ORIGINAL ARTICLE

OBSTRUCTIVE SLEEP APNOEA AND ROLE OF INTERLEUKIN-6 AS A CIRCULATORY BIOMARKER: A CROSS-SECTION STUDY

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Background: Despite reported link between OSA and systemic inflammation, results are indecisive so far for different inflammatory markers to evaluate the casual relation with OSA and its co-morbidities. Objective of this study was to assess the relationship of inflammatory cytokine Interleukin-6 with obstructive sleep apnoea (OSA) and its severity among Pakistani population. **Methodology:** A total of 180 subjects (55 without apnoea, 33 mild, 34 moderate, and 58 with severe apnoea) were analysed in this cross-sectional study, between Dec 2018 and Jan 2020 in Dow University Hospital, Karachi. Apnoea was confirmed and subjects were grouped on the basis of Apnoea Hypopnea Index (AHI) by overnight Polysomnography. Plasma IL-6 was analysed using enzyme-linked immunosorbent assay (ELISA). One-way ANOVA was used for comparison. Potential effect of age and BMI was controlled using ANCOVA and effect size was reported as partial eta squared. **Results:** Mean IL-6 levels were associated with severity of apnoea with 4.72±1.50 pg/ml, 21.08±6.83 pg/ml, 25.41±7.97 pg/ml and 26.96±7.39 pg/ml in no OSA, mild, moderate and severe OSA respectively, with statistically significant difference between all groups except between moderate and severe OSA. After controlling the potential effect of age and BMI still yielded a significant positive association between severity of OSA and IL-6 levels (effect size (partial η^2) of 59% (p<0.001)). **Conclusion:** Higher Interleukin-6 levels were observed in OSA and associated with its severity. It could provide a feasible method to improve timely diagnosis of OSA and can point toward presence of a more severe clinical phenotype.

Keywords: Sleep, Obstructive Sleep Apnoea, Interleukin-6, Inflammation

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INTRODUCTION

Obstructive Sleep Apnoea (OSA) is a condition associated with repeated episodes of partial or complete obstruction of upper airway, intermittent hypoxia during sleep, sleep fragmentation and excessive daytime sleepiness (EDS).[1,2] When there is complete obstruction of airway the event is called as apnoea, while in case of partial obstruction it is hypopnea. Number of apnoea-hypopnea episodes per hour of sleep is called as Apnoea Hypopnea Index (AHI) and indicates the severity of apnoea. On the basis of AHI, apnoea can be divided as mild (AHI= 5–10), moderate (AHI= 15–30) and severe apnoea (AHI ≤30) as mentioned by the American Academy of Sleep Medicine (AASM) revised scoring criteria.[2] Polysomnography is the gold standard diagnostic test for this condition which can not only measure AHI but also monitor time spent in each sleep phase, EEG, The ECG and many other sleep related parameters.[3]

Its prevalence is continuously increasing as a recent research described it as 9 to 38%.[1] The OSA has been observed to be associated with many adverse health outcomes and comorbidities like excessive day time sleepiness (EDS), impaired quality of life (QoL), increase occupational and motor vehicle accidents, oxidative stress, metabolic disorders, cognitive deficits, hypertension, coronary artery disease, diabetes, stroke, congestive heart failure, atrial fibrillation, cardiovascular events and mortality.[4–7] Common risk factors are advanced age, obesity, male gender, postmenopausal women and craniofacial dysmorphisms.[4] Continuously increasing prevalence, numerous adverse health outcomes and sudden death are the reason to increase OSA related research studies in past few decades to investigate about its aetiology, exact pathophysiology and other contributory factors, but results are still indecisive.

According to studies OSA has been found to be associated with increased systemic inflammatory responses and many of its related outcome can be explained by underlying inflammatory process that may contribute to a higher risk for end-organ morbidity.[3,8] Studies mentioned higher levels of proinflammatory mediator such as Interleukin-6 (IL-6), CRP and TNF-α in OSA and support the hypothesis for the relationship of OSA and inflammation.[9] There is a need to assess the casual relationship of OSA and systemic inflammatory pathways and to evaluate the circulatory biomarkers which can point toward the presence of OSA, its severity and related comorbidities.[8]

Interleukin-6 is one of the most significant inflammatory markers and an excellent proxy for the inflammatory/immune system.[10] It belongs to the category of four-helical cytokines and synthesised and secreted by many cells in body.[11] Physiologically it

involves in homeostasis, intracellular signalling, haematopoiesis, bone metabolism, liver metabolism, immune system coordination, proliferation and differentiation of B and T lymphocytes and neural development and survival.[10–12] Literature explains the complex biology of IL-6, as how one cytokine can have extremely different biological effects on different cells in different biological states.[11] It behaves as a cytokines as well as a myokine. It pays a role in different autoimmune diseases, diabetes, atherosclerosis, cancers, encephalitis, rheumatoid arthritis and reinforce different inflammatory states.[12] Studies also described its role in normal sleep physiology and higher circulatory levels with alterations in sleep duration and low quality of sleep.[13] Due to its vital role in haematopoiesis, immunomodulation, homeostasis and inflammatory processes, it's up-regulation or over-expression can disturb functions of multiple organ system.[14] Under normal condition, its levels are 1–5 pg/ml but during inflammation it can go through immense amplification and levels can rise up to 1,000 folds and extreme conditions can leads to sepsis IL-6 levels in µg/ml.[11] Its prognostic levels may varies from 5 to 100,000 pg/ml, depending on the nature and severity of the disease. Its multifunctional role describe the significance of its quantitative detection in assessing the severity of different diseased conditions.[14] We can conclude that elevated levels of IL-6 can be consider as the major alarm signal for the body.[11]

Studies described the elevated levels of IL-6 in OSA subjects.[10] It has been suggested that repetitive hypoxia, re-oxygenation and oxidative stress during OSA may lead to activation of inflammatory cells which increase the production of cytokines IL-6. IL-6 is a well-established risk factor for cardiovascular disorders and could be related to OSA related cardio-metabolic comorbidities. Studies described the elevated levels of inflammatory mediators IL-6 as well as concomitant reduction of anti-inflammatory mediators IL-10 so tilting the balance toward more proinflammatory status.[2,3,15] Studies also described the role of genetic variation as different genetic background in different population may account for variability in the results for IL-6 levels in different countries, which emphasizes for the need of current study in our population.[15]

Despite multiple related co-morbidities and complications, OSA is still an underdiagnosed and underestimated disease in our community due to lack of information not only in general public but also in our medical practitioners. We have only few OSA related research studies for our local population, which examined relationship of OSA with metabolic syndrome, disturbance in lipid profile, derangements in glucose metabolism and relationship with physical activity.[6,7,16,17] There is a dire need to evaluate a biomarkers like IL-6, which can point toward the presence of OSA, its severity and related comorbidities. It could provide a feasible and accessible method to improve the timely diagnosis of OSA and to identify patients who are at high risk of CVS complications and required immediate intervention. The objective of this study was to assess the relationship of inflammatory cytokine Interleukin-6 with obstructive sleep apnoea and its severity among Pakistani population.

MATERIAL AND METHODS

This cross-sectional study was conducted in Dow University of Health Sciences, Karachi, from Dec 2018 to Jan 2020. Study approval was taken from Ethical Review Board of the University. Study population were subjects coming to Sleep Laboratory, referred from many hospitals of Karachi as well as from many cities of Pakistan for the diagnosis of their OSA.

Inclusion criteria were subjects with snoring, witnessed apnoea or EDS (ESS Score >9), having indication for Polysomnography. The Epworth Sleepiness Scale (ESS) is a questionnaire which can tell us about chances of having EDS when score is >9.[18] Subjects with recent surgery, pregnancy or current serious illness were excluded.

Each subject arrived at 9 PM on their respective night booked for Polysomnography. Oral and written consent was taken with verbal explanation of procedure while approved performa was used to record anthropometric measurements, medical and personal history and medical examination. All subjects went through split-night polysomnography for their AHI and OSA diagnosis. Test was performed by using a multichannel polysomnographic machine (Philips Respironic Alice 5 and Alice 6). Polysomnographic data gave us recording for chest and abdominal wall movement, ECG, air flow and volumes, nasal pressure, Arterial oxygen saturation (Sa_{O2}), pulse waveform, the bilateral electro-oculogram (EOG), electroencephalo-gram (EEG), chin and anterior tibial electromyograms (EMG).[3] The OSA subjects were categorized on the basis of their AHI as mild (32 subjects), moderate (35 subjects) and severe apnoea (58 subjects) while a comparison group without apnoea was comprised of 55 subjects. Control group included age and gender matched subjects with ESS score <9, no sign and symptoms of apnoea and no EDS.

After OSA diagnosis they were analysed for IL-6 levels by taking blood sample in the morning, in a vacutainer tube containing EDTA. Centrifugation was performed to separate plasma and stored it at -80 °C until further processing for IL-6 analysis.

Enzyme-link immune-sorbent assay (ELISA) kits (DIA source, SA Belgium) was used with standard automated procedures, in Biochemistry Laboratory. Sample handling, temperature control and working procedures were managed according to manufacturer's

protocol. Dichromatic readings were taken by calculating the mean of duplicate determinations.

SPSS-20 was used for data analysis. Quantitative variables, e.g., age, weight, height, BMI and IL-6 were stated as Mean±SD, whereas qualitative variables were mentioned as frequency. Mean levels of IL-6 were compared by using independent sample *t*-test for two groups, while one way ANOVA was used to make comparison between sub-groups; $p \leq 0.05$ was considered as significant. Potential effect of age and BMI was controlled using ANCOVA and effect size was reported as partial eta squared.

RESULTS

Mean age of study group was 48.92±11.28 years (No OSA: 46.04±12.6, mild OSA: 47.88±10.41, moderate OSA: 51.80±12.39, severe OSA: 50.48±9.10). There were 110 (61%) males subjects and 70 (39%) females subjects while no statistically significant difference was present in the IL-6 levels between genders (p=0.77). Mean AHI score (apnoea score) for mild, moderate and severe apnoea groups and ESS score for all groups are mentioned in Table-1. Mean plasma IL-6 level for whole study group was 18.82±11.41 pg/mL. IL-6 levels for no apnoea group was 4.72±1.50 while mean levels for all three apnoea groups was 25.02±7.74 (p<0.0001).

Table-1 shows the means and standard deviations of IL-6 levels, age, and BMI of different groups. Mean IL-6 levels showed increasing trend with severity of apnoea with 4.72±1.50 pg/ml, 21.08±6.83 pg/ml, 25.41±7.97 pg/ml and 26.96±7.39 pg/ml in no OSA, mild, moderate and severe OSA respectively with statistically significant difference between all groups except between moderate and severe OSA. (Table-2 shows the mean differences and respective *p*-values in different groups). Despite there was non-significant difference of mean age between groups and statistically significant difference in the mean BMI scores between groups as both are showing an increasing trend according to severity of OSA, controlling for these two variables still yielded a significant positive association between severity of OSA and IL-6 levels. After controlling for the possible effect of BMI and age, the association between OSA severity and IL-6 was statistically significant with and effect size (partial η^2) of 59% (p<0.001).

Table-1: Comparison of IL-6 levels, age, ESS scores, AHI scores and BMI in different groups of obstructive sleep apnoea (Mean±SD)

Severity of OSA	IL-6 levels	Age	ESS Score	Apnoea score	BMI
No OSA (n=55)	4.72±1.50	46.04±12.6	3.38±2.24	0.00±0.00	24.97±5.68
Mild OSA (n=33)	21.08±6.83	47.88±10.41	11.09±3.45	10.25±3.72	32.98±5.93
Moderate OSA (n=34)	25.41±7.97	51.80±12.39	13.68±3.34	24.67±6.45	36.15±9.18
Severe OSA (n=58)	26.96±7.39	50.48±9.10	16.71±4.27	50.28±15.16	37.91±8.05

Table-2: Mean differences of IL-6 in different groups of obstructive sleep apnea

Severity of OSA	Severity of OSA	Mean Difference	*p*	95% Confidence Interval	
				Lower Bound	Upper Bound
No OSA	Mild OSA	-16.3570	<0.001	-19.062	-13.651
	Moderate OSA	-20.8309	<0.001	-23.511	-18.150
	Severe OSA	-22.2421	<0.001	-24.555	-19.930
Mild OSA	No OSA	16.3570	<0.001	13.651	19.062
	Moderate OSA	-4.4739	0.004	-7.476	-1.471
	Severe OSA	-5.8851	<0.001	-8.564	-3.206
Moderate OSA	No OSA	20.8309	<0.001	18.150	23.511
	Mild OSA	4.4739	0.004	1.471	7.476
	Severe OSA	-1.4111	0.295	-4.065	1.243
Severe OSA	No OSA	22.2421	<0.001	19.930	24.555
	Mild OSA	5.8851	<0.001	3.206	8.564
	Moderate OSA	1.4111	0.295	-1.243	4.065

DISCUSSION

Our findings are suggesting an association of plasma IL-6 levels with OSA and its severity in our population. Results are still significant even after controlling the potential effect of age and BMI.

Literature review reflects the variability in this association in different study populations. Gozal *et al*[3], examined a small group of polysomnographicaly diagnosed OSA children and found significantly higher levels of IL-6, independent to obesity. After treatment with tonsillectomy and adenoidectomy (T and A) IL-6 levels were decrease as those of controls. Huang *et al*[9] conducted their research in Taiwan and failed to get any significant difference with respect to IL-6 levels (p=0.104) in age and BMI matched case-control groups. A multicentre study[15] examined Spanish children and found significantly higher levels of IL-6 in OSA children as compare to age and BMI matched controls (p=0.009) and suggested a role of IL-6 in OSA related comorbidities. Motamedi *et al*[2], evaluated a young adult cohort with moderate to severe apnoea and determined higher IL-6 levels. As study population consist of young individuals and hypertension/CVDs were infrequent so they concluded

that higher levels of IL-6 in their study was most likely due to presence of oxygen desaturation. They proposed that repetitive hypoxia may induced vascular injuries, aggravation of existing inflammatory process and may lead to development of cardiovascular and cerebrovascular issues and cognitive deficits in OSA. A recently published meta-analysis[10] examined 63 studies (57 studies with adult population and 6 studies with children) and concluded that plasma/serum levels of IL-6 were significantly higher in majority of studies. Van Eyck *et al*[19], examined over-weight and obese children and couldn't get any association between interleukin-6 levels with severity of OSA. Study population of Tam *et al*[20] consisted on 44 mild apnoeic children. They evaluated multiple parameter of inflammation in OSA group with comparison to controls and failed to find any significant correlation between majorities of inflammatory mediators including IL-6 with OSA. Their negative findings could be due to the initial and mild form of apnoea in those children.

Studies reinforced that OSA induces a reversible low-grade systemic inflammatory response and increase inflammatory mediators such as IL-6 which may leads to further activation of multiple downstream pathways and further proceed for different end-organ morbidities, identified for OSA. Intermittent hypoxia, hypercapnia and sleep fragmentation are reported for activation of pro-inflammatory pathways along with down-regulation of anti-inflammatory markers, as exemplified by decline in IL-10 levels in some study.[3] IL-6 is considered as a well-recognized risk factor for CVDs and could be considered as an important link between OSA and CVDs. Studies explained correlation of IL-6 levels with the degree of hypoxia and sleep duration independent of obesity.[15]

The CVDs remains a highly prevalent cause of morbidity and mortality worldwide.[21] OSA can be considered as a modifiable risk factor for prevention of CVDs, related complications and to reduce huge economic burden on health care system. There is need to focus on all the contributing factors and related biomarkers which could have a possible role in the aetiology or treatment of OSA.

One important strength of current study is the analysis of our results after adjustment of age and BMI which could play a role as confounders. Future longitudinal studies are advisable to determine temporal relationship. Blood IL-6 levels could be a feasible and accessible method to improve the timely diagnosis of OSA and related morbidities and to decrease the burden of more serious complications.[2] Plasma IL-6 measurements may be used in OSA treatment follow-ups, when polysomnography is not available.

CONCLUSION

Higher Interleukin-6 levels were observed in OSA and associated with the severity of disease. Measurement of IL-6 levels could provide a feasible method to improve the timely diagnosis of OSA and can point toward the presence of a more severe clinical phenotype and required immediate intervention. It may also facilitate the implementation of better treatment options for OSA patients in our country. This study further supports the hypothesis that elevated levels of IL-6 in OSA could be the reason of inflammatory stress and related co-morbidities in these patients.

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AQ: Concept and designing of work, analysis and interpretation of data
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