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Pilot study of salivary butyrylcholinesterase, phosphodiesterase, thiols and cerulopalsmin in auditory neuropathy

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ABSTRACT

Objective: To estimate butyrylcholinesterase (BChE), phosphodiesterase (PDE), thiols and cerulopalsmin by non - invasive means in saliva a of subjects (both cases and controls) and correlated to their hearing sensitivity. **Methods:** Total of 13 subjects participated in this study. Among them 7 were having auditory neuropathy and 6 were healthy controls. Unstimulated saliva (10 ml) was collected from each participant. Ceruloplasmin , thiols, phosphodiesterase and pseudocholinesterase were estimated by colorimetric method in the salivary samples. **Results:** Salivary BChE and PDE levels were marginally elevated and protein thiols were marginally decreased in cases as compared to that of controls. Salivary ceruloplasmin was significantly decreased ($p = 0.022$) in cases as compared to that of controls. **Conclusions:** Saliva can be used as a potential noninvasive tool for evaluation of disorders.

1. Introduction

Auditory Neuropathy Spectrum Disorder (ANSD) is a broad terminology which includes disorders that are characterized by grossly abnormal or absent ABR (Auditory Brainstem Response) with preserved Outer Hair Cell (OHC) function as reflected by OAE (Oto Acoustic Emissions) and/or Cochlear Microphonics (CM)[1].The prevalence of such a disorder is reported to vary from 1.8% to as high as 11%[2]. However, there is a very little information available on the prevalence of such a disorder in Indian context.

Common causes of AN include hyperbilirubinemia, low birth weight, anoxia and otoferin mutation. The above mentioned causes either lead to a hearing loss by birth or in the immediate postnatal period. However, there is a sub group of AN individual who are reported to have normal hearing sensitivity till a particular age and following which they develop hearing loss and it is not associated with any disease condition. Although the exact site of lesion and pathophysiology of Auditory Dy-synchrony (AD) or Auditory Neuropathy (AN) is not yet completely understood, it may involve damage to the inner hair cells or damage to the nerve itself [3,4] Acquired copper deficiency in humans has been associated with a syndrome similar to the sub-acute combined degeneration [5,6] oxidative-nitrosative stress and reduced systemic antioxidant defence regulates the expression of genes that promote inflammatory reactions and neuronal dysfunction and is considered to play an important mediating role in pathogenesis of neuropathy [7 - 9].

Studies have shown that serum cholinesterase activity

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correlated with nerve conduction and toxic peripheral neuropathy [10] phosphodiesterase-5 inhibitor commonly used for erectile dysfunction, has been shown to have a beneficial therapeutic effect in the treatment of stroke, subarachnoid haemorrhage, and neurodegenerative disorders by enhancing angiogenesis and neurogenesis [11].

Hence we estimated these parameters by non - invasive means in saliva of subjects (both cases and controls) and correlated to their hearing sensitivity.

2. Materials and methods

The study was carried out after obtaining approval from the Institutional Ethics Committee. The subjects were cases of auditory neuropathy of either sex came to the outpatient department of ENT, Kasturba Hospital, Manipal from June 2009 to June 2010. Age and sex matched healthy controls were local people from Manipal. Both the controls and cases were included in the study after obtaining informed consent.

2.1 Study design: Prospective, case control study

2.1.1 Inclusion criteria

For cases: patients of either sex between 13 - 45 years with auditory neuropathy based on Pure tone audiometry, Auditory Brainstem Response and Oto Acoustic Emissions. For controls: healthy subjects between age of 13 and 45 years, who underwent an ENT examination to rule out all possible benign or malignant diseases with normal hearing sensitivity based on above investigations.

2.1.2 Exclusion criteria

For cases: Cases with hearing loss due to middle ear involvement; patients with serious medical and surgical illness; patients on long term medication.

For control: subjects with coexisting diseases; subjects on long term medications.

2.2 Sample collection:

Unstimulated saliva (10 ml) was collected from both cases and controls. Saliva was centrifuged at 10,000 rpm for 15 minutes to separate any suspended particles and mucin. Ceruloplasmin, thiols, phosphodiesterase and pseudocholinesterase were estimated by colorimetric method in the salivary samples.

2.3 Sample assays

2.3.1 BChE assay

Acylthiocholine is hydrolyzed by BChE to corresponding fatty acid and thiocholine. The rate of formation of thiocholine can be monitored by continuous reaction of thiol group with 5, 5′-dithio-bis-(nitro-benzoic acid)- DTNB to form a yellow anion that can be measured spectrophotometrically at 410nm. Enzyme activity was calculated by absorption coefficient of the product of chemical reaction, 5-thio-2-nitro-benzoate (1.36 x mmol -1 x min -1 x cm -1)[12].

2.3.2 Phosphodiesterase (PDE) Assay

Para-nitrophenyl phosphate (4-nitrophenyl phosphate) is hydrolysed by phosphodiesterase (PDE) to4-nitrophenol (4-hydroxynitrobenzene) and inorganic phosphate. The yellow color formed due to liberation of 4-nitrophenolate at pH 9 was measured spectrophotometrically at 400nm. Enzyme activity was calculated by absorption co-efficient of the product of chemical reaction, 4-nitrophenol (17,600 M-1x cm -1)[13].

2.3.3 Protein Thiols

Ellman's reagent or dithiobisnitrobenzoate (DTNB) readily undergoes a thiol-disulphide interchange reaction in the presence of free thiol. The 2- nitro 5 - thiobenzoate di-anion has a relatively intense absorbance at 412nm (mec: 13600 M-1 cm-1), which can be used to assess thiols [14].

2.3.4 Ceruloplasmin

When mixed with parphenyldiamine saliva forms a coloured complex which when read at 546 nm gives a measure of ceruloplasmin [15].

2.4. Statistical analysis

The data analysis was done by using SPSS statistic analyser software version 15. Data was summarized as median and interquartile range since it was skewed. Comparisons between cases and controls were done using Mann - Whitney test and p value < 0.05 was considered as significant.

3.Results

In this study, cases and controls were aged between 13 and 45 years of either sex. Out of them, 7 were having auditory neuropathy and 6 were healthy controls. Thus, a total of 13 subjects participated in this study.

Among the patient group, salivary BChE and PDE levels were marginally elevated (Figs: 1&2) and protein thiols were marginally decreased (Fig: 3) compared to those of controls. Salivary ceruloplasmin was significantly decreased (Fig: 4) (P = 0.022) in cases as that of controls (Table - 1).

Table: 1

Comparison of levels variables in saliva of controls and patients with auditory neuropathy

Variable	Control (n = 6)	Cases (n = 7)	*P value
BChE (U/L)	15.5 (9.42, 49.9)	33.3 (17.7, 55.5)	0.061
PDE (μ mol/L	7.5 (0, 83.4)	24 (0, 48.1)	0.94
Thiol (μ mol/L)	99 (44.4, 227.3)	51 (40.5, 72)	0.1
CP (mg/dL)	28.6 (3.1, 39.8)	3.08 (1.4, 4)	0.022

* Mann - Whitney test

BChE = Butyrylcholinesterase

PDE = Posphodiesterase

CP = Ceruloplasmin

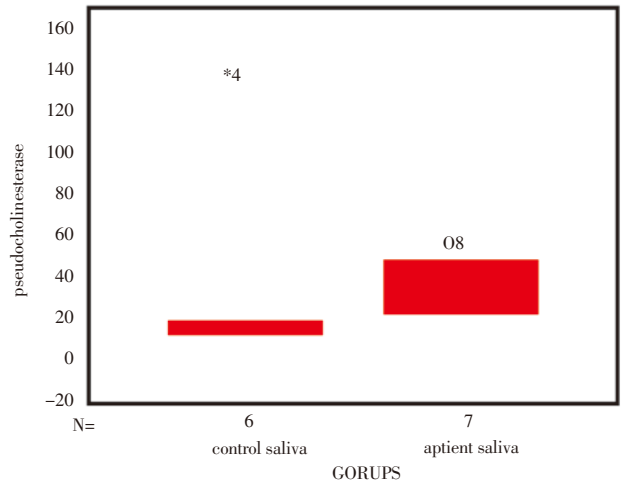

Fig1: Comparison of salivary butyrylcholiesterase between controls and cases

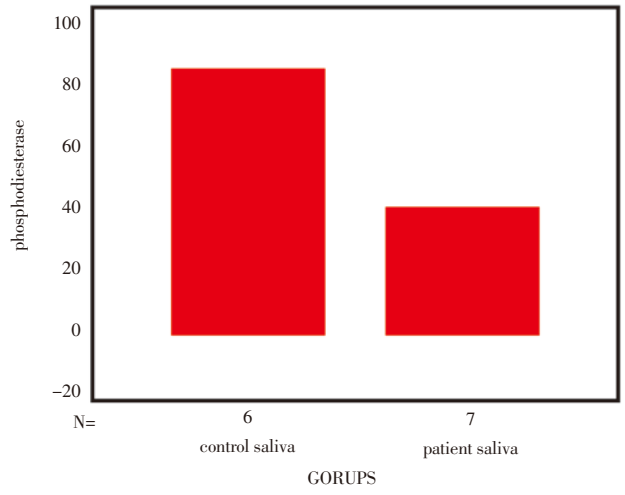

Fig2: Comparison of salivary phosphodiesterase between controls and cases

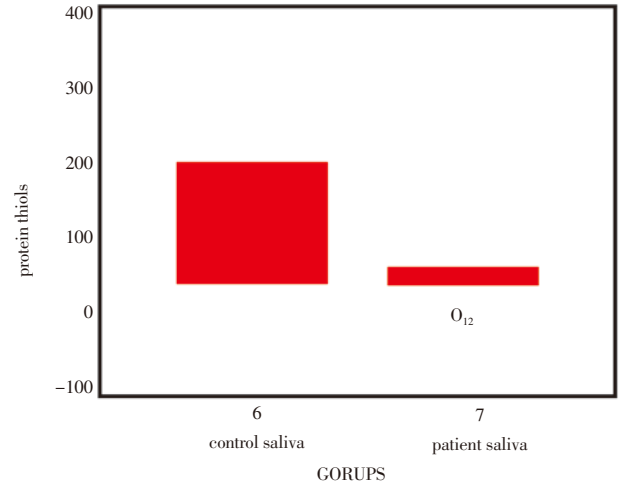

Fig 3: Comparison of salivary thiols between controls and cases

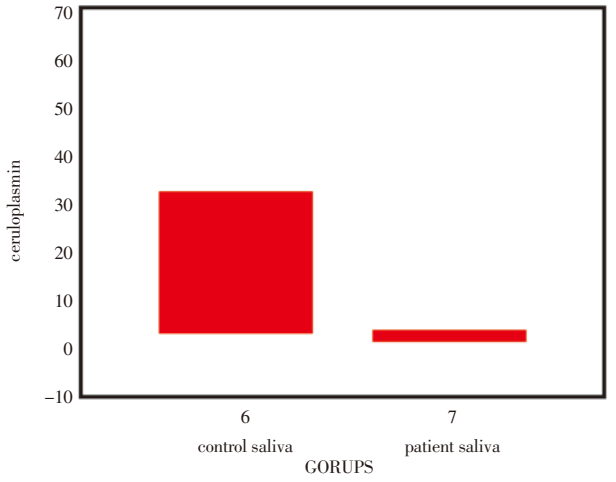

Fig4: Comparison of salivary ceruloplasmin between controls and cases

4. Discussion

Serum cholinesterase levels were shown to be reduced in a mild neuropathy [16]. Also acetylcholinesterase inhibitors protected neurons from glutamate-induced neurotoxicity, apoptosis and in the treatment of drug-induced neuropathy and in chronic constipation [17- 19]. We found a marginal increase salivary BchE indicating the same mechanism is also may be involved in the development and progression of this type of neuropathy.

2′, 3′-cyclic nucleotide 3′-phosphodiesterase enzyme is involved in myelin synthesis and is present in high levels in brain and peripheral nerves [20]. It is found almost exclusively in the myelin-producing oligodendrocyte cells in the central nervous system. Reduced PDE levels have been reported in various neurologic and demyelinating diseases, spinocerebellar ataxia with axonal neuropathy [21, 22]. Coffee which contains PDE inhibitor has been shown to facilitate recovery from diabetes-induced auditory neuropathy [23]. Sildenafil, a phosphodiesterase-5 inhibitor has been tried in the treatment of stroke and neurodegenerative disorders [23]. Thus there are reports regarding the roles of PDE in central nervous system disorders and treatment. We found a marginal increase in salivary PDE in cases as compared to that of controls indicating their possible role in pathophysiology of auditory neuropathy.

Acquired copper deficiency in humans has caused a syndrome similar to the sub-acute combined degeneration of vitamin B12 deficiency which improved after treatment with copper[5, 6]. Reduced systemic antioxidant defence is considered to play an important mediating role in onset and progression of diabetic neuropathy [7- 9]. We found a significant decrease in salivary ceruloplasmin in cases. Also there was a marginal decrease in serum thiols both of which indicating increased oxidative stress. Also decreased ceruloplasmin may

have led to decreased circulating copper in body which may have predisposed to neuropathy.

Conclusion: Thus, the causes of auditory neuropathy are very obscure and multiple factors are involved. Since this type of occurrence is very rare we could not get many cases in the study period. If this study is conducted in larger samples it may further substantiate our findings. Our study shows that, saliva is a potential non-invasive tool for evaluation of such disorders.

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