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Sialometry and sialochemistry - A diagnostic tool in Sjogren's syndrome.

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Abstract:
Aim & Objective: *The aim of the study is to assess the role of sialometry and sialochemistry as a diagnostic tool in Sjogren's syndrome by quantifying the levels of sodium, potassium, chloride, urea, calcium, phosphate, total protein, creatinine, albumin and estimating the flow rate of whole unstimulated saliva in patients with a positive diagnosis of Sjogren's syndrome in comparison with control group and thus utilizing the changes in the chemical levels in the diagnosis of Sjogren's syndrome .*
Materials & Methods: *A cross sectional study was done with 60 subjects divided into two groups, 30 in study group with mean age of thirty six years and 30 in control group with the mean age of forty three years. 9 among the study group had Primary Sjogren's syndrome and 21 had Secondary Sjogren's syndrome. Minor salivary gland biopsy was done to confirm the diagnosis of Sjogren's. Unstimulated whole saliva was collected and the chemical analysis was done using diagnostic kits to estimate sodium, potassium, chloride, total protein, albumin, creatinine, urea, calcium and phosphate.The changes in the levels were statistically analysed.*
Results: *Individuals with Sjogren's syndrome showed very high significant reduction (P=0.001) in the salivary flow rate (0.18ml/min) and salivary phosphate (6.49mg/ml) level. There was very high significant increase (P=0.001) in the levels of salivary Na+(131.40mmol/min), Cl-(132.09mmol/min),albumin(6.18mg/dl) and creatinine(0.60mg/dl). Levels of total protein(278.17)showed a significant increase(P=0.05).*
There was no significant change in the levels of potassium(9.37 mmol/l), calcium (5.05mg/dl) and urea(9.83mg/dl).
Conclusion*: This study has established that the sodium, chloride, phosphate level of saliva could be used to diagnose Sjogren's syndrome and to assess the prognosis of the disease.*
Clinical Significance: *To utilize sialometry and sialochemistry as non invasive and cost effective diagnostic tools in Sjogren's syndrome.*
Keywords: *cross sectional study,sialometry,sialochemistry,Sjogrens syndrome.*

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I. Introduction

Sjögren's syndrome (SS) is a systemic autoimmune exocrinopathy of unknown etiology, first presented by Hadden in 1888, then by Mikulitz in 1892 and later described by a Swedish Ophthalmologist Sjogren in 1933[1]. SS is characterised by the presence of lymphocytic inflammatory infiltrate confirmed by biopsy of the labial gland[2]. There are two forms of SS. Primary SS (pSS) is characterised by dry eyes and decreased salivation. The secondary SS (sSS) is associated with connective tissue disorder like rheumatoid arthritis and lupus erythematosus[3]. SS is more prevalent in middle aged women and elderly people[4]. The diagnosis of SS is based on American-European Consensus Criteria for SS[5]. Minor salivary gland biopsy is the highly used diagnostic procedure for the salivary component of SS along with sialometry, sialography, scintigraphy, magnetic resonance imaging, ultrasonography, sialochemistry, plasmatic and serologic markers[6].

Sialochemistry is an easy and non invasive method in the diagnosis of SS[1]. On measuring the salivary flow rate (sialometry) and by chemical analysis of saliva (sialochemistry) salivary gland dysfunction can be assessed. Various salivary gland diseases including SS can be diagnosed by sialochemistry [7,8]. There are two ways by which sialometry can be used as a diagnostic tool, collection of whole saliva which is combined secretions of all salivary glands and collection of glandular saliva that is gland specific saliva[9]. Attempts have been made to use saliva for the conclusive diagnosis of SS. In the present investigation, the value of sialochemistry in the study of SS was explored by comparative examination of a spectrum of salivary

components in a group of subjects with a positive diagnosis of SS with healthy control group matched for age and sex. The parameters that were quantified included salivary flow rate, sodium, potassium, chloride, phosphate, calcium, urea, total protein, creatinine and albumin.

II. Materials And Methods

This study was approved by the Institutional Ethics Committee. This cross sectional study was done among 60 subjects divided into two groups, 30 in study group with mean age of thirty six years and 30 in control group with the mean age of forty three years. 9 among the study group had Primary Sjogren's syndrome and 21 had Secondary Sjogren's syndrome. Minor salivary gland biopsy was done in the lower labial mucosa to confirm the diagnosis of Sjögren's syndrome under aseptic conditions. After anesthetizing the area with 2% lignocaine a wedge shaped incision was performed, such that the biopsy tissue contained atleast 3 to 4 minor salivary gland tissue. It was fixed in 10% formalin and submitted to the Department of Oral pathology for histopathological examination.

Collection Of Unstimulated Whole Saliva(UWS) :

The collection was made on non- fasting subjects between 8.00AM and 10.00AM. The subjects were instructed to refrain from eating and drinking 2 hours prior to collection. Initially the subject was asked to rinse the mouth with water in order to remove the food debris. The saliva was allowed to passively drain from the lower lip into a pre-weighed Eppendorf sample tube for 5 minutes. The volume was estimated by weighing the tube before and after collection. The samples were centrifuged at 4500 rpm for 15 minutes to remove debris and transferred to another Eppendorf tube and labeled.

Chemical Analysis:

Sodium was analysed by precipitating it as a triple salt with magnesium and uranyl acetate. The excess uranyl ions reacted with ferro cyanide in an acidic medium to develop brown colour. The intensity of the colour produced is inversely proportional to the concentration of sodium in the sample. The absorbance of sample and standard were measured against reagent blank at 540nm in a calorimeter. Potassium reacts with sodium tetraphenyl boron in a specially prepared buffer to form a colloidal suspension. The amount of the turbidity produced is directly proportional to the concentration of potassium in the sample. The absorbance of sample and standard were measured against reagent blank at 600nm in the calorimeter. Chloride ions combine with free mercuric ions and release thiocyanate from mercuric thiocyanate. The thiocyanate released combines with ferric ions to form a red brown ferric thiocyanate complex. Intensity of the color formed is directly proportional to the amount of chloride present in the sample. The absorbance of sample and standard measured against reagent blank at 540nm immediately. Albumin was estimated by Bromocresol green method by formation of an albumin/ bromocresol green complex at pH 4.2 and calorimetric measurement of absorbance at 540nm. Total protein was estimated by Biuret method. The absorbance of sample and standard were measured against reagent blank at 540nm. Estimation of urea was done by DAM method. Urea reacts with hot acidic diacetylmonoxime in presence of tiosemi carbazide and produces a rose purple coloured complex, which was measured calorimetrically. Phosphorus was estimated by Modified Gomorri's method. Estimation of salivary calcium was done by O.C.P.C method. Creatinine was estimated by Alkaline picrate method.

III. Results

A total number of 60 individuals were studied of which 30 were control group and 30 were study group with a positive diagnosis of SS. The age of the control and the study group varied from 21 to 60 years with an average of 41 years. Out of the 30 individuals 6 were males and 24 were females. The age of females in the study and the control group varied from 21 to 60 years the average being 43 years. The age of males varied from 26 to 51 the average being 36 years. (Table1) In the present study out of the 30 Sjogren's syndrome individuals 9 of them had primary Sjogren's syndrome including 1 male and 8 females. Among the secondary Sjogren's group 5 were males and 16 were females (Table 2) and the associated connective tissue disorder was rheumatoid arthritis among 5 males and 11 females and systemic lupus erytematosus in 5 females (Table 3). According to age wise distribution of SS 4 were in the age group of 21-30 years, 18 in the age group of 31-40 years, 6 in the age group of 41-50 and 2 in the age group of 51-60 years. (Table 4).

On comparing the flow rate, sodium, potassium, chloride, phosphate, calcium, urea, total protein, albumin and creatinine levels of UWS between control and the study group (Table 5) the following results were observed. The mean flow rate of 30 study group subjects was 0.18ml/min and that of 30 control group individuals was 0.55ml/min. Among the electrolytes Na+ in the UWS of the control group was 60.18 mmol/l and study group was 131.4 mmol/l, chloride level in the saliva of control group was with a mean of 45.18 mmol/l and that of study group was 132.09 mmol/l and potassium in the control group was 8.74 mmol/l and the study group was 9.37 mmol/l. Phosphate level in the control group was 13.31mg/dl and study group was

6.49mg/dl. Calcium of the control group was with a mean of 3.25 mg/dl and of study group was 5.05mg/dl. The mean of urea of the control group was 9.74 mg/dl and study group was 9.83 mg/dl. Total protein in the saliva of control group was with a mean of 2.31mg/dl and study group was 278.17 mg/dl. Albumin of the control group was with the mean of 0.5 mg/dl and of study group was 6.18mg/dl. Creatinine in the saliva of control group was with the mean of 0.09 mg/dl and study group was 0.6 mg/dl.

On comparing the variables of UWS between the females (Table 6) and the males (Table 7) of the control and the study group there was reduction in the flow rate of both females and the males of study group with the mean of 1.8ml/min. Sodium level was increased in both males and females of study group with the mean of 127.76mmol/l in females and 153.77 mmol/l in males. Chloride levels were significantly increased in both females and males with the mean of 129.16 and 144.44mmol/l respectively. Phosphate levels were reduced in both males (7.17mg/dl) and females (6.06mg/dl) among the study group. Total protein was increased in both males (267.33mg/dl) and females (280.88mg/dl). Albumin was increased in both males (6.67mg/dl) and females (6.19mg/dl). Creatinine level was increased in both males (0.58mg/dl) and females (0.59mg/dl) of the study group. There was no change in the potassium, calcium and urea levels.

Student 't' test and P value for comparing control group with study group for variables of UWS revealed (Table 8) showed a very high significant reduction in the flow rate of saliva ($P \leq 0.001$), very high significant increase in sodium, chloride, albumin, creatinine ($P \leq 0.001$). There was very high significant decrease in the levels of phosphate ($P \leq 0.001$). No significant change was evident in the levels of potassium, calcium and urea. Comparison of UWS between the pSS and sSS group of individuals (Table 9) revealed significant increase in the levels of sodium in the pSS group (168.15mmol/l) compared to sSS individuals (125.86mmol/l). Chloride levels in the pSS group (145.83mmol/l) were considerably increased than that of sSS group (133.314mmol/l). Total protein had a highly significant increase in pSS group (314.75mg/dl) compared to sSS (277.23 mg/dl) group.

IV. Discussion

Several methods are used to examine the oral component of SS including sialometry, sialochemistry,siaography, salivary gland scintigraphy, ultrasonography, labial salivary gland biopsy, plasmatic and serologic markers. Sialochemistry is an easy, non invasive method in the diagnosis of SS. Fox and spreight, 1996[10]; Sreenby and Zhu, 1996[11], Streckfus et al, 2001[12] conducted study to use saliva for conclusive diagnosis of SS. As in the studies by Ben Aryh et al[1], Mandel and Baurmash[13], Stuchell et al[14] the present study explored the value of sialochemistry in SS by comparative examination of a spectrum of salivary components in a group of subjects with a positive diagnosis of SS and a healthy control group. In the present study, the control group patients were thoroughly studied by clinical findings, routine blood and urine examination. All the patients were established free from other body diseases and hence the values obtained in the present study were attributed to SS. The present study has proved that the incidence of SS is more in females as was similarly observed by R.Jonsson et al[15]. The present study has proved a marked reduction in the flow rate of the individuals as with SS Kalk[16] , Stuchell[14] I.D.Mandel and H.Baurmash[13]. The present study has revealed that there is a very highly significant elevation ($P \leq 0.001$) in the sodium and chloride levels and reduction ($P \leq 0.001$) in the levels of phosphorus. The elevation of sodium and chloride and reduction of phosphorus is also seen in the different age groups and in both males and females. This observation is consistent with the reports of Burgeon and Emmelin[17]; Mandel and Baurmash[13]; Ben Aryeh[1]; Kalk[16].

The metaplastic epithelial cells and epimyoepitheial cell islands that replace the normal ductal cells are not capable of effectively resorbing the high sodium and chloride content of the primary secretion, despite the low flow rate. The clinical impression is that the cases with the most advanced changes exhibit the highest level of sodium and chloride, almost approaching blood levels. Although, the mechanism of phosphate transport in human salivary glands has not yet been characterized, there is no doubt that it is an active process and that striated duct cells are involved[17]. Metaplastic cells would seem to be incapable of active transport of phosphate. It is not unusual in advanced cases of SS to find virtually no phosphate in the parotid secretions.

The present study has revealed that there was no significant change in the potassium level of the control and SS subject of both sexes and all age groups, an observation similarly reported by Shannon and Prigmore[18]. In contrast, sodium potassium level is unaffected in SS. This would suggest that potassium is not secreted into ductal fluid purely in exchange for sodium. A high sodium and normal potassium concentration has also been reported in inflammatory salivary gland disease. In the present study there was no significant change in calcium concentration of SS individual compared to that of control group. The normal concentration of calcium in SS indicates that ductal cells are probably not strongly involved in calcium transport. Studies with the rat parotid gland slices suggested that calcium be secreted mainly with protein from the acinar cells[19].

In the present study, the urea level in the saliva of the control group and that of the SS individual were not significantly altered ($P>0.05$). There was also no change in the urea level among all age groups and in both males and females. The relatively normal concentration of urea in the diseased group can be considered as

evidence that the basement membrane in the ductal region is still functioning well. Urea is passively diffused from blood to saliva, and a defective basement membrane should result in leakage of serum components and higher than normal concentrations of urea[7].

There was a significant increase in albumin, creatinine and total protein. This was observed in all age groups and both in males and females. The significant elevation in total protein and albumin noted in patients with SS was not evident in the earlier study of Mandel and Baurmash[13]. But it was evident in the study of Stuchell[14]. The present data probably reflects a patient group with more advanced disease, possibly including case with some recurrent secondary inflammation. The degree of elevation in total protein, albumin and creatinine is valuable in assessing the extent of inflammatory change. In patients with SS who manifest active inflammation because of the susceptibility of the poorly functioning gland to ascending infection, it is often difficult to differentiate the sialochemical changes due to SS from those associated with inflammation. In SS patients, however, much of the elevation in sodium, chloride and the decrease in phosphate will persist after the inflammation subsides. Sialochemistry shows promise as an adjunctive aid in diagnosis and assessment of the degree of pathosis in SS. The elevation in sodium and chloride concentrations and a normal potassium level clearly differentiates sialadenosis. Modest elevation in albumin is due to inflammation. The degree of increase in sodium and chloride concentration and the decrease in phosphate appears to be a rough indication of the degree of pathologic change. In addition, nature of changes in salivary chemistry is helpful in understanding the pathogenic change resulting from the disease process. The elevations in sodium, chloride and the reduction in phosphste level have been proved statistically.

V. Conclusion

The study has proved that sialochemistry can provide a means of differentiating between normal and SS individuals. Increase in sodium, chloride concentrations and decrease in phosphate concentration among SS individual can be utilized in the diagnosis of SS. Though there was an increase in the albumin and creatinine level it is attributed to the inflammatory changes in the gland. This study has established that the sodium, chloride, phosphate level of saliva could be used to diagnose SS.

VI. Clinical Significance

Keeping these observations after establishing the SS and eliminating other disease as basis, the result of sodium, potassium and chloride could be standardized for diagnostic and prognostic purpose. Hence it is submitted that this study could be a useful basis for further elaborate study and it can be definitely established that these values can be used as diagnostic aid in SS.

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Table 1: Age and Sex distribution of cases

Sjogren's Syndrome (Mean)	Control group (Mean)	Variables	Sl.No.
41.41	41.41	Age	1.
35.67	35.67	Male	2.
43.43	43.46	Female	3.

Table 2. Sex wise distribution of primary and secondary Sjogren's syndrome

Female	Male	Diagnosis	Sl.No
08	01	Primary Sjogren's	1.
16	05	Secondary Sjogren's	2.

Table 3. Distribution of associated connective tissue disorder.

Female	Male	Connective Tissue Disorder	Sl.No.
11	05	Rheumatoid Arthritis	1.
05	-	Systemic lupus erythematosus	2.

Table 4. Age wise distribution of Sjogren's Syndrome

51-60 years	41-50 years	31-40 years	21-30 years	11-20 years	0-10 years
02	06	18	04	-	-

Table 5. Comparison of flow rate, Sodium, Potassium, Chloride, Phosphate, Calcium, Urea, Total Protein, Albumin and Creatinine levels of USW saliva between control and the study group

Sjogren's syndrome		Control		Variables	Sl.No
±Sem(Y)	Mean(Y)	± Sem(X)	Mean(X)		
0.0038	0.18	0.0088	0.55	Flow rate (ml/min)	1.
7.9053	131.40	2.6380	60.18	Sodium (mmol/l)	2.
0.5310	9.37	0.5624	8.74	Potassium(mmol/l)	3.
5.1963	132.09	3.4312	45.18	Chloride (mmol/l)	4.
0.4022	6.49	0.1855	13.31	Phosphate (mg/dl)	5.
0.2514	5.05	0.1357	3.25	Calcium (mg/dl)	6.
0.5881	9.83	0.7450	9.74	Urea (mg/dl)	7.
5.8350	278.17	1.9761	231.00	Totalprotein(mg/dl)	8.
0.4421	6.18	0.0510	0.50	Albumin (mg/dl)	9.
0.0253	0.60	0.0096	0.09	Creatinine (mg/dl)	10.

Table 6. Comparison of variables of UWS between control and the study group of females

Sjogren's syndrome		Control		Variables	Sl.No
±Sem(Y)	Mean(Y)	± Sem(X)	Mean(X)		
0.0041	0.18	0.0108	0.56	Flow rate (ml/min)	1.
7.9053	131.40	2.7296	62.10	Sodium (mmol/l)	2.
0.5611	9.44	0.5844	8.23	Potassium(mmol/l)	3.
5.7835	129.16	3.8259	43.88	Chloride (mmol/l)	4.
0.4094	6.06	0.2158	13.38	Phosphate (mg/dl)	5.
0.3042	5.21	0.1457	3.28	Calcium (mg/dl)	6.
0.5888	9.40	0.9252	9.70	Urea (mg/dl)	7.
8.0470	280.88	2.4575	232.38	Totalprotein(mg/dl)	8.
0.4921	6.19	0.0569	0.51	Albumin (mg/dl)	9.
0.0284	0.59	0.0830	0.18	Creatinine (mg/dl)	10.

Table 7. Comparison of variables of UWS between control and the study group of males

Sjogren's syndrome		Control		Variables	Sl.No
±Sem(Y)	Mean(Y)	± Sem(X)	Mean(X)		
0.0092	0.18	0.0225	0.57	Flow rate (ml/min)	1.
20.7498	153.77	6.1252	54.44	Sodium (mmol/l)	2.
1.2544	9.42	1.1932	10.42	Potassium(mmol/l)	3.
11.1100	144.44	8.0114	42.22	Chloride (mmol/l)	4.
1.1960	7.07	0.3844	12.87	Phosphate (mg/dl)	5.
0.9347	5.10	0.2907	2.95	Calcium (mg/dl)	6.
1.2462	12.11	1.4797	9.61	Urea (mg/dl)	7.
13.0682	267.33	4.9911	233.67	Totalprotein(mg/dl)	8.
0.8566	6.67	0.1327	0.58	Albumin (mg/dl)	9.
0.0654	0.58	0.0108	0.07	Creatinine (mg/dl)	10.

Table 8. Student 't' test and P value for comparing control group with study group for variables of UWS

Results	P-value	't' test	Variables	Sl.No.
VHS	P≤0.001	39.19	Flow rate (ml/min)	1.
VHS	P≤0.001	8.55	Sodium (mmol/l)	2.
NS	P>0.05	0.82	Potassium (mmol/l)	3.
VHS	P≤0.001	13.96	Chloride (mmol/l)	4.
VHS	P≤0.001	15.41	Phosphate (mg/dl)	5.
NS	P>0.05	0.9	Calcium (mg/dl)	6.
NS	P>0.05	0.09	Urea (mg/dl)	7.
S	P≤0.05	2.66	Total protein (mg/dl)	8.
VHS	P≤0.001	4.77	Albumin (mg/dl)	9.
VHS	P≤0.001	18.80	Creatinine (mg/dl)	10.

VHS-Very Highly Significant, NS-Not Significant, S-Significant

Table 9. Comparison of variable of UWS between pSS and sSS group individuals

sSS	pSS	Variables	S.No.
0.17	0.2	Flow Rate (ml/min)	1.
125.86	168.15	Sodium (mmol/l)	2.
8.68	10.12	Potassium (mmol/l)	3.
133.314	145.83	Chloride (mmol/l)	4.
6.84	6.56	Phosphate (mg/dl)	5.
5.09	6.08	Calcium (mg/dl)	6.
6.72	6.18	Urea (mg/dl)	7.
277.23	314.75	Total protein(mg/dl)	8.
6.21	7.25	Albumin (mg/dl)	9.
0.61	0.58	Creatinine (mg/dl)	10.

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