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ASSOCIATION OF URINARY 6 SULFATOXYMELATONIN LEVELS AND ENDOTHELIAL DYSFUNCTION IN HYPERTENSION SUBJECTS

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ABSTRACT

Melatonin is involved in a variety of diseases, like cancer, depression, hypertension, and diabetes; its secretion is influenced by environmental light. Melatonin, the principal product of the pineal gland, is metabolized in the liver mainly to 6-sulfatoxymelatonin As this substance is mostly excreted by urine, urinary 6sulfatoxymelatonin is considered a good index of melatonin production In vascular diseases, endothelial dysfunction is a systemic pathological state of the endothelium and can be broadly defined as an imbalance between vasodilatation and vasoconstriction substances produced by the endothelium. Endothelial dysfunction can result from and/or contribute to several disease processes, as occurs in hypertension, hypercholesterolemia, diabetes, and Behcet's_disease, and it can also result from environmental factors, such as from smoking tobacco products and exposure to air pollution. **Objective**: To investigate the association of Urinary 6 sulfatoxymelatonin levels and endothelial dysfunction in hypertension subjects. **Methods:** 63 hypertensive subjects without cause of any secondary hypertension. 35 subjects were control in this study, total of 98 subjects were studied. Subjects with secondary hypertension, past history of stroke, coronary artery disease, myocardial infarction were excluded from this study. **Results:** The systolic and diastolic blood pressure considered to be elevated in hypertensive patients and statistically significant when compared to the control group. Our study shows an significant

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difference between urine aMT6s and NO in hypertension subjects compare to control. **Conclusion**: The results suggest that melatonin and nitric oxide levels are low in subjects with hypertension, thus endothelial dysfunction precedes and predicts the development of hypertension.

INTRODUCTION

Hypertension is another name for high blood pressure. Blood pressure is the force exerted by the blood against the walls of the blood vessels. If, the blood pressure reading shows >160 it is said to be hypertensive crisis, on this condition the person should wait for 2 or 3 minutes and then repeat the test [1]. High blood pressure is one of the most common Non communicable disorders. Hypertension is present in 60–70% of the population over 60 years of age and may result in cardiovascular complication such as stroke, coronary heart disease, and heart failure.The most common type of hypertension can affecting 95% of primary hypertensive patients. It can be diagnosed when the systolic BP ranging between 140 and 160 mmHg and/or diastolic BP between 90 and 100 mmHg at three random checks and need medical monitoring and lifestyle changes [2]. Melatonin is involved in a variety of diseases, including cancer, insomnia, depression, dementia, hypertension, and diabetes; its secretion is influenced by environmental light. a pineal gland hormone secreted predominantly at night, is involved in not only regulation of circadian rhythm but also prevention of a variety of diseases, including cancer, insomnia, depression, dementia, hypertension, and diabetes [3,4]. Melatonin, the principal product of the pineal gland, is metabolized in the liver mainly to 6-sulfatoxymelatonin [5].As this substance is mostly excreted by urine, urinary 6sulfatoxymelatonin is considered a good index of melatonin production. Melatonin is primarily involved in the regulation of biological rhythms. In addition, melatonin exerts a modulatory action over the vascular tone, with several studies indicating that melatonin affects vascular smooth muscle contraction [6,7]. This effect could be homeostatically relevant to counteract the rise in arterial peripheral resistance brought about by neuroendocrine activation in CHF. Increased endogenous melatonin secretion might be associated with lower risk of these diseases; however, the determinants of the amount of endogenous melatonin secretion remain unclear. Physiologically, light exposure is the most important modifier of melatonin secretion in humans. Recently, photosensitive retinal ganglion cells and the photo pigment melanopsin were identified as primary receptors of environmental light, and the mechanisms underlying the association between light exposure and melatonin secretion are being solved [8]. Light exposure at night is a well-established and powerful suppressor of nocturnal melatonin secretion through the activation of the suprachiasmatic nucleus (SCN) of the hypothalamus, which contains the body's master biological clock [9]. Serum melatonin has a very short half-life and is rapidly metabolized, mainly in the liver. Measurements of the principal metabolite of melatonin, 6-sulfatoxymelatonin (aMT6-s), which is excreted in urine, reflect pineal function; various studies have shown a high correlation between measurements of aMT6-s in urine and plasma. In vascular diseases, endothelial dysfunction is a systematic pathological state of the endothelium and can be defined as an imbalance between vasodilating and vasoconstricting substances produced by the endothelium [10]. Endothelial dysfunction can result from contribute to several disease processes, as occurs in hypertension like, hypercholesterolemia, diabetes mellitus , septic shock, and Behcet's disease, and it can also results from environmental factors, such as from smoking tobacco products and exposure to air pollution [11].Endothelial dysfunction is more prevalent in shift workers, a group known to have a higher risk for cardiovascular diseases[12]. Nitric oxide is a molecular, chemical compound with chemical formula of ·NO [13]. In mammals including humans, nitric oxide is an important cellular signaling molecule involved in many physiological and pathological processes [14]. It is a powerful vasodilator with a short half-life of a few seconds in the blood. Long-known pharmaceuticals such as nitroglycerine and amyl nitrite were found to be precursors to nitric oxide more than a century after their first use in medicine. Nitric is essential for hepatic lipid metabolism during starvation [15]. NO is one of the known gaseous signaling molecules and is additionally exceptional due to the fact that it is a radical gas. It is a key vertebrate biological messenger, playing a role in a variety of biological processes [16]. Nitric oxide, known as an endothelium-derived relaxing factor (EDRF), is biosynthesized endogenously from L-arginine, and NADPH by nitric oxide synthase (NOS) enzymes. Reduction of inorganic nitrate may also serve to make nitric oxide. The endothelium of blood vessels can use the nitric oxide to signal the surrounding smooth muscle to relax, thus resulting in vasodilation and increasing blood flow. Nitric oxide is highly reactive, yet diffuses freely across

membranes [17]. Independent of nitric oxide synthase, an alternative pathway, coined the nitrate-nitrite-nitric oxide pathway, elevates nitric oxide through the sequential reduction of dietary nitrate derived from plant-based foods. Nitric oxide (NO) contributes to blood vessel homeostasis by inhibiting vascular smooth muscle contraction and growth, platelet aggregation, and leukocyte adhesion to the endothelium.Human subjects with atherosclerosis, diabetes, hypertension often show impaired NO pathways[18].

METHODS

The study design included two groups of human subjects as participants. A total of 98 subjects were studied. Among these 63 were diagnosed hypertensive patients without cause of any secondary hypertension. 35 subjects were control in this study. Hypertension was diagnosed when the systolic and diastolic pressure read 140 and 90 mmHg, respectively, at two to three random checks. Rajah Muthiah Medical College and Hospital, Annamalai University, Tamil Nadu, India. All the participants underwent a detailed clinical examination. Different parameters such as age, BP, Hb, and ECG were recorded. The study was approved by both research and ethics committee of our institute. A fully informed consent was obtained from these subjects before participation in the study.

Subject selection criteria

All volunteers enrolled in this study were aged between 30 and 65years. Subjects of both sexes were included. Women, being on a regular menstrual cycle were included in the study. Subjects with hypertension without any secondary causes were included in this study. Subjects with secondary hypertension, past history of stroke, coronary artery disease, myocardial infarction, and peripheral vascular disease with evidence of tissue injury or loss, renal diseases, diabetes mellitus, after infections, smokers, alcoholic and who with vitamin supplement, known to cause changes in antioxidant and lipid status, were excluded from the study. Serum lipid profile, FRAP Assay and serum TBARS, high sensitivity C reactive protein were estimated in these patients by standard procedures and the values were compared with healthy control subjects. Separated serum was used to analyze lipid profile, uric acid, Malondialdehyde (MDA), hsCRP were estimated in these patients by standard procedures and the values were compared with healthy control subjects. Total cholesterol, high density lipoprotein cholesterol (HDL-C), Triglyceride (TG), was estimated using Erba assay kits. Low density lipoprotein cholesterol (LDL-C) was calculated by Freidewalds formula.. MDA was measured using thio-barbituric acid (TBA). The mixture was heated at 100°C for 15 min, allowed to cool and centrifuged at 5000 rpm. Total anti-oxidant status was assayed by measuring FRAP (ferric reducing activity of plasma) Urine 6-sulfatoxymelatonin performed by ELISA kit method and Nitric Oxide was done by Griess reagent.

Statistical analysis

Fischer exact test has been used to find the significance of various parameters among cases and controls. Data presented are mean ± SD. Analysis of data was done by student t test. A 'p' value < 0.05 was considered significant.

RESULTS

The compared value of the study groups both hypertension and control subjects are shown in the table 1. Our data clearly show that significant increase in Total Cholesterol, Triglyceride, LDL-C, whereas HDL-C, antioxidants, were decreased among hypertensive compared to controls, marking ongoing oxidative stress in hypertensive patients. Table 2 shows an correlation between urinary melatonin and lipid profile. There will be a positive correlation between LDL cholesterol and Triacylglycerol, There will be a negative correlation between urinary melatonin and nitric oxide. Table 3 shows a correlation between Nitric oxide and lipid profile there will be a positive correlation between LDL cholesterol and Triacylglycerol. The association of blood pressure depend upon the combination of risk factors, such as age, gender, weight, physical activity, smoking, family history, serum cholesterol and diabetes mellitus.

Table 1. Comparison of study variables in test and control groups.

Variables	Test group	Control group	P value
FBS	91.30±9.22	86.66±5.83	0.002
BMI	25.2±0.3	24.3±0.4	0.17
SBP(mmHg)	158.15±10.46	121.39±8.03	0.001

Variables	Test group	Control group	P value
DBP(mmHg)	83.37±10.79	77.80±3.21	0.001
Triglycerides	126.97±29.29	111.78±23.91	0.002
Total cholesterol	189.71±25.99	175.95±23.42	0.002
HDL	43.73±2.79	54.34±4.72	0.004
LDL	89.28±19.90	81.83±13.51	0.022
FRAP Assay	180.20±58.72	223.71±64	0.001
Nitric Oxide	15.68±4.48	22.74±9.34	0.0001
Urinary Melatonin	224.73±75.16	289.8±80.71	0.0002

Table 2. Correlation between Urinary melatonin and Lipid profile.

Variables	r-Value	p-Value
Urinary Melatonin **Vs LDL**	0.442	0.0001
HDL	-0.287	0.0001
TAG	0.329	0.0008
TC	-0.029	0.0007
Nitric Oxide	-0.19	0.0002

Table 3. Correlation between Nitric Oxide and lipid profile.

Variables	r-Value	p-Value
NO vs LDL	0.062	0.0001
HDL	-0.069	0.0001
TAG	0.192	0.0008
TC	-0.468	0.0007

DISCUSSION

Our results clearly show the significant difference and correlation between the study groups. Among 98 subjects 63 were hypertensive and 35 were control subjects included in this study and the results were compared. High excretion levels of morning urinary aMT6s levels and low levels of nitric oxide levels were independently associated with a increased risk of developing hypertension. To our knowledge, this is the first prospective study to demonstrate an association between urinary melatonin levels with nitric oxide in hypertension. In this study, we found that low levels processing of urine samples also had only a modest effect on aMT6-s levels, with slightly lower levels of aMT6-s associated with developing hypertension. Thus, first spot morning urine melatonin measurements are a feasible biomarker in observational studies [19]. Physiologically, light exposure is the most important modifier of melatonin secretion in humans. Recently, photosensitive retinal ganglion cells and the photo pigment melanopsin were identified as primary receptors of environmental light, and the mechanisms underlying the association between light exposure and melatonin secretion are being solved [20,21]. Serum melatonin has a very short half-life and is rapidly metabolized, mainly in the liver. Measurements of the principal metabolite of melatonin, 6-sulfatoxymelatonin (aMT6-s), which is excreted in urine, reflect pineal function; various studies have shown a high correlation between measurements of aMT6-s in urine and plasma [22, 23, 24, 25]. The endothelium is not only the inert interface between circulating blood and the vessel wall but is also a major paracrine organ that plays critical roles in controlling vascular tone, inflammation, and smooth muscle cell proliferation. NO production can be controlled by the availability of substrates and cofactors, transcription of eNOS, mRNA stability of eNOS, subcellular localization of eNOS protein, enzymatic uncoupling, and posttranslational modifications [26,27]. NO is one of the few gaseous signaling molecules known and is additionally exceptional due to the fact that it is a radical gas. It is a key vertebrate biological messenger, playing a role in a variety of biological processes[28].The production of nitric oxide is elevated in populations living at high altitudes, which helps these people avoid hypoxia by aiding in pulmonary vasculature vasodilatation[29]. Nitric oxide (NO) contributes to vessel homeostasis by inhibiting vascular smooth muscle contraction and growth, platelet aggregation, and leukocyte adhesion to the endothelium. Humans with atherosclerosis, diabetes mellitus or high blood pressure often show impaired NO pathways[30]. A high salt intake was demonstrated to attenuate NO production in patients with essential hypertension, although bioavailability remains unregulated [31].

CONCLUSION

Our results shows the significant difference between the study group when compare to the control group, low urinary melatonin levels are associated with cause of hypertension and low melatonin production may be a pathophysiological factor for development of hypertension. Endothelial nitric oxide abnormality in hypertensive subjects shows an endothelial nitric oxide deficiency will be results in higher blood pressure. But, in contrast to these findings, we also know that endothelial NO deficiency can be precipitated by a variety of non hypertension related results that increase vascular oxidative stress, such as hypercholesterolemia. More studies are required to explore the potential mechanisms, and additional clinical studies are needed to confirm this association.

Conflicts of interest:
There are no conflicts of interest.

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