm)	Median (minimum–maximum)	0.3 (0.1–0.6)	0.8 (0.6–1)	2	<0.001
Serum Neopterin (nmol/L)	Mean±SD	7.21±1.84	15.63±3.2	11.76	<0.001
Serum hs-CRP (mg/dL)	Median (minimum–maximum)	2.4 (1.0–3.0)	4.4 (0.8–16)	162.5	0.006
Serum lipoprotein (a) (mg/dL)	Median (minimum–maximum)	7.0 (4.0–11.2)	10 (9.6–16.4)	52	<0.001
Plasma fibrinogen (mg/dL)	Median (minimum–maximum)	180.5 (160–220)	339 (229–620)	0.00	<0.001
Plasma plasminogen (mg/dL)	Mean±SD	10.05±2.27	12.05±1.57	3.69	<0.001

Statistically significant: $P \leq 0.05$.

liver with significant elevation of liver enzyme activities (AST, ALT and GGT). These enzymes showed a positive correlation with carotid-IMT, suggesting that NAFLD could be associated with subclinical atherosclerosis in young obese adults.

The Hoorn study,[41] conducted on a large population aged 50–70 years, proved that subjects with NAFLD and elevated ALT were liable to increased risk of future coronary heart disease over a 10-year follow-up period.

The association between NAFLD, its elevated biomarkers and the risk of atherosclerosis in obese subjects has been explained in previous studies. Targher *et al*.[42] reported that abdominal adipose tissue-derived adipocytokines may be implicated in this link. In addition, low-grade systemic and hepatic inflammation related to abdominal obesity could produce tumour necrosis factor-α and interleukin-6 which further amplify the inflammatory cascade by stimulating hepatic CRP production.[43] Kerner *et al*.[44] demonstrated elevated hs-CRP in patients with high ALT, which was in accordance with the findings in the obese subjects in the present study.

In conclusion, young adult males with abdominal obesity are liable to develop subclinical atherosclerosis and hence they are prone to increased risk of future cardiovascular events. Elevated serum levels of the inflammatory biomarkers neopterin, hs-CRP and plasma fibrinogen, as well as the increased activity of the hepatic transaminases (ALT and AST) and GGT may be useful predictors of subclinical atherosclerosis. Screening for subclinical atherosclerosis in obese subjects, especially those with NAFLD, is recommended and should be conducted from childhood and continued into adulthood. Young adults with abdominal obesity should receive early treatment to reduce atherosclerotic risk factors and be encouraged to modify their lifestyle and reduce their weight. □

Table 4. Correlation between carotid-IMT and some studied parameters in obese subjects.

	Pearson coefficient	P value
BMI	0.406	0.026
WC	0.416	0.022
WHR	0.481	0.007
Systolic blood pressure	0.456	0.011
Diastolic blood pressure	0.403	0.027
Total cholesterol	0.504	0.001
Triglycerides	0.589	0.001
HDL-cholesterol	–0.626	0.001
AST	0.404	0.027
ALT	0.603	0.001
GGT	0.0496	0.005
Neopterin	0.398	0.03
hs-CRP	0.49	0.006

BMI: body mass index, WC: waist circumference, WHR: waist:hip ratio. Statistically significant: $P \leq 0.05$..

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