Salivary Galectin 3 Levels in Schizophrenia Patients

Saba Shoukat[1], Humaira Fayyaz Khan[1], Muhammad Kashif[2], Shazia Ali[1], Syeda Nayab Qamar[1] and Madeeha Maqsood[1]

[1]*Islamic International Medical College, Rawalpindi, Pakistan*
[2]*Department of Psychiatry, Benazir Bhutto Hospital, Rawalpindi, Pakistan*

ABSTRACT

Objective: To compare the levels of galectin 3 in the serum and saliva of patients with schizophrenia and normal subjects.
Study Design: Cross-sectional analytical study.
Place and Duration of the Study: Physiology Department and Multifunctional Research Lab of Islamic International Medical College, in association with the Institute of Psychiatry Benazir Bhutto Hospital, Rawalpindi, from September 2022 to May 2023.
Methodology: There were 60 subjects in this study which included 30 Schizophrenia patients and 30 age and gender aligned healthy subjects. Clinically diagnosed patients of schizophrenia as per standards of diagnoses given in Diagnostic and Statistical Manual of Mental Disorders (fifth edition) were included. Unstimulated whole-mouth saliva was collected through the spitting method from the study subjects. Tetra acetic acid (EDTA) tubes were used to collect blood samples and to measure the association of galectin-3 between saliva and serum of schizophrenia patients. Enzyme linked immunosorbent assay (ELISA) was performed. Independent samples t-test and Pearson correlation were implemented.
Results: Mean salivary galectin 3 level were far more significant in schizophrenia patients as opposed to their healthy subjects having CI 95% (641.51 and 822.45, p-value <0.001). A positive association was observed between salivary and serum levels of galectin 3 in schizophrenia patients (p = 0.03).
Conclusion: Galectin 3 levels are raised in the saliva of schizophrenia patients and these levels are positively correlated with levels of galectin 3 in the serum of schizophrenia patients. Galectin 3 levels in the saliva can be an effective indicator in diagnostic confirmation of clinically suspected schizophrenia patients.

Key Words: *Galectin 3, Schizophrenia, Saliva, Serum level, Inflammation.*

How to cite this article: Shoukat S, Khan HF, Kashif M, Ali S, Qamar SN, Maqsood M. Salivary Galectin 3 Levels in Schizophrenia Patients. *J Coll Physicians Surg Pak* 2024; **34(02)**:202-205.

INTRODUCTION

Schizophrenia is a progressive mental illness with a diverse inherited and the neurological origin that affects the initial development of the brain. It manifests as a complex set of psychotic symptoms such as distorted perceptions, disorganisation, and behavioural and intellectual impairments.[1] Epidemiological studies estimate that the prevalence of schizophrenia is around 1% across the globe and is more common in males than females.[2] In Pakistan, the reported frequency of schizophrenia is 1.5%.[3] Unfortunately, the life expectancy of individuals with schizophrenia is 15 to 25 years, and their mortality rate is two to three-fold more substantial compared to the overall population.[4]

Schizophrenia manifests itself in patients as three unique kinds of manifestations: positive, negative, and cognitive. False beliefs, hallucinations, jumbled thoughts, anhedonia, asociality, blunted affect, and alogia are positive symptoms.[5,6]

Correspondence to: Dr. Saba Shoukat, Islamic International Medical College, Rawalpindi, Pakistan
E-mail: sabashoukat7586@gmail.com
Received: June 13, 2023; Revised: August 04, 2023; Accepted: December 28, 2023
DOI: https://doi.org/10.29271/jcpsp.2024.02.202

Cognitive symptoms are learning and attention disorders. These symptoms often appear in the late teenage years or early adulthood and last throughout a person's life.[7]

In the pathophysiology of schizophrenia, there is role of immunological dysfunction associated with higher amounts of inflammatory cytokines.[8] In order to comprehend the role of inflammatory processes in mental diseases, certain proteins have also been looked into as novel bioindicators.[9] Galectin 3 is one of them that is present in a wide range of tissues and organs and plays a role in cell movement, promoting growth, longevity, division, attachment, cell death, and immune defense. Neuroinflammation is critically impacted by galectin 3 and found in activated microglia. Microglial activation occurs as a result of an inflammatory insult or minor changes in brain homeostasis, resulting in the production of several inflammatory chemicals.[10] There have been studies of elevated levels of galectin 3 in various neurological and psychiatric diseases, including Alzheimer's diseases, multiple sclerosis, anxiety, depression, and cognitive and attention disorders.[11]

Studies are being conducted to find biological indicators for schizophrenia that aim to improve diagnosis and treatment response evaluations as most psychiatric disorders are diagnosed on clinical basis and no specific biochemical marker is available for its diagnostic confirmation. It is difficult for any

patients with mental illness to have invasive procedure for sampling, so saliva being non-invasive diagnostic fluid, is becoming increasingly popular alternative to blood-based analysis.[12]

If the levels of galectin 3 in the two groups show considerable variations, it could prove to be a valuable biomarker for the diagnosis of schizophrenia along with other clinical assessments. The identification of reliable biomarkers for Schizophrenia will significantly improve the diagnosis, prognosis and treatment of this debilitating mental illness.

The objective of the study was to measure the levels of galectin 3 in the saliva of schizophrenia patients and compare them to the levels of galectin 3 in the saliva of healthy subjects.

METHODOLOGY

The study was performed in the Physiology Department and Multifunctional Research Lab of the Islamic International Medical College, in association with the Institute of Psychiatry, Benazir Bhutto Hospital, Rawalpindi. It was a study with a cross-sectional analytical design, carried out from September 2022 to May 2023. It was conducted after sanction from the Institutional Review Committee of the study centre.

Sixty subjects were split into two separate groups, Group A (Healthy Subjects) and Group B (Schizophrenia patients) comprising 30 subjects in each group. Sample size was calculated according to the given formula: $Z=pq/e^2$. Clinically diagnosed patients of schizophrenia as per standards of diagnoses given in the Diagnostic and Statistical Manual of Mental Disorders and healthy subjects were recruited.

Patients with any comorbidities like oral disease, musculoskeletal diseases, cardiovascular disease, chronic kidney disease, neurological disorder, immunological disorder, chronic infection, and cancer, were excluded from the research.

Non-probability convenient sampling was done. Sample was taken after describing the process and purpose to all study subjects and after receiving written agreement from them. They were instructed to stay away from eating and drinking for two hours before the saliva collection, with the exception of plain water. A 2 ml of unstimulated whole mouth saliva was taken from each subject in 15 ml falcon tube by the spitting method. It was then instantly encased in an ice bath to reduce its proteolytic properties. After that, the samples were spun in a centrifuge for duration of 15 minutes at 2600 x g at 4°C to obtain the initial clarity, and then for an additional 15 minutes at 2600 x g at 4°C to purge germs and impurities from mucous membrane cells. Every sample was given a buffer to inhibit activity of protease. Following that, the samples were kept at -80°C until the ELISA analysis. For measuring correlation, a 3 ml of blood from schizophrenia patients was also drawn and placed in EDTA tubes. In order to separate plasma from blood cells the centrifugation process took place at 500 g for 5 minutes at 24°C. Then, until the ELISA procedure, each portion of plasma is kept at 80°C. The human galectin 3 ELISA-kit (Elabscience Biotechnology *Co. Ltd.,* Japan) was used to quantify the levels of galectin 3 in SI unit ng/ml.

The data were assessed using SPSS 27 and the findings were noted as mean ± standard deviation, frequency, and percentages. Independent sample student t-test was applied for comparison between the means of the two groups, and Pearson correlation was used to check if levels of salivary and serum galectin 3 were associated in schizophrenia patients (Group B). Statistical significance was considered as a p-value of 0.05 or less.

RESULTS

Age and gender related parameters are mentioned in Table I. Figure 1 shows comparison between the means of two study groups that revealed significantly higher salivary levels of galectin 3 in Group B. Independent sample student's t-test was applied. The mean levels of galectin 3 in saliva of Group A was 684±336 ng/ml and in Group B was 1324±74 ng/ml with highly significant p-value (<0.001).

A strong positive connection between salivary and serum levels of galectin 3 in Group B was seen as Pearson correlation coefficient is 0.389. These results were significant with p-value of 0.03. Correlation is significant at $p<0.05$.

Table I: Both research groups' age and gender related factors.

Parameters	Mean± Standard deviation (Group A)	Mean± Standard deviation (Group B)
Age (years)	27.3 ± 5.08	27.9 ± 5.08
Gender		
Male	22 (73.3%)	8 (26.7%)
Female	8 (26.7%)	8 (26.7%)

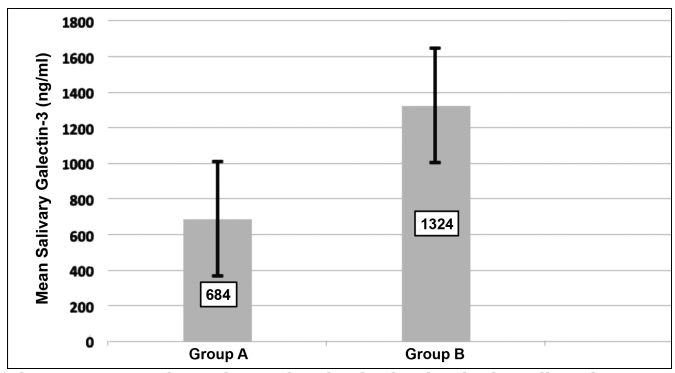

Figure 1: Comparison of mean levels of galectin 3 in the saliva of Group A (healthy subjects) and Group B (schizophrenia patients) using independent sample student's t-test.
***Comparison is highly significant with p<0.001.**

DISCUSSION

Neurodegenerative illnesses are burdensome, leading to cognitive decline, and are not totally treatable at the moment. One of the major clinical challenges in treating schizophrenia is the scarcity of cost-effective, easily accessible methods for moni-

toring, and early detection of high- risk individuals in the general population. Due to this diagnosis which is often made at an advanced point in the course of the disease after exhibiting noticeable symptoms that require psychiatric evaluation, this inhibits persons with schizophrenia from seeking initial therapy and adds to the disorder's poor prognosis.[13]

The current research was conducted to look into the occurrence of an important protein, galectin 3 in the saliva of schizophrenia patients and use it as a tool for its diagnosis. Galectin 3's existence in the saliva of schizophrenia patients aged 19-55 years was identified and compared to healthy subjects. The current study found schizophrenia patients had notably raised levels of salivary galectin 3 than healthy control. The results of this investigation correspond with earlier plasma-based studies. The present study findings agreed with those of Kajitani *et al*. who discovered elevated levels of serum galectin 3 in schizophrenia patients.[9] Another study done by Yuksel *et al*. discovered raised levels of galectin 3 in the serum of schizophrenia patients' siblings compared to normal subjects.[14] The results of this study are also consistent with another study that found elevated levels of galectin 3 in the serum of stable schizophrenia patients. Also, a positive correlation was found between galectin 3 and inflammatory cytokines i.e. TNF-alpha and IL-23 in remission phase.[15] Galectin 3 levels in the blood and cerebrospinal fluid were significantly higher in AD and compared to healthy people.[16] According to the latest studies, elevated levels of serum galectin 3 were significantly linked with ailment intensity in persons with idiopathic Parkinson's disease.[17] The results of the current study are also consistent with the study done by King *et al*. who found that a greater concentration of galectin 3 were linked to symptoms of depressive disorders.[18] Minisha *et al*. also found raised levels of galectin-3 in the saliva of COVID-19 patients.[19] Although Devic *et al*. employed saliva to measure levels of Alpha-Synuclein protein for the very first time in people suffering from Parkinson's disease.[20] To the best of the authors' information, this is the first research on the saliva of schizophrenia patients. The mean value of salivary galectin 3 in schizophrenia patients was 1324.36 ng/ml, whereas it was 684.06 ng/ml in the healthy controls with p-value <0.001. These findings are significant in establishing that levels of galectin 3 are notably higher in the saliva of schizophrenia patients than in healthy controls and due to non-compliance and invasiveness of CSF and blood in schizophrenia patients, saliva may be a better choice for checking galectin 3 levels.

Another observation in the current research was that a positive association was found between salivary and serum galectin 3 levels in schizophrenia patients. This suggests that if galectin 3 levels are raised in serum, they are also raised in saliva or the other way around. It also implies that alterations in galectin 3 levels in saliva may represent variations in the blood of people suffering from schizophrenia. Currently, there is no biochemical investigation available that can be used to expedite the diagnostic process.

The varying clinical characteristics of schizophrenia patients have sparked an increased interest in biomarkers as potential diagnostic and therapeutic indicators. There were a few constraints, like the fact that the results could not be applied to the whole community because of less sample size. It is necessary to perform more research on the levels of galectin 3 in schizophrenia patients, on a broader scope, and in different study contexts, in order to corroborate these observations and also to relate them to the intensity of symptoms.

CONCLUSION

Galectin 3 levels provide a glimpse of a new frontier in the diagnosis of schizophrenia. When combined with additional tests, clinical assessments, and patient history, it may reveal the potential for a decisive and comprehensive knowledge of this complicated neurological disorder.

ETHICAL APPROVAL:
An approval was obtained from the Ethical Committee before the commencement of the study (Riphah/IIMC/IRC/22/2067).

PATIENTS' CONSENT:
Informed consents were obtained from the patients to publish the data.

COMPETING INTEREST:
The authors declared no competing interests.

AUTHORS' CONTRIBUTION:
SS: Contributed to the conception and design of work, data collection, related literature search, and performed reference settings.
HFK: Contributed to the material and methods section, interpreted the data, and conducted the statistical analysis.
MK: Provided patients for sampling.
SA: Provided critical evaluation of work.
SNQ: Participated to the discussion portion.
MM: Added to conclusion portion.
All authors agreed to publish the final version of the manuscript.

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