Research Article

Can the Determination of Salivary Cotinine Level Be a New Method in Diagnosis and Follow-up of Childhood Tinnitus?

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Abstract

Background: There are many risk factors for childhood tinnitus such as hearing loss, exposure to high pitch sound, and passive smoking. Cotinine (C) is one of the metabolites of nicotine and is an important biochemical marker that reveals the objective and numerical indication of smoking exposure. Although there is a study investigating the role of urinary C levels in the etiology of tinnitus (T), the role of salivary C levels has not yet been elucidated.

Objectives: Therefore, this study aimed to investigate the risk factors of idiopathic subjective T in children in terms of passive smoking and whether the salivary C level can be used as a novel marker for monitoring and follow-up of T.

Methods: We retrospectively studied 1,245 children aged 7 - 15 years with T. We excluded 830 patients (66.5%) whose total tinnitus diagnosis was confirmed due to organic causes such as middle ear pathology and hearing loss. The remaining 415 (33.5%) patients with subjective T and 200 healthy individuals constituted the study and control groups. Complete blood counts, routine biochemical tests, and salivary C levels of children with T and controls were measured and their parents were also evaluated.

Results: In univariate analysis, parents' C, children's C, ALP, and erythrocyte levels were significantly associated with T (P < 0.001 in all). Regarding laboratory values, erythrocyte and serum ALP levels were significantly associated with T (P < 0.001 in both) in univariate analysis (OR, 0.99; 95% CI, 0.98 to 0.99 for erythrocyte and OR, 157.04; 95% CI, 44.7 to 551.6 for serum ALP level). No significant relationships were found between other parameters. These four parameters that were found to be significant in the univariate analysis showed meaningful associations with T in multivariate analysis (P < 0.01 for parents C and P < 0.001 for other parameters). It was also observed that as the C levels increased in the parents, the C level also increased in children.

Conclusions: There is a significant correlation between salivary C levels and parental salivary C levels in children. This suggests that the salivary C be used for evaluating the etiology of T in children and monitoring children with T exposed to cigarette smoke.

Keywords: Child, Cotinine, Passive Smoking, Tinnitus

1. Background

Tinnitus (T) is the sound of hearing in the ear or head such as ringing, clicking, hiss, roaring, or buzzing without external audible stimulus. Rarely, unclear voices or music can be heard, as well. It is a symptom rather than an illness and is associated with hearing loss, otological problems, neurological disorders, cardiovascular diseases, and head and neck traumas (1, 2). Tinnitus is one of the most common otological complaints that is seen in approximately 17% of the adult population and this ratio increases with age (3). Although T is thought to be a rare symptom in childhood, its incidence is not as low as expected (4). In the studies conducted in children, it was reported that T prevalence ranged from 4.7% to 46% in the general pediatric population and 23.5% to 62.2% in children with hearing loss (5). There are many risk factors in childhood T. Some factors have been prominent in recent years including hearing loss, exposure to high sound pitch, low socioeconomic level, history of ear infections, essential palatal tremor, and passive smoking (6, 7).

T is divided into two main groups: objective and subjective. Objective T is usually of vascular origin and occurs in about 10% of the patients. Perceived voice is also heard by the doctor during the examination of objective T. In the remaining 90% of patients who have subjective T, the voice is heard only by the patient. In addition, idiopathic cases who have no cause in subjective T constitute more than half of the population (8, 9). Since medical treatment of T in childhood is inconvenient and there is no evidence-based information about the monitoring and effectiveness of the treatment process, the assessment of modifiable factors such as nutritional status and social habits would be much more beneficial. Although a significant relationship

has been found between active smoking and subjective T in recent studies in adolescents and adults, there are not enough studies in the literature to evaluate the relationship between T and passive smoking using serological or salivary parameters, especially in the pediatric age group (10, 11).

Today, smoking is one of the most common factors that adversely affect human health. This negative phenomenon can turn children from non-smokers to passive smokers due to smoking in the home environment and has increased rapidly in recent years (12). In humans, 80% of nicotine is converted to cotinine (C) by microsomal cotinine-oxidation in the liver (13) and it can be measured in body fluids such as saliva, blood, urine, and milk. In several studies, it was shown that C is an important marker that shows the objective and numerical status of smoking exposure (14, 15).

2. Objectives

In a study conducted by Lee and Kim (10), they elucidated a correlation between T and smoking among adolescents and identified urinary C as a new novel marker for serial follow-up and monitoring. Therefore, in this study, we aimed to investigate the role of exposure to passive smoking in children in the etiology of T and whether the salivary C level can be used as a novel marker for monitoring and follow-up of T.

3. Methods

3.1. Data Collection

A retrospective controlled study was performed at the Otorhinolaryngology (Ear-Nose-Throat) Clinic of Bahat Hospital between 2007 and 2018. The power analysis revealed that 233 patients were required for each group, with a predetermined 5% type-I error level and 95% expected power. The inclusion criteria were children aged 7 - 15 years from both genders and with complaints of T. The exclusion criteria included middle ear pathology, hearing loss, head trauma, hypothyroidism treatment before the study, meningitis history, dehiscent jugular bulb history, and hypertension. A control group was selected among healthy children admitted to our clinic with the same age range.

Of 1,245 patients with no missing data included in our study, we detected middle ear pathology in 428 patients, hearing loss in 385 patients, head trauma in nine patients, history of treatment of hypothyroidism in four patients, history of meningitis in two patients, history of jugular bulb dehiscent in one patient, and hypertension due to renal pathology in one patient and thus, a total of 830 patients (66.5%) with definite T diagnosis were excluded from

the study. As a result, the remaining 415 (33.5%) patients with subjective T and 200 healthy individuals constituted our study and control groups. The inclusion/exclusion flow diagram is given in Figure 1.

3.2. Ethical Permission

The study was approved by the Non-Interventional Clinical Research Ethics Board of Istanbul Yeni Yuzyil University (Date: May 06, 2019 - Decision No: 2019/05). We obtained approved consent forms from all the families of children after explaining the study. This study was performed in accordance with the principles of the Helsinki Declaration.

3.3. Examination

All tympanic membranes (both right and left) of patients with tinnitus were examined by trained otologists and they were divided into two groups as intact and pathologic. In addition, pure-tone audiometry was measured for threshold values by experienced audiologists at 500, 1,000, 2,000, 3,000, 4,000, and 6,000 Hz. Hearing loss was defined as an average hearing loss of more than 30 dB at 500, 1,000, 2,000, and 3,000 Hz on a pure tone audiometer. To investigate the causes of T, we performed neck USG and temporal CT. A standard audiometer (Interacoustics AC40) and supra-aural TDH39 headphones were used for audiometry. The calibration of the Interacoustics AC40 Clinical Audiometer was performed up to 16 kHz by a technician experienced in calibrating audiometers. The accuracy of the measurements of the sound level meter was checked regularly using a sound level calibrator.

For the presence of T, the patients were asked whether they heard any sounds such as ringing, buzzing, hiss, humming, and roaring without any sound source. The age and sex of all patients were noted. Two tubes of blood were taken from all patients for examining the complete blood count, blood urea nitrogen (BUN), creatinine, glucose, liver enzymes (aspartate aminotransferase-AST and alanine aminotransferase-ALT), total cholesterol, triglyceride, alkaline phosphatase (ALP), lead (Pb), and mercury (Hg). Complete blood counts were determined in tubes with EDTA using an auto hematology analyzer (BC6800, Mindray) and routine biochemical tests were carried out using commercially available test kits.

The saliva was collected from all children and their parents with T in their mouths for evaluating the C levels. A sample of at least 1 mL was frozen within eight hours after collection for assessing the C concentration by gas-liquid chromatography.
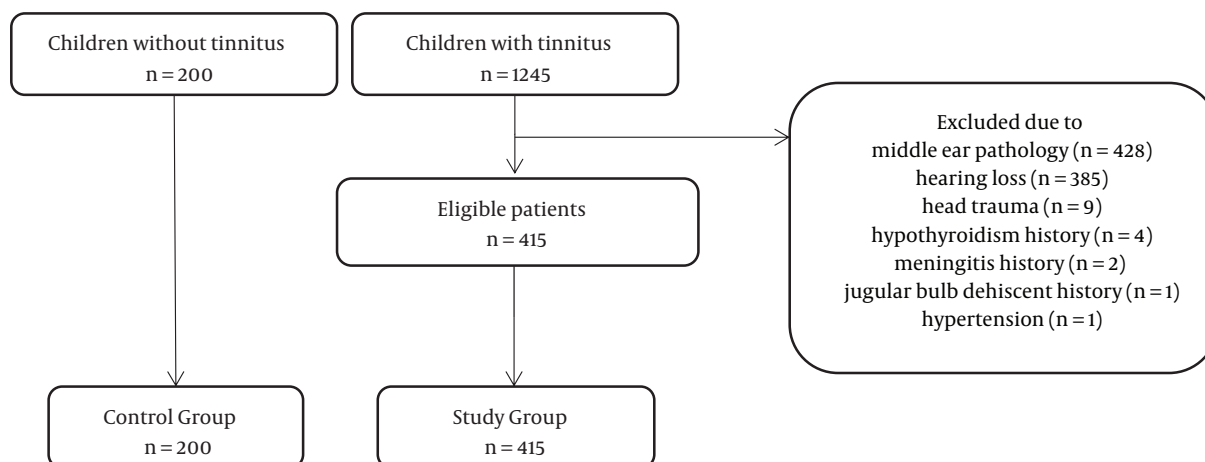
Figure 1. Inclusion and exclusion flow diagram of the study

3.4. Cotinine Analysis

The saliva was collected from all children and their parents with T in their mouths for evaluating the cotinine levels. An average of 3 mL salivary sample was centrifuged and then a clear liquid portion was taken. C measurements in the saliva were performed using the Enzyme-Linked Immuno sorbent Assay (ELISA) method, Salivary Cotinine Quantitative Enzyme Immunoassay kit, and ELISA Reader device (Beckman Coulter, model-Access 2, Germany) (16, 17).

3.5. Statistical Analysis

The analyses were done using SPSS 20.00 for Windows. First, the mean and standard deviation of all variables were calculated. After determining the clinical value ranges, adjusted odds ratios (AOR) were calculated. The relationships between T and children's age, gender, and biochemical parameters and parents' smoking status were analyzed using univariate and multivariate logistic regression analyses. Multiple linear regression analysis was used to evaluate co-linearity and variance inflation factors were evaluated. In the subgroup analysis, the relationship between the smoking levels of parents of the children with and without T was analyzed by the t test. The AOR and 95% confidence intervals (CIs) were calculated and $P < 0.05$ was accepted to indicate significance.

4. Results

4.1. Study Population

The study included 1,245 T patients with a mean age of 11.28 ± 1.57 years (range, 7 to 15 years) consisting of 415

(33.5%) idiopathic subjective T patients and 830 (66.5%) secondary ('not idiopathic') T patients including 428 (34%) patients with middle ear pathology, 385 (31%) patients with hearing loss, nine (0.7%) patients with head trauma, four (0.32%) patients with hypothyroidism history, two (0.16%) patients with meningitis history, one (0.08%) patient with jugular bulb history, and one patient with hypertension (Table 1). The mean age of 415 subjective T patients included in the study group was 12.05 ± 2.43 (range, 7 to 15 years) and the male to female ratio in all participants with T was 1.47 ± 0.50 (Table 2).

Table 1. Demographic Data of Children with Tinnitus

	Number	%
Idiopathic subjective tinnitus	830	66.5
Middle ear pathology	428	34
Hearing loss	385	31
Head trauma	9	0.7
Hypothyroidism history	4	0.32
Meningitis history	2	0.16
Jugular bulb history	1	0.08
Hypertension	1	0.08

4.2. Biochemical Analyzes

In univariate analysis, the parents' C, children's C, and erythrocyte levels in children with T (92.04, 9.58, and 5.191, respectively) were significantly higher than the levels in children without T (46.27, 4.873, and 4.819, respectively) (all $P < 0.001$). The serum ALP levels were significantly lower

Table 2. Univariate Regression Analysis of Risk Factors for Tinnitus

	Univariate Analysis				
	Tinnitus (+) Children	Tinnitus (-) Children	OR	95% CI	P Value
Age	12.05 ± 2.43	11.97 ± 2.43	1.01	0.91 - 1.12	AD.
Gender (F/M)	1.47 ± 0.50	1.49 ± 0.50	0.94	0.57 - 1.55	AD.
Parents cotinine	92.04 ± 19.04	46.27 ± 19.04	1.15	1.11 - 1.20	< 0.00
Children cotinine	9.58 ± 2.79	4.873 ± 2.76	1.32	1.22 - 1.43	< 0.00
ALP	366.30 ± 68.65	406.2 ± 68.65	0.99	0.98 - 0.99	< 0.00
Erythrocyte	5.191 ± 0.26	4.819 ± 0.25	157.04	44.7 - 551.6	< 0.00
Hb	13.59 ± 1.00	13.70 ± 0.95	0.88	0.68 - 1.14	AD.
Hct	42.12 ± 2.37	42.323 ± 0.86	0.92	0.75 - 1.13	AD.
WBC	11.24 ± 50.10	7.288 ± 0.56	1.01	0.92 - 1.10	AD.
Urea	39.16 ± 2.52	39.2 ± 2.62	0.99	0.90 - 1.09	AD.
Creatinine	1.309 ± 6.04	1.664 ± 8.51	0.99	0.96 - 1.02	AD.
Glucose	90.26 ± 3.03	90.15 ± 3.08	1.01	0.93 - 1.09	AD.
ALT	18.59 ± 4.10	18.82 ± 3.60	0.98	0.92 - 1.05	AD.
AST	18.58 ± 1.69	18.5 ± 1.57	1.02	0.88 - 1.19	AD.
LDL	164.9 ± 830.00	98.95 ± 4.48	1.02	0.95 - 1.09	AD.
TG	116.16 ± 15.17	115.2 ± 17.68	1	0.98 - 1.02	AD.
Pb	0.368 ± 0.07	0.367 ± 0.07	0.91	0.04 - 32.73	AD.
Hg	2.2188125 ± 8.36	2.458 ± 9.78	0.99	0.97 - 1.20	AD.

in children with T than in children without T (366.30 vs. 406.2; P < 0.001) (Table 2). Regarding laboratory values, erythrocyte and serum ALP levels were significantly associated with T (P < 0.001 in both) in univariate analysis (OR, 0.99; 95% CI, 0.98 to 0.99 for erythrocyte and OR, 157.04; 95% CI, 44.7 to 551.6 for serum ALP level). No significant relationships were found between other parameters. These four parameters that were found to be significant in the univariate analysis showed meaningful associations with T in multivariate analysis (P < 0.01 for parents' C and P < 0.001 for other parameters) (Table 3). The C levels of parents and the C levels of children were evaluated with each other. It was observed that as the C levels increased in the parents, the C level also increased in children (Figure 2).

5. Discussion

In 66.5% of the patients, the cause of T was detected to be 34% middle ear pathology, 31% hearing loss, and 1.5% trauma, hypothyroidism, meningitis, dehiscent jugular bulb, and kidney pathology. In 33.5%, the etiology could not be determined, so they were evaluated as idiopathic subjective T. In multivariate analysis, the parents' and children's salivary C, erythrocyte, and ALP levels were found to be associated with T. In the literature, there is only

one study evaluating the C levels and other blood parameters of patients with T. According to a study conducted by Lee and Kim (10) in adolescents, blood tests and urinalysis showed significant correlations between T and erythrocyte, ALP, and urine C. It was also stated that the urine C level was the only parameter associated with T. Moreover, they found that smoking was correlated with T. It was seen that our findings were in line with this study.

The ALP level is a marker of bone formation and osteoblast activity. The serum ALP level increases starting from childhood, peaks in puberty, and decreases at an older age. It is thought that nicotine in cigarettes has a toxic effect on osteoblast function and thus causes a decrease in basal osteoblastic activity and leads to bone loss with a direct effect (18, 19). Lee and Kim (10) also found low levels of ALP and high levels of C in adolescents with T. In our study, the ALP level was found to be significantly lower in children with T with higher salivary C than in the control group. However, this reduction in ALP is complex and controversial in terms of the cause and effect relationship. Further specific studies need to be planned on this subject to better evaluate this result.

Although the effect of smoking on middle ear and hearing is controversial in children, studies have shown that it has a significant effect on T and smoking has been identi-
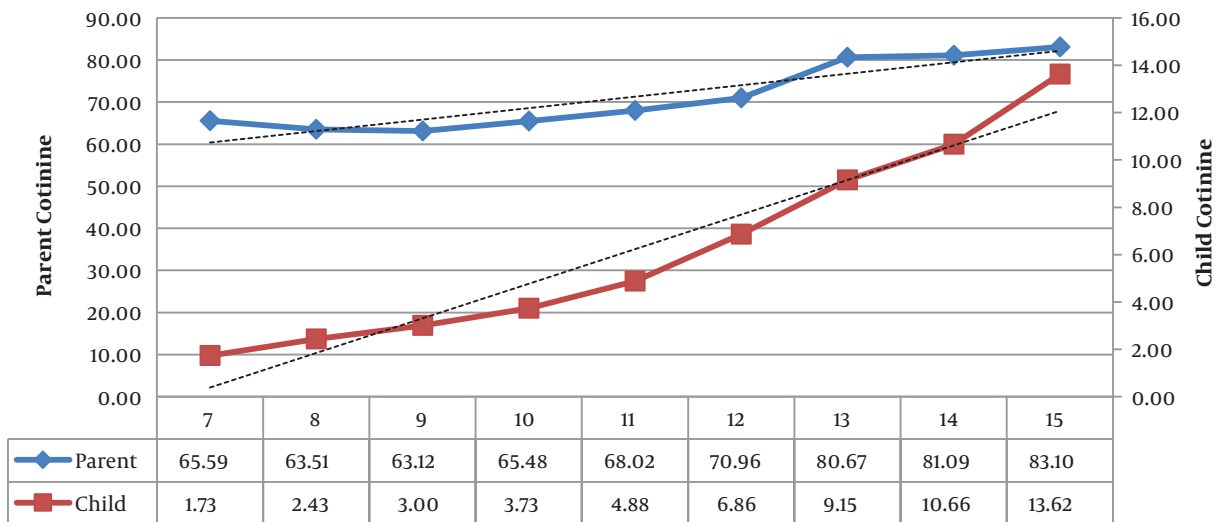
Figure 2. Cotinine levels of children with tinnitus and their parents

Table 3. Multivariate Regression Analysis of Risk Factors for Tinnitus

	Multivariate Analysis			
	OR	**%95 CI**	**P Value**	**VIF**
Parents' cotinine	0.51	0.08 - 0.01	< 0.01	2.06
Children's cotinine	0.32	0.02 - 0.04	< 0.00	2.57
ALP	-0.38	00.00 - 0.00	< 0.00	1.75
Erythrocyte	1.18	0.18 - 0.39	< 0.00	1.33

fied as a risk factor for T in both adults and children. Several recent large cohort studies have confirmed that direct and indirect smoking is an important risk factor for T in children (20-23). Hearing loss is not the only pathophysiological factor in T and it is possible that cigarettes develop T by a mechanism different from that of hearing loss (24, 25). Smoking has proven to have significant side effects on the hearing system. The most important ones are hypoxia at the cochlear level, atherosclerotic changes in vascular structures, and decreases in blood viscosity (7, 10). Nicotine and its toxic metabolites also impair the activity of the hearing cortex and cause a decrease in otoacoustic emissions (26, 27). In addition, the negative effects of smoking on the hearing system are more pronounced in children who are growing up (10, 28). Our finding concerning a significant relationship between passive smoking and T in children supports these results. Based on our study, we think that smoking may be directly related to T.

The half-life of cigarette nicotine is approximately two hours while that of C is 18 hours (13, 29). Following smoking, the C concentration reaches the highest level and remains stable in body fluids for a longer period than nico-tine and it is found at higher concentrations (30). The C measurement in body fluids is considered to be the best indicator for smoking because it provides 96% sensitivity and 99% specificity (31). The determination of C in the saliva has advantages since it has shown to be a non-invasive method easily obtained from children that gives highly sensitive results about the smoking effect (22, 32-34). Our study showed that the saliva C levels were significantly correlated with T. The C level was significantly higher in children with T (+) than in the T (-) group. Similarly, the C values were significantly higher in parents of children with T (+) than in parents of children with T (-). In our literature search, we found no study using the saliva cotinine biomarker for the evaluation of childhood T. In this respect, we think that the saliva C level can be used as a novel biomarker for monitoring and follow-up of T.

In recent years, many studies have been conducted on the effects of passive smoking on children in the home environment. They have found a significant decrease in lung function, asthma, lower respiratory tract infections such as broncho-pneumonia, and middle ear and sinus diseases in children, especially in the preschool period (35,

36). Smoking also causes protein oxidation and DNA damage by increasing the oxidative stress in the body due to free oxygen radicals in its composition (37). This increases the risk of chronic obstructive lung disease, lung cancer, leukemia, lymphoma, diabetes, cardiovascular diseases, and inflammatory diseases (38-40). Recently, the C level has been used as a biomarker for various smoking-related health problems, including cardiovascular diseases (41). We think that the salivary C level may be an important marker for pediatric T. In addition, saliva C should be included in the initial assessment because it reflects smoking status and poses a potential risk factor for T. According to our results, it would be beneficial for physicians to check the salivary C levels of children presenting with T complaint. In this way, physicians may have information about the exposure of children and parents to smoking.

5.1. Conclusions

- Meta-analysis studies show a statistically significant relationship between smoking and T. However, these studies cannot give evidence of causality. More evidence is needed to produce more accurate results. Nevertheless, we believe that passive exposure to cigarette smoking may also cause T.

- A significant relationship was found between children's and parents' salivary C levels.

- The level of salivary C is thought to be an important marker to explain the etiology of T in children.

- Since passive smoking is a known risk factor for T in children, the serial monitoring of salivary C levels should be used in the diagnosis and follow-up of T but further studies must be conducted to confirm these results.

Acknowledgments

Footnotes

Conflict of Interests: The author declares no conflict of interest to estate.

Informed Consent: We obtained approved consent forms from all the families of children after explaining the study. This study was performed in accordance with the principles of the Helsinki Declaration.

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