

Acute Phase Reactants (Proteins) in Schizophrenia

Okasha, T., Elgamel, O. and Ashry, H.

Abstract

A great number of studies show biological alterations in patients with schizophrenia, but many of these data are conflicting. Schizophrenia is a vastly heterogeneous disorder, most likely not caused by one etiological factor, but rather due to a complex network of different, interacting pathogenic influences. There are changes occurring in the immune system as well as the acute phase reactants. This study was carried out on 25 patients diagnosed as non paranoid schizophrenia and 10 controls. The results showed that there is no difference in the scores of the patients and controls. These results show that the process of schizophrenia is more on an immunological level than on an inflammatory level. Further in depth studies on these changes in recommended.

Introduction

Acute-phase reactants (proteins) are a class of plasma proteins whose plasma concentrations increase (positive acute phase proteins) or decrease (negative acute phase proteins) in response to inflammation. This response is called the acute-phase reaction. The levels of these proteins alter in response to tissue injury, inflammation, malignancy and psychological conditions.

Local inflammatory cells (neutrophil granulocytes and macrophages) secrete a number of cytokines into the bloodstream, most notable of which are the interleukins IL-1, IL-6 and IL-8, and TNF-alpha.

The liver responds by producing a large number of acute-phase reactants, most notable of which are: C-reactive protein (CRP), mannose-binding protein, alpha 1-antitrypsin, alpha 1-antichymotrypsin, alpha 2-macroglobulin, some coagulation factors (Fibrinogen, prothrombin, factor VIII, von Willebrand factor, plasminogen), complement factors, ferritin, serum amyloid P component, serum albumin concentrations fall in acute disease states. For this reason albumin is sometimes

referred to as a negative acute phase protein (Pepys and Hirschfield, 2003).

Measurement of acute phase proteins is a useful marker of inflammation.

1) CRP is a member of the class of acute phase reactants as its levels rise dramatically during inflammatory processes occurring in the body. It is thought to assist in complement binding to foreign and damaged cells and affect the humoral response to disease. It is also believed to play an important role in innate immunity, as an early defense system against infections. C-reactive protein is a test of value. Marked rises in CRP reflect the presence and intensity of inflammation.

ESR provides a non-specific screening test for the presence of an acute phase reaction.

Although the ESR and CRP may be valuable indicators of an acute phase response, normal results do not exclude active disease.

2 Mannose-binding proteins is a soluble factor in the human body that binds mannose residues to pathogens. It is part of the immune system's defenses against

bacteria. It is produced in the liver as a response to infection, and is part of many other factors termed acute phase proteins. Mannose-binding protein may also be referred to as mannan binding lectin.

3 Alpha 1-antitrypsin or α_1 -antitrypsin (A1AT) is a serine protease inhibitor (serpin). It protects tissue from enzymes from inflammatory cells, especially elastase, and is present in human blood at 1.5 - 3.5 gram/liter. A1AT is a 52 kDa serine protease inhibitor, and in medicine it is considered the most prominent one, given the fact that the words α_1 antitrypsin and protease inhibitor (P_i) are often used interchangeably.

4 Alpha 1-antichymotrypsin is a alpha globulin glycoprotein and serpin

5 Alpha-2 macroglobulin is a large plasma protein found in the blood. It is produced by the liver, and is a major component of the alpha-2 band in protein electrophoresis.

6 Fibrin is a protein involved in the clotting of blood. Fibrin is made from its zymogen fibrinogen, a soluble plasma glycoprotein that is synthesized by the liver.

7 The complement system is a biochemical cascade of the immune system that helps clear pathogens from an organism. It is derived from many small plasma proteins that work together to form the primary end result of cytolysis by disrupting the target cell's plasma membrane. The actions of the complement system affects both innate immunity and acquired immunity. Activation of this system leads to cytolysis, chemotaxis, opsonization, immune clearance, and inflammation, as well as the marking of pathogens for phagocytosis.

8 Serum Amyloid P component (SAP) is the identical serum form of Amyloid P

component (AP). AP is thought to be an important contributor to the pathogenesis of a related group of diseases called the amyloidoses. (Retrieved from <http://en.wikipedia.org/wiki/>).

A great number of studies show biological alterations in patients with schizophrenia, but many of these data are conflicting. Schizophrenia is a vastly heterogeneous disorder, most likely not caused by one etiological factor, but rather due to a complex network of different, interacting pathogenic influences. Variable clinical pictures may reflect different etiological factors. In a comprehensive theory of the origin of schizophrenic disorders, genetic and environmental influences cause changes in neuronal development which result in functional alterations of different neurotransmitter systems. Immunological research in schizophrenia was initially based on the "infection hypothesis" which was triggered by observing schizophrenia-like psychoses after influenza pandemic. Numerous immunological studies focusing on antibodies against specific viruses, unspecific antibodies and different other immune-phenomena were carried out in schizophrenia patients. Although the variability of the results from these studies is strikingly high, subgroups of patients with schizophrenia show an activated inflammatory response system with increased levels of proinflammatory cytokines and acute phase proteins. Furthermore, some investigations find changing activities in the T-cell system with a shift of TH-1 to an increased TH-2 activity. Endocrinological factors which may play a relevant role in the etiopathogenesis of schizophrenia include sex hormones and all changes caused by stress or other influences which are directly related to the HPA-axis. Alterations of the

immune and the endocrinological systems might be caused by environmental factors like infections or exogenous stress. Due to the intensive interaction between the central nervous system, the immune system and different hormones the "development of a pathology" like schizophrenia can be seen in an integrative but multifactorial fashion. The clinical manifestation, the severity and the course of the disease might then be modulated by genetic vulnerability, the time of the "primary insult" -- which could be an infection or psychological stress -- and its neuronal localization and intensity. Different compensatory and decompensatory mechanisms in later life very likely play a crucial role for the further course of the disorder (Sperner, 2005)

In this study we tried to evaluate the levels acute phase proteins in a sample of Egyptian patients suffering from schizophrenia.

Subjects and Method

This study was carried out at the Institute of Psychiatry, Ain Shams University Hospitals over a period of 5 months. The study included 25 patients (18 males and 7 females), as well as 10 controls (4 females and 6 males). The inclusion criteria for the patients were:

Inpatients at the Institute of Psychiatry Ain Shams University Hospitals.

Ages between 21 and 41 years

Both males and females patients were included

Patients were diagnosed as suffering from non-paranoid schizophrenia according to the ICD-10 Research and Diagnostic Criteria (1993) using the ICD-10 symptom checklist (1994). Non-paranoid schizophrenia was chosen as most of the

studies show that these forms of schizophrenia are richer in structural brain changes as well as brain imaging changes and genetic findings which will lead them to have more immunological and inflammatory changes, while the paranoid form is more environmentally determined.

All laboratory tests were done within 48 hours of admission after being diagnosed and before starting treatment.

All patients were not taking any medication for at least 6 weeks and did not receive any ECT sessions at least 6 months prior to joining the study.

Informed consent was taken from patients or their families to join the study.

The entire patient group had no co-morbid medical illness, or co-morbid axis 1 psychiatric diagnosis, or substance use disorder.

A control group selected from the employees of the institute of psychiatry were matched to age, sex and educational level of the patient group and had no medical illness, or psychiatric morbidity assessed by the general health questionnaire (GHQ) (Goldberg, 1988) in its Arabic version (Okasha, 1988). The entire control group gave their consent to participate in study.

ESR, C reactive protein, Alpha 1 antitrypsin, Fibrinogen and Complement 3 were evaluated for all patients and controls, however, Haptoglobin, Alpha 1 antichymotrypsin and Ceruloplasmin from the acute phase reactants were not assessed due to unavailability of the kits at the time of the study.

All laboratory investigations were carried out at the Institute of Psychiatry laboratory,

where ESR was measured in mm/hr at 20 degrees + or - 3 degrees.

C₃ and AAT were estimated by Radial immuno diffusion (RID) plates (manufactured by Biocientifica S.A.) Serum samples were collected and stored at - 20°C using Berne Method (1974).

C- reactive protein was detected by Latex Seralogy Test (Avitex) from omega diagnostics LTD when latex suspension coated with antibodies to human CRP is mixed with serum, clear agglutination is seen within 2 minutes (Ward, 1975).

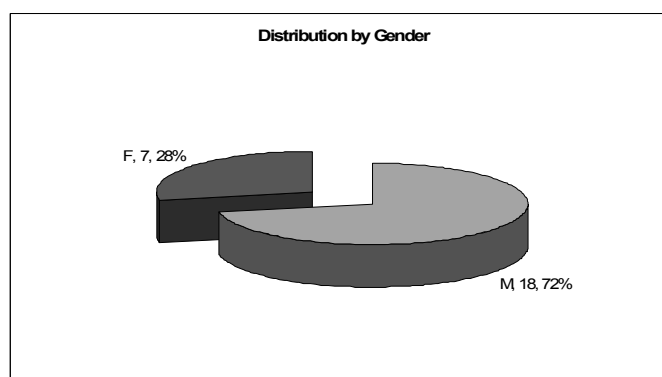
Erythrocyte sedimentation rate was done using the Westergren method.

Fibrinogen was assayed by Multifibrin U test (Dadebehring) using fibrintimer.

Results

In this study the mean age for the patient group was 27.56 (± 4.37), while that for the control group was 27.30 (± 4.40). Out of the 25 patients 18 were males (72%) and 7 were females (28%), while in the control group, out of the 10 controls 6 males (60%) and 4 females (40%) (Figures (1) and (2) respectively).

Figure (1)



Regarding the ESR levels the mean level in the patient group was 14.84 (± 11.14), while that of the control group was 13.30 (± 11.68) with no significant difference.

Comparing the results of both groups as regard the acute phase reactants, we found that the C reactive protein was negative in 24 patients out of the 25 and was also negative in the control with no significant difference.

The fibrinogen mean level result was 2.88 g/l (± 1.60) in the patient group and 2.64 g/l (± 0.89) in the control group with no significant differences between both groups.

There was also no significant difference between both groups regarding the mean level of C3, which was 144.80 mg/dl (± 30.33) in the patient group and 145.10 mg/dl (± 52.12) in the control group.

Similarly, no significant differences were found between both groups regarding the mean level of Alpha 1-antitrypsin (A1AT), which was 173.96 mg/dl (± 33.43) in the patient group and 173.10 mg/dl (± 44.25) in the control group (Table (1) and Figure (3)).

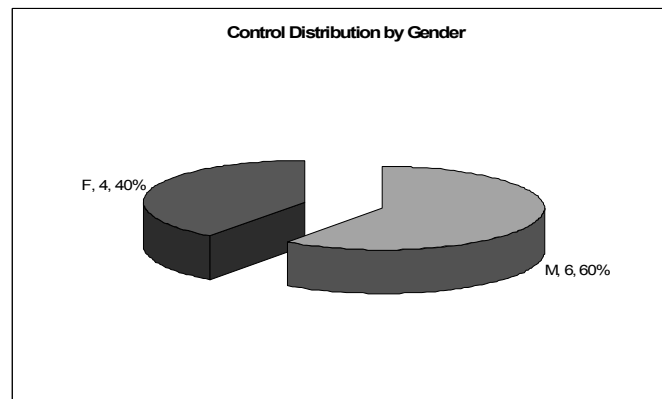
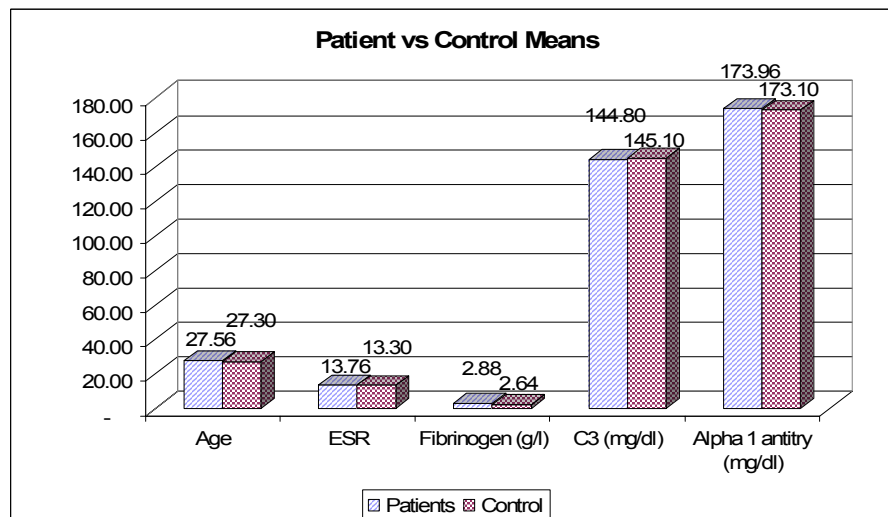
Figure (2)

Table (1) shows the comparison of the different mean levels of acute phase reactants (ESR, fibrinogen, C3 and A1AT) between both the patient and the control groups.

Item	Patient group	Control group
ESR	14.84 (± 11.14)	13.30 (± 11.68)
Fibrinogen (g/l)	2.88 (± 1.60)	2.64 (± 0.89)
C3 (mg/dl)	144.80 (± 30.33)	145.10 (± 52.12)
A1AT (mg/dl)	173.96 (± 33.43)	173.10 (± 44.25)

Figure (3) shows the differences in mean levels of age, ESR, fibrinogen, C3 and A1AT in both the patient group and the control group with no significant difference between both groups.

Figure (3)

Discussion

A working model to understand schizophrenia would help understanding the process of the disorder. It is suggested that DNA, gene expression, viruses, toxins, nutrition, birth injury and psychological experiences all play a role in the aetiology of schizophrenia. These aetiological factors lead to the pathophysiology of the disorder mainly affecting the brain development which includes neuron formation, migration, pruning, and apoptosis. This will in turn lead to affection of the neural connectivity and communication causing impairment in the fundamental cognitive process (thinking) causing impairment in the second order cognitive processes which include attention, memory and language. All this will lead to the appearance of the symptoms of schizophrenia. This working model helps us take into account all the different factors that may be involved in the schizophrenia process (Okasha, 2006).

There is a growing body of opinions affirming schizophrenia is a spectrum disease covering several conditions of different aetiology. Various studies have recently shown immunological changes in schizophrenia, and an immune pathogenetic hypothesis has gained acceptance. In a study carried out by Mazzarello *et al.* (2004), they analyzed with a relatively wide approach the immunological dysfunction in schizophrenia, focusing in particular on lymphocytes morphology and subset distribution. They performed in peripheral blood samples of 24 schizophrenic patients, assessment of acute phase proteins and immunological variables and found an increased serum CRP concentration (mg/ml), which is different from the results of our study since the timing of sampling was different in both studies. Also, the

difference in patient sample where they included all subtypes of schizophrenia, while in our study non-paranoid schizophrenia was chosen as most of the studies show that these forms of schizophrenia are richer in structural brain changes as well as brain imaging changes and genetic findings which will lead them to have more immunological and inflammatory changes, while in comparison the paranoid form of schizophrenia is more environmentally determined.

An acute phase protein (AP) response has been reported in major depression. In order to examine whether an AP response occurs in other psychiatric disorders, such as schizophrenia and mania, Maes *et al.* (1997) measured plasma acute phase proteins such as haptoglobin (Hp), immunoglobulin G (IgG), IgM, fibrinogen (Fb), complement component 3 (C3C), C4, alpha 1-antitrypsin (alpha 1 AT), alpha 1-acid-glycoprotein (alpha 1S) and hemopexin (Hpx), in 27 schizophrenic, 23 manic, 29 major depressed and 21 normal subjects. Schizophrenic patients had significantly higher plasma Hp, Fb, C3C, C4, alpha 1S and Hpx than normal controls. Manic subjects showed significantly higher plasma Hp, Fb, alpha 1S and Hpx than normal volunteers. Depressed subjects had significantly higher plasma Hp, Fb, C3C, C4 and alpha 1S than normal controls. Overall, the above disorders in AP reactants were more pronounced in schizophrenic than in depressed subjects. No significant differences in the above AP reactants could be found between normal volunteers, and schizophrenic, manic or depressed patients who underwent chronic treatment with psychotropic drugs. The results suggest that not only major depression but also schizophrenia and mania are accompanied

by an AP response, and that the latter may be suppressed by (sub) chronic treatment with psychotropic drugs.

Chiu and his colleagues (1999) studied a common polymorphism in the alpha1-antichymotrypsin (ACT) gene which is associated with Alzheimer's disease. ACT is also a trophic factor in the hippocampal neurons. In order to examine if the ACT gene plays a role in the pathogenesis of schizophrenic disorders, patients (n = 175) and control subjects (n = 114) were genotyped for ACT. The results demonstrated no association between schizophrenia and cognitive deficit in schizophrenia and ACT polymorphism. The data suggest that the ACT gene is not of major importance for the genesis of schizophrenia. In our study we were not able to study the antichymotrypsin since the laboratory kits were unavailable but from the negative results we reached in the other acute phase proteins we can say that the results would have been similar for antichymotrypsin.

In a study carried out by Wong et al. (1996) measuring the changes in the concentration of some serum acute phase proteins (alpha 1-antitrypsin, alpha 2-macroglobulin, complement C3, haptoglobin, ceruloplasmin, transferrin, albumin and hemopexin, thyroxine-binding globulin, retinol-binding globulin, plasminogen and Gc-globulin) are reported in two separate series of Chinese, male schizophrenic patients and healthy controls. In the first series, 41 healthy blood donors and 98 schizophrenic patients in different stages of the disease were investigated. The second series consists of a random sample of 50 acutely ill schizophrenic patients and a second group of healthy subjects. The concentrations of these serum proteins were

measured by rocket immunoelectrophoresis in agarose gel. Increased levels of serum alpha 1-antitrypsin, alpha 2-macroglobulin, haptoglobin, ceruloplasmin, and thyroxine-binding globulin were observed in both series of patients when compared to their respective controls. Albumin, transferrin and retinol-binding protein levels were reduced in patients in both series. Hemopexin levels were increased only in the acutely ill patients while complement C3 was decreased in the chronically ill patients. No changes were observed in the Gc-globulin levels of all groups of patients. With the exception of complement C3, the changes observed in the levels of these serum proteins were appropriate for that of an acute phase response. Differences from our study are due to the different laboratory methods used and the sample was carried out on Egyptian patients who have a different ethnic background and may show a different response to environmental stressors.

We can conclude from this study that the acute phase proteins are not the main changes taking place in patients with schizophrenia as there is no acute inflammatory response, but rather an earlier and more subtle immunological change which does not directly affect the acute phase proteins or elevate them to a level which can be considered as an acute inflammatory response. The differences in the results obtained from different studies suggests that there is a deficiency in the process of investigating the acute proteins, also there has been a dramatic shift to studying the different genes involved in schizophrenia and their polymorphism.

Limitations of the study

The limitation of this study rests in three main domains, first the number of patients

was limited in order to generalize these findings on all patients, secondly the patients should be in acute relapse of schizophrenia when being assessed, and thirdly a wider evaluation of the acute phase proteins and immune system changes should be carried out in future studies.

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Authors

Okasha T.

Assistant Professor of Psychiatry,
Institute of Psychiatry,
Ain Shams University

Elgamel O.

Consultant Clinical Pathology,
Institute of Psychiatry,
Ain Shams University.

Ashry H.

Specialist Clinical Pathology,
Institute of Psychiatry,
Ain Shams University.

Address of Correspondence:

Dr. Tarek A. Okasha
3, Shawarby Street, Kasr El Nil, Cairo,
Egypt.
E-mail: tokasha@internetegypt.com

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