Role of membrane cholesterol and lipid peroxidation in regulating the Na+/K+-ATPase activity in schizophrenia

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INTRODUCTION

The Na+/K+-ATPase (NKA) is a ubiquitous enzyme that maintains the Na⁺ and K⁺ gradients across the cell membrane by transporting three Na⁺ out and two K⁺ into the cell. Oxidative stress and membrane lipid composition play important roles in regulating NKA activity.

Background: Na+/K⁺-ATPase (NKA) activity is compromised in several neuropsychiatric disorders. Oxidative stress and membrane lipid composition play important roles in regulating NKA activity.

Aims: The present study was undertaken to evaluate the effects of oxidative stress-induced membrane lipid damage and membrane cholesterol composition on NKA pump activity in schizophrenia.

Settings and Design: It was a hospital-based, cross-sectional, observational study in 49 cases and 51 controls for 1 year.

Materials and Methods: NKA pump activity in red blood cell membrane, serum levels of thiobarbituric acid reactive substances (TBARS), protein carbonyl (PC) adducts, and cholesterol were measured by standard spectrophotometric techniques in newly diagnosed schizophrenia patients by Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision criteria. Membrane cholesterol was analyzed by chloroform and isopropanol extraction followed by measuring the cholesterol concentration by spectrophotometric technique.

Statistical Analysis and Results: Mean values for NKA pump activity, membrane cholesterol level, and serum cholesterol levels were significantly lower in the case group (P < 0.001). The activity of NKA pump was found to be directly correlated to membrane cholesterol level rather than with the serum cholesterol values. Although the NKA pump activity showed inverse relationship with the serum values of TBARS and PC products both, on multiple linear regression analysis, it was found to be significantly positively dependent on the membrane cholesterol (β = 0.268, P = 0.01) and negatively dependent on the serum TBARS (β = −0.63, P < 0.001) levels only.

Conclusion: Reduced membrane cholesterol and oxidative stress-induced damage to membrane lipids play crucial roles in decreasing the NKA activity in schizophrenia. Hence, for a better prognosis and treatment, measures are required to maintain optimum levels of cholesterol in neuronal tissues along with a proper control on oxidative stress.

Key words: Lipid peroxidation, membrane cholesterol, Na+/K⁺-ATPase activity, schizophrenia
the cell. It is necessary for proper cellular function since it helps to preserve the ionic gradients across the cell membrane and thus the membrane potential and osmotic equilibrium of the cell. Structural variations of this pump are notable which is supposed to contribute significantly in its regulation by different modulators. This pump has one principal catalytic subunit termed α subunit, one sugar-rich auxiliary β subunit, and sometimes a γ subunit. The α subunits have 10 transmembrane segments known as FYXD segments, with variable binding affinity for different regulator or modulators, particularly the lipid molecules. Several lipids have been found to influence its activity through binding at different sites of this oligomeric protein pump. Phospholipids such as phosphatidylcholine (PC) and phosphatidylethanolamine enhance the activity of the pump by promoting the conformational change from E1 to E2 (P) by reacting with the surface charges of this integral membrane protein[2] while some other such as phosphatidylserine (PS) helps in stabilizing the pump by binding critical Ile and Lys residues in the hydrophobic core of the FYXD α helices[3,4]. Moreover, a specific interaction of this pump with 18:0/18:1 PS (1-stearoyl-2-oleoyl-sn-glycero-3-phospho-L-serine) and cholesterol was found to help its thermal stability significantly.[5] However, so far, cholesterol has been suggested to be the only membrane lipid component that is found to be resolved in NKA structures.[2] Maintenance of native NKA structure is strongly dependent on cholesterol.[6,7] The molecular structure of the shark rectal gland NKA in the E2 (P) conformation reveals a key cholesterol molecule bound at the interface of the β transmembrane helix and helices 3 and 7 of α.[8] However, interestingly, reflection of the vital role of cholesterol in maintaining this optimum membrane protein function has not been found to be consistent with its serum level as reported by several studies, and some of them, including some recent ones, have put doubts on the link between serum cholesterol level and psychiatric disorders as they could not find out any significant correlation between the two.[9,10] Moreover, studies have reported that there is no correlation between the serum and red blood cell (RBC) membrane cholesterol levels, and some have found even an increase in serum cholesterol levels with decreased RBC membrane cholesterol that is accompanied with an inhibition of the sodium pump activity.[11] These incongruent observations necessitated exploration of the role of membrane cholesterol itself in maintenance of the NKA activity. Although many studies reported a direct correlation between the membrane cholesterol and NKA activity,[12] some have observed an increased value of membrane cholesterol with decreased NKA activity, particularly in cases of metabolic syndrome.[13] Hence, significant lacunae still exist regarding the relationship between alterations in membrane cholesterol and NKA activity in different pathophysiological conditions including neuropsychiatric disorders.

The potential link between membrane cholesterol and NKA activity is reflected by their linkage with several neuropsychiatric disorders including schizophrenia. Decreased membrane fluidity in RBC ghost membranes has been found to correlate well with the severity of psychosis and increased frequency of relapse in schizophrenia.[14] Cholesterol, being a key lipid component in the CNS, is a major mediator of membrane fluidity, permeability, and neurotransmission.[15] Several cholesterol receptors involved in transport of cholesterol into the cells and their membrane have been found to play crucial roles in the evolution of several neurodegenerative and neuropsychiatric disorders such as Alzheimer's disease and schizophrenia. On the other hand, alterations in the activities of NKA in both downward and upward directions have been observed in schizophrenia that was found to be significantly associated with oxidative stress also.[16,17] Oxidative stress-induced membrane lipid damage has been found to affect the NKA activity significantly in several neuropsychiatric disorders including schizophrenia.[18] In animal models, effective treatment with antipsychotics was found to prevent the lipid peroxidation-induced reduction in NKA activity.[19]

From all these observations and their lacunae, we hypothesized that any potential alteration in the NKA activity in schizophrenia patients may be dependent on a complex interplay between the membrane cholesterol concentration and oxidative stress-induced reactive oxygen species (ROS) molecules. Moreover, these changes in the membrane cholesterol might reflect their own intracellular metabolism that may not be always reflected by their plasma levels. Accordingly, we undertook the present study to assess any potential changes in the NKA activity in schizophrenia patients and to analyze its dependence on the biochemical parameters such as serum cholesterol, RBC membrane cholesterol, and ROS-induced damage to the membrane lipids and cellular proteins.

MATERIALS AND METHODS

Study design
The present study was performed as a hospital-based, observational, case–control study from March 2015 to December 2016 in an urban metropolitan area.

Selection of study population
Selection of cases
We selected newly diagnosed cases of schizophrenia on convenience basis from the Outpatient Department of Psychiatry of a Tertiary Care Medical College and Hospital according to specific inclusion and exclusion criteria. The inclusion criteria were (1) diagnosed cases of schizophrenia according to Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision guidelines, (2) both male and female patients in the age group in 17–60 years

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with equal preference, and (3) patients belonging to similar socioeconomic status and ethnicity. Exclusion criteria were (1) patients suffering from any other neuropsychiatric disorder, (2) schizoaffective patients or any mixed type of psychiatric disorder, (3) patients with history of any drug addiction, chronic smoking habit, or alcohol addiction, (4) patients with any metabolic or endocrine disorders such as diabetes mellitus or diseases of the pituitary, thyroid, or adrenal gland, and (5) patients with any malignant or any chronic inflammatory condition.

Selection of control subjects
Control subjects were selected from the age-matched healthy subjects accompanying the patients to the psychiatry OPD. First-degree relatives were not considered to avoid any genetic predisposition. To ascertain the similar nutritional and economical status, both cases and control subjects were selected from the similar socioeconomic status with the similar ethnic background.

Ethical considerations
Informed consents were taken from all participants following the protocol and guidelines of the Helsinki declaration 1975, revised in 2000. Before starting, the study was approved by the institutional ethical committee.

Measurement of biochemical parameters
Measurement of Na\(^+\)/K\(^+\)-ATPase activity
NKA activity was measured in the RBC membrane as it is already known to correlate well with that of the neuronal tissues.\[^{20}\] The enzyme was measured in the RBC membrane in following three steps:

Preparation of red blood cell hemolysate
The method consisted of following steps as described by Noori \textit{et al.}\[^{21}\]. The whole blood was centrifuged at 4°C, 450 g for 15 min to obtain the packed red cells and then diluted in 25 volumes of 0.011 mol/L Tris-HCl buffer at pH 7.4 for getting its hemolysate. The hemolyzed cells were centrifuged for 30 min at 12,000 rpm at 4°C to obtain the membrane pellet. The membrane pellet was resuspended in 30 ml of 0.011 mol/L Tris-HCl buffer and the centrifugation step was repeated thrice. The final concentration of the membrane suspension was adjusted to about 4 mg protein/ml of Tris buffer for optimum assay of the enzyme activity and was stored at −20°C until the assay was performed.

Measurement of Na\(^+\)/K\(^+\)-ATPase activity
The enzyme was assayed by linked enzyme system with the sequence of reactions which is based on an ATP-regenerating system followed by conversion of phosphoenolpyruvate to pyruvate and then to lactate with oxidation of NADH. The linear rate of NADH oxidation correlates to the hydrolysis of ATP. One unit (1 U) of ATPase represents 1 µmol of NADH oxidation per minute. A suitably diluted sample was added to the reaction mixture and once a good linear rate was established, ouabain, the inhibitor of NKA, was added followed by measurement of the rate of the reaction. The NKA activity was taken to be the difference between these two rates (ΔA/min).

\[ ΔA/min \text{ for NKA} = ΔA/min \text{ before addition of ouabain} − ΔA/min \text{ after addition of ouabain}. \]

The value was compared against a standard curve of ADP to obtain the enzyme activity in units or milliunits (1 µmol/min = 1 Unit).

Final expression of the enzyme activity
RBC membrane protein was measured by Lowry method using Folin-Ciocalteu reagent, and the membrane NKA activity was finally expressed in IU/mg of tissue protein.

Measurement of serum thiobarbituric acid reactive substances
Serum thiobarbituric acid reactive substances (TBARS) was measured as a marker of lipid peroxidation through a reaction system that enables the lipid peroxides to react with thiobarbituric acid at boiling temperature in acidic medium. After precipitating the proteins out from 0.5 ml with 10% trichloroacetic acid (TCA), the reaction mixture was acidified with 0.05M H\(_2\)SO\(_4\) and was kept in boiling water for 30 min after adding 2.5 ml thiobarbituric acid. After cooling, the chromogen was extracted with 4 ml n-butanol, and its color was measured at 532 nm in a spectrophotometer. The absorbance was converted into TBARS concentration in nmol/ml by calibrating against a standard curve prepared with the 2.5, 5, 7.5, 10, and 12.5 nmol/ml of 1’ 1’ 3’ 3’ tetraethoxypropane obtained from Fluka, Germany.

Measurement of protein carbonyl products
Carbonylation of serum proteins was measured with the modified Levin’s method.\[^{22}\] First of all, a precipitate was obtained by adding 0.5 ml 10% TCA to 1 ml serum and centrifuging it at 5000 rpm for 5 min. After discarding the supernatant, 0.5 ml of 10 mmol/L 2,4 DNPH in 2 mol/L HCl was added to the precipitate. After 30 min incubation with vigorous mixing every 10 min, 0.5 ml of 10% TCA was added and the mixture was centrifuged at 5000 rpm for 5 min. The supernatant was discarded; the precipitate was washed with the 750 µl of protein washing solution (2 g SDS, 50 mg EDTA in 100 ml of 80 mmol phosphate buffer, pH 8.0), and the reaction mixture was incubated at 37°C for 10 min in water bath. The supernatant was collected and measured at 370 nm taking 2M HCl as blank. The protein carbonyl (PC) content was calculated by multiplying the absorbance of the reaction mixture with its molar extinction coefficient (21 × 10\(^5\) L/mol). Finally, the expression of carbonylated proteins was converted to nmol/mg of serum protein.

Measurement of membrane cholesterol
Cholesterol in the RBC membrane was measured through the reaction of commercially obtained CHOD-PAP
reagent (ERBA diagnostic, Mannheim, Germany) with the cholesterol in dry lipid extracts of the RBC membrane.\[^2\,^3\] The total procedure was done in following steps:

**Obtaining dry residues of the red blood cell membrane**

After washing erythrocytes three times with physiological saline, the packed cells were hemolyzed by mixing with distilled water in 1:2 ratio. A 0.5 ml of hemolysate was transferred to a stoppered glass tube, and 5 ml of isopropanol was added drop by drop slowly with vigorous mixing on a vortex. After incubating the mixture for 1 h at room temperature, 3.2 ml of chloroform was added to it drop by drop in a similar way. The reaction mixture was stored in stoppered glass tubes in dark overnight at room temperature. Thereafter, the contents were transferred to 15 ml centrifuge tubes and centrifuged at 3000 rpm for 10 min. Supernatant was obtained and 600 µl of it was evaporated at 60°C. Each sample was evaporated in triplicate to remove the bias of individual variations.

**Measurement of cholesterol in the dry membrane residue**

The dry residues were completely dissolved in 2 ml of CHOD-PAP reagent with the aid of nonionic detergent 0.5% Triton-X. After incubating for 30 min at room temperature, reaction mixture in the tubes was vortexed vigorously until all the dry residues were converted into homogeneous solution. Absorbance of the reaction mixture was measured at 500 nm at spectrophotometer. Values of individual samples were calculated by comparing their absorbance with that of a cholesterol calibrator (5.17 mmol/l) treated in the same way. Final concentration was expressed as mmol/mg of total protein.

**RESULTS AND STATISTICAL ANALYSIS**

The data obtained were first checked for normal distribution and then compared for the significance of difference between the mean values of case and control groups by independent Student's t-test. The strength of association between the study parameters in the case group was assessed by bivariate Pearson's correlation study. The predictive values of different study parameters on the NKA activity were analyzed by multiple linear regression study. All statistical analyses were done with the SPSS 17 software for Windows from IBM, USA. P value was considered statistically significant at a level <0.05 for a 95% confidence interval.

**Tests for normal distribution of the data obtained**

From the tests for normal distribution (Smirnov–Kolmogorov’s test and Shapero–Wilk’s test, data not shown in the tables), data appeared to follow the normal distribution pattern. Their overall distribution has been shown through the Box-Whisker plot in Figure 1a and b that also suggests that the data are approximately normally distributed.

**Tests for significance of mean value differences**

In the independent t-test, these were reflected by the t-values and their corresponding P values in Table 1. The results exhibited that in the case group, NKA activity was found to be significantly compromised with substantial increases in the oxidative stress-induced lipid and protein damage markers, i.e., serum TBARS and PC adducts, respectively. On the other hand, cholesterol levels in both serum and membranes showed significant decrease in the schizophrenia patients in comparison to healthy controls.

**Test for strength of association between study parameters in the case group**

After observing significant lower values for the NKA activity, membrane cholesterol, and serum cholesterol in the case group with significantly raised oxidative stress-induced cell damage markers, we performed the Pearson's bivariate correlation analysis to explore any possible association between the individual study parameters in the case group. The results in Table 2 showed that decrease in the NKA activity was linearly correlated (correlation coefficient \( r = 0.396, P = 0.005 \)) with reduction in membrane cholesterol only without showing any such relationship with the serum cholesterol \( r = 0.197, P = 0.176 \). Similarly, the

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**Figure 1:** (a) Box-Whisker plot showing the distribution of different study parameters in the case and control groups. (b) Box-Whisker plot showing distribution of membrane cholesterol among the cases
Table 1: Independent t-test showing comparison between the mean values of study parameters between case and control groups

|                       | Mean±SD                      |
|-----------------------|------------------------------|
| Case (n=49)           |                              |
| Na⁺/K⁺-ATPase activity| 3.75±0.98                    |
| Serum TBARS           | 5.77±0.76                    |
| Serum protein carbonyl | 1.87±0.36                    |
| Serum cholesterol     | 3.43±0.72                    |
| Membrane cholesterol  | 122.40±19.78                 |
| Control (n=51)        |                              |
| Na⁺/K⁺-ATPase activity| 28.99±5.52                   |
| Serum TBARS           | 3.07±0.63                    |
| Serum protein carbonyl | 1.19±0.14                    |
| Serum cholesterol     | 4.65±0.76                    |
| Membrane cholesterol  | 183.63±34.23                 |

P value was considered to be statistically significant for P<0.05 at 95% of CI. SD – Standard deviation; CI – Confidence interval; TBARS – Thiobarbituric acid reactive substances

Table 2: Bivariate Pearson correlation analysis showing the strength of relationship between the study parameters in the case group

| Na⁺/K⁺-ATPase activity | Membrane cholesterol | Serum TBARS | Serum protein carbonyl | Serum cholesterol |
|-------------------------|----------------------|-------------|------------------------|-------------------|
| r                       | 1                    | 0.396*      | 0.197                  | −0.699*           |
| P                       | 0.005                | 0.176       | 0.000                  | 0.802             |

Membrane cholesterol

| r                       | 0.396*                | 1            | 0.071                  | −0.206             |
| P                       | 0.005                 | 0.629        | 0.156                  | 0.198              |

Serum cholesterol

| r                       | 0.197                 | 0.071        | 1                      | −0.112             |
| P                       | 0.176                 | 0.629        | 0.442                  | 0.644              |

Serum TBARS

| r                       | −0.699*               | −0.206       | −0.112                 | 1                   |
| P                       | <0.001                | 0.156        | 0.442                  | 0.694              |

Serum protein carbonyl

| r                       | 0.037                 | −0.187       | 0.068                  | −0.058             |
| P                       | 0.802                 | 0.198        | 0.644                  | 0.694              |

*P value was considered to be statistically significant for P<0.05 at 95% of CI. CI – Confidence interval; TBARS – Thiobarbituric acid reactive substances; PC – Protein carbonyl

activity of this enzyme was found to be inversely associated significantly (r = −0.699, P < 0.001) with rise in lipid peroxidation only without showing any such relationship with protein carbonylation (r = 0.037, P = 0.802).

Test for finding out the dependence and predictive values of study parameters

As the bivariate analysis only shows a bidirectional relationship between two individual parameters, to ascertain the relative importance of predictive values of the study variables taken together on the NKA activity, we performed the multiple linear regression analysis. In this test, we tested for the relative significance of the predictive values of serum cholesterol, membrane cholesterol, serum TBARS, and PC adducts on the dependent factor NKA activity. Results showed that NKA activity was significantly decreased only with decreased membrane cholesterol (regression coefficient β = 0.268, P = 0.013) and increased lipid peroxidation (β = −0.630, P < 0.001) without any such effect of serum cholesterol or PC adducts on it. This indicated strong negative and positive predictive effects of membrane cholesterol and membrane lipid peroxidation only on the NKA activity, respectively. This has been evident also from the interactive plot [Figure 2].

DISCUSSION

Link between reactive oxygen species generation and Na⁺/K⁺-ATPase activity in schizophrenia patients

In the present study, significant reduction in the level of NKA is found to be associated with increased production of ROS, particularly the lipid peroxidation product TBARS. Due to high metabolic rate, the brain consumes about 25% of oxygen in spite of consisting of only 2% of the total body weight. However, due to reduced capability of cell regeneration, low antioxidant defense, high amount of polyunsaturated fatty acids (PUFAs), and high content of metal ions such as iron, zinc, copper, and manganese, the brain tissues are more susceptible to ROS-induced damage as compared to other body organs. Increased dopaminergic activity in schizophrenia is a potential source of free radicals in the brain tissues including the prefrontal cortex, one of the major sites of the brain affected in schizophrenia. Enzymatic metabolism of dopamine itself generates significant amount of hydrogen peroxide that culminates in production of ROS such as dopamine quinones and superoxide. Increased ROS further damage the dopamine transporters on cell membrane, thus leading to accumulation of more dopamine in synaptic regions that in turn accentuates generation of ROS. Moreover, increased dopamine levels are reported to reduce antioxidant levels of glutathione as much as 40% in the brain tissues which is already compromised in several regions of the brain in schizophrenia patients. Thus, the upregulation of ROS production along with a downregulation of the antioxidant defense interplay together to cause a substantial drift of the redox balance toward an oxidative stressful condition in schizophrenia that has been evident in our present study too [Table 1]. Moreover, a compromised NKA activity also contributes to an increased oxidative stress to some extent. Recently, a particular NaKtide sequence in the α subunit of NKA has been reported to restrict ROS generation via Src-mediated pathway explaining the increased ROS production when
NKA activity is compromised. This bilateral association of oxidative stress-induced lipid and protein damage with decreased NKA activity has been strengthened in our bivariate correlation analysis where a significant negative correlation was found between the NKA activity with serum TBARS and PC adducts [Table 2]. Two-way association between them was further specified through the multiple linear regression analysis [Table 3] where the significance of predictive values of lipid peroxidation, PC adducts, and membrane cholesterol was analyzed together on the NKA activity. It showed that when the effects of all these study variables were considered together on the NKA activity through the multivariate analysis, only membrane cholesterol levels and oxidative stress-induced lipid peroxidation showed significant predictive values on the pump activity (β = −0.633 and −0.044, P < 0.001 and 0.670, respectively). It revealed that among several confounding factors, these two play relatively more crucial roles in regulation of the NKA activity.

Role of membrane cholesterol in maintenance of the stability of Na+/K+-ATPase activity

It is known for long that lipids play a major role in regulation of NKA activity. Different distributions of saturated and unsaturated fatty acids in these lipids have been found to alter the sodium pump activity by changing the lateral compression pressure in the hydrophobic core of this membrane protein. In most studies, the activity and turnover of this protein pump are found to be correlated to the content of PUFA, particularly C22:6. Furthermore, the alteration of the ω-6:ω-3 fatty acids in the brain and heart has been reported to alter the NKA pump activity significantly. All these knowledge are further proving useful for applying necessary dietary changes required for maintaining an optimum NKA pump activity in these tissues.

Cholesterol plays a major role in modulating the membrane fluidity; however, according to studies at molecular levels, the stabilizing effect of membrane cholesterol on G protein-coupled receptors and thereby on the signal transduction cascades of important neurotransmitters such as serotonin has been conclusively proved. Lower levels of cholesterol have been found to be associated with reduced 5 HT1A, 5 HT7, and serotonin receptor activity in several psychiatric disorders. Furthermore, cholesterol has been found as the major lipid that is directly involved in the structure of NKA pump. Along with other lipids, its effect has been variably described depending on the site preferred for its binding. Binding of similar lipids such as PS and cholesterol has been found to be stabilizing if bound to a particular site whereas binding of PC and cholesterol at a different site is reported to be inhibitory. Studies of purified detergent-soluble recombinant αβ or αβ FXYD NKA complexes revealed separate functional effects of phospholipids and cholesterol with characteristic structural selectivity. The observations suggest that these different effects are exerted at separate binding sites for phosphatidylserine/cholesterol. All these reports point to a central role of direct and specific interactions of different phospholipids and cholesterol molecules in determining both stability and molecular activity of the NKA which explains possible implications of changes in the membrane lipid composition in physiological regulation of this integral membrane transporter.

Membrane cholesterol, by virtue of its ability to compact the saturated fatty acid side chains of membrane phospholipids, contributes significantly to the formation of membrane rafts and hence in maintenance of the optimum function of integral membrane proteins. In congruence to the significant predictiveness of the membrane cholesterol on the sodium pump activity in our study, its vital role in maintaining normal neuropsychological functions has been reiterated by recent findings in schizophrenia patients that reported marked polymorphisms of the CAV1 genes affecting expression of the membrane caveolin proteins which target cholesterol to membrane. Caveolin, like all integral membrane

![Figure 2: Interactive plot showing the dependence of Na+/K+-ATPase activity on membrane cholesterol and serum thiobarbituric acid reactive substances activity](image)

**Table 3: Multiple linear regression analysis showing the relative dependence of Na+/K+-ATPase activity on oxidative stress parameters and cholesterol levels in membrane and serum in the schizophrenia patients**

| Model | Unstandardized coefficients | Standardized coefficients | t | P |
|-------|-----------------------------|---------------------------|---|---|
|       | B                          | SE                        | β |    |
| Constant | 8.493                       | 2.735                     | 3.105 | 0.003*  |
| Serum TBARS | −1.641                     | 0.266                     | −6.161 | <0.001* |
| Serum protein carbonyl | 0.002                      | 0.005                     | 0.044 | 0.670    |
| Membrane cholesterol | 0.031                      | 0.012                     | 0.268 | 2.583 | 0.013* |
| Serum cholesterol | 0.009                      | 0.008                     | 0.104 | 1.038 | 0.305    |

*P value was considered to be statistically significant for P < 0.05 at 95% of CI. CI – Confidence interval; TBARS – Thiobarbituric acid reactive substances; SE – Standard error
proteins, interacts with bilayer lipid components and helps them to form a surrounding annulus that solvates the exposed transmembrane segment of membrane proteins. It enables the annular lipids to restrict their mobility relative to “free” lipids so that their variable hydrophobic acyl chains may alter their conformation to accommodate the width of the hydrophobic surface of the protein and optimize their functions.\textsuperscript{39} Hence, it is evident that any significant reduction in their amount or damage to their structure may cause detrimental changes in the functions of the concerned membrane protein. Interestingly, the decrease in NKA activity has been reported to cause a reduction in the level of intracellular magnesium level that further interferes with the intracellular cholesterol metabolism\textsuperscript{40} and consequently its integration into cell membrane. Overall, it results in a vicious cycle leading to a compromised NKA activity and membrane cholesterol in a synergistic manner.

The lack of predictability of the serum cholesterol levels for providing consistent information for an optimum NKA activity is underscored furthermore from the results of our study where serum cholesterol, in spite of showing a positive correlation with the erythrocyte membrane cholesterol, did not exhibit a statistically significant $P$ value ($r = 0.071, P = 0.689$, [Table 3]). It signified that despite some degree of free exchange of cholesterol with the plasma, total content of RBC membrane cholesterol is more prominently determined by an equilibrium between the cholesterol efflux and influx across the membrane and intracellular cholesterol transport, all of which occur via several enzymatic and biochemical pathways within the cells independent of the circulating plasma.\textsuperscript{41-43} Keeping on track with our study, some recent studies have also corroborated this view of membrane cholesterol content being independently regulated from the plasma cholesterol.\textsuperscript{39}

The findings of our present study have significant implications in neuronal tissue also. As cholesterol synthesis is essential for development of neuronal and glial cells since early period and is essential for myelin sheath production around the oligodendrocytes, both of its total pool and concentration is much higher in CNS than several other organs.\textsuperscript{44} Maintenance of this higher concentration inside the cell is not totally dependent on the blood levels of cholesterol due to presence of a tight capillary junctions around the neurons. Hence, changes in serum levels of cholesterol may not be always reflected in the intracellular cholesterol concentration in the brain cells.\textsuperscript{44} With this knowledge and the observations of our study, we suggest that serum cholesterol is not a dependable parameter for ascertaining the membrane cholesterol levels and their regulatory role on the NKA activity in the schizophrenia patients.

Interplay of decreased membrane cholesterol and increased lipid peroxidation on Na\textsuperscript{+} K\textsuperscript{+} pump activity

Thus, from a holistic viewpoint, results of the present study signify that both membrane cholesterol and lipid peroxidation function as significant determining factors for maintaining an optimum NKA activity in the neuronal tissues. Previously, it was found that an elevated lipid peroxidation with decreased membrane cholesterol aggravates the pathology of intracerebral hemorrhage.\textsuperscript{45} Keeping on track, we suggest that these two play a synergistic effect on the severity of the schizophrenia that is reflected by a significant dependence of the NKA activity on both increased lipid peroxidation and reduced membrane cholesterol in the present study. These findings are furthermore strengthened by the fact that human postmortem brain tissues of person

\[ \text{Figure 3: Graphical summary} \]
dying of another neuropsychiatric disorder, Huntington's disease, revealed marked reduction in membrane cholesterol in several brain areas along with significantly raised lipid peroxidation markers.[46]

Limitation of the present study

However, our results should be viewed regarding its limitation that it could not involve the techniques for analyzing oxidative stress-induced changes at the molecular levels of the membrane lipids surrounding the sodium pump in schizophrenia patients. Hence, in conclusion, we suggest that for a better prognosis and management of this major neuropsychiatric disorder, a successful control on oxidative stress and optimization of the membrane cholesterol level both should be addressed with more explorative investigations and research further. This may include the delineation of the lipid peroxidation-induced structural changes in the cholesterol and lipid molecules adjacent to the sodium pump at a supramolecular level by more recent techniques.

CONCLUSION

Changes in the sodium pump activity are important determinants of maintenance of membrane potentiality and hence neurotransmission. Other than the normal physiological stimuli such as stimulated nerve impulse propagation and exercise, altered heterodimerization of these membrane proteins due to changes in the membrane cholesterol concentration plays a significant contributory role.[47,48] Furthermore, the effect of lipid peroxidation on this pump activity is more detrimental in comparison to cellular protein oxidation. In this study, we have shown that among different potential parameters, membrane cholesterol concentration and membrane lipid peroxidation play most vital contributory roles for regulating the NKA activity in schizophrenia patients [Figure 3].

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Skou JC, Esmann M. The Na, K-ATPase. J Bioenerg Biomembr 1992;24:249-61.
2. Haviv H, Habeck M, Kanai R, Toyoshima C, Karlish SJ. Neutral phospholipids stimulate Na, K-ATPase activity: A specific lipid-protein interaction. J Biol Chem 2013;288:10073-81.
3. Haviv H, Cohen E, Lifshitz Y, Tal DM, Goldshleger R, Karlish SJ. Stabilization of Na(+), K(+)-ATPase purified from Pichia pastoris membranes by specific interactions with lipids. Biochemistry 2007;46:12855-67.
4. Mishra NK, Peleg Y, Cirié E, Belogus T, Lifshitz Y, Voelker DR, et al. FXyD proteins stabilize Na, K-ATPase: Amplification of specific phosphatidylinositol-protein interactions. J Biol Chem 2011;286:9699-712.
5. Habeck M, Haviv H, Katz A, Kapri-Pardes E, Aycinena S, Shevchenko A, et al. Stimulation, inhibition, or stabilization of Na, K-ATPase caused by specific lipid interactions at distinct sites. J Biol Chem 2015;290:4829-42.
6. Yeagle PL, Young J, Rice D. Effects of cholesterol on (Na+, K+)-ATPase ATP hydrolyzing activity in bovine kidney. Biochemistry 1988;27:6449-52.
7. Cornelius F. Cholesterol modulation of molecular activity of reconstituted shark Na+, K(+)-ATPase. Biochim Biophys Acta 1995;1235:205-12.
8. Shinoda T, Ogawa H, Cornelius F, Toyoshima C. Crystal structure of the sodium-potassium pump at 2.4 A resolution. Nature 2009;459:446-50.
9. Ergün UG, Uguz S, Bozdemir N, Güzel R, Burgut R, Saatçio E, et al. The relationship between cholesterol levels and depression in the elderly. Int J Geriatr Psychiatry 2004;19:291-6.
10. John S, Dharmawardh K, Matagi MV. Study on association between lipid profile values and psychiatric disorders. J Clin Diag Res 2014;8:WC04-6.
11. Kumar AR, Kurup PA. Membrane Na+K+ ATPase inhibition related dyslipidemia and insulin resistance in neuropsychiatric disorders. Indian J Physiol Pharmacol 2001;45:296-304.
12. Lança MJ, Machado M, Ferreira AF, Quintella BR, de Almeida PR. Structural lipid changes and Na+(+)+K(+)(-)-ATPase activity of gill cells' basolateral membranes during saltwater acclimatisation in sea lamprey (Petromyzon marinus L.) juveniles. Comp Biochem Physiol A Mol Integr Physiol 2015;189:67-75.
13. Zibro A, Duchnowicz P, Mulik A, Koter-Michalak M, Broncel M. Oxidative damages in erythrocytes of patients with metabolic syndrome. Mol Cell Biochem 2013;378:267-73.
14. Yao JK, van Kamen DP. Red blood cell membrane dynamics in schizophrenia. I. Membrane fluidity. Schizopr Res 1994;11:209-16.
15. Ghaemi SN, Shields GS, Hegarty JD, Goodwin FK. Cholesterol levels in mood disorders: High or low? Bipolar Disord 2002;2:60-4.
16. Corti C, Xuereb JH, Crepaldi L, Corsi M, Michiein F, Ferrugati F. Altered levels of glutamatergic receptors and Na+K+ATPase-a1 in the prefrontal cortex of subjects with schizophrenia. Schizopr Res 2011;128:7-14.
17. Petronijevic ND, Micic DV, Duricic B, Marinkovic D, Paunovic VR. Substrate kinetics of erythrocyte membrane Na, K-ATPase and lipid peroxidation in schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry 2003;27:431-40.
18. Banerjee U, Dasgupta A, Rout JK, Singh OP. Effects of lithium therapy on Na+K+ATPase activity and lipid peroxidation in bipolar disorder. Prog Neuropsychopharmacol Biol Psychiatry 2012;37:56-61.
19. Solbit KJ, da Luz Oliveira R, Rosenberg DB, Savio LE, Scherer EB, Schnitz F, et al. Na+K+ATPase activity and oxidative status in zebrafish brain: Reversal by antipsychotic drugs. J Neural Transm (Vienna) 2012;119:661-7.
20. Suham M, Na, K-ATPase: Ubiquitous multifunctional transmembrane protein and its relevance to various pathophysiological conditions. J Clin Med Res 2010;2:1-17.
21. Noori S, Zafar H, Mehbobb T. Biochemical effectiveness of cocoa powder on electrolytes homestasis, liver and cardiac specific enzymes and renal functions. Pak J Nutr 2009;8:882-6.
22. Levine RL. Carbohydrate modified proteins in cellular regulation, aging, and disease. Free Radic Biol Med 2002;32:790-6.
23. Macchia T, Mancinelli R, Barbini DA, Taggi F, Micatuno A, Cantafora A. Determination of membrane cholesterol in normal and pathological red blood cells. Clin Chim Acta 1991;199:59-67.
24. Fleckenstein AE, Voit TJ, Riddle EL, Gibb JW, Hanson GR. New insights into the mechanism of action of amphetamines. Annu Rev Pharmacol Toxicol 2007;47:681-98.
25. Kim HK, Andreazza AC, Yeung PY, Isaacs-Trepanier C, Young LT. Oxidation and nitration in dopaminergic areas of the prefrontal cortex from patients with bipolar disorder and schizophrenia. J Psychiatry Neurosci 2014;39:275-85.
26. Grima G, Benz B, Barpura V, Cuñod M, Do KG. Dopamine-induced oxidative stress in neurons with glutathione deficit: Implication for schizophrenia. Schizopr Res 2003;82:219-24.
27. Gysin R, Kraftsk R, Boulat O, Bovet P, Conus P, Ornerie E, et al. Genetic dysregulation of glutathione synthesis predicts alteration of plasma thiol redox status in schizophrenia. Antioxid Redox Signal 2011;15:2003-10.
28. Singh OP, Chakraborty I, Dasgupta A, Datta S. A comparative study of oxidative stress and interrelationship of important antioxidants in haloperidol and olanzapine treated patients suffering from schizophrenia. Indian J Psychiatry 2008;50:171-6.
29. Wan Y, Haller S, Sharpino A, Malhotra N, Tian J, Xie Z, et al. Ouabain-stimulated trafficking regulation of the Na+K-ATPase and NHE3 in renal proximal tubule cells. Mol Cell Biochem 2012;367:175-83.
30. Turner N, Else PL, Hulbert AJ. Doses of dopamine content of membranes determines molecular activity of the sodium pump: Implications for disease states and metabolism. Naturwissenschaften 2003;90:521-3.
31. Turner N, Haga KL, Else PL, Hulbert AJ. Scaling of Na+, K+ ATPase molecular activity and membrane fatty acid composition in mammalian and avian hearts. Physiol Biochem Zool 2006;79:522-33.
32. Gerbi A, Maixent JM, Barbez O, Jamme I, Pierlovisi M, Coste T, et al.
Alterations of Na, K-ATPase isoenzymes in the rat diabetic neuropathy: Protective effect of dietary supplementation with n-3 fatty acids. J Neurochem 1998;71:732-40.

33. Gerbi A, Barbe O, Raccah D, Coste T, Jamme I, Nouvelot A, et al. Alteration of Na, K-ATPase isoenzymes in diabetic cardiomyopathy: Effect of dietary supplementation with fish oil (n-3 fatty acids) in rats. Diabetologia 1997;40:496-505.

34. Patra SM, Chakraborty S, Shahane G, Prasanna X, Sengupta D, Maiti PK, et al. Differential dynamics of the serotonin1A receptor in membrane bilayers of varying cholesterol content revealed by all atom molecular dynamics simulation. Mol Membr Biol 2016;32:127-37.

35. Shrivastava S, Pucadyil TJ, Paila YD, Ganguly S, Chattopadhyay A. Chronic cholesterol depletion using statin impairs the function and dynamics of human serotonin(1A) receptors. Biochemistry 2010;49:5426-35.

36. Cornelius F, Habeck M, Kanai R, Toyoshima C, Karlish SJ. General and specific lipid-protein interactions in Na, K-ATPase. Biochim Biophys Acta 2015;1848:1729-43.

37. Simons K, Ikonen E. How cells handle cholesterol. Science 2000;290:1721-6.

38. Najafipour R, Heidari A, Alizadeh SA, Ghafelebashi H, Rashvand Z, Javadi A, et al. Association between upstream purine complexes of human caveolin-1 gene and schizophrenia in qazvin province of iran. Iran Red Crescent Med J 2014;16:e21484.

39. Lee AG. Lipid-protein interactions in biological membranes: A structural perspective. Biochim Biophys Acta 2003;1612:1-40.

40. Kurup RK, Kurup PA. Isoprenoid pathway-related membrane dysfunction in neuropsychiatric disorders. Int J Neurosci 2003;113:1579-91.

41. Namazi G, Pourfarzam M, Jamshidi Rad S, Movahedian Attar A, Sarrafzadegan N, Sadeghi M, et al. Association of the total cholesterol content of erythrocyte membranes with the severity of disease in stable coronary artery disease. Cholesterol 2014;2014:821686.

42. Gottlieb MH. Rates of cholesterol exchange between human erythrocytes and plasma lipoproteins. Biochim Biophys Acta 1980;600:530-41.

43. Fielding CJ, Fielding PE. Intracellular cholesterol transport. J Lipid Res 1997;38:1503-21.

44. Dietzchy JM, Turley SD. Thematic review series: Brain lipids. Cholesterol metabolism in the central nervous system during early development and in the mature animal. J Lipid Res 2004;45:1375-97.

45. Chen HH, Zhou JF. Low cholesterol in erythrocyte membranes and high lipoperoxides in erythrocytes are the potential risk factors for cerebral hemorrhagic stroke in human. Biomed Environ Sci 2001;14:189-98.

46. Kreilaus F, Spiro AS, McLean CA, Garner B, Jenner AM. Evidence for altered cholesterol metabolism in Huntington's disease post mortem brain tissue. Neuropathol Appl Neurobiol 2015. doi: 10.1111/nan.12286.

47. Giraud F, Ciaret M, Bruckdorfer KR, Chailley B. The effects of membrane lipid order and cholesterol on the internal and external cationic sites of the Na⁺-K⁺pump in erythrocytes. Biochim Biophys Acta 1981;647:249-58.

48. Mahmoud YA. Stabilization of trypsin by association to plasma membranes: Implications for tryptic cleavage of membrane-bound Na, K-ATPase. Biochim Biophys Acta 2005;1720:110-6.