



CLOZAPINE INDUCED NEUTROPHIL CYTOTOXICITY IN SCHIZOPHRENIC PATIENTS.

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ABSTRACT

The risk of potentially life threatening idiosyncratic reactions, limited the use of clozapine therapy, in the treatment refractory schizophrenia. The present study aims to investigate the neutrophil cytotoxicity in the clozapine treated schizophrenic patients, as it is still prescribed frequently for psychiatric patients in Pakistan. The assessment was focused on the hematological and absolute indices; serum iron profile, cell viability and morphological examination of peripheral blood smear, in the clozapine treated schizophrenic patients (n=56) by using standardized laboratory techniques and automated analyzers. The striking finding is the presence of neutrophil cytotoxicity; observed most frequently in the peripheral blood smear of the clozapine treated patients. All the stages of cytotoxic effect like vacuolation in cytoplasm, nuclear fragmentation and chromatolysis was observed in the neutrophil during differential counting with significant decrease in neutrophil count suggesting agranulocytosis. Our data, raised concerns about hematological side effects of clozapine treatment in order to provide hematological safety leading to decrease morbidity and mortality related with this drug.

KEYWORDS: Neutrophil Cytotoxicity, Clozapine, Schizophrenia, Agranulocytosis, Neutropenia.



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INTRODUCTION

Clozapine, an atypical antipsychotic drug used for the treatment refractory schizophrenia, carries a high risk of inducing agranulocytosis in 1 to 2% of patients¹⁻², neutropenia and thrombocytopenia in 1 to 3% of patients; and anemia, Leukocytosis and thrombocytosis in less than 1% of patients³⁻⁵. The actual mechanism of clozapine-induced agranulocytosis is not known, but a toxic serum factor with characteristics of an antibody was hypothesized as a plausible mechanism⁶ along with limitations⁷.

Clozapine undergoes extensive metabolism in several species, including man and rodent bile, principally to the stable metabolites like demethylclozapine, clozapine N-oxide and several other glucuronide and sulfate conjugates⁸. In humans clozapine was metabolized to a reactive intermediate by hepatic cytochrome P450 enzymes⁹⁻¹⁰, myeloid cells and peripheral blood PMN to a radical cation and then nitrenium ion by the enzyme myeloperoxidase (MPO)¹¹⁻¹³. However, in rodents it undergoes extensive bioactivation by *in vivo* detection of several glutathione (GSH) conjugates^{7, 14}. The nitrenium ion has been implicated in the pathogenesis of the agranulocytosis. The interaction of the nitrenium ion with a cellular macromolecule may leads to either direct toxicity or indirect immune-mediated toxicity, although the exact mechanism by which toxicity occurs is unclear^{7, 14, 15-17}.

The high incidence of recurrence of agranulocytosis in patients re-challenged with the drug¹⁸⁻¹⁹, indicates genetic predisposition in the pathogenesis of this disorder¹⁵. Different mechanisms are probably involved, but to date there was no clear evidence of an immune or direct toxic effect of the drug or its metabolites on cells of the myeloid lineage²⁰⁻²². In addition, one of the possible hypothesized mechanism underlying clozapine-induced agranulocytosis involves the covalent modification of neutrophils/bone marrow proteins by clozapine, either by a direct toxicity or via immunological

mechanism. For instance, the alteration in the function of the proteins that are responsible for survival of the cells, by the covalent binding of clozapine, lead to cell death, or it might be possible that the immune system could recognize this complex, generating an antibody- or a cell-mediated response against the cells. Gardner et al., (1998)²³ reported clozapine-modified polypeptides (49 and 58-kDa) from neutrophils in patients treated with clozapine. Wasti et al., (2006)²⁴ has also reported clozapine induced neutrophil cytotoxicity in rats following chronic clozapine treatment. The toxic effect of drug was observed on neutrophils with condensation and subsequent breakdown of chromatin material however the mechanism by which metabolites of clozapine cause neutrophil toxicity remains to be investigated. With reference to our previous finding of clozapine induced neutrophil cytotoxicity in an animal model²⁴, the aim of present study is to investigate the hematological variations and cytotoxicity in the schizophrenic patients on the chronic clozapine treatment.

MATERIALS & METHODS

The blood samples were collected after obtaining written informed consent from the patients at different psychiatric hospital all over Karachi. All patients (both genders M/F) met the criteria to the diagnosis of schizophrenia according to the DSM-IV (Diagnostic and Statistical Manual of Mental Disorders IV) and were particularly on clozapine treatment (n=56, run in duplicate), additionally as an active control, haloperidol treated patients was taken in order to verify our results, according to the normal clinical practice for more than 12 weeks. However, healthy individuals (n=44) were taken as control group, selected at random with no previous history of any psychiatric disorder, any other common diseases such as diabetes, cardiovascular diseases, bacterial or viral infection or on any type of medication. The diagnostic criteria were established on the basis

of the positive and negative syndrome scale (PANSS) according to the Diagnostic and Statistical Manual (DSM) IV. A group of psychiatrist reviewed the medical record and where necessary, interviewed the patients. The records of the schizophrenic patients were also used to determine the sex, age of the patients, age at the first onset of illness, duration of illness, duration of antipsychotic treatment, current daily antipsychotic drug dosage. Automated chemistry analyzer (Automated Analyzer STAT Lab 300 Plus) was used to determine hematological parameters, including hemoglobin, hematocrit, RBC and differential counts, absolute indices, Red cell Distribution Width (RDW). While morphological features of RBCs by peripheral blood smear was performed using standard Leishman's staining methods. Additionally, Serum Iron Concentration (SIC), Total Iron Binding Capacity (TIBC) (Roche Ltd.) and the serum ferritin level (Randox Ltd.) were also determined in the schizophrenic patients and control group. The cell viability was performed to determine the number of apoptotic and non-apoptotic cells by differential count. Differential diagnosis on morphological examination of blood smear was also done by using Differential Contrast Electron Microscopy by Nikon (Japan). Statistical analysis was performed using standard statistical software (SPSS version 16.0). All data are expressed as mean \pm SD. The data were also tested using paired t-tests with the significance level set as $p < 0.05$.

RESULTS

The present study focused on the hematological, biochemical and morphological assessment, in the schizophrenic patients chronically treated with an atypical antipsychotic drug clozapine (n=56) with respect to normal controls (n=44). However, the haloperidol treated patients were incorporated as an active control in the present study (detailed data not shown). The socio-demographic and clinical features of schizophrenic patients chronically treated with clozapine and haloperidol are

summarized in Table-1. No significant difference was observed in the two treatment groups on age, age at 1st onset of illness, duration of treatment and dosage of antipsychotic drug treatment. Significant ($p < 0.001$) positive correlation of PANSS score was observed in both haloperidol and clozapine treated schizophrenic patients with % positive ($r = 0.79$ and 0.815) and negative symptoms ($r = 0.69$ & 0.820) respectively. The age at 1st onset of illness shows significant ($p < 0.05$) positive correlation ($r = 0.49$) in case of haloperidol treated patients with PANSS, however negative correlation ($r = -0.62$) was observed in clozapine treated schizophrenic patients. While no significant correlation was observed with other variables (Table-2). The assessment of extra pyramidal (EPS) side effects of the typical antipsychotic drug haloperidol was evaluated as reported in the history profile of patient on different stages during treatment. Significant differences in the side effects profile of haloperidol and clozapine at their clinically effective dosage were observed (Table-3). Extra pyramidal syndrome EPS like Dystonia, Parkinsonism, Akathisia, and Tardive Dyskinesia were significantly more common in patients treated with haloperidol. In contrast, agranulocytosis was significantly more common in the clozapine treated patients.

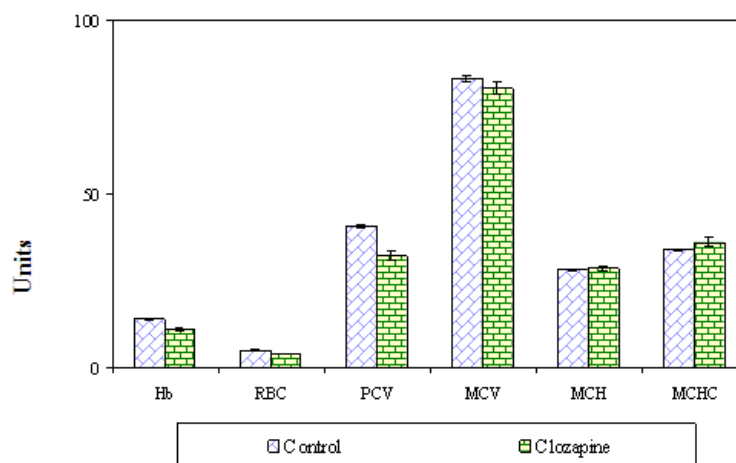
The hematological profile of the schizophrenic patients (n=56) treated with clozapine shows slight decrease in hemoglobin concentration (11.26 ± 1.85), RBCs counts (3.9 ± 0.61), PCV (32.16 ± 7.95) and MCV (80.50 ± 8.72) except slight increase in MCH (28.63 ± 3.16) while MCHC values (36.1 ± 6.66) are not attributed to anemia (Figure-1). Moreover, the hematological profile of the haloperidol treated patient's which was taken as an active control group; shows significant decrease in the hematological and absolute indices suggesting the presence of anemia. Analysis of peripheral blood smear allows interpretation of multiple visible characteristics of the red blood cells (RBC), including shape, size and pigmentation. No characteristic changes in RBCs morphology was observed subsequent to clozapine treatment in the

schizophrenic patients (Figure-2). Iron profile is a useful biochemical marker to confirm iron deficiency when other findings are inconclusive. In case of schizophrenic patients treated with clozapine, no significant changes were observed in the iron profile except marked decrease in Ferritin (33.9 ± 22.71) level (Figure-3). However, characteristic presence of anemia with changes in RBC's morphology and Iron profile was observed in haloperidol treated patients. The striking finding of the present study is the presence of neutrophil cytotoxicity frequently present in the peripheral blood smear of the clozapine treated schizophrenic patients. All the stages of cytotoxic effect like vacuolation in cytoplasm, nuclear fragmentation and

chromatolysis was observed in the neutrophil during differential counting (Figure-4), indicating that some cytotoxic metabolites may be involved in the pathogenesis of clozapine induced neutrophil cytotoxicity. Table-4 summarizes the total and differential WBC count in the schizophrenic patients following chronic treatment of clozapine. significant increase ($p < 0.05$) was observed in WBC count and lymphocyte count contrary to the neutrophil count, confirming the presence of agranulocytosis/ neutropenia in the schizophrenic patient chronically treated with clozapine as compared to their respective controls.

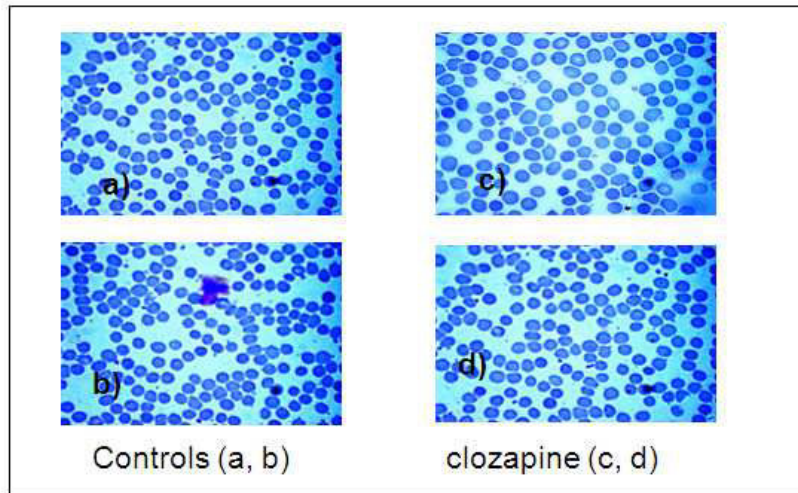
Figure 1

Comparison of Hematological indices in clozapine treated schizophrenic patients.



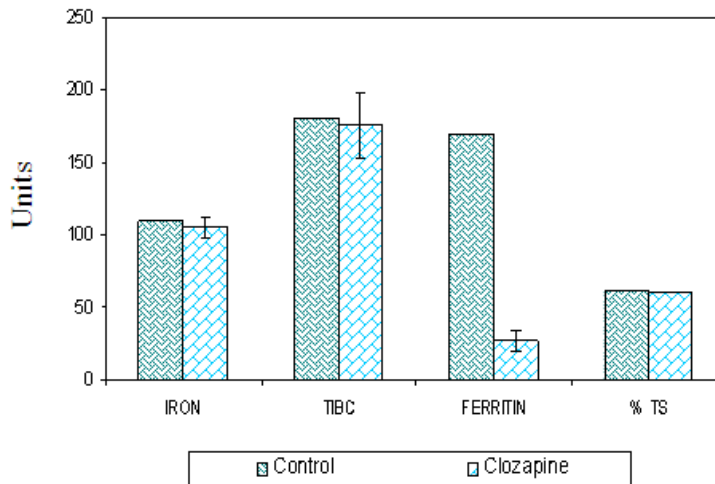
Hb- hemoglobin (g/dl), RBC- Red blood cell concentration (Cu.mm), PCV-Packed cell volume (%), MCV-Mean cell volume (fl), MCH-Mean cell hemoglobin (pg), MCHC- mean cell hemoglobin concentration (%), RDW-Red cell distribution width (%).

Figure 2
Light microscopy of Leishman's stained slides of peripheral blood smear of clozapine treated schizophrenic patients.



The peripheral blood smear of the control (a & b), and clozapine treated patients (c-d) individual, show Normocytic and Normochromic morphology of RBC.

Figure 3
Comparison of Iron profile of clozapine treated schizophrenic patients with normal controls.



Iron profile: Iron ($\mu\text{g/dl}$), TIBC-Total iron binding Capacity ($\mu\text{g/dl}$), Ferritin (ng/dl), %age TS-Transferrin saturation (%).

Figure 4

Neutrophil cytotoxicity in schizophrenic patients on clozapine treatment showing all three stages of toxicity i.e. vacuolation in cytoplasm, nuclear fragmentation and chromatolysis.

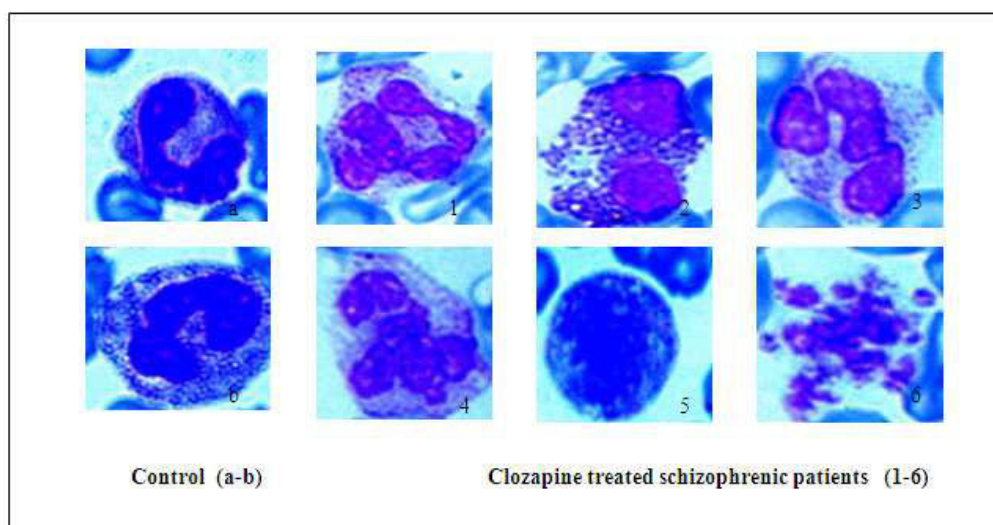


Table 1
Demographic and clinical features of schizophrenic patients treated with haloperidol and clozapine.

Variables	Schizophrenic Patient's	
	Haloperidol	Clozapine.
Age (years)	29.07±9.05	27.0±7.10
Age at 1 st onset of illness	24.07±7.48	21.09±3.71
Duration of treatment	3.66±3.03	3.53±4.01
Dosage of AP drugs (mg/day)	22.30±8.39	22.4±36.2
Positive symptoms (%PS)	40.47±9.77	39.36±11.05
Negative symptoms (%NS)	56.37±8.26	69.31±11.92
PANSS	48.41±6.74	55.71±10.79

• Values are Mean± S.D.

• PANSS- positive and negative symptoms scores.

Table 2
Correlation between PANSS and other clinical parameters.

Variables	Haloperidol	Clozapine
Age (years)	r = 0.26 (ns)	r = -0.27(ns)
Age at 1 st onset of illness	r = 0.49**	r = -0.62**
Duration of treatment	r = -0.013(ns)	r = 0.27(ns)
Dosage of AP drugs (mg/day)	r = -0.10(ns)	r = -0.09(ns)
Positive symptoms (%PS)	r = 0.79***	r = 0.815***
Negative symptoms (%NS)	r = 0.69***	r = 0.820***
Hemoglobin	r = 0.178(ns)	r = 0.026(ns)
Serum iron	r = 0.072(ns)	r = -0.073(ns)

* Correlation analysis (r) (p<0.05) **, (p<0.001)***, ns- not significant.

Table 3
Antipsychotic drugs and side effects profile.

Adverse affects	Haloperidol	Clozapine
EPS like (Dystonia, Parkinsonism, Akathisia, Tardive Dyskinesia)	+++++	+
Sedation	++	+++++
Hypo-tension	+	+++++
Agranulocytosis	—	++++

- The severity of side effects are represented by symbol +/_.
- Haloperidol (Dose-2-200mg/day)
- Clozapine (Dose-5-60mg/day)

Table 4
The total and differential WBC count in the schizophrenic patients following chronic treatment of clozapine.

Experimental Groups	Controls(n=44)	Clozapine (n=56) Treated Schizophrenic Patients.
WBC	7525±1541	8040±2177*
Lymphocytes	34±12.33	50.05±20.82 *
Neutrophils	47.5±16.6	35.23±20.27*.
Eosinophils	4.0±2.02	2.69±2.055*
Monocytes	2.0±1.20	1.53±0.647*

* The results were reported as Mean ±S.D.

* Significance of differences: P<0.05 – Student's t-test.

DISCUSSION

The present study aims to investigate clozapine induced neutrophil cytotoxicity in schizophrenic patients. Our study showed adverse effects of the medication. Consistent with our previous findings (Wasti et al., 2006)²⁴ there are certain parallels between the findings in human and rats on long-term clozapine treatment regarding neutrophil cytotoxicity, in all experimental animals treated with pure or commercial preparations of clozapine. However, the findings of the present study shows that more than 40 % of clozapine treated schizophrenic patients go on to develop cytotoxicity suggesting additional risk factors in its development. Although in this study we did not attempt to identify directly the reactive metabolites of clozapine, but it is interesting to note all stages of cytotoxic effect like vacuolation in cytoplasm, nuclear fragmentation and chromatolysis in the neutrophil during differential counting (Figure-4) which has not been reported so far. Clozapine is commonly assumed to have a cumulative incidence of 0.8% per year of agranulocytosis²⁵⁻²⁷. Consistent with the reported side effects of clozapine treatment such as agranulocytosis and/or neutropenia, our results suggests significant increase ($p < 0.05$) in WBC count and lymphocyte count with an significant decrease in the neutrophils count, confirming the presence of agranulocytosis/ neutropenia in clozapine treated schizophrenic patient (Table-4). The mechanism underlying cytotoxicity is not yet delineated. However it has been proposed that bioactivation of clozapine to a reactive metabolite²³ and the subsequent covalent binding of this reactive intermediate to both human and rat neutrophils as the plausible explanation for a role in clozapine-induced neutrophil cytotoxicity. Hepatic cytochrome P450 enzyme¹⁰, MPO and peripheral blood PMN²⁹ are observed in human (Invitro) to metabolized clozapine into a reactive

intermediate possibly nitrenium metabolites that cause cytotoxicity at therapeutic drug concentration. Previous studies³⁰⁻³¹ have demonstrated, that clozapine becomes covalently bound to human neutrophils during their maturation or after release of the neutrophils into peripheral circulation. There is evidence to support this contention; for example; it is possible that the covalent binding of clozapine to neutrophils in patients treated with clozapine is not due to reactive metabolites generated by the neutrophils but rather to clozapine reactive metabolite produced in the liver and then released into the blood. Observation on rodent⁷ demonstrates that in vivo clozapine undergoes hepatic bioactivation to a reactive intermediate excreted as glutathione conjugates¹⁴. Hence a probable mechanism by which neutrophils become covalently modified by the reactive metabolite is the release of clozapine reactive metabolites by the liver. In conclusion, this study also emphasized on the potential hematological complication of the antipsychotic drugs, prescribed to the patients of schizophrenia and other disorders. The baseline data of our population, with limitation due to the sample size; will help in determining hematological disorders; which is critical in order to design better screening procedures, dose monitoring and to provide hematological safety leading to decrease morbidity and mortality related with atypical antipsychotic drugs.

CONTRIBUTORS

Author Dr. Afshan Zeeshan Wasti conducted clinical study, undertook statistical analyses and manuscript write-up. Author Prof. Dr. Nikhat Ahmed designed, supervised the study and finalized the manuscript. All authors contributed to and have approved the final manuscript.

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CONFLICT OF INTEREST

None of the authors have a conflict of interest to declare.

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