Fractional protein study in chronic schizophrenics – A Case control study

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Abstract

Background: Schizophrenia is an illness of adolescence, it is called a cancerous disease in psychiatry. It is not a single disease probably comprises a group of disorders with heterogeneous etiologies. Still researchers do not know exact etiology of its occurrence. It presents a challenge of enormous proportions to medical research with failure of psychogenic model and reported increased incidence seen in the family with strong genetic history made researchers to explore biochemical factors. It is thought that there exists an innate metabolic error, an abnormal protein factor contributing in development of psychotic illness.

Aim: Present study was undertaken to explore the concentration of various protein fractions in chronic schizophrenics. To examine the fractions of protein which are abnormal in these cases in comparison to healthy individuals.

Methods: Study participants were in two groups, healthy individuals acting as a control and study group formed by chronic schizophrenics. Blood collected with all precaution was subjected for Electrophoresis and Biurate Reaction to find out fractional proteins and analysis was compared. Statistical Analysis was done using student 't' test.

Results: In chronic schizophrenia patients, increase in Alpha-1 fraction of protein and decrease in Beta fraction of protein was observed as compared to healthy control group.

Conclusion: Observation shows there exists an abnormal protein fraction in chronic schizophrenia patients. The present study findings show an abnormal globulin levels in the chronic schizophrenia. Decrease in albumin levels in study group was found but it was not grossly significant as compared to control. Significant increase in Alpha –1 fraction in chronic schizophrenic and no rise is seen in Alpha -2 fraction. But both Alpha -1 and Alpha -2 showed significant rise in acute schizophrenia cases. The Beta fraction found to be significantly decreased in chronic cases as compared to control group. In conclusion serum albumin fractions are an important indicator of chronic schizophrenia status which has the potential of marker in pathophysiology of disease process.

Key words: Chronic schizophrenia, Protein fraction, Electrophoresis, Biurate Reaction**.**

Introduction

Schizophrenia disorder maintains a challenge for etiological research even today. Different immunological abnormalities have been noted in patients suffering from Schizophrenia[1]. It was observed that when normal individual consumes amphetamine a psychostimulant in high doses produces a toxic psychosis, which simulates schizophrenic psychosis. This observation generated hopes of research that an abnormal level of a serum protein, along with other psycho- social factor may be contributing to aetiopathological process of this illness. The serum proteins in association with psychosis have been explored in previous studies.

Abnormal activity in septal region of brain in psychotic phase is consistently recorded in electroencephalogram of schizophrenic patients. When rhesus monkeys were given intravenous injection of a globulin fraction of sera of schizophrenic patient EEG changes recorded were similar to schizophrenics. This observation suggested a possible relation between abnormal protein and clinical manifestation

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of schizophrenia. Research by Bergen,[2] Heath R.G.[3,4], Lozovsky[5] has shown that abnormal protein is Alpha and Beta globulins. Frohman[6] in his studies found that abnormal protein fraction was Alpha Globulin. Similar observation was made by Lozovsky[5] and Pennel et al[7].

Elevations of Gamma globulins and increased incidence of rheumatoid factor like activity in patients suffering from chronic schizophrenia is observed in previous European studies[7-9].

Some researchers think schizophrenia is an autoimmune disorder and S19 like macroglobulin which has antibody like activity, must be working as an autoimmune factor in the pathogenesis of functional psychosis. Heath et al,[3,4,10] Edward Domino,[11] found increased Alpha -2 globulin and decreased plasma cholinesterase activity. In study it is seen that chronic schizophrenia is associated with hyper gamaglobinemia, but a good number of researchers failed to replicate. Kety[12] suggested serum protein abnormality in schizophrenia can be due to various nonspecific factors such as food, drugs, infection. Moreover it has been found that phenothiazine group of drugs is able to reduce the serum gamma globulin content in a study by Saunder and Muchmore.[13] Incidence and prevalence of schizophrenia are roughly same worldwide. Lifetime prevalence of schizophrenia is about 1% in United States. Healthcare cost and indirect costs of schizophrenia every year in United States alone are estimated to be in the tens of billions of dollars[1]. In this background a case control study was undertaken to find out abnormal serum proteins fraction in chronic schizophrenics when compared with healthy individuals.

Aim

To study the difference in levels of serum protein fractions in chronic schizophrenics and healthy subjects and which protein fraction levels are altered in chronic schizophrenia patients.

Materials and Methods

***Study design*:** Case control study

Sampling method: Universal sampling

Study setting: Department of Psychiatry in Khaja bandanawaz institute of medical sciences, Gulbarga.

Participants: This study consists of two groups. First group is formed by healthy individuals acting as control and the second group is the study group formed by chronic schizophrenia patients. Total no. in control group is 50 and in study group 25. To avoid discrepancy between two groups of study, the control was selected from the relatives of the patients coming along with the patients at the time of hospitalization. Hereditary factor and absent mental illness in family was also taken in consideration while selecting patients.

Data collection: After taking, permission from the Ethical Committee of college study was conducted in the Department of Psychiatry in Khaja bandanawaz institute of medical sciences, Gulbarga. Written consent was obtained from each participant regarding willingness in participating in the study after explaining the study to them in their own language. The study group was diagnosed to have chronic schizophrenia according to ICD-10 criteria by a Psychiatrist. By pilot study using Beta value for both study groups, we calculated sample size as 25 for study group and 50 for control.

Selection criteria for study were:

1. Chronic schizophrenia patients satisfying ICD 10-criteria were selected in the study group.
2. Patients age was between 17-50 yrs.

   Above 50 yrs excluded because after 50 yrs of age clinical picture was likely to be contaminated by other physical conditions and it is seen that co morbid physical condition may influence the protein pattern of sera, which would bias our sample.

   Lower age limit was kept 17 yrs because considerable variation in serum gamma globulin has been observed during 12-16 yrs of age even in healthy subjects and their values are quite different from those seen in later age group.
3. Drug free period of 12-14 weeks was ensured prior to enrolling in study.

   Because phenothiazines are known to decrease globulins and increase albumin and such changes last for 10 weeks [13].
4. A through physical examination and necessary laboratory investigations like complete blood count, chest X-ray, ECG, Urine examination were done to rule out possible evidence of physical illness.

***Procedure*:** When individual was found to be eligible for inclusion in this study, 5 ml of venous blood was collected, taking all aseptic precaution in plane AP tube. Blood was allowed to clot, serum was separated in another small tube. Subsequently sera subjected first for Paper Electrophoresis using Watman no. 1-chromatograph paper second for Biuret reaction,

using barbitone buffer of PH 8.6 and ionic strength 0.05. Good separation was obtained by 14-16 hours run using 150 volts and 12 mA current strength per centimeter width of paper. Paper strips after 16 hours run taken out and immediately immersed in alcoholic dye for 30 minutes, and then washed repeatedly in 0.5% acetic acid solution for 10 minutes and then subjected for drying in oven at a temperature between 90 degree centigrade to 105 deg. Centigrade for 10 minutes to get fixed protein bed on strips. Bromophenyl blue was used as dye.

Evaluation: Dried strips then scanned on a photoelectric densitometer. Optical density recorded at every 2 millimeter length. A graph of optical density was drawn. Showing 5 peaks corresponding to albumins, Alpha-1, Alpha 2, beta and gamma globulins. Then strips were cut with respective separate bands in five test tubes. 5ml of N/20 NaOH was added to each test tube and mixed. Reading on photoelectrical calorimeter using filter, A, Leitz or 520 Mu was taken. From this optical density percentage the total proteins of each fraction was calculated. Total proteins estimation was done by Biurate reaction. Then chart is prepared from standard protein bovine solution at 540mu filter. From above readings globulin value is calculated by subtracting albumin from total proteins.

Statistical analysis: Statistical analysis done by using student 't' test and Chi square test. Mean and Standard deviation calculated by using Excel and Open-Epi software.

Results

A total of 75 participants were included in the study. Results obtained showed (Table 1) there was decrease in Albumin levels in chronic schizophrenia patients (mean-2.38) compared to healthy individuals (mean-2.56) in control group. It was observed (Figure 1, 2) that increase in Alpha-1 fraction of serum globulin was seen in chronic schizophrenics (mean-0.38) compared to healthy controls (mean-0.33). There was decrease in Beta fraction of serum globulin in chronic schizophrenics (mean-0.93) compared to healthy controls (mean-1.08).

Table 1: Serum protein fractions with their quantitative estimation in chronic schizophrenics and controls.

Protein fraction	Control group		Chronic schizophrenia group	
	Total	Mean	Total	Mean
Albumin	128.02	2.56	59.64	2.38
Alpha-1	16.53	0.33	9.55	0.38
Alpha-2	32.44	0.64	15.04	0.60
Beta	54.89	1.08	23.44	0.93
Gamma	78.95	1.57	37.88	1.51

Table 2: Student 't' test values and probability

Protein fraction	't' value	Probability
Albumin	1.93	-
Alpha-1	2.10	<0.001
Alpha-2	0.92	-
Beta	2.46	<0.001
Gamma	0.70	-

The protein fraction results (Table 2) show Alpha-1 fraction in chronic schizophrenics was significantly [t value=2.10, p = < 0.001] increased compared to healthy controls. Though there was decrease in Alpha-2 fraction in study group but it was not significant compared to control group. Decrease in Beta fraction of serum globulin in chronic schizophrenia patients was significant [t value=2.46, p = < 0.001] compared to healthy controls.

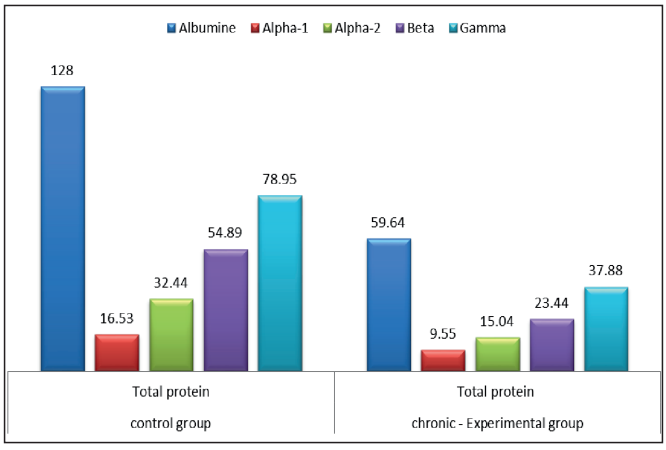

Figure 1: Total values of serum protein fractions in control and chronic schizophrenia group

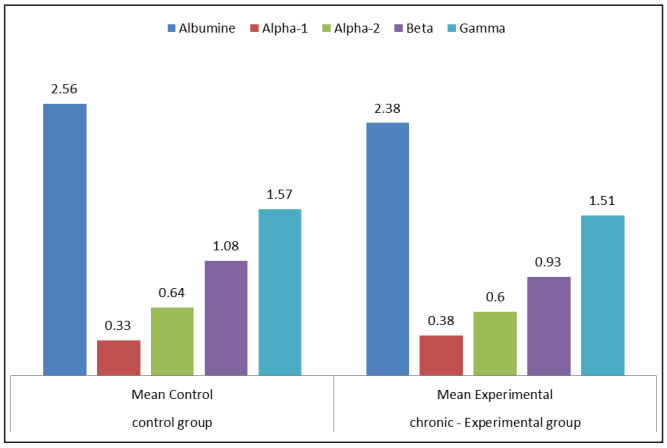

Figure 2: Mean values of serum protein fractions in control and experimental group

Discussion

A comparative study of protein fraction, between healthy individuals and chronic schizophrenics was carried out. In present study as compared to control group significant increase in Alpha -1 fraction and decrease in Beta fraction of serum globulin was found. In earlier study increase in Alpha -1 fraction of serum globulin in acute cases of schizophrenia was also noticed. Alpha -2 fraction was also increased in acute cases but in this study it was same in both in chronic schizophrenia patients and control group. Beta fraction was significantly decreased in chronic schizophrenia cases while it was normal in control group. The Gamma fraction did not show appreciable differences in chronic schizophrenics as compared to the controls.

There was difference in total Albumin value (Table 1) in healthy control group and chronic schizophrenia patients. This finding of the present study showing decrease in albumin was observed irrespective of the diagnostic sub groups. This is concurrent with the general notion held by other workers Consbruch and Faust[14] and Rudraprakash[15]. A decrease in the albumin level has also been reported by Fessel[7] who correlated it with a nonspecific stresses. As it is known, untreated chronic schizophrenics are seen in agitated state most of time. In present study decrease in albumin may be for the same reason. As nutrition also plays a role in protein levels, stress factor cannot be the only cause. Moreover majority of subjects in the study were from low socio economic class. However our finding regarding albumin are in consistent with the reports of other authors.

Previous studies have a good deal of evidence that Alpha globulin is consistently elevated in schizophrenia[2,6,11,16]. In present study also Alpha-1 fraction was found to be significantly high. However, Alpha -1 elevation in schizophrenia is not consistent with previous research, by an Indian study by Rudraprakash et al[15] but findings are agree with report of Kuruvilla et al[17] in which Alpha-2 globulin was not significantly raised. Increased psychomotor agitation was associated with highest rise in Alpha-2 fraction.[16,18] Most of our chronic patients were not agitated. Increase in gamma globulin is seen in schizophrenia patients as shown in studies by Gammack et al,[19] Fessel,[8] Solomen et al,[20] Rudraprakash. [15] However, present study did not confirm this widely documented findings. Like present study, another Indian study by Kuruvilla[17] also did not get a significant rise in gamma fraction.

In addition to above observation we found significant decrease in Beta globulin in chronic schizophrenics. Our findings did not agree with Gammack et al[19] who found significant elevations in Beta and Gamma globulin. Janik and Popisilova[21] found elevated Beta globulin in paranoid schizophrenia. Present study agreed with previous research by Lando and Fessel[8] about the abnormality in the serum globulins in Schizophrenia but with differing results in fraction of the abnormal protein. Currently there is extensive research involved in the elucidation of a possible protein metabolic disorder in Schizophrenia. It is stated that the abnormal protein produced due to aberration in the amino acid metabolism, causes production of abnormal metabolite which leads to the schizophrenia. Or it is this abnormal protein fraction acting as antibody to the specific areas of brain and the resulting antigen- antibody reaction, which is responsible for development of Schizophrenia.

Presumably, a protein may be responsible for the abnormal antigen in the serum of schizophrenia patients. Different research groups have isolated an identical plasma protein characteristic of schizophrenia or psychotic process. Most of them have demonstrated various globulin abnormalities especially of the Alpha and Gamma fraction of serum proteins. A rise in the Alpha fraction is seen consistently in most of studies. The authors of present study agree with other worker's findings of abnormal globulin levels in the Schizophrenia.

Limitations:

Present study had its limitations, as study participants were sampled from one tertiary care hospital hence applicability of study findings across populations is

limited.

Conclusion:

In this comparative study authors found decrease in albumin levels in study group but it is not grossly significant as compared to control. There was significant increase in Alpha –1 fraction in chronic schizophrenics and no rise was seen in Alpha -2 fraction in chronic schizophrenics. The Beta fraction though found to be decreased in chronic cases, the difference is not significant. Before concluding the authors would like to quote the prophecy of Teller and Denber[22], who themselves have tried to coordinate genetic, physiological, and biochemical concepts in causation of mental illness, which provides a final note of optimism.

They say "the future will show that molecular biology not only holds the key to illness with physical symptoms, such as cancer, but to mental illness as well."

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