

## THE ROLE OF PLASMA MELATONIN AND VITAMINS C AND B12 IN THE DEVELOPMENT OF IDIOPATHIC TINNITUS IN THE ELDERLY

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### SUMMARY

**Background:** To determine the correlation between plasma levels of melatonin, vitamin C and vitamin B12 and the presence of tinnitus among elderly subjects with unexplained subjective tinnitus.

**Method:** Prospective involving apparently healthy elderly with subjective tinnitus and those without. Plasma levels of melatonin, vitamin C and vitamin B12 were determined using high performance liquid chromatography and correlation determined by comparing subjects with and without tinnitus.

**Result:** There were 139 elderly subjects (78 females and 61males), the mean(SD) range of the age was 66.9years (0.77) 60-98years. Of these 58.3% had tinnitus. The mean (SD) range of the plasma levels of melatonin was 11.2 pg/mL(4.2) 5.1 pg/mL - 30.2 pg/mL while that of Vitamin C was 0.7 µmol/L (0.1) 0.3 µmol/L - 1.2 µmol/L, and vitamin B12 was 43.0pmol/L (3.1) 25.4 pmol/L- 71.6pmol/L . Comparing the plasma levels of the markers between elderly with and those without tinnitus, the plasma levels of melatonin (p=0.01) and vitamin B12 (p=0.03) were significantly lower among the elderly with tinnitus compared to those without, while the difference in the plasma level of vitamin C (p=0.6) was not.

**Conclusion:** Low plasma melatonin and vitamin B12 have significant correlation with the development of subjective idiopathic tinnitus among the elderly. This finding suggests the need for the trial of correction of these markers in the reversal or control of tinnitus.

**Key Words:** tinnitus, elderly, plasma, melatonin, vitamin B12, vitamin C, correlation

### INTRODUCTION

Tinnitus affects about 10-33% of the elderly, its diverse etiological factors and negative effects on disability and quality of life of the sufferers has necessitated the need for effective and efficacious treatment.<sup>1-3</sup> Various treatment modalities have been proposed, in spite of these treatment strategies the pharmacological treat-

ment of tinnitus has progressed little in the past decades and evidence of proven efficacious pharmacotherapy is not available.<sup>1,4,5</sup> This may be evidence of poor understanding of the pathogenesis of tinnitus and the complex interactions of the aetiological factors resulting in tinnitus.

Animal experiments has demonstrated that tinnitus could be central<sup>6,7</sup> or peripheral due to the effect on the cochlea, a direct effect on outer hair cells and slower effect on inner ear cells and/or cochlear nerve<sup>8-10</sup> With the use of Lidocaine, suppression of tinnitus has been reported in 60-73% of the ears and changes in the magnitude of tone-burst otoacoustic emission reported in 60% of the ears.<sup>9-15</sup> The effectiveness of lidocaine may suggest that other drugs with similar structure and mechanisms of action may prove beneficial in the treatment of tinnitus.<sup>15</sup> However, the relative ineffectiveness of other neurotropic drugs have been attributed to the multiplicity of the pathological processes underlying tinnitus, uncertainty regarding the anatomic location of the lesion, and a lack of understanding of the physiological mechanisms giving rise to tinnitus.<sup>1,4</sup>

This is a report of our pilot work on the role of immunobiologic factors on the development of subjective tinnitus among the elderly. We have chosen melatonin because it is a neurohormone which is centrally produced in the pineal gland and it helps in examining the hypothesis of the central origin of tinnitus; in addition the role of antioxidative scavenging was assessed by including vitamin C and vitamin B12.

### METHOD

#### *Study Design*

This was a prospective study of immunobiology of subjective tinnitus among apparently healthy elderly subjects. Subjective tinnitus was assessed by asking respondents if they had a sensation of noise in the ear without an external source of sound.

The participants were drawn from the community tagged to an out-reach programme organized for the detection and prevention of illnesses among the elderly. Included in the study were all elderly men and women above 60 years of age who had no known medical condition while the exclusion criteria involved those with history of diabetes, stroke, hypertension, ear diseases, exposure to noise and ototoxic drugs such as aminoglycosides antibiotics, diuretics; ear infections, ear trauma or ear surgery.

#### ***Participants Recruitment***

Recruitment commenced by obtaining informed consent from the subjects. The participants have had the opportunity of repeated physical examinations by physicians in the outreach programme and undergone few routine clinical investigations including blood glucose, and urinalysis. Prior to enrolment, participants were taken through an already prepared questionnaire aimed at eliciting otological and general medical conditions. Participants were also asked about history suggestive of allergy and use of some notable medications such as: aminoglycosides, diuretics and 4-amino-quinolines antimalarial drugs.

History of exposure to excessive noise such as working (as)/living near (to) blacksmith shop, radio room/disco room, welding shop etc for at least 2 hours per day for at least 5 days a week was also obtained. General and otorhinolaryngological examination was thereafter carried out and 20 millilitres of whole blood was taken from the antecubital vein, immediately spun and separated for estimation of vitamin B12, vitamin C and melatonin.

The diagnosis of the medical condition were based on simple definitions.<sup>16, 17</sup> Diabetes was taken as elevated blood glucose with presence of either of polyuria, polydipsia, polyphagia or complications or those on treatment already by physicians, hypertension defined as elevated blood pressure beyond 140/90mmHg or those already on treatment by physician and stroke referred to as weakness of the limb(s) or slurred speech in a patients with hypertension.

In this study exposure to noise referred to close contact with loud noise such as grinding machine, megaphone speaker, blacksmith workshop etc for not less than 2hours daily either at work or home; history of ototoxic medications referred to consumption of a drug in normal or high doses for at least one week particularly known ototoxic drugs whether prescribed by physicians or quack and ear surgery was taken as any operative intervention of the ear.

We based Meniere disease on a history of vertigo, hearing loss, tinnitus with nausea and/or vomiting; otosclerosis on history of hearing loss confirmed to be conductive with an intact tympanic membrane and arteriosclerosis was defined by the presence of thickened arteries confirmed by feeling the walls of the radial artery or locomotor brachialis – observing the pulsation of the brachial artery at the elbow. The Oyo State Research Ethical Review Committee approved the study.

#### ***Quantitative Assay of Melatonin***

Sample was prepared by pipetting 10.5ml of the plasma sample to a set of vials, this was diluted with 10.0ml of ultrapure water and 2.0ml of 0.1% of benzene acetic acid was added and swirled. To this was added 5.0ml of a mixture of methanol and Sulphuric acid and then shaken for 5.0 minutes. Sodium sulphate solution was added and finally shaken for 10.0minutes. The mixture was then transferred to a set of centrifuge tubes and samples were centrifuged for 15.0 minutes at the speed of 5000 revolutions per minute (rpm). The sample solution was transferred to another set of sterile vials and analyzed on the High Performance Liquid Chromatography.<sup>18</sup>

#### ***Quantitative Assay of Vitamin B 12***

Into a clean beaker, 1.0ml of the sample and 25ml of 0.2 N HCL was added and warmed on a water bath for 30 minutes, then cooled and adjusted the pH to 6.0 using NaOH. This was followed by adding 1N HCL to lower the pH to 4.5, then transferred into a set of 50.0ml centrifuge tubes, shaken for 30minutes and centrifuged for the period of 20.0minutes at 2000rpm. The supernatant was collected and vitamin B12 determined by High Performance Liquid Chromatography.<sup>18</sup>

#### ***Quantitative Assay of Ascorbic acid***

Sample was prepared by pipetting 1.5ml of the plasma into a centrifuge tubes and to this was added 10.0.ml of freshly prepared 0.5% oxalic acid and 10.0ml of a mixture of ultrapure water, acetone and dichlorophenolmidophenol solution. The mixture was shaken on a mechanical shaker for 30minutes and then centrifuge at 1500rpm for 20minutes. The supernatant was separated into a set of vials and the determination of the vitamin C was done with High Performance Liquid Chromatography.<sup>18</sup>

As quality control measure, control and standard sera were included in the analysis at every sera assay to ensure reliability and quality of the procedure. An initial pilot study was conducted to test all instruments and this was followed by a preliminary statistical analysis to detect outliers and correct factors.

**Data analysis**

The main outcome variables were the serum levels of melatonin, vitamin B12 and vitamin C in elderly subjects with report of subjective tinnitus and the control subjects were selected among the elderly without tinnitus.

Economic status was assessed by taking an inventory of household and personal items including chairs, clock, bucket, radio, television set, fans, stove or cooker, car and telephone. The list was composed of 21 such items.

Respondents' economic status was categorized by relating each respondent's total possessions to the median number of possessions of the entire sample. Thus, economic status was rated low if its ratio to the median was 0.5 or less, low-average if the ratio is 0.5 – 1.0, high-average if it was 1.0 – 2.0, and high if it was over 2.0. Residence was classified as rural (less than 12,000 households), semi-urban (12,000 – 20,000

households) and urban (greater than 20,000 households). The data were initially explored using the Stata software; variables were analyzed by unpaired *t* test both for equal and unequal variance using the variance ratio function of the Stata software to determine the appropriate use of the Satterthwaite's correction for the degrees of freedom. Level of statistical significance was at  $P < 0.05$  for all the analyses.

**RESULT**

The subjects included 139 elderly subjects (78 females and 61males), the mean(SD) range of the age was 66.9years (0.77) 60-98years. Of these 81(58.3%) had tinnitus. Tables 1 shows the sociodemographic and lifestyle factors among the subjects, it reveals that there was no significant difference between elderly persons with tinnitus and those without tinnitus in the gender, economic status, educational level, residence and history of lifetime alcohol or tobacco use.

**Table 1** Univariate analysis of the sociodemographic and life-style correlates between the subjects with and without tinnitus

Characteristics	Elderly subjects with Tinnitus (n=81)	Elderly subjects without Tinnitus (n=58)	p-value
<b>Sociodemographic</b>			
<b>Age (years)</b>			
60-64	24(29.6%)	19(32.8%)	0.3
65-69	20(24.7%)	15(25.9%)	
70-74	17(21.0%)	11(19.0%)	
75-79	12(14.8%)	7(12.1%)	
≥80	8(9.9%)	6(10.3%)	
<b>Gender</b>			
Female	45(52.4%)	33(59.8%)	0.4
Male	36(39.4%)	25(40.2%)	
<b>Western Education</b>			
Tertiary	8(4.9%)	7(7.6%)	0.2
Secondary	12(9.2%)	11(10.9%)	
Primary	24(17.5%)	16(20.1%)	
None	37 (37.5%)	24(42.4%)	
<b>Economic Status</b>			
High	9(8.2%)	6 (5.4%)	0.2
High-average	13(27.7%)	12 (32.6%)	
Low-average	26(34.8%)	18(34.8%)	
Low	33(21.2%)	22 (27.2%)	
<b>Residence</b>			
Urban	21(22.1%)	14(15.8%)	0.4
Semi-urban	23(39.0%)	17(47.8%)	
Rural	37 (30.7%)	27 (36.4%)	
<b>Lifestyle correlate</b>			
<b>Alcohol drinking, ever</b>			
No	47(49.7%)	31(52.7%)	0.8
Yes	34(41.1%)	27(45.1%)	
<b>Cigarette smoking, ever</b>			
No	44(52.9%)	33(50.5%)	0.7
Yes	37(38.9%)	25(48.4%)	

Among the subjects, the mean (SD) range of the plasma levels of melatonin was 11.2 pg/mL(4.2) 5.1 pg/mL - 30.2 pg/mL while that of Vitamin C was 0.7 μmol/L (0.1) 0.3 μmol/L - 1.2 μmol/L, and vitamin B12 was 43.0pmol/L (3.1) 25.4 pmol/L- 71.6pmol/L . Table 2 compares the plasma levels of melatonin, Vitamin C and vitamin B12 between elderly with and those without tinnitus. It shows that the plasma levels of melatonin (p=0.01) and vitamin B12 (p=0.03) were significantly lower among the elderly with tinnitus compared to those without, while the difference in the plasma level of vitamin C was not.

**Table 2** Analysis of the plasma levels of Melatonin, Vitamin C and vitamin B12 between elderly with and those without tinnitus

Characteristics	Elderly subjects with Tinnitus (Mean + SD)	Elderly subjects without Tinnitus (Mean + SD)	P value
Melatonin	11.1 pg/mL +3.4	13.1 pg/mL + 5.6	0.01
Vitamin B12	41.3 pmol/L ± 5.3	47.0 pmol/L ± 3.2	0.03
Vitamin C	0.7 μmol/L ± 0.3	0.9 μmol/L ± 0.5	0.09

**DISCUSSION**

The generation of tinnitus have been linked to damage to the central and peripheral auditory systems, even in cases where an impairment could not be detected by audiometry.<sup>19 - 22</sup> Impairment of the auditory system cannot be detected by audiometry if it only causes tinnitus without an accompanying hearing loss. In this study, the main finding is that low plasma melatonin and vitamin B12 have significant correlation with the development of tinnitus among the elderly. Melatonin is a neurohormone produced centrally by the pineal gland; it regulates the sleep-wake cycle by inducing sleepiness and reducing body temperature through its effects on the circadian clock.<sup>23, 24</sup> Several workers have reported that melatonin is useful in the treatment of tinnitus, even in cases associated with sleep disturbance.<sup>24-28</sup>

Concerning vitamin B12, our finding is supported by the report of Shemesh et al.<sup>29</sup> They reported that the incidence of vitamin B12 deficiency is significantly higher among patients with tinnitus and noise-induced hearing loss (47%) compared with those with noise induced hearing loss alone and normal subjects who exhibited vitamin B12 deficiency in 27% and 19%, respectively. In addition they reported some improvement in tinnitus and associated complaints in 12 patients following vitamin B12 replacement therapy. These suggest a relationship between vitamin B12 deficiency and dysfunction of the auditory pathway; hence they recommended that routine vitamin B12 serum levels be determined when evaluating patients for chronic tinnitus.

The presence of tinnitus as the only features in these subjects with low plasma vitamin B12 suggest that perhaps tinnitus may be one of the early features of the various neurological abnormalities associated with B12 deficiencies. In this case, the tinnitus is thought to be due to auditory nerve malfunction. Our impression is that correction of the plasma levels of these markers may lead to reversal of tinnitus, however, this needs to be studied in the future. Among other studies of the role of biochemical markers in tinnitus, Baguley<sup>30</sup> identified endogenous dynorphins to be responsible for the excitatory function of glutamate within the cochlea, thus, mimicking the action of sodium salicylate in increasing spontaneous neural activity. Similarly, Simpson and Davies<sup>9</sup> postulated that persistent tinnitus was due to role of serotonin and they suggested that

tryptophan (5HT) may use a reduction in auditory filtering ability and in tinnitus habituation. Other workers<sup>31-35</sup> have also found that Zinc influences the biophysiological function of neural membranes and transmission such that in the cochlea, levels of zinc are higher than any other organs of the body and the patients showed a significant decrement in their tinnitus after taking zinc. All these reports might be evidence that research into the biology of tinnitus is still evolving and so the application of the findings in the treatment of this condition.

Notably, the values for the serum levels of these biomarkers from this study are low compared with previously published figures. The mean serum levels of vitamin B12 of 151.0-300.0 pmol/L<sup>36</sup>, serum vitamin C value greater than 28μmol/L<sup>37</sup> and melatonin peak value of 70.6+/-14 pg/mL<sup>38</sup> were reported as normal. In our study Melatonin ranged between 5.1 pg/mL and 30.2pg/mL while serum Vitamin C level was between 0.3 μmol/mL and 1.2 μmol/mL and vitamin B12 ranged between 25.4 pmol/L and 71.6 pmol/L.

The low figures in this study may be further evidence of the prevalent low level of nutritional health in our population compared to others. However, the method of storage and assay may also account for the difference in the values reported. However, we conclude from this study that low plasma melatonin and vitamin B12 have significant correlation with the development of subjective idiopathic tinnitus among the elderly.

This finding suggests the need to study the role of correction of these markers in the reversal or control of tinnitus.

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