Original

Estimation of Salivary Electrolyte concentration in Depression–An Ex-Vivo study

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ABSTRACT:

Background: Depression is considered to be one among the psychological issue that influences approximately higher than 1 croreindividualsin India with a prevalencerate of 31.2 for every 1000 persons.The Autonomic nervous system in the Salivary gland constrains the quantity and quality of Saliva. Depression causeshyperactivity of hypothalamic-pituitary adrenal [HPA] axis and Sympathetic-Adrenal-Medullary(SAM) bring about salivary gland hypofunction.Electrolytealterationof saliva affects oral hygiene and more prone to develop oral infection.

Aim: Estimation ofElectrolyte levels of unstimulated whole saliva in Depressive individuals.

Materials &Methods: This observation study includes a total of 100 subjects-Group I (normal individuals) & Group II (depressive individuals). The study participants of Group I were selected based on convenient sampling method and group IIby using Hospital Anxiety & Depression scale for assessment of Depression, under the guidance of Psychiatrist. A wholeunstimulated saliva was collected from the study participants and subjected to Electrolyte analysis (Sodium, Chloride and Potassium) by the method called Ion-selectiveElectrode usingEasy lyteanalyzer. The obtained value were statistically analysed using parametric t-test.

Result: The Parameters showed statistically significant raised levels of Sodium and Chloridelevels andthere was no statistical differencein Potassium level.

Conclusion: Depressive patients have the risk of developing salivary gland hypofunction probably to a higher extent, this could reflect with altered salivary composition also. Electrolyte analysis of saliva showed significant alteration in sodium and chloride. The patient with altered salivary composition may experience numerous sequelae. By assessing the oral health status of depressive individuals by periodic monitoringof sialochemical value and helping them by providing proper prophylactic and interventional therapy to restore oral health status.

Keywords: Depression, Electrolyte,HPA axis, Neuropsychology, Sialochemistry, Sympatho-Adrenal medullary system.

Introduction:

Depression is the most common mental disorder influences approximately 280 million people all around the World.[1,2,3]Globally, India has one of highest prevalence of mental disordersapproximately 56 million Indians are affected by depression.[4]Depression in the early stages induces various adaptational responses of physiologic systems with particular increasing activities in the HPA axis as well as SAM system.[5,6]Sympathetic-adrenal-medullary (SAM) hyperactivityin chronic depression may resulting in altered salivary composition which were usually controlled by the nervous systems located in salivary glands.[7,8]

Brown in the year 1970 thoroughly reviewed the literature on the application of salivary measurements to psychophysiology and found that the salivary secretion in depressed patients secrete was much less than normal subjects.[9]Salivary gland dysfunction may leads transient to severe impairments of oral health.[8,10]Darani D and Gopakumar in 2011 in their study states that patients with xerostomia are found to have higher salivary concentration of sodium, chloride and total protein.[11] Alteration in salivary electrolyte concentration were noticed periodontitis, dental caries etc.,[12]

Reviewing the literature, depression itself or antidepressant medication affects the salivary secretion.They are various studies revealed that there is a decrease in salivary flow rate, while there are only limited studies that evaluated other parameters like Sodium, Potassium, Chloride etc. The altered salivary composition equally affects oral health[8, 10,11] of depressiveindividuals.Major ions like sodium, potassium, chloride, calcium and bicarbonate are few of the important contributors of the osmolarity of saliva. Calcium and phosphate neutralize acid that would otherwise compromise tooth mineral integrity. It is also proposed that in decreased flow rate, the ductal cells may undergo more reabsorption of Na+ and makes the final secretion with less Na+ concentration. In depressive individuals there is diminished membrane transport because of which electrolyte concentration may be increased. Urea is another buffer which is a product of amino acid and protein catabolism. They increase the salivary pH by releasing ammonia and carbon dioxide when hydrolyzes by bacterial urease. Increase in urea decreases the caries incidence but ammonia is

potentially cytotoxic to gingival tissues and initiates periodontal diseases[13].Hence, the present study was carried out to analyze the electrolyte alteration in depression. By assessing the electrolyte value of saliva and providing prophylactic interventional therapy along with antidepressant therapy may be helpful in restoring oral health status of depressive individuals.

Materials & Methods:

Study Setting and Design: This Observational study was conducted at Mahatma Gandhi Medical College & Hospital and Indira Gandhi Institute of Dental Sciences, Sri BalajiVidyapeeth, Pillaiyarkuppam, Puducherry.Theduration of the study was 6 months during the period of January-June in the year 2015. Sample collection was carried out in the Department of Psychiatry and laboratory works were undertaken at the Clinical Biochemistry Department. The clinical psychiatrist was an observer for patient selection and Clinical biochemist was a quality controller for this study.

Sampling Criteria:

This study includes 100 research participants between range of 18-50 years of age. Since the age range of 18-50 years was selected to avoid variations in salivary gland secretion though, it was found to be more active in younger individuals and least active in elderly individuals. Systemic conditions that could affect the quality and quantity of saliva were excluded from the study[14].

The sample size was determined by using

$$n = \frac{2\sigma^2 (Z_{1-\beta} + Z_{1-\alpha/2})^2}{(\mu_1 - \mu_2)^2}$$

Since, $\sigma^2 = \frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}$

$z_{1-\alpha/2}$ =1.96 & $z_{1-\beta}$ = 1.28 with significance level α = 0.05, power = 90%

The value obtained from the key article [15].

Mean [μ_1] = 28.15 Standard deviation [σ_1] = 22.42

Mean [μ_2]=15.94 Standard deviation [σ_2] = 1.11

$N_1 = 50$ $N_2 = 50$ (Total Number of Samples taken in key article)

N = 36,Hence the sample size was increased to 50 subjects/group and sampling method was based on selection criteria.

Inclusion criteria:

Subjects between the age range of 18-50 years were included in the study.Subject's Depression and Anxiety levels were assessed by using a Hospital Anxiety & Depression Scale**[HADS]**. For patients with HADS value D≥8 and A≥8, the Depressive disorder was confirmed using DSM-IV or ICD-10 systems guidelines by a clinical psychiatrist.[HADS - A self-assessment scale that was developed to detect the state of Anxiety, Depression and Emotional distress. The scale contains a total of 14 questions; each category has 7 questions for & every question a score from 0-3 can be given (3 indicates higher symptom frequencies -Whelan-Goodison et al., 2009). At last the summation of scores of anxiety & depression was doneindividually. The summation scores of anxiety & depression separately (A, D represented respectively) might range from 0 to 21 categorized as follows:Normal 0–7, Mild 8–10, Moderate 11–14, Severe 15–21.Scores for the entire scale (emotional distress) range from 0 to 42, with higher scores indicating more distress.[16]]

Excluding criteriaincludessystemic disorders like diabetic mellitus, Systemic hypertension, auto immune disorders etc or under any other medication that affectsecretion of saliva, and radio/chemotherapy in head and neck region in last 6 months.

A total 100 research participants into 2 groups as follows:

1. Group I (normal individuals) - Subjects who were scored a depression value were less than or equal to 8
2. Group II (depressive individuals) -Subjects who were scored a depression value more 8

Ethical Approval and Informed consent:

This research work was approved by the Institutional Review Board (IRB ref no: IGIDSIRB2014 NDP03PGUGOPM & 26.12.2014) and Institutional Ethical Committee,

Indira Gandhi Institute of Dental Sciences (Ref no IGIDSIEC2015 NDP03PGUGOPM & 21.01.2015). Informed Consent form was obtained from the subjects who were willing to participate in this study.

Collection and Transportation of samples:

Unstimulated whole salivary samples were collected from the research participants, between 8am-12 pm, based on spitting method illustrated byNavazesh in 1993**.**The individuals were asked to refrain from eating, drinking (except water), tooth brushing, practice physical exercises or be under high physical stress for at least 1 hour before sample collection. The subjects were instructed to wash their mouths thoroughly with deionized water and asked to sit in a relaxed position for 5minutes. The saliva was then allowed to accumulate in the mouth and then to expectorate into a sterile plastic container. This should be done once in every 60 seconds over a period of 5 minutes.[17]Collected sample was then transported to the Clinical chemistry laboratory, MGMCRI, SBV, Puducherry, immediately for processing. Processing was carefully monitored by a trained Biochemist.

Processing and Estimation of Electrolytes:

Salivary samples were centrifuged at 3200 rpm for 10 minutes; Supernatant fluid was collected and Salivary Sodium, Potassium & Chloride levels were estimated by Ion selective electrode principle, Easylyteanalyzer (Medica).

Statistical Analysis: Total 50 samples from each group (I, II) were subjected to statistical analysisusing Statistical Package for Social Sciences (SPSS)version 16.0. IBM,United States.Power calculation of study was 90%.Mean, Standard deviation was assessed, and 95% confidence interval was used for describing the data. Unpaired t-test is used to compare the parameters (Sodium, Potassium, Chloride) of group I (Control subjects) with group II (Depressive patients).[Table/Fig-2]: Showing statistical data of unstimulatedsalivary Sodium, Potassium, Chloride levels.

Result:

The Parameters were statistically analyzed and showing statistically significant raised levels of Sodium (p=0.03)and Chloride(p=0.02) levels and there was no statistical difference in Potassium level (p= 0.38).

Estimation of Salivary Sodium:

[Table/Fig-2]: Shows the mean unstimulated salivary sodium levels of Group I (11.4±5.23mEq/L), Group II (13.6±5.75mEq/L).While comparing the unstimulated whole salivary sodium level, it was found to be higher in group II than in group I and statistically significant difference between group I and group II (p=0.03).

Estimation of Salivary Potassium

[Table/Fig-2]: Shows the mean unstimulated salivary potassium levels of Group I (24.4±5.34mEq/L), Group II (25.4±6.3mEq/L). While comparing the unstimulated whole salivary potassium level, it was found to be slightly higher in group II than in group I. But **Table/Fig-2** did not show any statistically significant difference between group I and group II (p=0.38).

Estimation of Salivary Chloride

[Table/Fig-2]: Shows the mean unstimulated salivary chloride levels of Group I (20.3±5.58mEq/L), Group II (24.1±9.39mEq/L). While comparing the unstimulated whole salivary chloride level, it was found to be higher in group II than in group I. **Table/Fig-2** showing statistically significant difference between group I and group II (p=0.02).

Discussion:

Depression is common mental disorder which disturbing normal life and impacts negatively on oral health-related self care behaviours and also have a detrimental effect on oral health[18]. Anti-depressants are the drugs which readily prescribed for depressive patients to elevate mood in depressive disorders can effectively affect salivation: aside from influencing the amount of saliva, they may also alter the composition of the saliva. Salivary gland secretion is controlled by both sympathetic (noradrenergic- α & β adrenoceptors) and parasympathetic

(muscarinic cholinoceptors) nervous systemsand their nerve terminals located in salivary glands areaffected by the depressivedisorder and/or by Anti-depressants drugs[4,8]. Nederforscategorised that altered salivary composition one of form salivary gland hypofunction and have equal impacts on oral health such as mucositis, burning sensation, glossodynia, dysphagia, difficulty in speech, altered taste sensation and oral candidiasis.[9,19,20]

Sialochemistry is a useful means of chronologically monitoring quantitative changes of chemicals that are present in Saliva.[21] It is based on presumed relationship with intra glandular transport processes such as sodium, potassium, chloride.intra cellular synthesis such as protein, α-amylase and diffusion of plasma constituents such as urea.[21,22]

Electrolyte reabsorption, secretion could be carried out by the striated and excretory ducts of salivary gland.This process is regulated by the nervous system and mineralocorticoids produced by the adrenalmedulla.The sympathetic innervations have more significant function of controlling electrolyte transport in the ducts due to larger number of cAMP-regulated Cl–channels in luminal cell membrane.The final electrolyte composition of saliva varies, depending at high flow rates; saliva is in contact with the ductal epitheliumfor a shorter time, and Sodium (Na+) and Chloride (Cl-) ion concentrations increases and the Potassium concentration (K+) decreases. At low flow rates theelectrolyte concentrations change in the inverse directions.[23]

Electrolyte such as sodium, potassium and chloride are the important contributors in maintaining the osmolarity of saliva. The decreased salivary flow rate made the ductal cells to raised reabsorption of Sodium ions (Na+) and makes the final secretion with less Na^+ concentration. In depressive individuals there is diminished membrane transport because of which electrolyte concentration may be increased.(14,24,25)

In our study showed that the concentration of sodium (p=0.03)and chloride (p=0.02) was significantly higher in Depressive patients than in controls. It suggests that depressive individuals was found to be secretes low salivary flow rate(17)that can influence the striated and excretory ductal cell, which had more time for absorption of the sodium and chloride were comparatively less reabsorbed indicating a possible dysregulation in the absorption

mechanism in the ductal cells. Possible alteration in these electrolytes especially, the altered Na level have tendency to altered taste sensation[26] and also found that levels of sodium was significantly increased in dental caries patients.[13,27]

Disparity noticed in the analyzed parameters of the present study when compared with other studies might be due to difference in saliva sampling, study population and parameters used. There were only limited studies done in this area, and also most of the studies used stimulated saliva for sialochemical analysis. However further studies with large sample size and estimation of gland specific electrolyte analysis has to be initiated to add more information to the existing scientific evidence.

Conclusion:

Depressive patients have the risk of developing salivary glandhypofunction probably to a higher extent, this could reflectwith altered salivary electrolyte composition also.Electrolyte analysis of salivashowed significant alteration insodium and chloride.The present study, an attempt was made to assess and bring about a comparison of electrolyte alteration in unstimulated whole saliva of normal individuals (Group I), depressive patients (Group II).By assessing the oral health status of depressive individuals by periodic monitoring of sialochemical value and helping them by providing proper prophylactic and interventional therapy to restore oral health status.

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- **Author contributions:** We hereby certify that submitted work is equally contributed by all the authors above mentioned.LVL, UG and VN contributed to study conception, study design, data collection, analysis, interpretation, and manuscript preparation;RR, SA,

contributed equally in writing the paper following planning, researching and interpreting the results. RR&SK contributed in writing the paper, editing, and submitting the paper.

Ethical Policy and Institutional Review board statement:This research work was approved by the Institutional Review Board (IRB ref no: IGIDSIRB2014 NDP03PGUGOPM) and Institutional Ethical Committee, Indira Gandhi Institute of Dental Sciences (Ref no IGIDSIEC2015 NDP03PGUGOPM).

Patient Declaration of Consent: The authors certify that all the participants have provided their written consent to participate in the research and to publish the analyzed data. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity.

Data Availability Statement: Data are available upon a valid request to the corresponding author.

List of Abbreviations: HPA- Hypothalamic-Pituitary Adrenal

SAM-sympathetic-adrenal-medullary

HADS- Hospital Anxiety & Depression Scale

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[Table/Fig-1]: Materials and Armamentarium required:

S.No.	Armamentarium	Model number	Company name	Country name
i)	Sterile plastic container	-	Lab tech Medico Pvt Ltd, Kerala	India
ii)	Centrifuge	R-8C Laboratory Centrifuge	REMI	Vasai (India)
iii)	Easelyte Plus	Na/K/Clanalyzer	Medica	Europe
S.No.	**Reagants**	**Expiry date**	**Company name**	**Country name**
i)	Electrolyte assay kit Na/K/Clanalyzer-Solution Kit	**2017/04**	Medica	Europe

[Table/Fig-2]: Showing statistical data of Unstimulated Salivary Sodium, Potassium, Chloridelevels.

Salivary Parameters (Variables)	N	Mean ± *Standard deviation*		ConfidenceInterval (95%)		t value	Df	*p* value
		Group I	Group II	Lower	Upper			
Sodium (mEq/L)	50	11.4±5.23	13.6±5.75	0.265	4.634	2.225	98	0.03
Potassium (mEq/L)	50	24.4±5.34	25.4±6.3	-1.294	3.344	0.877	98	0.38
Chloride (mEq/L)	50	20.3±5.58	24.1±9.39	0.725	6.878	2.459	79.8	0.02
N- Total number of sample, S: Significant,df: Degrees of freedom								

[Table/Fig-3] Showing comparison of mean values of study parameters between Group I and II.