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ORIGINAL ARTICLE

Obstructive Sleep Apnea and Cardiovascular Risk Markers (Fibrinogen & Gamma Glutamyl Transferase) in Obese Males

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ABSTRACT

Aim: To determine and compare gamma glutamyl transferase (GGT) and fibrinogen among obese males with and without obstructive sleep apnea (OSA). Second objective was to investigate correlation between blood pressure and GGT.
Methodology: Sixty-four obese males aged 20-45 years with BMI > 25kg/m^2 were included by convenience sampling. The study was conducted, after obtaining ethical approval from IRB, at the Department of Physiology, Post Graduate Medical Institute, Lahore from August 2014 to May 2015. Participants having acute or chronic inflammatory conditions were excluded. Participants were screened for OSA by Berlin and STOP BANG questionnaires. Diagnosis of OSA was made by overnight portable pulse oximetry. The participants were divided into two groups. Group I had 32 obese males with OSA. Group II contained 32 obese males without OSA. After an overnight fasting of 10-12 hours blood samples were drawn. Serum fibrinogen and GGT were measured by spectrophotometer. The data was analyzed using SPSS-22. Quantitative variables were compared between the two groups by Mann-Whitney U test. Spearman correlation was used to correlate blood pressure and GGT among the participants.
Results: Fibrinogen was significantly raised (p=0.015) in obese males with OSA. Systolic blood pressure (p=0.003), diastolic blood pressure (p=0.001) and mean arterial blood pressure (p<0.001) showed strong positive correlation with GGT in obese males with OSA.
Conclusion: Proinflammatory, procoagulant and proatherogenic marker fibrinogen levels were significantly raised in obese otherwise healthy males with OSA. Oxidative stress marker GGT showed strong positive correlation with blood pressure in obese males with OSA.
Keywords: Fibrinogen, gamma glutamyl transferase, inflammation, obstructive sleep apnea, oxidative stress

INTRODUCTION

Obstructive sleep apnea (OSA) is defined as transient breathing cessation during sleep because of obstruction of upper airway. These apnea episodes which are repeated and frequent lead to disintegration of sleep and fall in oxygen saturation level.[1] Clinically, OSA presents with snoring, excessive daytime sleepiness, witnessed apneas and nocturnal choking.[2] Moderate–severe OSA has been observed in about 13% adult males and 6% adult females. OSA is associated with poor quality of life, daytime somnolence, impaired cognition, increased risk of automobile accidents and a variety of cardiovascular diseases[3].

Some mechanisms that may increase the risk of cardiovascular diseases in OSA patients are changes in the intrathoracic pressure, frequent intermittent hypoxia and stimulation of the central nervous system. These factors lead to mild inflammation, oxidative stress, increased sympathetic tone, altered vasomotor function and atheroma formation in the blood vessels.[4] Fibrinogen, a component of coagulation pathway and an acute phase reactant, participates in two processes of atherosclerosis: inflammation and thrombosis. Therefore, its screening has the potential of cardiovascular risk prediction.[5] Hypoxemia in OSA triggers the release of inflammatory factors and also increases fibrinogen levels that alter the micro milieu of blood resulting in increased blood coagulability which predisposes to increase cardiovascular disease[6].

Gamma glutamyl transferase (GGT) is proinflammatory, proatherogenic and oxidative stress marker. It can be used for the evaluation of "vessel at risk" along with C-reactive protein and fibrinogen.[7] Serum GGT is also important in predicting cardiovascular diseases in obstructive sleep apnea[8] Various studies demonstrate correlation between increase GGT levels and cardiac mortality and non-fatal myocardial infarction. Increased GGT level is also a useful oxidative stress marker in OSA patients.[9] Serum GGT is positively related to cardiovascular risk markers like CRP and fibrinogen. Serum GGT level is also associated with other markers of oxidative stress in hypertension, obesity, dyslipidemia, metabolic syndrome, diabetes mellitus and fatty liver disease. Higher GGT levels, even within its normal range, are considerably associated with a 10-years risk of cardiovascular diseases in men. These higher levels are also associated with diabetes mellitus, incident hypertension, metabolic syndrome and fatty liver disease in men[10].

There seems dearth of knowledge on fibrinogen, GGT and OSA in Pakistan. Comparison of cardiovascular risk markers (fibrinogen and GGT) among young otherwise healthy obese males with and without OSA may help in further understanding the pathophysiology of cardiovascular risk associated with OSA at Asian specific BMI cutoff for obesity. Therefore, we planned this study. Our first objective was to determine gamma glutamyl transferase (GGT) and fibrinogen levels among obese males with and without OSA. Second objective was to investigate correlation between blood pressure and GGT.

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METHODOLOGY

This was a comparative cross-sectional study that was conducted from August 2014 to May 2015 after permission from IRB. Study setting was the Department of Physiology at Postgraduate Medical Institute (PGMI), Lahore. Following formula was used to calculate the sample size:

$n = (\sigma^2 1+\sigma^2 2)\ (Z_{1-\alpha/2}+Z_{\beta})^{\ 2} / (\mu 1-\mu 2)^2$

Where σ1=10.0, σ2=22.0, μ1=28.0, μ2=40.0

$Z_{1-\alpha/2}$=1.96, where 1- α/2 = 0.975 or 95% level of confidence

Z_{β}= 0.841, where β = 0.10 or at 90% power of the study

Values were placed in the formula and we got n=31.9≈32 for each group[9].

Total sample size was 64. All the participants were obese males which were divided into two groups. Group I contained participants (n=32) who were suffering from OSA and the participants in group II (n=32) did not have OSA. Non-probability convenience sampling was done and participants were recruited according to the selection criteria. The subjects recruited were

students and employees of PGMI, Lahore. The study included all male subjects with age limit 20-45 years and BMI more than 25 kg/m^2 i.e., obese according to Asian specific criteria for obesity. Exclusion criteria of the study was any acute infection at the time of recruitment, chronic inflammatory disease, history of upper respiratory infection during last one-month, chronic use of sedative or anxiolytic drugs, smoking, COPD e.g., asthma, and alcoholism.

The study was approved by the ethical review committee of the PGMI, Lahore. The potential participants were approached and informed about the scope of the study. Then, the interested participants signed the consent form before data collection. All the study participants were screened for OSA by STOP BANG and Berlin Questionnaires to find low-risk and high-risk individuals. Definitive diagnosis of OSA was studied among the participants using overnight portable pulse oximetry. The subjects having oxygen desaturation index of ≥4% for 5-15 events per hour were included in the OSA group i.e., group I[11].

After about 12 hours of overnight fasting, 6 ml venous blood was drawn using aseptic technique. Blood was dispensed into yellow top serum vial and blue top vial containing sodium citrate for fibrinogen. Blood was centrifuged at 3000 rpm for ten minutes. We extracted serum and stored it at -40˚C until assayed. The plasma level of fibrinogen was determined by Clauss method. We used a kit manufactured by Wiener Lab to measure the fibrinogen level. Principle of Clauss method is that the time needed for clotting in dilute plasma is inversely proportional to the fibrinogen level in plasma in the presence of high thrombin concentrations. The clotting time obtained is compared with a standard preparation of fibrinogen[12]. GGT in serum was determined by colorimetric method using kit manufactured by Human, Germany.

IBM-SPSS version 22 was used to enter and analyze the data. Kolmogorov Smirnov test was used to check the distribution of data. Non-normally distributed quantitative variables were summarized by median (IQR). Non-normally distributed quantitative variables were compared between the two groups using Mann-Whitney U test. Correlation between blood pressure and GGT was determined by Spearman's correlation. *p*-value <0.05 was considered significant.

RESULTS

In group I median (IQR) of fibrinogen was 440 (356-470) mg/dl and in group II it was 375 (330- 410) mg/dl. Comparison of fibrinogen levels between the groups showed a significant difference (*p*=0.015). In group I median (IQR) of GGT was 42.75 (28.68–77.55) Units/L and in group II it was 36.25 (19.73–51.08) Units/L. No significant difference (*p*=0.127) was found for GGT between the two groups (Table I).

A positive correlation was observed between serum GGT and systolic blood pressure (Rho= 0.508, *p*= 0.003), diastolic blood pressure (Rho= 0.577, *p*= 0.001) and mean arterial pressure (Rho= 0.645, *p* <0.001) in group I. No significant correlation of serum GGT with systolic blood pressure (Rho= -0.005, *p*= 0.980), diastolic blood pressure (Rho= 0.056, *p*= 0.762) and mean arterial pressure (Rho= 0.019, *p*= 0.917) was found in group II (Table II).

Table I: Comparison of fibrinogen and GGT among the study groups

Variables	**Group I (**Obese males with OSA) (n=32)	**Group II** (Obese males without OSA) (n=32)	***p*-value**
Fibrinogen (mg/dl) [a]	440 (356–470) [b]	375 (330–410)	0.015*
GGT (Units/L) [a]	42.75 (28.68–77.55)	36.25 (19.73–51.08)	0.127

[a] Comparison done by Mann-Whitney U test [b] Values are given as median (IQR) *Significant

Table II: Correlation between blood pressure and gamma glutamyl transferase in obese males with and without OSA

	Serum GGT inGroup I (Obese males with OSA) (n = 32)		**Serum GGT in Group II** (Obese males without OSA) (n = 32)	
	Spearman's Rho	***p*-value**	**Spearman's Rho**	***p*-value**
Systolic Blood Pressure	0.508	0.003*	-0.005	0.980
Diastolic Blood Pressure	0.577	0.001*	0.056	0.762
Mean Arterial Pressure	0.645	<0.001*	0.019	0.917

*Significant

DISCUSSION

We have found significantly raised (*p*= 0.015) fibrinogen levels in obese males with OSA. There are studies that are in favor of our results that fibrinogen level is raised in OSA.[13] However, a study failed to reveal the difference of fibrinogen levels between two groups of obese individuals.[14] Raised fibrinogen levels in OSA are suggestive of tendency for coagulation and atherosclerosis.[13] Nocturnal hypoxia and sleep disturbances in OSA leads to increased fibrinogen levels and other inflammatory markers.[15] These hypoxic events also increase sympathetic activity and hence procoagulant disturbance in OSA.[16]

Fibrinogen, a main clotting protein related with inflammation, is an important marker for cardiovascular risk. Increased levels of systemic inflammatory markers like fibrinogen and its correlation with OSA in subjects without known comorbidities suggest the independent involvement of inflammation in OSA.[17] In our study the raised fibrinogen level in OSA group is independent of smoking and alcohol consumption as has also been reported in literature.[15] According to Fibrinogen Studies Collaboration a long term rise in fibrinogen level of 100mg/dl above normal doubles the risk of cardiovascular diseases. Increase fibrinogen levels are highly important in middle aged, healthy population for predicting cardiovascular risk.[15,18] Fibrinogen increases thrombogenesis and atherogensis by effecting platelet aggregation, endothelial cell injury and plasma viscosity.[19] Fibrinogen and its degradation products stimulate smooth muscle proliferation and migration and damage blood vessels.[19] Fibrinogen directly induces atherosclerosis formation because it is not only a prothrombotic and proinflammatory factor but also an indicator of ongoing acute process associated with inflammation[20].

Our study has found a strong positive correlation between GGT and systolic blood pressure (*p*=0.003), diastolic blood pressure (*p*=0.001) and mean arterial pressure (*p*<0.001) in group I only. Literature also supports this correlation of GGT with blood pressure.[21] Raised GGT has as an important role in predicting CVD in OSA and its levels are directly proportional to the severity of OSA.[8, 22] In the present study although values of GGT in group I are higher than in group II within normal reference range but we have failed to find statistically significant difference (*p*=0.127) of GGT among the study groups. Due to positive correlation of GGT and blood pressure and higher values of GGT in group I, values if followed for long time may exhibit a significant difference between the groups. This is supported by a population-based cohort study in which longitudinal GGT change has been assessed in an average period of 6.9 years and serial GGT measurement prospectively followed up for a median of 10.2 years. They found a longitudinal increase in GGT even within its normal range, independent of baseline level. These raised GGT levels increase the risk of fatal cardiovascular disease in younger age.[23]

GGT is considered important in predicting cardiac diseases in OSA patients. The main contributing factor in the development of cardiac complications in OSA is oxidative stress.[9] There are some mechanisms that support the supposition that GGT activity reflects the risk of CVD. GGT is an oxidative stress marker that is related to subclinical chronic inflammation. Both of these mechanisms are important for atherosclerotic cardiovascular disease. Another

possible mechanism is the strong correlation of GGT with several atherosclerotic risk factors like metabolic syndrome, hypertension and diabetes.[10] The oxidative stress mediated by GGT has a central role in atherosclerotic plaque formation. GGT is positively related with oxidative and inflammatory reactions contributing to atheromatous plaque formation. Significantly higher GGT levels are found in acute coronary syndrome (ACS) that are indicator of oxidative stress in ACS patients without liver disease and alcohol abuse. GGT is an accurate cost-effective test that can be used as an adjuvant biomarker to identify potential risk of coronary atherosclerosis[24].

Cross-sectional nature of the study, non-probability sampling and limited sample size are some of the limitations that may hinder the generalizability of the results. Prospective studies are required to study the cause-and-effect relationship of OSA, fibrinogen and GGT. Strength of study is that use of simple laboratory biochemicals in resource poor settings may help the clinicians to lessen the incidence of cardiovascular disease risk and its progression in obstructive sleep apnea patients. Researchers have to recognize that OSA, fibrinogen and GGT are in need of dedicated research to broaden the knowledge base in Pakistan.

CONCLUSION

Intermittent hypoxia of sleep apnea can lead to significantly higher levels of proinflammatory, procoagulant marker fibrinogen in obese males with OSA. Higher values of GGT in OSA group are indicative of oxidative stress and GGT show strong correlation with blood pressure in OSA group labeling it a cardiovascular risk marker in OSA.

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