



## 'AN ANALYSIS OF SERUM CALCINEURIN ACTIVITY IN DRUG NAÏVE SCHIZOPHRENIA PATIENTS'

### Medical Science

**Nivedita S. Shireshi**

Research Scholar, Department of Biochemistry, Karnatak University Dharwad

**Dr.K.S.Devaraju**

Associate Professor, Department of Biochemistry, Karnatak University Dharwad

### ABSTRACT

Calcineurin is a Ca-calmodulin dependent protein phosphatase, which plays as connecting link between dopaminergic and glutamatergic neurotransmitter systems and their dysregulation in schizophrenia. The main objective of this study was to examine the potential role of calcineurin in schizophrenia. Calcineurin levels were estimated in 15 schizophrenia patients in the age group of 18-45 who were attending outpatient department of psychiatry, Dharwad institute of mental health and Neurosciences, Dharwad in Karnataka, India. The results of the present study showed significant ( $\geq 0.001$ ) decreased activity of serum calcineurin in schizophrenia patients compared to those of normal subjects. The observations thus suggest impaired calcineurin activity in schizophrenia patients.

### KEYWORDS:

Schizophrenia, calcineurin, drug naïve, PANSS score, calmodulin

#### Introduction

Schizophrenia is a complex neuropsychiatric disorder which characterized by an array of spectrum of symptoms, which includes delusions, hallucinations, disorganized speech or behavior and impaired cognitive ability.<sup>(1)</sup> This disease occurs at an early age and becomes chronic and devastates the life style of the affected person and becomes disabling disorder for many patients and their families.<sup>(2)</sup> The prevalence of schizophrenia is 4.3 to 8.7 million people in India and 2.2 million in USA and 6 to 12 million in china.<sup>(3)</sup> Males tend to experience their first episode of schizophrenia in their late 20's or early 30's.<sup>(4)</sup> While in women the mean age of onset of schizophrenia is 25-35.<sup>(5)</sup> However the women seem to have two peaks in the age of onset of disease the first after menarche and the second once they are over 40.<sup>(6)</sup> Recent hypothesis of schizophrenia pathophysiology model describes the role of protein phosphatases in schizophrenia which regulate the cellular processes by dephosphorylating the proteins.<sup>(7)</sup>, particularly the calcineurin the protein phosphatase 2B which is a key regulatory molecule involves in numerous signaling pathways by removing phosphate groups that have been added to proteins by various kinases and involves in turning on and off the cellular processes and there by controls the cellular processes. Particularly in neurons, calcineurin regulates phosphorylations elicited by both glutamatergic and dopaminergic signaling making it particularly interesting molecule in schizophrenia since it acts as a connecting link between the dopaminergic and glutamatergic neurotransmitter systems which are dysfunctional in schizophrenia.<sup>(8,9)</sup> An attempt is made to investigate the serum calcineurin in Schizophrenia patients and evaluate the potentiality of calcineurin as biomarker for the schizophrenia.

#### MATERIALS AND METHODS:

##### Patients' recruitment:

This case-control study has been approved by Ethical committee of Dharwad Institute of Mental Health and Neurosciences (DIMHANS). All patients taking part in this study were meeting DSM V diagnostic criteria. Patients were recruited for the study from the outpatient department of psychiatry, Dharwad Institute of mental health and neurosciences (DIMHANS) Karnataka, India and Control subjects were recruited based on the following criteria.

##### Inclusion Criteria for patients:

1. Patients diagnosed as schizophrenia
2. Aged between 18 – 45 yrs
3. Either gender

##### Exclusion Criteria for patients:

1. Co-morbid psychiatric disorders
2. Co-morbid substance use disorder except nicotine use
3. Co-morbid chronic medical disorders (Diabetes, Hypertension, Tuberculosis, etc.)(Based on the Case history of the participants)
4. Co-morbid developmental disorders (Mental retardation, autism)

##### For Controls:

1. Age and sex matched

2. No co-morbid Psychiatric disorders or substance use disorders.
3. No co-morbid medical disorders (Diabetes, Hypertension, Tuberculosis etc.) Based on the case history of the participants)

Socio demographic details of patients and control subjects were obtained by specially designed proforma. Consent form is obtained from all the subjects participated in this study.

##### Assessment of Clinical Profiles:

The severity of clinical symptoms in patients with Schizophrenia was assessed by the Positive and Negative Syndrome Scale (PANSS). Which consists of 30 items, in that 7 items consist of positive symptom scale, 7 items consist of negative symptoms scale and the remaining 16 items consist of a general psychopathology scale. The rating points 2 to 7 of each items corresponds to incremental levels of symptom severity.

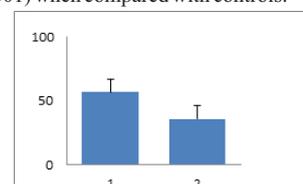
##### Calcineurin (CaN) measurement in Serum:

The drawn blood (10 ml) was taken in a centrifuge tube and allowed to clot for 30 minutes and centrifuged at 3000rpm and supernatant serum was stored in cryo vials and kept at -200c for the assessment of calcineurin activity in serum of patients and control groups.

Calcium-calmodulin dependent phosphatase activity of CaN present in the samples was assayed according to the procedure of Wang et al<sup>(10)</sup>. in the presence and absence of trifluoroperazine (TFP) 150µm ol/L. The assay was conducted in a reaction mixture containing 250Mm/L tris (pH 7.2), 250mM/L morpholino ethane sulphonic acid (MES pH 7.0), 10Mm/L MnCl<sub>2</sub>, 2.4Mm/L paranitrophenyl phosphate (p-NPP). The serum was added to the assay mixture. Reactions were initiated by addition of p-nitro phenyl phosphate followed by incubation for 10min at 300C. The reaction was terminated by the addition of 13% dipotassium hydrogen phosphate. Absorbance was measured spectrophotometrically at 405nm. The difference between the amounts of p-nitro phenol released in the presence and in the absence of TFP indicated the activity of the calcineurin in both the groups.

##### Results:

Statistical analysis is done by SPSS 16.0 version soft ware using student t-test. The serum calcineurin activity was expressed as µmols of paranitrophenol released per mg of protein present in the serum showed for drug naïve schizophrenia patients 40.87±3.147 and for controls 59.13±4.578. It is indicated that the decrease in calcineurin levels in drug naïve schizophrenia patients was significantly decreased ( $P \geq 0.001$ ) when compared with controls.



**Socio demographic details:**

No. of Patients	Male	Female	Age (female)	Age (Male)	Positive symptoms	Negative symptoms
15	8	7	30.21±6.078	28.43±9.86	17.8±8.37 (male)	29.85±6.88 (male)
					15.18±5.27 (female)	26.68±6.87 (female)
No. of controls						
15	08	07	25±7.083	30±8.756		

**Discussion:**

Calcineurin is a calcium and calmodulin dependent serine/threonine protein phosphatase and is also known as protein phosphatase 2B which involves in various cellular processes<sup>(11)</sup>. It also mainly involved in cognition and working memory.<sup>(12)</sup> Dysregulation of calcineurin leads to impairment in working memory, attention deficit, aberrant social behavior and several other abnormalities which are the characteristics of schizophrenia.<sup>(13)</sup> Calcineurin It directly controls the cAMP response element binding protein (CREB) which plays an important role in the cognition and memory.<sup>(14)</sup> It regulates Growth associated protein43(GAP43) dephosphorylation which is involved in neuronal plasticity and memory.<sup>(15)</sup> Genetic association studies of several population showed that genetic variation in PP3CC gene encoding for a gamma unit of catalytic subunit of calcineurin leads to Schizophrenia.<sup>(16)</sup> In calcineurin Knockout mice model, Calcineurin deficiency impairs the ability of neurons in Prefrontal cortex(PFC) to efficiently mobilize and recycle synaptic vesicles during high-frequency activity, leading to a disruption of high-frequency neuronal and network activities in PFC that are required for working memory leads to impairment in working memory of Schizophrenia.<sup>(17)</sup> In our study the serum calcineurin activity has been measured, as per our knowledge this is the first report which is based on the peripheral, blood based estimation of calcineurin in schizophrenia since the calcineurin has been estimated in the other pathological conditions such as diabetes mellitus, mental retardation, Down's syndrome, leukemia, ischemia<sup>(18,19,20,21,22)</sup>, and found to be significantly decreased in all pathological conditions, we have excluded the co-morbid psychiatric, and other diseased conditions to avoid the factors which might responsible for the decrease in the calcineurin activity and reflects the activity of calcineurin in Schizophrenia disorder conditions.

**Conclusion:** These findings provide further evidence for the systemic nature of schizophrenia and give added validity to the concept that calcineurin in peripheral system also varied in schizophrenia and this can be used to develop blood based biomarker for the schizophrenia.

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