ORIGINAL ARTICLE

Haematological Markers of Obese Male Subjects with and without Obstructive Sleep Apnea

SHAGUFTA KHALIQ[1], ATTIQA KHALID[2], HIFZA NOOR LODHI[3], *KOMAL IQBAL[4], MUNIZA SAEED[3]*
[1]Postgraduate Medical Institute(PGMI), Lahore. Currently working at Lahore Medical and Dental College, Lahore.
[2]Lahore Medical and Dental College, Lahore.
[3,4,5]Postgraduate Medical Institute/Ameer ud din medical college /Lahore General Hospital
Correspondence to Dr. Shagufta Khaliq, Senior Lecturer, Email: shaguftakhaliq30@gmail.com, Tel.: 00923064409748

ABSTRACT

Background: Obstructive sleep apnea (OSA) is a breathing disorder. Intermittent hypoxia, central nervous system stimulation and intrathoracic pressure changes of OSA initiate oxidative stress and systemic inflammation. Inflammation is the central part of atherogenic process and patients with OSA have higher levels of inflammatory biomarkers.
Aim: To compare the nonspecific inflammatory markers of cardiovascular risk in obese male population with and without OSA.
Study design: Comparative Cross Sectional Study
Methods: Sixty four male subjects were recruited for this. Thirty two in group I (Obese Male with Obstructive Sleep Apnea) and thirty two in group II (Obese Male without Obstructive Sleep Apnea). After taking informed consent, they were screened for OSA by using Berlin questionnaire, stop bang questionnaire and overnight pulse oximetry. Six ml blood sample was taken by using aseptic technique for estimation of complete blood count by haematology analyser. *P*-value < 0.05 was considered statistically significant.
Results: Comparison of both WBC related and RBC related haematological markers showed statistically non significant results.
Conclusion: Non specific haematological markers are not raised in obese otherwise healthy subjects with OSA.
Keywords: Obstructive sleep apnea, cardiovascular risk, non specific inflammatory marker

INTRODUCTION

Obstructive sleep apnea (OSA) is a sleep related breathing disorder. It is associated with complete or incomplete difficulty in airflow in spite of an ongoing effort to breathe because of relaxation of the pharyngeal muscles. These frequent apnea episodes result in oxygen desaturation, sleep fragmentation and excessive daytime sleepiness[1]. OSA has been associated with many adverse outcomes like hypertension, obesity, diabetes mellitus and cardiac abnormalities[2]. The risk factors for OSA are obesity, age, gender, menopause, craniofacial abnormalities, smoking, alcohol use, and family history[3]. Obesity, a low grade systemic inflammation, is related with endothelial dysfunction. Sleep apnea also increases systemic inflammation in obese individuals and increase the risk of cardiovascular disease in obese individuals with OSA[4].

OSA pathophysiology has not been fully explained. It is suggested that repeated hypoxia episodes of OSA leads to the development of low grade systemic inflammation and oxidative stress. It is also responsible for altered inflammatory, metabolic and immune responses. According to literature low grade chronic inflammation within microvasculature play an important role in OSA related cardiovascular problems[5]. Hence, detection of inflammatory markers in OSA may be helpful for cardiovascular risk prediction in OSA subjects. Inflammation is the basis of atherosclerosis and OSA patients have raised levels of inflammatory markers. White blood cell count (WBC) in the circulation has been considered as one of a few biomarkers for cardiovascular risk prediction. In literature relationship between WBC count and fatal coronary artery disease have been noted but still controversy exist. It provides assessment of inflammatory status but its predictive ability has not been fully explored[6]. Raised white blood cell count is strong in predicting coronary risk in patients of both genders, whether they have coronary disease or not[7]. Neutrophil Lymphocyte Ratio (NLR) is a new indicator of subclinical inflammation both in cardiac and non cardiac disorders[8]. Increased hermatocrit in OSA can also contribute to cardiovascular risk[9]. Red blood cell Distribution Width (RDW), a measure of the variability in size of the circulating erythrocytes, is related with cardiovascular comorbidities[10].

Pakistan offers a good opportunity to study the risk of cardiovascular diseases in relation to OSA.

Received on 24-03-2019
Accepted on 14-07-2019

MATERIALS AND METHODS

A cross-sectional comparative study was carried out at Physiology Department Postgraduate Medical Institute, Lahore. The study involved 64 obese males comprising of two groups

Group I Obese males with obstructive sleep apnea (n = 32)
Group II Obese males without obstructive sleep apnea (n=32)
All male & female patient between age :20 – 45 years having BMI more than 25kg/m^2 were included in the study.

Exclusion Criteria

- Any acute febrile illness or infection at the time of recruitment
- History of (H/O) upper respiratory tract infection within past four week
- H/O chronic inflammatory disease
- H/O chronic anxiolytic or sedative use
- H/O chronic respiratory diseases like asthma, COPD
- Smoking
- Alcoholism

After subject selection, written informed consent of the volunteer subjects was taken on a consent proforma.

Obstructive Sleep Apnea (OSA) screening: All subjects who met the inclusion criteria were screened for obstructive sleep apnea by three screening tools:
i. Berlin Questionnaire
ii. STOP BANG Questionnaire
iii. **Overnight portable pulse oximetry (**Spirodoc pulsox) was done on all subjects whether they were high risk or low risk according to questionnaires and those subjects were included in obstructive sleep apnea group who had ≥4% ODI 5-15 events/hour.

Blood collection: By using aseptic technique six ml of blood sample was drawn from antecubital vein after 10 – 12 hours of fasting. It was dispensed into purple top EDTA vial for CBC.

White blood cell count(WBC) & hematocrit

White blood cell counts (WBC) and Hematocrit were measured using automated hematology analyzer(Sysemex hematology analyzer) performed at the clinical laboratory of the Lahore general hospital.

Normal Range: WBCs 4- 11 × 10^3/ cu mm (Barret et al., 2010)

Measurement of NLR: Neutrophil lymphocyte ratio (NLR) was calculated as a simple ratio between the absolute neutrophil and lymphocyte count. Both neutrophil and lymphocyte were obtained from the same automated blood sample. Neutrophil lymphocyte ratio was computed for each subject.

Normal Range: 0.78- 3.53 (Forget et al., 2017)

Statistical analysis: The data was entered and analyzed using IBM-SPSS version 22. The data was checked for normal distribution by Kolmogorov Smirnov test. Normally distributed variables were presented by mean ± standard deviation. Median IQR was taken for non-normally distributed quantitative variables. Independent Sample 't-test' was applied to compare normally distributed quantitative variables between the two groups. Mann Whitney U test was applied to compare non-normally distributed quantitative variables between the two groups. A p value of < 0.05 was considered statistically significant

RESULTS

We measured and compared simple, inexpensive easily available cardiovascular risk markers WBC, NLR , hematocrit, RBC, RDW and hemoglobin between obese males with and without OSA. Comparison of white cell related parameters in Table 1 show non significant results and comparison of RBC related hematological parameters in Table 2 also show statistically non significant results. Comparison of white blood cell related hematological parameters between the study groups

Table.1 Comparison of White blood cell related hematological parameters between the study groups

Variables	Group I	Group II	*p*- value
WBCs[b] (per cubic mm)	7.7±1.8[d]	6.9±1.3[d]	0.060
NLR[a]	1.56(1.39 –.89)[c]	1.39(1.1–1.9)[c]	0.265

Values are given as median (IQR)[c] and mean ± SD[d]
[a] Comparison by Mann- Whitney U test
[b] Comparison by Independent Sample "t-test"
p< 0.05 is considered significant
WBC: White Blood Cells
NLR: Neutrophil Lymphocyte Ratio

Comparison of Red Blood cell related hematological parameters among study population

Table.2 Comparison of Red Blood Cell related hematological parameters among study population

Variables	Group I	Group II	*p*- value
RBCs[b] (per cubic mm)	4.77±0.41	4.7±0.68[d]	0.65
Hemoglobin[b](g/dl)	14.04 ± 0.97	13.98 ± 0.81[d]	0.76
RDW[b]	12.76 ± 0.82	13.22 ± 1.58[d]	0.153
Hematocrit[a] (%)	42.3 (40.6 – 43.3)[c]	42.8 (40.0 – 44.2)[c]	0.804

Values are given as median (IQR)[c] and mean ± SD[d]
[a] Comparison by Mann- Whitney U test, [b] Comparison by Independent Sample "t-test"
RBC: Red Blood Cell, RDW: Red cell Distribution Width
p< 0.05 is considered significant

DISCUSSION

The variables used in this study are simple and inexpensive. A universally available test Complete Blood Count (CBC) can be used for cardiovascular risk prediction.

Cardiovascular disease is one of the greatest cause of death globally. Many factors increase the risk of cardiovascular disease(CVD) and obesity is one of them. Obstructive sleep apnea (OSA) also increases the risk of CVD. This study has been done to find out whether simple, inexpensive hematological markers are more in obese with OSA than obese without OSA.

Obstructive sleep apnea is a low level inflammation that exist both at systemic and tissue level.[11]Local inflammation is a strong predictor of endothelial health. The total number of WBCs and each subtype have been reported as predictors of Coronary Heart Disease. All blood cells are involved almost in the development and progression of atherosclerosis[12].

The leukocyte count has emerged as a marker of inflammation and its raised levels are predictor of future cardiovascular events.[13] Though higher values of WBC's within normal range are present in group I but the present study has failed to show statistically significant difference of WBC (*p*= 0.060) between the groups. Increase leukocyte count affects CHD through several pathological mechanisms that cause inflammation, oxidative and proteolytic damage to the endothelial cells, increase coagulation, thrombosis and infarction.[14] Neutrophil lymphocyte ratio(NLR) of peripheral blood is another indicator of systemic inflammation as well as coronary artery disease and its risk.[15] Our study has shown no significant results for NLR (*p*= 0.265). The results are consistent with a study which has reported that NLR is not reflective of the severity level of systemic inflammation in OSA subjects[16].

Hematocrit plays an important role in blood coagulability because of its effect on blood viscosity and platelet aggregation. Hypoxic individuals frequently have increased hematocrit.[17] Increased blood coagulability due to changes in rheological properties of blood is an important factor linking OSA and cardiovascular complications[18]. OSA subjects have increased hematocrit levels because of hypoxia and there is positive correlation of hematocrit with OSA severity.[19] Current study fails to show statistically considerable difference (*p*=0.804) of hematocrit between the study groups. Our results are similar with a study in which hematocrit was not raised in patients with OSA[20]. There are controversial results for

hematocrit in OSA. Some studies have reported increased hematocrit in OSA and its positive correlation with disease severity [19] but Reinhart et al in 2002 found no relation between hematocrit and severity of OSA[21]. Recently hematocrit is labelled as a useful marker of response to Continuous Positive Airway Pressure (CPAP) treatment in OSA[22].

In the present study RBC related hematological parameters are also studied. we found a considerably non significant result for Red Blood Cells(RBC) (p = 0.65) and Hemoglobin (0.76). But the mean value of hemoglobin(14.04 ± 0.97) is higher in obese with OSA group as compared to obese without OSA (13.98 ±0.81). Our results are in line with a study conducted by Radhika G., et al in USA, in which they have also found out negative result for polycythemia in patients of OSA.[23] The low prevalence of polycythemia may be attributed to neocytolysis, wherein transition from sustained hypoxia to normoxia leads to overcorrection of polycythemia due to transient expansion of mitochondria-generating reactive oxygen species, with preferential destruction of young red cells made in hypoxia.[24] In literature many researchers have observed erythropoiesis which is characterized by increase in number of red blood cells, increase in hemoglobin and red cell distribution width in patients of OSA.[22] The persistent RDW increase in cardiovascular diseases has been attributed to the effective stimulation of erythropoiesis by erythropoietin (EPO), a hormone secreted during hypoxic events, which promotes the release of enlarged RBCs from bone marrow[25]. During OSA there are multiple episodes of hypoxia which leads to increase in erythropoietin release and thus cause erythropoiesis, hence increasing the risk of CVD in patient of OSA.[26] But the present study fails to show significant results for RDW (p= 0.153). A community cohort study in Taiwan also concluded that elevated RDW values are associated with increased risk of mortality but not with the development of cardiovascular disease in general population[27].

In short, present study fails to show significant difference of nonspecific, simple inexpensive hematological variables in obese young otherwise healthy male population with OSA.

CONCLUSION

Nonspecific hematological markers (WBC, NLR, Hematocrit, RBC, hemoglobin, RDW) are not significantly different between the two study groups.

Conflict of interest: Declared none

REFRENCES

1. Ephros HD, Madani M, Yalamanchili SC. Surgical treatment of snoring; obstructive sleep apnoea. Indian J Med Res., 2010;131:267–76.
2. Punjabi NM. The epidemiology of adult obstructive sleep apnea. Proc Am Thorac Soc., 2008; 5(2):136–43
3. Kapsimalis F, Kryger M. Sleep breathing disorders in the U.S. female population. J Womens Health (Larchmt) 2009;18(8): 1211–1219
4. Tintinger GR, Pretorious L, Labadarios D. Obstructive sleep apnea and obesity. S. Afr. J. Clin. Nutr.,2011; 24(4): 174-77.
5. Korkmaz M, Korkmaz H, Kucuker F, Ayyildiz SN, Cankaya S. Evaluation of the association of sleep apnea- relatedsystemic inflammation with CRP, ESR and neutrophil- to-lymphocyteratio. Med. Sci. Monit., 2015; 21: 477-81
6. Horne BD, Anderson JL, John JM, Weaver A, Bair TL, Jensen KR,. Which white blood cell subtypes predict increased cardiovascular risk?. Journal of the American College of Cardiology. 2005 May 17;45(10):1638-43.
7. Majid M, Fatem, O. Components of the complete blood count as risk predictors for coronary heart disease. Tex. Heart Inst. J., 2013; 40: 17-29.
8. Lou M, Luo P, Tang R, Peng Y, Yu S, Huang W, He L. Relationship between neutrophil-lymphocyte ratio and insulin resistance in newly diagnosed type 2 diabetes mellitus patients. BMC endocrine disorders. 2015 Dec;15(1):9.
9. Choi JB, Loredo JS, Norman D, Mills PJ, Ancoli-Israel S, Ziegler MG, Dimsdale JE. Does obstructive sleep apnea increase hematocrit? Sleep Breath. 2006;10(3):155-60.
10. Lappegård J, Ellingsen TS, Skjelbakken T, Mathiesen EB,. Red cell distribution width is associated with future risk of incident stroke. Thrombosis and haemostasis. 2016;115(01):126-34.
11. Arnaud C, Dematteis M, Pepin JL, Baguet JP, Levy P. Obstructive sleep apnea, immuno- inflammation and atherosclerosis. Semin. Immunopathol., 2009; 31(1):113 - 25.
12. Mohammad M, Omid F, Components of the Complete Blood Count as Risk Predictors for Coronary Heart Disease In-Depth Review and Update Tex Heart Inst J. 2013; 40(1):17
13. Kounis NG, Soufras GD, Tsigkas G, Hahalis G. Whiteblood cell counts, leukocyte ratios, and eosinophils as inflammatory markers in patients with coronary artery disease. Clin. Appl. Thromb. Hemost., 2014; 21(2): 139-43.
14. Madjid M, Awan I, Willerson JT, Casscells W. Leukocyte count and coronary heart disease. Implications for riskassessment. J. Am. Coll. Cardiol., 2004; 44: 1945 -1956.
15. Balta S, Celik T, Mikhailidis DP, Ozturk C, Demirkol S, Aparci M, LyisoyA.The relation between atherosclerosisand the neutrophil lymphocyte ratio. Clin. Appl. Thromb. Hemost., 2015; 22(5): 405 - 11
16. Korkmaz M, Korkmaz H, Kucuker F, Ayyildiz SN, Cankaya S. Evaluation of the association of sleep apnea- related systemic inflammation with CRP, ESR and neutrophil- to- lymphocyte ratio. Med. Sci. Monit., 2015; 21: 477-81
17. Dikmenoglu N, Ciftic B, Ileri E, Guven SF, Seringec N, Aksoy Y, Ercil D. Erythrocyte deformability, plasmaviscosity and oxidative status in patients with severe obstructivesleep apnea syndrome. Sleep Medicine, 2006; 7: 255-61.
18. Al Lawati NM, Ayas NT. Hypercoagulability: Another potential mechanism of obstructive sleep apnea related cardiovascular disease? Lung, 2008; 186: 195-96.
19. Liak C, Fitzpatrick M. Coagulability in obstructive sleep apnea. Can. Respir. J., 2011; 18(6): 338-48.
20. Yousef AM, Alkhiary W. The severity of obstructive sleep apnea syndrome is related to red cell distribution width and hematocritvalues. J. Sleep Disord. Ther.,2015; 4: 192 - 99.
21. Reinhart, WH, Oswald, J, R, Walter, R and Kuhn, M. Blood viscosity and platelet function in patients with obstructive sleep apnea syndrome treated with nasal continuous positive airway pressure. Clin. Hemorheol. Microcirc*.,* 2002. **27**:201.
22. Feliciano A, Linhas R, Marco R, Cysneiros A, Martinho C, Reis RP, Penque D, Pinto P, Barbara C. Hematological evaluation in males with obstructive sleep apnea before and after positive airway pressure. Rev. Port. Pneumol.,2017; 23(2): 71-78
23. Radhika G, Krishna MS, Jihyun S, Josef T, Prchal. Polycythemia Is Rarely Caused By Obstructive Sleep Apnea. Blood, 2016; 128:2444
24. Song J, Song K, Sundar R Regulation of erythropoies is after normoxic return from chronic sustained and intermittent hypoxia J. Appl. Physiol., 2017;123:1671-75
25. Söderholm M, Borné Y, Hedblad B, et al. Red cell distribution width in relation to incidence of stroke and carotid atherosclerosis: a population-based cohort study. PLoS One 2015;10:e0124957
26. Sánchez-Chaparro MA, Calvo-Bonacho E, González Quintela A, et al. Higher red blood cell distribution width is associated with the metabolic syndrome: results of the Ibermutuamur Cardiovascular RIsk assessment study. Diabetes Care 2010;33:e40.
27. Chen PC, Sung FC, Chein KL, Hsu HC, Su TC, Lee YT. Red blood cell distribution width and risk of cardiovascular events and mortality in a community cohort in Taiwan. Am. J. Epidemiol., 2010. 171(2): 214-220.