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A Study of Optical Densities of Salivary Proteins in Schizophrenia

Dr K.V.Rami Reddy[1] Associate Professor; Dr U. Raghava Rao[2] Assistant Professor ;Dr G.Suresh Kumar[3],Assistant Professor ; Dr Jagadish Kanta[4]
Post Graduate Department Of Psychiatry.

Abstract:
Background: *Biomarkers are useful in diagnosing many illnesses, the biomarker (salivary proteins) which are used in this study might help in confirming the diagnosis of schizophrenia.*
Hypothesis: *Variations in optical densities of salivary proteins in schizophrenia are diagnostic.*
Aims & Objectives: *1) To record optical densities of salivary proteins in schizophrenia.*
2)To compare the optical densities of salivary proteins in schizophrenia with salivary proteins of individuals not having any psychiatric disorder.
Method: *This study is conducted at Government Hospital for Mental Care, Visakhapatnam in collaboration with the Biochemistry Department, Andhra University, Visakhapatnam.The Salivary samples of 2-3ml in 70 schizophrenia patients and 40 controls were taken. The salivary samples were processed and 10 samples of 50 micro-liters each were taken at a time and run under 12% SDS-PAGE with a low molecular weight protein marker as a reference. The bands were numbered and molecular weights were taken using Gel-Doc technique. The optical densities were identified using buffer elution method and compared for variations with the controls.*
Results & Conclusion: *There is a change in optical densities of salivary protein's which is significant in the regions of 20.1 – 40.3kDa (p=0.014) and in 14.3 – 20.1kDa (p=0.01) but the change in the region of 43 – 97.4 kDa was not significant (p=0.069).*
Keywords*: Optical density, saliva, schizophrenia*.

I. Introduction

Saliva is a plasma ultra-filtrate that includes specific salivary proteins produced by three major salivary glands (parotid, sub-mandibular and sub-lingual) and other smaller glands. 65 - 70% of unstimulated saliva is produced by submandibular gland. Under stimulated conditions (citric acid, paraffin wax) 50% of saliva is produced by parotid gland. Salivary protein changes were identified in various medical and surgical illnesses. As the collection of saliva is non-invasive it becomes easy to collect the sample

Hypothesis: - Variations in optical densities of salivary proteins in schizophrenia are diagnostic.

Aims & Objectives: - 1) To record optical densities of salivary proteins in schizophrenia.
2) To compare the optical densities of salivary proteins in schizophrenia with salivary proteins of individuals not having any psychiatric disorder.

II. Method

The study was conducted at Government Hospital for Mental Care, Visakhapatnam in collaboration with Biochemistry department, Andhra University, Visakhapatnam. After the approval of institutional ethics committee and informed consent samples of saliva was collected from January 2014 Samples were collected from 70 schizophrenia patients and 40 controls.

Inclusion Criteria of Cases
-**Male**
-Age >18
-Diagnosed as schizophrenia as per ICD-10 on risperidon
-No substance use
- No current medical or surgical illness.

Exclusion Criteria
-**Female**
-Age <18
-Substance use
Each patient is given a code

After taking socio-demographic profile a through physical examination is done. The saliva containers will be labeled by codes.

Collection Of Saliva

Subjects should not eat within 60 minutes prior to sample collection. For recovery of salivary glands, alcohol, caffeine, and dairy products should also be avoided. Resting saliva can be collected avoiding any chemical (i.e., acids), physical (i.e., pressure, warm, cold), biologic (i.e., taste, chewing), and psychologic (i.e., imagination of a meal) stimulation.

Whole saliva can be collected simply by drooling into a vial with forward tilted heads or by allowing the saliva to accumulate in the mouth and then expectorate it into a vial after rinsing the mouth.

After collection of saliva samples, transported to Biochemistry department, Andhra University, Visakhapatnam in cold boxes.

Preparation of saliva samples should be done in 1-1.5 hours after the collection, if necessary it can be stored in freezer at -20c.

Preparation Of Saliva Samples

- To prepare the samples of saliva for analysis in each it is added 1 / 2 (from its volume) solution containing 100 mM Tris (pH 7.5), 7% sodium dodecyl sulfate, 2% mercaptoethanol, 0.02% Bromophenol blue, 20% glycerol.
- Mix thoroughly by shaking, and incubated for 10 min at room temperature (20 0 C).
- Then the product is centrifuged for 5min at 5000 rpm.

Electrophoresis

- 20 µl of saliva is used for analysis by electrophoresis in 12% sodium dodecyl sulphate polyacrylamide gel (SDS-PAGE) by the method of Laemmli.

Visualisation Of Proteins In Electropheretic Gels

This is performed by using the dye Coomassie brilliant blue R250.

1- Place the gel in the dye solution at ~ 1 hour.
2- Remove dye solution (it can be used several times).
3- Quickly rinse the gel with water.
4- Wash the gel in destaining solution until a clear picture of the protein band appears.

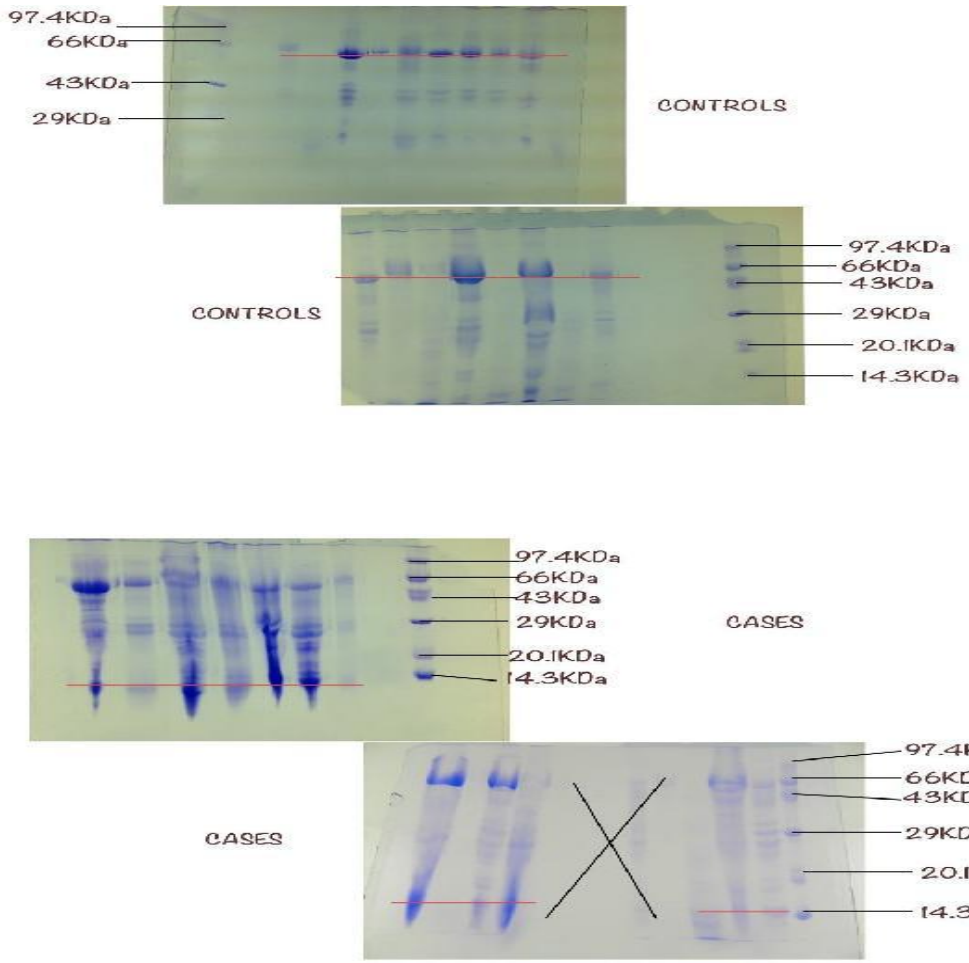
Standard Marker

- Bio-Lit midrange 1 protein marker (BLM001) 14-94 kDa was used as standard marker inSDS-PAGE.
- All the gels are photographed by Gel Documentation method and molecular weights are determined.
- Optical density can also determined by using buffer elusion method.

III. Results

Out of 70 samples of schizophrenia
20 - samples were run on10% SDS PAGE
10 - lost due to voltage fluctuations
7 - lanes did not appear
33 lanes appeared

• Out of 40 lanes of controls 8 lanes did not appear and 32 lanes appeared.



OPTICAL DENSITIES OF SALIVARY PROTEINS IN CONTROLS (Abs)

CODE	43.0 - 97.4 KDa A	20.1 - 43.0 KDa B	14.3 - 20.1KDa C
N1	0.15	0.13	0.12
N2	0.14	0.15	0.08
N3	0.17	0.13	0.06
N4	0.11	0.11	0.06
N5	0.12	0.14	0.15
N6	0.16	0.11	0.04
N7	0.10	0.13	0.10
N8	0.15	0.09	0.09
N9	0.16	0.12	0.06
N10	0.21	0.12	0.09
N11	0.15	0.12	0.04
N12	0.17	0.12	0.06
N13	0.09	0.11	0.09
N14	0.11	0.15	0.05
N15	0.19	0.06	0.04
N16	0.08	0.11	0.06

CODE	43.0 - 97.4 KDa A	20.1 - 43.0 KDa B	14.3 - 20.1KDa C
N17	0.09	0.12	0.04
N18	0.14	0.10	0.06
N19	0.11	0.11	0.10
N20	0.12	0.12	0.05
N21	0.12	0.08	0.05
N22	0.12	0.12	0.06
N23	0.08	0.12	0.12
N24	0.15	0.11	0.04
N25	0.11	0.12	0.05
N26	0.15	0.10	0.06
N27	0.12	0.10	0.04
N28	0.10	0.13	0.10
N29	0.15	0.12	0.06
N30	0.16	0.13	0.05
N31	0.16	0.11	0.10
N32	0.17	0.12	0.06

OPTICAL DENSITIES OF SALIVARY PROTEINS IN SCHIZOPHRENIA PATIENTS (Abs)

CODE	43.0 - 97.4 KDa D	20.1 - 43.0 KDa E	14.3 - 20.1KDa F
S1	0.15	0.10	0.12
S2	0.10	0.11	0.12
S3	0.09	0.09	0.11
S4	0.08	0.09	0.09
S5	0.19	0.12	0.11
S6	0.07	0.10	0.12
S7	0.09	0.12	0.05
S8	0.14	0.12	0.08
S9	0.10	0.13	0.11
S10	0.13	0.10	0.07
S11	0.09	0.12	0.09
S12	0.09	0.15	0.14
S13	0.09	0.07	0.11
S14	0.01	0.12	0.15
S15	0.09	0.11	0.13
S16	0.08	0.08	0.05
S17	0.07	0.14	0.15

CODE	43.0 - 97.4 KDa D	20.1 - 43.0 KDa E	14.3 - 20.1KDa F
S18	0.09	0.10	0.11
S19	0.10	0.07	0.07
S20	0.05	0.11	0.10
S21	0.07	0.10	0.16
S22	0.09	0.07	0.09
S23	0.16	0.12	0.09
S24	0.08	0.10	0.14
S25	0.08	0.10	0.09
S26	0.08	0.10	0.10
S27	0.09	0.09	0.11
S28	0.13	0.11	0.09
S29	0.09	0.12	0.08
S30	0.09	0.08	0.12
S31	0.09	0.07	0.08
S32	0.07	0.12	0.11
S33	0.09	0.10	0.11

CONTROLS

	43.0 - 97.4 KDa A	20.1 - 43.0 KDa B	14.3 - 20.1KDa C
MEAN ± SEM	0.135 ± 0.006	0.12 ± 0.003	0.07 ± 0.005

	43.0 - 97.4 KDa A	20.1 - 43.0 KDa B	14.3 - 20.1KDa C
SD	0.0322	0.0181	0.0282

SCHIZOPHRENIA

	43.0 - 97.4 KDa D	20.1 - 43.0 KDa E	14.3 - 20.1KDa F
MEAN ± S.E.M	0.09 ± 0.006	0.10 ± 0.002	0.10 ± 0.004

	43.0 - 97.4 KDa D	20.1 - 43.0 KDa E	14.3 - 20.1KDa F
SD	0.0269	0.0199	0.0326

BASIC STATISTICS OF OPTICAL DENSITIES(A,B,C,D,E,F)

STUDENT T TEST

SNo	GROUP	MEAN		STANDARD DEVIATION		STANDARD ERROR		SIGNIFICANCE
		X1	X2	S1	S2	S.E1	S.E2	P
1	A vs D	0.135	0.09	0.0322	0.0269	0.006	0.006	0.009
2	B vs L	0.12	0.10	0.0181	0.0199	0.003	0.002	0.014
3	C vs I	0.07	0.10	0.0282	0.0326	0.005	0.004	0.01

Molecular Weight (kDa)	PROTEINS
97.4 - 66.0	Lactoferrin, Albumin
66.0 - 43.0	Alpha amylase
43.0 - 29.0	Basic proline rich proteins
29.0 - 20.1	Acidic proline rich proteins
<20.1	Others low molecular proteins

There is change in optical densities in all the regions of salivary proteins
There is decrease in optical densities in the region of 43 - 97.4 k.Da but it was not significant (p= 0.069).
The increase in the optical densities of salivary proteins in the second region 20.1 – 43 KDa was significant (p= 0.014). The increase in optical densities in the region 14.3 - 20.1k.Da was significant (p = 0.01).

IV. Discussion

The composition of salivary proteins consists of among the 70 samples from the patient taken the first 20 samples were done on 10% SDS PAGE because, the salivary proteins were low molecular proteins and they can be visualized at lower concentrations of gel and also to make a standardization of gel for the further study, but the proteins did not get separated.

Due to voltage fluctuations 10 in patient samples and 8 in controls were not seen and 7 lanes did not appear in cases which might be due to inappropriate mixing of sample buffer.
Finally using first 20 samples the procedure was standardized to 12% SDS PAGE.

Females were not included in the study because of swings in mood during menstrual periods may change the composition of proteins similarly in the cases of substance abuse other illnesses.
As the sample was collected after tacking informed consent below 18 years were excluded.

The samples were prepared and the on the same day and the electrophoresis was run and it takes 24 – 48 hours to get the final results of a single gel.

The obstacles faced during the study were voltage fluctuations, power supply, proper maintenance of chemical concentrations & pH and presence of single machine which has to be shared.

Reports of similar studies are rare in existing literature. Venkatesh G et al., in 2011 analyzed average mean O.D of 50-60 k.Da, 30-40 k.Da, 14-20 k.Da , and they were 0.086±0.005, 0.063±0.003 and 0.332±0.013 respectively. When compared with the healthy volunteers the O.D were, 0.213±0.003, 0.113±0.005 and 0.071±0.004 respectively.

The proteins of mol.wt 50-60 k.Da had strongly decreased in schizophrenia patients and in healthy volunteers.

In the current study there were contrasting results the proteins in the region 43.0-97.4 kDa decreased but they were not significant and the other proteins like proline rich proteins increased significantly when compared with controls. In the study conducted by Venkatesh G et al there were no significant changes in the other regions and the samples taken in that study were only 20.

Limitations Of The Study
The sample collected is small.
The results would have been better using mass spectroscopy.
All the patients were on treatment and the change in composition might be due to the drugs. It would have been better if all were drug naive.
Comparing the results with the other psychiatric diseases like depressive disorder or bipolar affective disorder would have been useful.
In this study subclinical syndromes or diseases were not identified which might have brought the changes.

Future Directions
If the results were due to change in structure of proteins can be identified using MALDI- TOF technique.

Abreviations:
OD – Optical Density
kDa – kilo Dalton
Abs – Absorbance
MALDI-TOF - Matrix-assisted laser desorption/ionization – Time of Flight

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