ORIGINAL RESEARCH PAPER
VOLUME-6 | ISSUE-5 | MAY - 2017 | ISSN No 2277 - 8179 | IF : 4.176 | IC Value : 78.46
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

MIF gene polymorphism and MIF levels in Asian Indian with Obstructive Sleep Apnea

Pulmonary Medicine

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ABSTRACT

Obstructive sleep apnea (OSA) is associated with increased risk for cardio-metabolic dysfunction. Macrophage migration inhibitory factor (MIF) has been shown to play a key role in inflammation. We hypothesized that increase serum MIF levels and MIF variant frequencies may have associated with OSA and this may affect cardio-metabolic risk. 200 subjects, 100 with OSA and 100 without OSA were evaluated. Serum MIF levels was determined using ELISA. Sequencing was carried out to checkMIF-173 G/C gene polymorphism using Big Dye Terminator v3.1 Cycle Sequencing Kit on an ABI Genetic Analyzer 3730. Serum MIF levels were elevated in subject with OSA, it correlated with severity of OSA and this correlation was independent of age and obesity. MIF gene frequency of CC, GG and GC was 3%, 75% and 22% in subject with OSA, while it was 4%, 56% and 40% in subjects without OSA respectively. Serum MIF level are elevated and associated with severity of OSA. Up- regulation of MIF may play a role in the progression of cardio-metabolic risk. We recommend further studies to focus on MIF as a potential marker and screening tool for OSA.

KEYWORDS:

Obstructive sleep apnea; Genetic polymorphism; macrophage migration inhibitory factor; Asian Indian

1. Introduction

Obstructive sleep apnea (OSA) syndrome is a potential serious disorder, characterized by episodes of complete or partial pharyngeal obstruction, and thus increased resistance to airflow and cessation of breathing during sleep [1]. Chronic OSA induces mitochondrial dysfunction, tissue hypoxia, counter-regulatory hormones, altered adipocytokine patterns and increases the risk for Type II diabetes mellitus (DM II) as well as cardiovascular disease [2-3]. Several factors have been reported to be associated in the pathogenesis of OSA, including obesity, craniofacial abnormality, alcohol and ethnicity [4-6].

Inflammation is a potential mechanistic link between cardiovascular disease and OSA. However, there is limited evidence regarding elevation of inflammatory markers in OSA and a direct association with cardiovascular disease. In vitro studies demonstrate that hypoxia, a key characteristic of OSA, increases cellular production of pro-inflammatory cytokine macrophage migration inhibitory factor (MIF) [7]. Pro-inflammatory cytokines induce MIF production which reduces the sensitivity of cells to the anti-inflammatory actions of glucocorticoids [8].

MIF is an important pro-inflammatory multifunctional cytokine involved in many acute and chronic inflammatory disorders including autoimmune disease, arthrosclerosis, wound healing, metabolic disorders and cardiovascular disease [9-10]. Further, MIF has a chemokine-like function and promotes the migration and recruitment of leukocytes into infection and inflammatory sites [11]. Furthermore, MIF plays a pivotal role in the pathogenesis of inflammatory disorders by promoting and amplifying involved inflammatory reactions such as monocyte/macrophage survival, MAPK signalling and inflammatory cytokine release [9]. A recent study showed that the plasma levels of MIF are elevated and associated with OSA severity [12].

The MIF gene maps to chromosome 22q11.2 in humans. There are four polymorphisms that have been reported in the human MIF gene [13]. The +254 T/C (rs2096525), +656 C/G (rs2070766) are located in introns and thus do not affect the coding sequencing of MIF gene, whereas -173 G/C(rs755622), -794 $CATT_{5-8}$microsatellite (rs5844572) are located within the promoter region of MIF. Previous studies have shown that polymorphisms in promoter influence the induced transcription activity of MIF and -173C allele is associated with greater production of MIF protein [14].

The aim of this study was to look for correlation, if any, between serum MIF levels and single nucleotide polymorphisms (SNPs) in subjects with and without OSA. We hypothesized that OSA may be associated with increased serum MIF levels and that the presence of specific SNPs in MIF gene may affect the cardio metabolic risk in Asian OSA population. To the best of our knowledge, no study has looked for a correlation of MIF gene with OSA.

2. Materials and methods

2.1. Subjects

A total of 200 subjects, aged between 15 to 70 years were recruited from outpatient department, All India Institute of Medical Sciences (AIIMS), New Delhi, India. Of these, 100 were newly diagnosed with OSA (78 males, 22 females) and 100 were without OSA (54 males, 46 females). All subjects underwent a clinical evaluation prior to the study, in order to rule out acute or chronic inflammatory diseases, i.e. coronary heart disease, chronic obstructive pulmonary disease, upper airways resistance syndrome, periodic limbs movement and narcolepsy. Pregnant women and patients with a known history of DM, hypertension or any chronic liver or kidney disease were excluded.

2.2. *Ethical Statement*

The study was approved by the Institutional Ethics Committee of All

India Institute of Medical Sciences, New Delhi (AIIMS, Ethical Ref No-IEC/NP-167/2011, on 23 August 2011) and written informed consent was obtained from all the study subjects after explaining the objectives of the study.

2.3. Overnight Polysomnography

All subjects underwent overnight full- montage digital polysomnography (Alice 5 Diagnostic Sleep System, Philips-Healthcare, United States) using standard techniques [15], and OSA was classified according to apnea hypopnea index (AHI). The duration of polysomnography was at least six hours in the sleep laboratory. A sleep technician was present throughout the study and followed patient behaviour and confirmed sleep position by the infrared camera inside the room. The recordings were analysed with 60 second epoch, and sleep stages were scored according to the standard criteria [16].

Diagnosis of OSA was made on the basis of international classification of sleep disorders [17]. Apnea was defined as the cessation of airflow ≥10 s and hypopnea was defined as a transient reduction of breathing ≥10 s associated with either an oxygen desaturation of ≥3%or an arousal [18]. AHI was defined as the number of apneas and hypopneas/hour of sleep. Based on the American Academy of Sleep Medicine recommendations, subject with an AHI ≥5/hour were considered to have OSA and it was classified as mild OSA, in those with AHI of ≥5 to <15, as moderate OSA in those with AHI of ≥15 to <30 and severe OSA in those with AHI of ≥30/hour. Subjects with an AHI <5/hour were diagnosed as not having OSA. Polysomnography was conducted in single laboratory and analysis was done by a single expert. The Epworth sleepiness scale was used to check excessive daytime sleepiness [19].

2.4. MIF Elisa

All samples were collected in a fasting state between 6 to 7 am. Serum MIF levels were measured using the quantikine human MIF kit (R&D systems, USA). As per protocol, 100 µl of assay diluent and 50 µl of diluted standard or samples were added to a microplate pre-coated with capture antibody. Samples were incubated for two hours and then given four washes. 200 µl of MIF conjugate was added to each well for another 2 hours on the shaker. Color was developed by incubating with substrate solution for 30 minutes at room temperature. Stop solution was used to terminate the reaction and absorbance was read by a microplate reader set to 450 nm.

2.5. DNA Extraction and genotyping of the MIF –173 G/C polymorphisms

Peripheral blood samples were collected in vacutainer tubes containing EDTA (Becton Dickinson, Franklin Lakes NJ, USA).All DNA samples were extracted using QIAmp DNA blood kit (Qiagen, Germany) according the manufacturer's protocol. The MIF-173 G/C polymorphism was detected by PCR amplification of a 366 bp. DNA fragment was produced by PCR using primers forward, 5-ACTAAGAAAGACCCGAGGC-3, and reverse, 5-GGGGCACGTTGGTGTTTAC-3. PCR was performed in a total volume of 25 µl [150 ng of genomic DNA, 1.25 units of Taq DNA polymerase (Invitrogen, USA), 0.4 µmol of each primer, 200 µmol of each deoxynucleoside triphosphate (dNTPs), and 1.5 mM $MgCl_2$]. Thermocycling was performed under the following conditions: an initial denaturation at 94°C for 3 minutes followed by a three step PCR program: 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute for 40 cycles. A final cycle of 72°C for 5 minutes completed the reaction. A 6 µl final reaction consisted of amplified PCR product (5µl) mixed with 6X Loading buffer (1µl) were resolved on a 3% agarose gel and visualized using UV transillumination. After agarose electrophoresis purified amplicons were then subjected to bi-directional Sanger sequencing by using BigDye® Terminator v3.1 Cycle Sequencing Kit (ABI, USA) on an ABI Genetic Analyzer 3730 (ABI, Japan). All the sequencing results were analyzed using Sequencing Analysis 5.2. Genotyping analysis is performed using Variant Reporter® Software v2.0 by using reference sequences of MIF (Gene ID: 4282) NG_012099.1 obtained from Gen Bank.

2.6. Statistical analysis

Data on demographic characteristics, biomarkers and genotypes for MIF -173G/C was collected in subjects with and without OSA (100 each). Distribution of subjects as per age, sex, BMI categories, biomarker levels and genotypes was expressed in terms of number and percentage. All statistical analyses were performed using stata 12.0 software. The statistical significance of each categorical variable with study groups was determined using Pearson's chi-square test or Fisher exact test depending upon cell frequencies. Age, BMI and serum MIF levels of the subjects were also summarised and mean ± SD and Student's *t*-test was used to compare mean values between the two groups. Serum MIF levels and genotypes were analysed using multivariate ANOVA. Statistical significance was evaluated at 5% level.

3. Results

The clinical and demographic characteristics of the 200 subjects are shown in Table 1. BMI (mean ± SD) was significant higher in subjects with OSA (P<0.003). Serum MIF levels were significant higher in subjects with OSA when compared to subjects without OSA, and 52% had MIF levels higher than 10 ng/ml as compared to 22% in subjects without OSA, P<0.0001. Further the mean of serum MIF level was significant higher (17.3 ±16.7ng/ml) in subjects with OSA as compared to subjects without OSA (8.6±10.9 ng/ml) P<0.0001 (Table 1 & Figure1). The overall frequencies of MIF-173G/C in subjects with OSA, CC, GG and GC genotyping was 3% (n=3), 75% (n=75) and 22% (n=22), while it was 4% (n=4), 56% (n=56) and 40% (n=40) in subjects without OSA. Also, subjects with OSA showed significantly lower variant allele frequency when compared to subjects without OSA (p <0.017), and this association become even more apparent when alleles G and C were compared in subjects with and without OSA (P <0.015).

Table1: Characteristic of subjects with and without obstructive sleep apnea.

Variables	Subjects without OSA (n=100)	Subjects with OSA (n=100)	P- Value
Age in years [n (%)]			
Mean ±SD	41.3±10.4	49.3 ±10.6	
Gender [n (%)]			
Male	54 (54)	78 (78)	
Body mass index (Kg/m2) [n (%)]			
Mean ± SD	30.8±6.4	33.7±7.0	<0.003
Serum Macrophage migration inhibitory factor (ng/ml) [n (%)]			
≤ 10	78 (78)	48 (48)	<0.0001
10.1 – 20	16 (16)	32 (32)	
≥ 20.1	6 (6)	20 (20)	
Mean ±SD	8.6±10.9	17.3 ±16.7	<0.0001
Macrophage migration inhibitory factor – 173 [n (%)]			
CC	4 (4)	3 (3)	<0.017
GG	56 (56)	75 (75)	
GC	40 (40)	22 (22)	
Allele			
G	152	172	<0.015
C	48	28	

SD-standard deviation, P-probability, OSA-obstructive sleep apnea, BMI-body mass index, MIF-macrophage migration inhibitory factor.

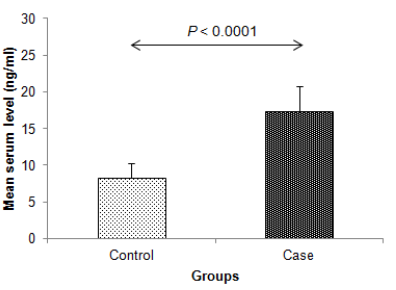

Figure 1: Comparison of mean serum MIF levels in subjects with (Case) and without (control) OSA.

Table 2 Characteristic of subjects with and without severity of OSA groups.

Variables	Subjects without OSA, (n=100)	Mild OSA (n=14)	Moderate OSA (n =34)	Severe OSA (n =52)	P- Value
Serum MIF (ng/ml) [n (%)]					
≤ 10	78 (78)	8 (58)	23 (68)	17 (32)	<0.005
≥10.1	22 (22)	6 (42)	11 (32)	35(68)	
Mean ±SD	8.6±10.9	11.8±13.2	15.7±16.3	21.2±18.1	<0.0001
MIF – 173 [n (%)]					
CC	4 (4)	0 (0)	1 (3)	2 (3.8)	0.127
GG	56 (56)	10 (71.4)	28 (82.3)	37 (71.2)	
GC	40 (40)	4 (28.6)	5 (14.7)	13 (25)	

As shown in table 2, more subjects with severe OSA had a mean serum MIF levels more than 10 ng/ml as compared to the mild and moderate OSA (P <0.005). Further, the mean ±SD of serum MIF levels were elevated (11.8 ± 13.6 ng/ml in mild OSA, 15.7± 16.3 ng/ml in moderate OSA and 21.2± 18.1 in severe OSA) and correlated significantly with increasing severity of OSA, P <0.0001 (Table 2 & Figure 2). The association between MIF-173 genotypes with severity of OSA showed an insignificant correlation (P =0.127).

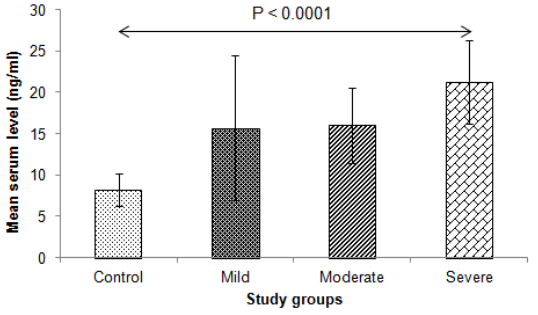

Figure 2: Comparison of mean serum MIF levels with and without severity of OSA.

4. Discussion

Our interest was to evaluate the association of MIF-173G/C promoter polymorphisms with serum MIF levels in Asian Indian OSA populations. To the best our knowledge this is the first case control study that reports the genotypic and allelic frequencies of MIF-173 G/C and serum MIF levels in OSA. One previous study has reported a frequency of -173 G/C MIF in psoriatic arthritis patients with a frequency of the 55% for the -173G allele and 45% for the -173C allele in western Mexico population [20]. Another study has reported a frequency of the 74 % of the G allele and 26 % of the A allele in MIF gene rs10433310 in children with OSA in United States [21]. However, we observed a higher frequency of the -173 G allele (86%) and a lower frequency of the -173 C allele (14%) in our population. This difference may be influenced by racial differences between the population studies [22], but could also be attributed to the sample size and the criteria for inclusion in each study.

We observed that serum MIF level was significantly higher in subjects with OSA. Also, a significant association between the severity of OSA and MIF serum levels emerged. Our findings are in agreement with the other published study in which the presence of OSA was not only a risk factor for elevated plasma MIF levels but the later also correlated with severity of OSA [12]. In case of the -173G/C MIF polymorphism a significant difference in the allele frequency distribution was found.

MIF is an essential, upstream component of the inflammatory cascade, and will up-regulate TNF-α secretion by macrophages [23], and other cytokines such as IL-1 and IL-6. MIF is a significant cytokine because of its upstream action on the immune cells and due to a possible feedback loop between MIF and TNF-α [24]. Several studies have suggested that MIF has a role in both experimental and human atherosclerosis [25-26, 23]. In an animal model, deficiency of MIF attenuated atherogenesis in low density lipoprotein receptor-deficient mice [27]. It has been suggested that MIF may directly affect endothelial-monocyte adhesion because MIF triggers monocyte arrest under flow conditions on aortic endothelial cells exposed to oxidized LDL [28]. Herder et al reported a strong positive association between systemic concentrations of MIF and impaired glucose tolerance and type II diabetes [29]. Another study showed that the MIF genotype (rs1007888) CC was associated with elevated circulating MIF levels and diabetes [30]. In a recent study [12], MIF was strongly associated with the hypothalamic pituitary adrenal axis and was postulated to play a key role in regulation of the inflammatory response [31]. MIF may also play a dual role in optimizing inflammatory activity (i.e. increase IL-8 and TNF-α level), while indirectly inhibiting maximal anti-inflammatory glucocorticoid activity [32]. Childhood OSA has also been associated with higher plasma MIF, high sensitive C - reactive protein (hsCRP) and fasting insulin levels and it promotes cardio metabolic risk [21]. We showed not only an elevation of MIF levels in OSA, but our data also suggests that some of the variance in metabolic and cardiovascular risk associated with OSA may be accounted for a specific allelic variant in the MIF gene. Our findings are similar to that reported in the US population [12].

5. Conclusion

We have demonstrated a significant association between the MIF promoter polymorphism in subjects with OSA. Serum MIF levels were elevated in subjects with OSA and correlated with the severity of OSA and this was independent of age and obesity. Up- regulation of MIF in OSA may be important for the development of an inflammatory process that may play a crucial role in the progression of cardiovascular disease and stroke. MIF may serve as a potential marker for OSA. Future studies on MIF may examine whether successful treatment of OSA has the potential to normalize elevated levels of MIF.

Acknowledgments: The authors greatly appreciate the role of subjects who participated in this study. This study was funded by Indian Council of Medical Research (ICMR) 5/4/1-6/11-NCD-II, New Delhi, India. Author would also like to thank Mr. Jitendra Sharma, Mr. Jitendra Kumar, Mr. Omendra Sharma and Mr.Punnu lal for technical help in conducting the sleep study.

Conflict of Interest: The authors declare that there is no conflict of interests regarding the publication of this paper.

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