STUDY OF SOME BIOCHEMICAL AND GENETIC ASPECTS OF SCHIZOPHRENIC DISORDERS


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Summary
Molecular genetics and Biochemistry have been devoted to establish the genetic contribution to aetiology of schizophrenia. The biochemical changes in brain neurotransmitters may contribute to the pathogenesis of schizophrenia. The human platelets contain monoamine oxidase (MAO) which is similar in many physiochemical properties to that of the brain, the similarity was also established between brain catechol-O-methyltransferase (COMT) and acetylcholinesterase (AChE) and that of RBCs. So, this study was directed towards monitoring the platelet MAO and RBCs, COMT and AChE as possible indices for the CNS cellular events. The present study was carried out on 144 subjects classified into normal control group free of any psychiatric manifestation and schizophrenic patients group. Assessment of the changes in neurotransmitters metabolism, was tested e.g. that of catecholamine and acetylcholine by determination of the activity of the enzymes involved in its catabolism e.g. MAO, COMT and AChE either by fluorimetric method or colorimetric method. Our results indicated a highly significant reduction in platelets MAO activity among schizophrenic patients than control group \((P<0.001)\). Concerning the COMT activity, there was no statistical significant difference between control and patients group. Assessment of AChE activity indicated a significant reduction in patients group \((P<0.02)\). So, the changes in cholinergic activity in relation of that catecholamine may play a role in the explanations of schizophrenic dysfunction. The genetic contribution was conducted by phenotyping of group specific component (Gc) and phosphoglucomutase I (PGM1) as genetic makers of schizophrenia using isoelectrofocusing techniques. In the present study analyzing the distribution of different Gc genotypes among control and schizophrenic groups demonstrated the increase of Gc 2-1 genotype frequency among schizophrenics \((P<0.001)\) with a relative risk factor of \(RR= 2.56\). There was significant difference in distribution of PGM11+1+ between normal control group and schizophrenic group \((P<0.001)\). No correlation could be detected between MAO, COMT, AChE enzyme activity and Gc genotypes or PGM1 phenotypes.
Introduction

Schizophrenia is a primary chronic progressive disorders characterized by a peculiar disorganization of thought, behavior and affect, with frequent hallucinic of delusion, hallucination, ideas of reference or persecution(1). It is still unclear as to whether schizophrenia is a single disorder or group of disorders. Some behavioral scientists believe that schizophrenia is a clinical syndrome with multiple etiologies(2). Many studies have been devoted to establish the genetic contribution to the etiology and pathogenesis of disease.

Family studies, biochemical genetic, and molecular biology have been utilized to elucidate the genetic basis of schizophrenia. They proved that genes are involved in producing the illness(3,4). Gc is a genetic markers which was associated with schizophrenia.(5).

It has been suggested that biochemical changes in brain neurotransmitters may contribute to pathogenesis of schizophrenia(3). Assessment of the changes in metabolism of neurotransmitters can be tested by determination of the activity of the enzymes involved in their catabolism such as monoamine oxidase (MAO), catechol-O-methyltransferase (COMT) and acetylcholinesterase (AChE)(6,7).

It has been demonstrated that chronic schizophrenic patients have significantly lower platelets MAO than normal control subjects with no significant difference between chronic paranoid and chronic undifferentiated schizophrenic patients(7).

The aim of present work was designed to study:

1. Some of the biochemical aspects of schizophrenic disorder by assessment of the enzymes responsible for catecholamine catabolism namely, MAO ,COMT, and AChE activities as an index to cholinergic dysregulation.(1)

2. Some genetic aspects of schizophrenic disorder through assisting group -specific protein (Gc), phosphoglucomutase enzyme polymorphism (PGM1).

3. Correlation between; Gc and PGM1 phenotypes and MAO, COMT and AChE activities.

Material and Methods

The study was conducted upon 144 individuals of matched age and sex. They were divided into two groups:

A. Control group: composed of 72 normal volunteers who were clinically free from any psychiatric disorder, none of them was taking any medication.

B. Patients group: this group comprised 72 schizophrenic patients. The diagnostic criteria of DSM-III R were followed in the diagnosis of the cases.

The samples were taken from all individuals after taking their consent.

Methods

A. Sample collection:

Ten ml blood were collected by venipuncture into heparinized tubes. The
platelets were separated for measuring MOA enzyme activity. Packed RBCs were used for measuring activities of COMT and AChE enzymes and typing of PGM1. Plasma was used for typing Gc protein.

B. Methods for measuring enzymes activity

Determination of platelet monoamine oxidase activity was carried out according to method of McEntire et al (8). This method had been based on the fluorimetric measurement of 4-hydroxyquinoline (4-OHQ) produced by enzymatic oxidative deamination of Kynuramine. MAO activity is expressed as 4-hydroxyquinoline nM/h/10^8 platelets.

The catechol-O-methyltransferase (COMT) was determined according to method of Axerod and Tomchick (9). This method was based on the fluorimetric measurement of metanephrine produced by enzymatic transmethylation of epinephrine by COMT in presence of S-adenosyl-L-methionine. COMT is expressed as mM/h/ml of packed RBCs.

Assay of acetylcholinesterase (AChE) was performed by the modified colorimetric method of Ellman (10). This method was based on the measurement of yellow color intensity of 5-thio-2-nitrobenzoate anion at 412 nm. This compound was developed by the reaction of thiocholine released by enzymatic hydrolysis of acetylcholine by acetylthiocholinesterase and DTNB. AChE activity is expressed as SH μM/min/ml of packed RBCs.

C. Typing of Gc and PGM1

Gc typing was performed by isoelectric focusing according to method of Westwood and Werrett (11). The sample was treated before application by adding 50 μl of 1% glycine to 450 μl sample and centrifuged at 3000 rpm at 4°C for 10 min to get rid of any salt which may affect protein solubility and particles which hinder the movement of the protein. The run was done on 2117 LKB isoelectrofocusing system. After completion of the run, the gel was immersed in staining solution. The Gc bands appeared as strong white precipitate immediately, seen best on black background with lateral light according to Hoste (12).

Isoelectrofocusing of phospho-glucosamylase (PGM1) was performed according to method of Turowska and Nowicka (13). The sample was prepared by adding 300 μl packed RBCs to 600 μl water, over heated and centrifuged at 1900 g for 10 min at 4°C, and the supernatant was used. The gel was stained according to the method of Pasteur et al, (14).

Results

Table (I) shows the mean ± standard deviation of three enzymes activities of the control and patients groups. MAO activity was lower in patients compared to control. The result was statistically significant (P<0.001). No statistical difference could be detected when activity of COMT in control compared to patients, on other hand there is a significant decrease in the activity of AChE of patients compared to control (P<0.02).

Table (II) shows results of the correlation coefficient between three enzyme activities which did not reveal any level of significance.
Table (I): Mean±SD of MAO platelets, COMT and AChE RBCs activities in control group and schizophrenic patients.

<table>
<thead>
<tr>
<th></th>
<th>MAO (nM/h/10^8 platelets)</th>
<th>COMT (µM/h/ml packed RBCs)</th>
<th>AChE (SH µM/min/ml packed RBCs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>10.36±2.04</td>
<td>1.56±0.36</td>
<td>133.47±5.79</td>
</tr>
<tr>
<td>Patients group</td>
<td>4.95±2.29</td>
<td>1.6±0.33</td>
<td>10.82±3.73</td>
</tr>
<tr>
<td><strong>T</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td>&lt;0.001</td>
<td>NS</td>
<td>&lt;0.02</td>
</tr>
</tbody>
</table>

NS = not significant

Table (II): Correlation coefficient of three enzymes in schizophrenic patients.

<table>
<thead>
<tr>
<th></th>
<th>MAO (nM/h/10^8 platelets)</th>
<th>COMT (µM/h/ml packed RBCs)</th>
<th>AChE (SH µM/min/ml packed RBCs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAO</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>R</strong></td>
<td></td>
<td>0.2172</td>
<td>0.1087</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td></td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>COMT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>R</strong></td>
<td>0.2172</td>
<td></td>
<td>0.1595</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>NS</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>AChE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>R</strong></td>
<td>0.1087</td>
<td>0.1595</td>
<td>-</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>NS</td>
<td>NS</td>
<td>-</td>
</tr>
</tbody>
</table>

Table (III): Gc genotypes distribution in schizophrenic patients and control subjects

<table>
<thead>
<tr>
<th></th>
<th>Patients N=72(%)</th>
<th>Controls N=72(%)</th>
<th>RR</th>
<th>X^2</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gc 1-1</td>
<td>48(66.67)</td>
<td>67(93.05)</td>
<td>0.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gc 2-1</td>
<td>18(25)</td>
<td>3(4.17)</td>
<td>2.56</td>
<td>12.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gc 2-2</td>
<td>6(8.33)</td>
<td>2(2.78)</td>
<td>1.9</td>
<td>1.461</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>Total</td>
<td>72(100%)</td>
<td>72(100%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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Table (IV): Distribution of PGM1 phenotypes among schizophrenic patients and control subjects

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Patients N=72(%)</th>
<th>Controls N=72(%)</th>
<th>RR</th>
<th>X²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1+1-</td>
<td>40(55.66)</td>
<td>25(34.72)</td>
<td>1.7</td>
<td>8.55</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1+1-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-1-</td>
<td>2(2.70)</td>
<td>1(1.39)</td>
<td></td>
<td>1.23</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>1+2+</td>
<td>25(34.72)</td>
<td>34(47.25)</td>
<td>0.56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1+2-</td>
<td>(0)</td>
<td>6(8.94)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2-</td>
<td>(0)</td>
<td>(0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2-</td>
<td>2(2.70)</td>
<td>1(1.39)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2+2+</td>
<td>(0)</td>
<td>3(2.78)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2+2-</td>
<td>2(2.70)</td>
<td>1(1.39)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-2</td>
<td>1(1.3)</td>
<td>2(2.70)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>72(100)</td>
<td>72(100)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The distribution of Gc genotypes in patient and control was shown in table III. Gc 2.1 and Gc 2-2 genotypes were detected more frequently in Schizophrenic patients; 25% and 8.33% versus 4.17% and 2.78% respectively in control and the relative risk was 2.56 and 1.9 respectively. P value of Gc 2-1 was significant (P>0.001) while P value of Gc 2-2 did not reach a significant level.

The distribution of PGM1 genotypes in patient and control was shown in table IV, PGM1 1+1+ was detected more frequently in the patients; 55.66% versus 34.72% respectively in control and the relative risk 1.7. P value was significant (P>0.01).

Discussion

In the present work the activities of three enzymes: MAO, COMT and AChE were measured. Platelets MAO activity were significantly reduced in all schizophrenic patients as compared with control subjects. This finding is agreeable with results obtained by many workers.(15,16,17) It seems likely that reduction in platelets MAO are related to the disease itself rather than the presence or absence of specific symptoms.(16) Also, it is not clear whether MAO reduction in schizophrenic patients is secondary to other psychological differences or represents a primary difference. It has been proposed a molecular aberration of MAO in schizophrenic patients, but this postulation was not be ascertained(16). Another postulation was the alteration of Michaelis constant (Km) for platelets MAO in schizophrenic patients.(16) The stability of low platelets MAO activity over two weeks and over 8 weeks implies that MAO activity may provide general indicator of vulnerability to schizophrenia(17). Furthermore, studies of MAO activity in schizophrenic twins and in schizophrenic
patients with their first degree relatives have shown a remarkably consistent degree of genetic control. However, the mode of inheritance of platelets MAO is still not clear(17,18).

Consistently, the reduced platelets MAO activity could be in accordance with either the dopamine or transmethylation hypotheses of schizophrenia. Hence this reduction could produce an excess of dopamine and or contribute to an increase in methylated tryptamines(19). Also, low MAO activity could yield an excess of phenylethylamine, being another proposed endogenous psychotogen which might produce an amphetamine-like psychosis. On the other hand, the observed decline in platelet MAO activity rather than being a simple reflection of central MAO activity may also influence the course of schizophrenia by acting in the periphery to facilitate the accumulation of toxic amines such as octopamine. In turn, increased blood octopamine concentration could facilitate its entry into CNS where it may function as false neurotransmitter.(19)

Therefore, lowered platelet MAO activity may contribute to the pathogenesis of schizophrenic syndrome.(20)

In the view of dopamine hypothesis of schizophrenia, and the similarity of peripheral COMT and that of the brain, considerable interest in erythrocyte COMT has been generated. In the current data, no significant difference has been revealed, which are in agreement with other workers(21,22).

The increased activity of COMT in schizophrenic patients cannot be attributed to a change in the intrinsic properties of the enzyme but was explained by a considerable overlap among individual samples. Data on increased COMT activity in schizophrenics described by White et al(23) and Mattaysse and Baldesarini (24) might be ascribed to diagnostic and sample differences or to different procedures of assay.

Based on the postulation that altered acetylcholine (Ach) activity usually develops in relation to second neurotransmitter underlying the several pathological neuropsychiatric disorders, many attempts to assess the cholinergic systems in schizophrenia tend to study CSF AChE. Reports have indicated that AChE in CSF cannot distinguish between control subjects and schizophrenics(25). It was concluded that CSF -AChE may not reflect central cholinergic function. So, the peripheral AChE activity of RBCs may prove better candidate for trait markers of central cholinergic function. Determination of serum pseudocholinesterase showed significant increase when acetylcholine and benzolycholine and butyrylcholine were used as substrates. The ratios of the hydrolysis rates of the different substrates showed significant decrease in acetyl/butyryl but benzoyl/acetyl was unsignificant changes.

The present study tended to assess AChE activity in RBCs because it has many properties similar to that of brain(26). The present data identified a significant reduction in AChE in schizophrenic patients compared to normal control subjects. Our results are in accordance with results of Muravich(27) who suggested that true cholinesterase may have a prognostic significance in evaluation of the treatment efficiency and stability of remissions.

Since cholinergic mechanisms have
been proposed to be involved in the coordination of component of behavior in the behavior patterns which are centered in the septohippocampal system,(28) so close interactions of catecholaminergic and cholinergic systems may be assumed to be responsible for the moderation of some secondary symptoms in schizophrenia. However, they are unlikely to be the primary eliciting cause of the disease. This assumption would be supported by the accumulation of evidence implicating neuropeptides to be co-localized within midbrain dopamine neurones e.g. AChE in the pathogenesis and pharmacotherapy of schizophrenia.(29).

With selective attention mechanisms that have been strongly influenced by septo-hippocampal cholinergic activity, it was speculated that abnormal changes of ACh metabolism could result in one of the cognitive disturbance of schizophrenia.

The correlation coefficients of the three enzyme activities were performed in the control as well as patients. No significant correlation could be detected either in the control or in the patients. One assumes that the change in the activity of these enzymes has different mechanisms. Our results show that, there is a significant increase of the Gc 2-1 in the schizophrenic patients compared to normal. A number of investigators (5&30) studied the relationship between Gc protein as genetic marker for schizophrenic patients(5&30). Our results are in agreement with results of Book et al, who found a positive association between Gc-2-1 and schizophrenic patients (5). On other hand, Lange (31) reported a significant increase of the Gc 1-1 among the schizophrenic patients. In contrast to the previous report, Beckman and Perris (32), Rudduck et al, (33) and Fananas et al (34) did not find any association between Gc protein and schizophrenic patients. The difference between our results and results of Lange (31) and other workers (32,33&34) may be due to differences in ethnic groups. Analysis of the distribution of PGM1 genotypes among schizophrenic patients and normal controls showed significant increase in the frequency of PGM 1+1+ in schizophrenic patient than control (P<0.001). McGuffen and Stut (35) failed to detect any evidence for a linkage between PGM1 1+1+ and schizophrenia.

The correlation coefficients of MAO, COMT, and AChE activities and Gc 1-1 and PGM1 revealed unsignificant correlation. So, one can assume that the changes in the activities of the enzymes and genetic markers have different mechanisms.

In view of the results of the present study, we recommend using molecular biology techniques such as restriction fragment length polymorphism, square specific probe, sequence specific oligonucleotides probe and DNA sequence of genes code for MAO, COMT, AChE, Gc and PGM proteins in order to get a good idea about etiology of schizophrenia.

References

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