UNIVERSITY of TRIPOLI

Faculty of Science, Zoology Department

INCIDENCE OF AGRANULOCYTOSIS AS AN ADVERSE EFFECT OF ANTIPSYCHOTIC DRUGS IN LIBYIAN PATIENT (AL-RAZI HOSPITAL)

A Thesis Submitted in Partial Fulfillment for the Requirement of the Degree of Master of Science in Zoology

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الإدارة العامة للدراسات العليا والتدريب بالجامعة

قـــــرار لجنـــــة المناقشـــــة والحـــــكم

الكلية العلوم القسم علم الحيوان

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أنا د. **سهيرة امحمد أبوراوي**،عضو هيئة تدريس بكلية **الصيدلة** والمشرفة على رسالة الطالب **محمد أحمد محمد موسى**، بتاريخ 2017 /3/22ف .

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أمنة

Dedication

This thesis is dedicated to **my father,** who taught me that the best knowledge is that which is learned for it own sake, and to **my mother** ,who taught me that the hardest tasks can be accomplished if it is done one step at a time.

Last, this work is dedicated to **my wife** and **children** for their patient during the accomplishment of this work.

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Summary

Schizophrenia is a common psychiatric disorder of high incidence, affecting approximately 1% of the world population. The essential neurotransmitter pathology of schizophrenia remains poorly defined, despite huge advances over the past half-century in identifying neurochemical and pathological abnormalities in the disease. Even though Genetics is considered as an important contributory factors, Antipsychotics may produce agranulocytosis. The mechanisms underlying the development of agranulocytosis have not been completely identified. Granulocyte-macrophage colony stimulating factor (GM-CSF) is one of a family of glycoprotein cytokines that have potent effects in stimulating proliferation, maturation, and function of hematopoietic cells. GM-CSF acts as a potent growth factor both in vitro and in vivo, stimulating proliferation and maturation of myeloid progenitor cells, giving rise to white blood cells..

The objectives of this study were to estimate the incidence of drug-associated agranulocytosis in newly diagnosed schizophrenic Libyan patients and to evaluate their risk factors and outcomes. The study includes: Screening for the expression and function of circulating leukocyte granulocyte-macrophage colony-stimulating factor receptor (CD116); also screening of patient's biochemistry and hematology. Screening for the expression and levels of GM-CSF, biochemistry and hematology were done using specific diagnostic kits for each.

GM-CSF expression were decreased after antipsychotic treatment for one month and continued to decrease after two months' treatment. GM-CSF expression starts to increase after the two-month treatment and continue increasing to the levels of healthy or newly diagnosed schizophrenic patients or after chronic treatment.

Complete blood counts were not changed compared to the normal levels. **Liver function** showed transient increase in ALK after one and two months' treatment. All other parameters were not changed compared to normal levels. **Kidney function** showed that Urea and creatinine levels were within the normal range during the different treatments. Concerning **Lipid profile**, while all other parameters were within normal range, LDL levels was increased after one , two months of treatment and after chronic administration of the antipsychotic drugs .

الملخص

مرض الفصام من الامراض الشائعة والتي تقدر نسبة حدوثها بحوالي 1% من السكان حول العالم، ولازال المسبب العصبى لهذا المرض غير مكتمل المعرفة بالرغم من الدراسات العديدة التي اجريت فى نصف القرن الماضي لمعرفة التغيرات المرضية والكيميائية لهذا المرض . تعتبر الوراثة من العوامل المهمة في هذا المرض. تتسبب الادوية النفسية في نقص شديد في كرات الدم البيضاء لكن الالية غير معروفة. ينتمي العامل المحفز لكرات الدم البيضاء والبلعمية الي أحد البروتينات السكرية (السيتوكينات) التى لها تأثير فعال في تحفيز نمو ونضج ووظائف الخلايا الدموية. يعمل هذا المحفز لكرات الدم البيضاء والبلعمية كعامل نمو قوي سواء في المختبر أو في الاجسام الحية، حيث يحفز نمو ونضج الجذعات الدموية بالنخاع التي تتحول الي الخلايا الدموية البيضاء.

تهدف هذه الدراسة الي معرفة حدوث النقص الحاد لكرات الدم البيضاء الناتج من تناول الادوية النفسية للمرضى الليبيين المصابين بمرض الفصام (حديثى التشخيص) ، ولتقييم هذه العوامل والمخاطر أشتملت هذه الدراسة فحص النشاط والوظيفة لمستقبلات العامل المحفز لكرات الدم البيضاء والبلعمية، وكذلك فحص الجوانب الدموية والكيمياء الحيوية باستخدام كواشف تشخيصية خاصة (Kits).

أظهرت التجارب المعملية نقص في العامل المحفز لكرات الدم البيضاء والبلعمية بعد شهر واحد من تناول الادوية النفسية، ويستمر النقص طيلة الشهر الثاني من العلاج. يبدأ العامل المحفزلكرات الدم البيضاء والبلعمية فى الزيادة بعد الشهر الثاني من تناول الادوية النفسية، ويستمر فى الزيادة طيلة الشهر الثالث من العلاج حتى يصل لمستويات طبيعية، بما في ذلك المرضى الجدد الذين لم يتلقوا العلاج والمرضى المزمنيين.

لم يظهر الفحص الكامل لدم مرضى الفصام أي تغيرات مقارنتا بالأشخاص الطبيعيين؛ بينما أظهر فحص وظائف الكبد زيادة طفيفة في الفوسفاتيز القلوية(ALK)، بينما لم تظهر أي تغيرات بالمؤشرات الاخرى لوظائف الكبد وذلك مقارنتا بالاشخاص الطبيعيين. لقد كانت مؤشرات وظائف الكلى ضمن المستوبات الطبيعية أثناء فترة العلاج. أظهرت نتائج الدراسة لمستويات الدهون أرتفاعاً في مستوى البروتين الدهنى منخفض الكثافة (LDL) بعد الشهر الأول والشهر الثاني من العلاج لحديثي الاصابة بالمرض و المرضى المزمنيين الذين يتناولوا الأدوية النفسية لمدة طويلة.أما مستويات البروتين الدهني عالي الكثافة(HDL) للمرضى المزمنيين فكانت منخفضة، رغم أن المؤشرات الأخري كانت طبيعية.

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LIST OF ABBREVIATION

abbreviation	***terms***
LFTs	Liver Function Tests .
SZ	Schizophrenia.
TG	Triglyceride.
LDL	Low Density Lipoprotein.
HDL	High Density Lipoprotein.
ALK	Alkaline Phosphate.
GGT	Gamma-Glutamyl Transferase.
GPT	Glutamate Pyruvate Transaminase .
GOT	Glutamate Oxaloacetate Transaminase .
WBC	White Blood Cell
RBC	Red Blood Cell
HGB	Hemoglobin
HCT	Hematocrite Value
PLT	Platelet
FCM	Flow cytometry
IL-3Ra-chain	InterLeukin 3 Receptor, alpha-chain
CD123	InterLeukin 3 Receptor, alpha-chain

LIST OF ABBREVIATION continous

abbreviation	***terms***
IFCC	International Federation of clinical Chemistry
CD116	Cluster of Differentiation 116
G-CSF-R	granulocyte colony-stimulating factor receptor
CD	Cluster designation or cluster differentiation
MoAb	Monoclonal Antibody
GMRα	GM-CSF receptor α Chain is also known CD116
CD123	InterLeukin 3 Receptor, alpha-chain
CD116	Granulocyte–Macrophage Colony-Stimulating Factor Receptor alpha-chain

Chapter I

Introduction

Schizophrenia is considered a common psychiatric disorder with high incidence. The pathological neurotransmitters involved in this disease remains poorly defined, although there were lots of studies in identifying neurochemical and pathological abnormalities in the disease (Kehrer, et al. 2008). This disease is manifested by impairments in perception and/or expression of reality; also showed an extensive withdrawal of interest from people and outside world. Schizophrenic patients are not dangerous or violent (Goldner, et al.2002; Kehrer, et al. 2008).

Schizophrenic symptoms are divided into positive and negative categories. Positive symptoms (altered behaviors) such as hallucinations (hearing voices), delusions, extreme emotions, incoherent thoughts and speech and excited motor activity. While negative symptoms (lack of behaviors) such as emotion, speech, social interaction (Lasley, 1997). The onset of symptoms is late teens and early twenties in men, while in women at age forty to fourty five (Häfner, et al. 1989 &1993; Castle, et al. 1991; Hambrecht, et al. 1993; Seeman, 1996; Seeman, 2007).

In schizophrenia, there is an increase in dopamine activity in the brain. Drugs that antagonize dopamine action provide an effective treatment for the disease, by reducing the intensity and frequency of the symptoms. Schizophrenic treatment has two phases: an Acute phase, where higher doses might be necessary followed by chronic phase, which could be life-long. Drugs take 2-4 weeks to produce effect (Carlsson and Lindquist, 1963; Meltzer and Stahl, 1976; Creese, et al. 1976; Davis, et al. 1991).

Antipsychotic drugs are classified according to structure into two class: The first-generation antipsychotics (typical antipsychotics) such as chlorpromazine, haloperidol, fluphrnazine; the Second-generation antipsychotics (atypical antipsychotics) such as clozapine, quetiapine, olanzapine, risperidone (Baldessarini and Frankenburg, 1991; Salzman, 2005; Muench and Hamer, 2010).

Genetic and environmental factors may act in combination to produce schizophrenia (Harrison and Owen, 2003). The important contributing factors are genetics, environment, neurobiology, psychological and social processes (Becker and Kilian, 2006). Schizophrenia may occur equally in both sexs, although the onset is earlier in men with the peak ages of onset 20–28 years for

males and 26–32 years for females (Castle, et al. 1991). Genes that may cause schizophrenia includes: Colony-Stimulating Factor 2 Receptor alpha-chain (CSF2RA), Interleukin 3 Receptor alpha-chain (IL3RA) and Granulocyte–Macrophage Colony-Stimulating Factor Receptor alpha-chain (GM-CSF) (Harrison and Owen, 2003; Becker and Kilian, 2006; Lencz, 2007).

Antipsychotic treatment is associated with adverse effects include: weight gain, diabetes, hyperprolactinemia, decrease brain volume, tardive dyskinesia, tachycardia, hypotension. Treatment also associated with significant risk of serious blood disorder, such as agranulocytosis, a potentially dangerous reduction of white blood cells number in the body. Patients with agranulocytosis risk need blood checks to catch the condition early if it does occur (Meltzer et al., 1989; Fischer et al., 1991; Alvirb et al., 1993; Bourin, et al.2001; Iqbal, et al. 2003; Husain et al., 2006; Essali, et al. 2009).

Agranulocytosis is a decrease of neutrophil count to less than 500 cells\mm³; risk of this life-threatening adverse effect is highest during the first 4 months of antipsychotic administration, even thought, it can occur after 11 years of continuous treatment. The mechanism of drug-induced agranulocytosis is unknown; now there are new methods of treating agranulocytosis that stimulate granulopoiesis using granulocyte and granulocyte-macrophage colony-stimulating factors (Weide, et al.1992; Gerson, et al. 1992; Barnas, et al. 1992; Jose, 1993; Pollmächer et al., 2000; VanStaa, et al. 2003; Sedky, et al. 2005).

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a glycoprotein family of cytokines, it is a potent growth factor on myeloid progenitor cells. It stimulates the proliferation and differentiation of hemocytoblast and regulates the production and functional activities of mature granulocytes. Granulocyte-macrophage colony-stimulating factor bind to its receptor (GM-CSFR), which is known by CD116 leading to stimulate the production of white blood cells (Nicola, et al. 1979; Gasson, et al. 1984; Nicola and Metcalf, 1985; Sieff, et al. 1985; Donahue, et al. 1986; Grabstein, et al. 1986; Nienhuis, et al. 1987; Valent et al., 1990; Gasson, 1991; Eder, et al. 1994; Jokhi, et al. 1994; Jubinsky, et al. 1994; Ronco, et al. 1994; Wognum, et al. 1994; Jubinsky, et al.1994; Wognum**,** et al. 1994; Browning, et al. 1995; Agis et al., 1996; Stacchini , et al. 1996; McClure, et al. 2003; Schernthaner et al., 2005; Lencz et al., 2007; Suzuki, et al. 2008).

Since 1950, it is known that phenothiazines have immunomodulatory effects; chlorpromazine and atypical compound, clozapine, influence the production of cytokines. Cytokines, as a peptide, has pleiotropic functions and are pivotal humoral mediators of inflammation and infection; they play an important role in hematopoiesis and autoimmunity. Antipsychotic drug may improve schizophrenia through its effect on cytokines in CNS; therefore, the effects of antipsychotic drugs on cytokine may explain the immune-mediated side effects of these drugs as agranulocytosis (Pollmächer et al., 2000).

Numerous antipsychotic drugs have an increased risk of agranulocytosis, according to the observations of individual patients, but few studies have evaluated its incidence. Agranulocytosis is characterized by severe neutropenia with a sudden onset of signs and symptoms of bacterial infection, as fever, malaise, and oropharyngeal or anorectal lesions (VanStaa, et al. 2003).

Clozapine, as atypical antipsychotics, is used for the treatment of schizophrenia (Baldessarini and Frankenburg, 1991). It is useful in cases that resist other drug therapy, also it is free of extra pyramidal side effect (Meltzer et al., 1989; Iqbal et al., 2003). However, its use has been limited because it produces agranulocytosis in about 1% of patients (Alvirb et al., 1993).

Hematologic records are important for patients with agranulocytosis; poly morphonuclear-leukocyte confirms agranulocytosis episode with the count below 500 cells per cubic millimeter. Hematologic monitor is important to prevent complications and deaths due to agranulocytosis (Jose, 1993).

Flow cytometer technique showed that the expression of CD123 (Interleukin 3 Receptor, alpha-chain) and CD116 Granulocyte–Macrophage Colony-Stimulating Factor Receptor alpha-chain was decreased during mast cell development (Schernthaner et al., 2005). It was suggested that immature Mast Cell progenitors express various cytokine-binding sites as IL-3R and GM-CSFR during differentiation and maturation; cytokine receptors expression was decreased over time and disappear, leading to mature human tissue Mast Cell lack IL-3R and GM-CSFRs (Valent et al., 1990; Agis et al., 1996).

Clozapine-induced agranulocytosis may due to an indirect mechanism involving reactive oxygen intermediates (Husain et al., 2006). Increasing the risk of agranulocytosis with age and reducing

incidence after the first six months of treatment provide additional guidelines for the prescription and monitoring clozapine treatment (Alvirb et al., 1993).

Granulocyte-macrophage colony stimulating factor, as cytokine, is potent growth factor both in vitro and in vivo, also has potent stimulating proliferation, maturation, and function of hematopoietic cells; it stimulates the proliferation and maturation of myeloid progenitor cells, leading to a rise of neutrophilic and eosinophilic granulocytes and monocytes levels (Gasson, 1991; Lencz et al., 2007). Important role of GM-CSF in CNS repair through the expression of brain-derived neurotrophic factor, a trophic factor associated with psychiatric illness. Evidences of the roles of GM-CSF and IL-3 in CNS, as neuroprotection, communication across the blood–brain barrier, and neurotransmitter modulation in, particular, acetylcholine and GABA levels (Lencz et al., 2007).

Human Granulocyte-Macrophage Colony-Stimulating Factor Receptor (hGMCSFR-M1) antibody reacts with subunit (GM-CSFR) of the human GM-CSFR complex; this 75-85 kD subunit of GM-CSFR is known as CD116 (Eder, et al. 1994; Jokhi, et al. 1994; Jubinsky, et al. 1994; Ronco, et al. 1994; Wognum, et al. 1994; Browning, et al. 1995; Stacchini, et al. 1996). The inhibition of neutrophil migration, increased neutrophil phagocyte activity and antibody-dependent cytotoxicity and increased monocytes tumoricidal activity were enhanced by GM-CSF (Jubinsky, et al.1994). White blood cells production is stimulated by GM-CSFR (CD116) which is located on myeloblast, mature neutrophil, but not on any erythroid or megakaryocytic lineage cells (Nicola and Metcalf, 1985).

Receptor of GM-CSF is a heterodimer composed of α chain and β chain subunits; these subunits are present in IL-3 and IL-5 receptors. α Subunit is a binding site for GM-CSF (McClure, et al. 2003); β chain is involved in signal transduction (Eder, et al. 1994; Geijsen, et al. 2001). By flow cytometry, GM-CSF-Rα and GM-CSF-Rβ were demonstrated on the cell surface of peripheral blood leukocytes (Suzuki, et al. 2008).

Aim of the work:

The objectives of this study were to estimate the incidence of drug-associated agranulocytosis in newly diagnosed schizophrenic Libyan patients and to evaluate their risk factors and outcomes. The study was conducted for patient's data from the Al-RAZI Hospital. The study includes: Screening for the expression and function of circulating leukocyte granulocyte-macrophage colony-stimulating factor receptor (CD116); also, screening of patient's biochemistry and hematology.

Chapter II

Materials and Methods

2.1. Materials:

Chemicals for FACS Calibur flowcytometer: The human Granulocyte-Macrophage Colony-Stimulating Factor Receptor complex (hGMCSFR-M1) antibody was purchased from Becton Dickinson, Tullastrasse, Heidelberg, Germany. **Alcohol** 75% (ethyl alcohol used as antiseptic, disinfectant) purell product, UK (purchased from Milton Keynes). **Sheath** fluid was purchased from Becton Dickinson GmbH, Tullastrasse, Heidelberg, Germany. **Cell back**, is normal saline, used as diluent, which was obtained from sysmex Europe, Bornbach Norderstedt, Germany. **Stromatolyser®-WH** was purchased from sysmex europe, Bornbach, Norderstedt, Germany. **Cell clean** from sysmex europe, Bornbach ,orderstedt, Germany.

Chemicals for kidney function tests: Urea Reagents, Creatinine Reagents, Uric reagents and electrolytes reagent (sodium and potassium) were purchased from Roche Diagnostics Mannheim, Germany.

Chemicals for lipid Profile tests: Cholesterol reagent, Triglycerides (TG) reagent, Low Density Lippoprotein (LDL) reagents, High Density Lipoprotein (HDL) reagents. Roche Diagnostics Mannheim, Germany.

Chemicals for liver function tests: Alkaline phosphatase (ALK) reagents, Gamma-Glutamyl Transferase (GGT) reagent, Glutamate Pyurvate Transaminase (GPT or ALT), Bilirubin reagents (working solutions), and Glutamate Oxaloacetate Transaminase (GOT or AST) reagents were purchased from Roche Diagnostics Mannheim, Germany.

Chemicals for haematology tests: Cell back, Stromatolyser®-WH and Cell clean were purchased from Sysmex corporation, Wakinohama-Kaigandori, Chuo ku, Kobe, Japan.

Instruments:

Sysmex.kx-21n Analyzer: Sysmex corporation1-5-1 Wakinohama-Kaigandori, Chuo ku, Kobe 651-0073, Japan. **ROCHE DIAGNOSTICS-COBAS INTEGRA® 400 PLUS:** Roche Diagnostic GMmbH, Sandhofer Strabe Mannheim, Germany. **Abbott Diagnostics - ARCHITECT c8000:** Abbott GmbH Diagnostika Max-Planck-Ring 2,65205 Wiesbaden,

Germany. **Heraeus centrifuge (ROCHE Company):** Ludwig-Wagner-Str, Wiesloch, Germany. **Becton-Dickinson FACS Calibur flowcytometer:** purchased from Becton Dickinson GmbH. Tullastrasse Heidelberg, Germany**.**

Tools:

BD microlance™3: (0.6×25mm) from Becton Dickinson S.A FRAGA (HUESCAl), Spain. **BD Discardit™II:** (syringe 5ml) from Becton Dickinson S.A FRAGA (HUESCAl), Spain.**BD Vacutainer®: (**K2E 5.4mg, plus blood collection tubes) Red blood3.0ml made in Loughborough, UK.**BD Vacutainer®:** (SST™II Advance, plus blood collection tubes)White blood Loughborough, UK. **ICE CHEST:** container from sitra company, Tunisia. C**up sample:** specified for Cobas integra 400 and Architect C8000. Abbott.

Patients:

Blood samples of healthy people (n=51; 26 males and 25 females) are taken from different places in Tripoli and schizophrenic patients (n=71; patient for acute administration: n=8; 4males, 4 females and patients for chronic administration: n=63; 35 males, 28 females).

2.2. Methods:

Sampling:

The participants were recruited from AL-RAZI Hospital in Tripoli-Libya and from different places in Tripoli during the period (2010-2011).

Three participants' groups were studied: group 1, (n=51) healthy persons without any disease; group 2, (n=12) newly diagnosed schizophrenic patients before any treatment with a follow up after one, two and three months; group 3, (n=63) schizophrenic patients after chronic treatments and follow up for one month later.

GM-CSF expression evaluation:

Blood samples (3 cc) are withdrawn from patients or volunteers in EDTA tube. Human Granulocyte-Macrophage Colony-Stimulating Factor Receptor complex (hGMCSFR-M1)

antibody is used according to the instruction. Expression and levels of hGMCSFR-M1 were evaluated using flowcytomete technique following the instruction of hGMCSFR-M1 antibody kids.

Biochemistry tests:

Blood (5cc) was collected from the vein in Silica (Clot Activator)/Gel tube. The biochemical tests for, uric acid and electrolytes (sodium and potassium), kidney function tests (urea and creatinine), liver function tests (alkaline phosphatase, Glutamyl transferase, glutamate Pyruvate transferase, bilirubin direct, glutamate oxaloacetate transferase), and lipid profile (cholesterol, triglyceride, low density lipoprotein, high density lipoprotein), were carried out using Roche Diagnostics Mannheim, Germany.

Haematology tests:

Complete blood picture was done automatically using Sysmex.kx-21n Analyzer. White Blood Cell count (WBC), Red Blood Cell count (RBC), The hemoglobin level (HG), Hematocrit level (HCT), Platelet count (PLT) were collected from output CBC result.

Chapter III

Results

3.1. Granulocyte-macrophage colony-stimulating factor expression and levels

Granulocyte-macrophage colony-stimulating factor (GM-CSF) expression was not changed in newly diagnosed, as schizophrenic, patients before starting treatments (p=0.884) but was significantly decreased after one-month (p=0.023) and two months (p=0.007) treatment compared to GM-CSF expression of healthy volunteers. While GM-CSF expression after three months' treatment, chronic treatments (years) or even one month after chronic treatment did not show any changed (p>0.05) compared to GM-CSF expression of healthy volunteers (table 3-1, figure 3-1).

GM-CSF expression after treatment for one or two months was decreased significantly compared GM-CSF expression of the schizophrenic patients before starting treatment (p=0.07, 0.021 respectively). GM-CSF expression of schizophrenic patients after three months treatment, also after chronic or one month after chronic treatment did not show any changes compared to schizophrenic patients before starting treatment (p>0.05) (table 3-1, figure 3-1).

Table 3-1:

GM-CSF expression in healthy and schizophrenic patients.

Treated groups	GM-CSF Mean ± S.E	*P* Compared to healthy	*P* Compared to schizophrenic without treatment
Healthy	97.7 ± 4.06		
schizophrenic without treatment	96.0 ± 21.26	0.884	
Acute one-month treatment	66.2 ± 4.08	0.023	0.07
Acute two-month treatment	51.6 ± 5.55	0.007	0.021
Acute three-month treatment	64.3 ± 7.41	0.119	0.171
Chronic (years)	109.4 ± 5.38	0.151	0.264
Chronic one month Later	108.4 ±7.65	0.249	0.333

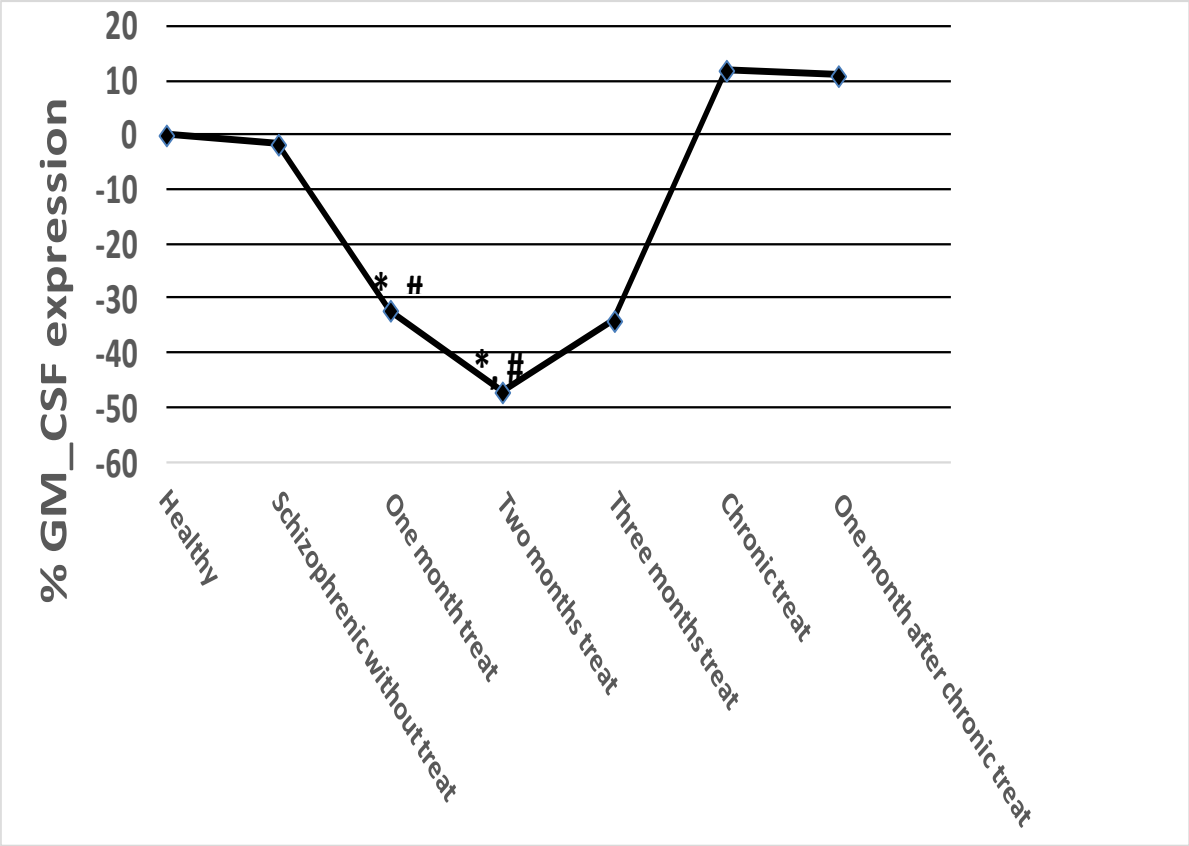

Figure 3.1. Percentage changes of GM-CSF expression in healthy and schizophrenic patients.

3.2. Hematology Tests

3.2. 1. White Blood Cell

White Blood Cell (WBC) was increased significantly after treatment for one-month (p=0.049) or even after chronic (years) treatment (p=0.016) compared to WBC of healthy volunteers; although the levels were within the normal range. While WBC after two, three months' treatment and chronic (years) even after one month later did not show any changed compared to WBC of healthy volunteers (table 3-2).

White Blood Cells Count were not changed in patients with treatment for one, two and three months after diagnosed as schizophrenic patients, or even chronic(years) and chronic one month later compared to the untreated patients at $p>0.05$ (table 3-2).

Table 3-2:

White Blood Cell in healthy and schizophrenic patients.

Treated groups	WBC Mean ± S.E	*P* Compared to healthy	*P* Compared to Schizophrenic without treatment
Healthy	6.5000±0.250		
Schizophrenic without treatment	7.7167±0.619	0.098	
Acute one month treatment	8.2125±1.187	0.049	0.634
Acute two month treatment	6.7714±0.448	0.768	0.384
Acute three month treatment	6.3333± 0.664	0.902	0.348
Chronic (years)	7.6032±0.338	0.016	0.588
Chronic one month later	6.8400±0.410	0.572	0.293

White Blood Cell units: Range for ♂ (n=124) 2.6-8.8 $X10^3/\mu l$,
Range for ♀ (n=117) 3.1-10.3 $X10^3/\mu l$.

3.2.2. Red Blood Cell:

Red blood cells count was decreased significantly in schizophrenic patients with chronic (years) treatments ($p=0.034$) compared to RBC of healthy volunteers, although they were within the normal range. While RBC of schizophrenic patients without treatment and after one, two, three months treatment or even of chronic and one month later did not show any changed compared to RBC of healthy volunteers (table 3-3).

Red blood cells count was not changed in patients with treatment for one, two and three months after diagnosed as schizophrenic patients, or even chronic (years) and chronic one month later compared to the untreated patients at $p>0.05$ (table 3-3).

Table 3-3:

Red Blood Cell count in healthy and schizophrenic patients.

Treated groups	Red blood cells Mean ± S.E	*P* Compared to healthy	*P* Compared to Schizophrenic without treatment
Healthy	4.7869±0.062		
Schizophrenic without treatment	4.6192±0.171	0.301	
Acute one month treatment	4.7350±0.261	0.787	0.615
Acute two months treatment	4.4614±0.235	0.111	0.511
Acute three months treatment	4.8400±0.354	0.859	0.498
Chronic (years)	4.5843±0.062	0.034	0.826
Chronic one month later	4.6060±0.091	0.143	0.941

RBC Units: Range for ♂(n=124) 3.6-5.3X106/ µl, Range for ♀(n=117) 3.2-4.6 X106/ µl.

3.2. 3. Hemoglobin:

The hemoglobin levels were not changed in all groups of schizophrenic patients (before and after treatment) compared to hemoglobin levels of healthy volunteers and compared to hemoglobin levels of schizophrenic patients before starting treatment (table 3-4).

Table 3-4:

Hemoglobin levels in healthy and schizophrenic patients

Treated groups	HGB Mean ± S.E	*P* Compared to healthy	*P* Compared to Schizophrenic without treatment
Healthy	13.6196± 0.232		
Schizophrenic without treatment	13.4667±0.516	0.790	
Acute one month treatment	14.0750±0.726	0.503	0.456
Acute two month treatment	13.5429±0.721	0.915	o.929
Acute three month treatment	14.4667±1.26	0.425	0.386
Chronic (years)	13.3492±0.236	0.422	0.835
Chronic one month later	13.5040±0.326	0.791	0.953

HGB units: Range for ♂(n=124) 11.3-15.7g/dl, Range for ♀(n=117) 9.9-13.6g/dl.

3.2.4. Hematocrit:

Hematocrit levels were not changed in all groups of schizophrenic patients (before and after treatment) compared to hematocrit levels of healthy volunteers also compared to hematocrit levels of schizophrenic patients before starting treatment (table 3-5).

Table 3-5:

Hematocrite levels in healthy and schizophrenic patients

Treated groups	HCT Mean ± S.E	*P* Compared to healthy	*P* Compared to Schizophrenic without treatment
Healthy	40.5843± 0.6537		
Schizophrenic without treatment	40.4750±1.1464	0.940	
Acute one month treatment	42.1000±1.798	0.381	0.434
Acute two month treatment	40.3286±1.601	0.889	0.946
Acute three month treatment	42.6000±2.635	0.456	0.469
Chronic (years)	40.0206±0.597	0.511	0.751
Chronic one month later	41.0040±0.764	0.705	0.740

HCT UNITS: Range for ♂(n=124) 32.6-47.5%, Range for ♀(n=117) 30.2-42.3%.

3.2.7. Platelet Count:

Platelet count (PLT) was increased significantly in newly diagnosed schizophrenic patients before starting treatments (p=0.012) compared to PLT count of healthy volunteers. While PLT count after one, two and three months treatment or even of chronic (years) treatment and of chronic one month later did not show any change compared to the PLT count of healthy volunteers (table 3-6). All the PLT count levels were within the normal levels.

PLT count, after treatment for two month or after chronic treatment, were decreased significantly compared to PLT count of schizophrenic patients without treatment (p=0.031, 0.039 respectively). Platelet count were not changed in patients after one month or three months treatment as schizophrenic patients and of chronic one month later compared to the untreated patients at p>0.05. All the PLT count in different groups are within the normal range (table 3-6).

Table3-6:

Platelet count in healthy and schizophrenic patients

Treated groups	PLT Mean ± S.E	*P* Compared to healthy	*P* Compared to Schizophrenic without treatment
Healthy	250.5588±10.284		
Schizophrenic without treatment	319.2500±35.028	0.012	
Acute one month treatment	261.7500±21.368	0.727	0.137
Acute two month treatment	232.0000±11.680	0.586	0.031
Acute three month treatment	225.3333±40.596	0.615	0.086
Chronic (years)	264.0159±11.745	0.398	0.039
Chronic one month later	281.2000±15.256	0.139	0.201

PLT UNITS: Range for ♂(n=124) 134-377X10³/µl, Range for ♀(n=117) 128-434X10³/µl.

3.3. Kidney Function Tests:

3.3.1. Serum Urea levels:

Urea levels were decreased significantly in chronic (years) schizophrenic patients (p=0.012) compared to urea levels of healthy volunteers. While urea levels of newly diagnosed, after one month, two months, three months treatment and one month after chronic patients did not show any change compared to urea levels of healthy volunteers; urea levels in different groups were within the normal levels.

Urea levels of the schizophrenic patients before starting treatment, after one, two, three months treatment and after chronic treatments for years or treatment one month after chronic treatment were not changed, the levels were within the normal range (table 3-7).

Table 3-7:

Urea levels in healthy and schizophrenic patients.

Treated groups	Urea Mean ± S.E	*P* Compared to healthy	*P* Compared to Schizophrenic without treatment
Healthy	19.0 ±1.46		
Schizophrenic without treatment	14.4± 2.05	0.129	
Acute one month treatment	21.0 ±3.69	0.597	0.131
Acute two month treatment	14.7± 3.37	0.257	0.948
Acute three month treatment	19.0± 9.00	0.991	0.529
Chronic (years)	15.5± 1.16	0.012	0.994
Chronic one month later	15.9± 1.86	0.195	0.650

Urea units: normal level 10-50mg/dl**;** *significantly different from healthy at $p \leq 0.05$.

3.3.2. Serum Creatinine levels:

Serum creatinine levels were not changed in different treated groups compared to serum creatinine levels of healthy volunteers; all levels were within the normal range. Also there is difference in the levels of serum creatinine in all treated groups compared to untreated patients (table 3-8).

Table 3-8:

Creatinine levels in healthy and schizophrenic patients.

Treated groups	Creatinine Mean ± S.E	*P* Compared to healthy	*P* Compared to schizophrenic without treatment
Healthy	0.6± 0.04		
schizophrenic without treatment	0.5 ±0.08	0.816	
Acute 1 month treatment	0.6 ±0.13	0.964	0.899
Acute 2 month treatment	0.5 ±0.07	0.805	0.939
Acute 3 month treatment	0.6± 0.20	0.959	0.945
Chronic (years)	0.6± 0.03	0.897	0.752
Chronic 1 month later	1.0± 0.54	0.515	0.706

Creatinine units: normal levels 0.5-1.5 mg/dl.

3.3.3. Serum Uric acid level

Uric acid levels did not show any change in different treated groups compared to uric acid levels of healthy volunteers or schizophrenic patients before starting treatments; All the levels of uric acid were within the normal levels in different treated groups (table 3-9).

Table: 3-9:

Uric acid levels in healthy and schizophrenic patients.

Treated groups	Uric acid Mean ± S.E	*P* Compared to healthy	*P* Compared to Schizophrenic without treatment
Healthy	4.2 ±0.19		
Schizophrenic without treatment	4.7± 0.55	0.327	
Acute one month treatment	5.0 ±0.82	0.138	0.604
Acute two month treatment	5.2± 0.67	0.081	0.460
Acute three month treatment	4.3 ±0.69	0.898	0.697
Chronic (years)	4.3 ±0.16	0.835	0.385
Chronic one month later	4.4± 0.32	0.615	0.584

Uric acid units: normal levels 2.0-7.0 mg/dl.

3.3.4. Serum Sodium levels:

Newly diagnosed schizophrenic patients, patients after one, two, three months treatment or even after chronic treatment and one month after chronic treatment did not show any changes in serum sodium levels compared to serum sodium levels of healthy volunteers (table 3-5).

Sodium levels were not changed in patients with treatment for one month, two months and three months after diagnosed as schizophrenic patients and also of chronic treatments for years or even after one month of chronic treatment compared to the untreated patients (table 3-5).

Table 3-10:

Sodium levels in healthy and schizophrenic patients.

Treated groups	Sodium Mean ± S.E	*P* Compared to healthy	*p* Compared to schizophrenic without treatment
Healthy	144.4 ±0.98		
Schizophrenic without treatment	141.5 ±1.49	0.176	
Acute one month treatment	140.5± 1.82	0.148	0.754
Acute two month treatment	141.6 ±2.38	0.332	0.974
Acute three month treatment	140.6 ±4.33	0.337	0.835
Chronic (years)	142.8± 1.00	0.054	0.892
Chronic one month Later	142.4 ±1.14	0.258	0.724

Sodium units: normal level 144-157mmol/l.

3.3.5. Serum Potassium level:

Serum potassium levels did not show any change in newly diagnosed, as schizophrenic patients before starting treatments and after treatment for one month, two months, three months, also after chronic treatments (years) or even after one month compared to serum potassium levels of healthy volunteers (table 3-11).

Serum potassium levels of schizophrenic patients treated for one month, two months, three months, even after chronic treatments and one month after chronic administration were not changed compared to the serum potassium levels in newly diagnosed untreated patients. Serum potassium levels in different treatments were within the normal levels (table 3-11).

Table 3-11:

Potassium levels in healthy and schizophrenic patients.

Treated groups	potassium mean ± S.E	*P* Compared to healthy	*P* Compared to schizophrenic without treatment
Healthy	4.7± 0.13		
schizophrenic without treatment	4.8 ±0.49	0.741	
Acute one month treatment	4.4± 0.48	0.499	0.424
Acute two month treatment	4.0± 0.18	0.122	0.121
Acute three month treatment	4.1± 0.29	0.346	0.302
Chronic (years)	4.6± 0.11	0.521	0.789
Chronic one month later	5.1± 0.31	0.203	0.519

Potassium units: normal level 3.7-5.5mmol\l.

3.4. Lipid Profile Tests:

3.4.1. Serum Cholesterol level:

Although serum cholesterol levels were increased significantly in schizophrenic patients after treatment for one month (p=0.042) and chronic treatment after one month later (p=0.036) compared to serum cholesterol levels of healthy volunteers, but the levels were within the normal range. While Cholesterol of newly diagnosed as schizophrenic patient before starting treatment and after treatment for two months and after three months treatment or even chronic treatments (years) did not show any change compared to healthy volunteers (table 3-12).

Serum cholesterol levels did not show any significant difference in all different treatments compared to the patients without treatments. All serum cholesterol levels were within the normal range (table 3-12).

Table 3-12:

Cholesterol levels in healthy and schizophrenic patients.

Treated groups	cholesterol mean ± S.E	*P* Compared to healthy	*P* Compared to schizophrenic without treatment
Healthy	160.4 ±4.27		
schizophrenic without treatment	159.7±8.47	0.953	
Acute one month treatment	189.0±10.95	0.042	0.082
Acute two month treatment	179.3±11.7	0.117	0.242
Acute three month treatment	196.3±13.09	0.101	0.123
Chronic (years)	170.0±5.42	0.17	0.374
Chronic one month later	180.0±7.42	0.036	0.121

Cholesterol units: normal level 50-200mg/l.

3.4.2. Serum Triglyceride level:

Serum triglyceride levels were increased significantly in schizophrenic patients after treatment for one (p=0.028), two (p =0.016) and three months (p =0.014) or even chronic treatments (years) (p =0.002) and chronic after one month later (p =0.013) compared to serum triglyceride levels of healthy volunteers. While serum triglyceride levels of schizophrenic patients without treatments did not show any changed compared to serum triglyceride levels of healthy volunteers (table 3-13).

There was a significant increase in serum triglyceride levels after treatment for one (p =0.036), two (p =0.024) and three months (p=0.015) when compared to triglyceride of schizophrenic patients without treatment. Also triglyceride level of chronic treatment (years) schizophrenic patients (p=0.024) and chronic one month later (p=0.038) were increased significantly compared to schizophrenic untreated patients. All levels of serum triglyceride were within normal range (table 3-13).

Table 3-13:

Triglyceride levels in healthy and schizophrenic patients.

Treated groups	TG Mean ± S.E	*P* Compared to healthy	*P* Compared to schizophrenic without treatment
Healthy	99.26±8.26		
schizophrenic without treatment	92.00±16.93	0.737	
Acute one month treatment	159.28±24.6	0.028	0.036
Acute two month treatment	161.62±31.75	0.016	0.024
Acute three month treatment	198.33±29.61	0.014	0.015
Chronic (years)	140.07±9.01	0.002	0.024
Chronic one month later	141.91±14.45	0.013	0.038

Triglyceride units: normal level 50-200mg/l.

3.4.3. Serum Low Density Lipoprotein level:

Serum low density lipoprotein levels (LDL) in schizophrenic patients after treatment for one (p=0.004), two months (p=0.033) and chronic one month later (p=0.006) were increased significantly compared to serum LDL levels of healthy volunteers. While serum LDL levels of newly diagnosed as schizophrenic patient before starting treatment and chronic treatments (years) did not show any change compared to serum LDL levels of healthy volunteers (table 3-14).

Serum low density lipoprotein levels were not changed in patients after treatment for one month and two months or even after chronic treatment (years) and chronic one month later compared to the patients without treatments at p>0.05 (table 3-14).

Table 3-14:

Low density lipoprotein level in healthy and schizophrenic patients.

Treated groups	LDL Mean ± S.E	*p* Compared to healthy	*P* Compared to Schizophrenic without treatment
Healthy	96.63±5.07		
Schizophrenic without treatment	111.14±18.84	0.278	
Acute one month treatment	154.66±21.97	0.004	0.055
Acute two months treatment	147.50±6.5	0.033	0.166
Acute three months treatment	Lost	lost	lost
Chronic (years)	106.96±5.2	0.177	0.758
Chronic one month later	122.05±6.82	0.006	0.45

Low density lipoprotein units: normal level 10-100mg/l.

3.4.4. Serum High Density Lipoprotein level:

Serum high density lipoprotein (HDL) levels were decreased significantly in schizophrenic patients after chronic treatment (years) (p=0.003) compared to serum HDL levels of healthy volunteers. While serum HDL levels after one, two, and three months treatment or after diagnosed as schizophrenic patients without treatment or even after chronic one month later did not show any changed compared to serum HDL levels of healthy volunteers (table 3-15).

Serum levels of High density lipoprotein were not changed in patients after treatment for one month, two months and three months, also chronic treatment (years) and chronic one month later compared to the patients without treatments at p>0.05 (table 3-15).

Table 3-15:

High density lipoprotein level in healthy and schizophrenic patients.

Treated groups	HDL Mean ± S.E	*P* Compared to healthy	*P* Compared to schizophrenic without treatment
Healthy	57.48±2.25		
Schizophrenic without treatment	56.63±3.89	0.863	
Acute one month treatment	51.14±5.81	0.78	0.504
Acute two months treatment	49.75±3.33	0.346	0.31
Acute three months treatment	46.50±12.5	0.303	0.373
Chronic (years)	48.94±1.96	0.003	0.115
Chronic one month later	56.52±2.69	0.795	0.983

High density lipoprotein units: normal level 55-110 mg/l.

3.5. Liver Function Tests:

3.5.1. Serum Alkaline Phosphatase Levels

Serum alkaline phosphatase (ALK) levels were increased significantly in newly diagnosed schizophrenic patients before starting treatments (p=0.04) or even after treatment for one (p=0.016) compared to serum ALK levels in healthy volunteers; although the levels were within the normal range. After two months, serum ALK levels was increased significantly (p=0.000) compared to healthy serum ALK levels. While serum ALK levels after three months treatment, chronic treatment or even after chronic one month later did not show any changed compared to serum ALK levels of healthy volunteers (table 3-16).

The levels of ALK did not show any changed (p>0.05) after treatment for one month, two and three month or even after chronic(years) and after chronic one month later compared to serum ALK levels of schizophrenic patient without treatment (table 3-16).

Table 3-16:

Alkaline phosphatase level in healthy and schizophrenic patients.

Treated groups	ALK Mean ± S.E	*P* Compared to healthy	*P* Compared to schizophrenic without treatment
Healthy	71.95±2.77		
schizophrenic without treatment	102.27±23.13	0.04	
Acute one month treatment	113.14±19.76	0.016	0.057
Acute two month treatment	139.50±55.49	0.000	0.801
Acute three month treatment	87.50±2.50	0.6	0.621
Chronic (years)	87.47±3.54	0.087	0.591
Chronic one month later	89.60±4.84	0.10	0.496

Alkaline phosphatase units: normal level 40-129U/l .

3.5.2. Serum Gamma-Glutamyl Transferase level

Serum γ-Glutamyl Transferase (GGT) levels were increased significantly in schizophrenic patient after treatment for one month (p=0.017) and two months (p=0.034) compared to serum GGT levels of healthy volunteers. While serum GGT levels in newly diagnosed as schizophrenic patient and after three months treatment as schizophrenic patients did not show any changed compared to GGT level of healthy volunteers. All serum levels of GGT of treatment did not show any significant changes except after two months treatment (increase) compared to newly diagnosed schizophrenic patients, but all the levels were within the normal range (table 3-17).

Table 3-17:

γ-Glutamyl Transferase level in healthy and schizophrenic patients.

Treated groups	GGT Mean ± S.E	*P* Compared to Healthy	*P* Compared to Schizophrenic without treatment
Healthy	22.02±2.85		
Schizophrenic without treatment	23.18±5.81	0.889	
Acute one month treatment	49.00±19.34	0.022	0.053
Acute two month treatment	51.57±18.82	0.004	0.018
Acute three month treatment	56.00± one parameter	-----	-----
Chronic (years)	31.00±3.71	0.101	0.35
Chronic one month later	29.54±6.15	0.367	0.543

γ-Glutamyl Transferase units: normal level: 10-66 U\l .

3.5.3. Serum Glutamate Pyruvate Transaminase levels

Serum Glutamate Pyruvate Transaminase (GPT) levels were increased significantly in schizophrenic patients after treatment for one (p=0.038) and two months (p=0.012) compared to serum GPT levels of healthy volunteers. While serum GPT levels of schizophrenic untreated patients, after three months treatment and chronic treatments (years) and even one month after chronic treatment did not show any changes compared to GPT level of healthy volunteers. All the levels were within the normal range (table 3-18).

All different treatments did not show any significant difference in the serum levels of GPT compared to schizophrenic untreated patients; all the levels were within the normal range (table 3-18).

Table 3-18:
Glutamate Pyruvate Transaminase levels in healthy and schizophrenic patients.

Treated groups	GPT Mean±S.E	*P* Compared to healthy	*P* Compared to Schizophrenic without treatment
Healthy	19.35±2.92		
Schizophrenic without treatment	15.50±2.31	0.52	
Acute one month treatment	27.87±5.83	0.038	0.08
Acute two month treatment	28.33±4.58	0.012	0.09
Acute three month treatment	20.33±4.48	0.228	0.62
Chronic (years)	20.44±1.68	0.23	0.35
Chronic one month later	23.00±3.16	0.06	0.18

Glutamate Pyruvate Transaminase units: normal level 5-41U\l**.**

3.5.4. Serum Bilirubin level

Serum Bilirubin levels were decreased significantly in schizophrenic patients after chronic (years) treatment (p=0.00) and chronic one month later (p=0.00) compared to serum Bilirubin levels of healthy volunteers. While serum Bilirubin levels in newly diagnosed as schizophrenic patients before starting treatment, after one month, two months and after three months treatment did not show any changed compared to serum bilirubin levels of healthy volunteers; although all the lserum levels were within the normal range (table 3-19).

Patients after chronic (years) treatments (p=0.008) and chronic one month later (p=0.036) showed a significant decrease in serum Bilirubin levels compared to serum Bilirubin levels of schizophrenic patients without treatment. Serum Bilirubin levels were not changed in patients with treatment for one month, two months and three months after diagnosed as schizophrenic patients, compared to the untreated patients at p>0.05. All the serum levels of bilirubin were within the normal range (table 3-19).

Table 3-19:

Bilirubin level in healthy and schizophrenic patients.

Treated groups	bilirubin Mean ± S.E	*P* Compared to healthy	*P* Compared to Schizophrenic without treatment
Healthy	0.666±0.08730		
Schizophrenic without treatment	0.722±0.20600	0.766	
Acute one month treatment	0.625±0.2136	0.768	0.71
Acute two month treatment	0.571±0.21900	0.181	0.49
Acute three month treatment	1.250±0.950	0.783	0.12
Chronic (years)	0.300±0.0223	0.000	0.008
Chronic one month later	0.350±0.02850	0.000	0.036

Bilirubin units: normal level: 0.0-1.3mg\l.

3.5.5. Serum Glutamate Oxaloacetate Transaminase level:

Serum Glutamate Oxaloacetate Transaminase (GOT) levels did not show any change in newly diagnosed schizophrenic patients before starting treatments, after treatment for one, two and three months or even after chronic (years) and chronic one month later compared to serum GOT levels of healthy volunteers; all the serum GOT levels were within the normal range (table 3-20).

All different treatments showed no significant difference in serum GOT levels compared to schizophrenic patients before starting treatment at $p>0.05$, all the levels were within the normal range (table 3-20).

Table 3-20:

Glutamate Oxaloacetate Transaminase level in healthy and schizophrenic patients.

Treated groups	GOT Mean ± S.E	*P* Compared to healthy	*P* Compared to Schizophrenic without treatment
Healthy	19.13±1.225		
Schizophrenic without treatment	22.27±2.316	0.297	
Acute one month treatment	18.60±1.503	0.898	0.445
Acute two month treatment	22.00±3.193	0.496	0.955
Acute three month treatment	17.33±1.201	0.734	0.395
Chronic (years)	18.60±1.266	0.768	0.213
Chronic one month later	20.95±2.394	0.45	0.692

Glutamate Oxaloacetate Transaminase units: normal level: 5-38U\l

Chapter IV

Discussion

Schizophrenia is a severe neuropsychiatric disorder that represents the 18^{th} leading cause of years lived with disability globally (Whiteford et al., 2013) and has an estimated point prevalence of 0.5% to 1.0% (Tandon et al., 2008). Functional and structural disconnectivity are among the most reproducible neurophysiological abnormalities associated with schizophrenia (Burns et al., 2003; Whalley et al., 2005; Liang et al., 2006; Begre and Koenig, 2008; Konrad and Winterer, 2008; Hoptman et al., 2010; Qiu et al., 2010; Whitford et al., 2011; Shi et al., 2012a,b; Curčić-Blake et al., 2013; Rane et al., 2013; Straube et al., 2013; Tepest et al., 2013).

Antipsychotic drugs affect the cytokine network. Hence, it is plausible that the influence of antipsychotics on the cytokine systems may be responsible for their clinical efficacy in schizophrenia (Drzyzga et al., 2006). It was suggested that both clozapine and haloperidol, at concentrations within the therapeutic range, may exert immunosuppressive effects (Song et al., 2000).

Granulocytopenia (decrease in number of granulocytes) and agranulocytosis (a condition in which the bone marrow does not make enough neutrophils) are severe side effects of antipsychotic therapy. Patient suffers severe agranulocytosis that developed after 7 weeks of antipsychotic drugs treatment is presented. After antipsychotic drugs discontinuation, treatment with granulocyte-macrophage colony-stimulating factor (GM-CSF), a glycoprotein, that has been shown to stimulate the proliferation of precursor cells in the bone marrow and their differentiation into granulocytes and macrophages, was initiated. Treatment with GM-CSF may lower the risks associated with antipsychotic drugs -induced agranulocytosis. This indicate that the levels or the expression of GM-CSF was lower than the normal (Barnas et al., 1992). Oren et al. found that, clozapine-induced agranulocytosis is usually reversible after discontinuation of the drug. A patient who developed agranulocytosis after termination of clozapine therapy responded to treatment with GM-CSF (Oren et al., 1993). In this study, GM-CSF expression and levels were decreased in the first 8 weeks of the antipsychotic treatment. It is probable that antipsychotic therapy reduces the expression and the levels of GM-SCF in the first 8 weeks. The increase in the levels or expression after 8 weeks may be due to tolerance toward antipsychotic effect or until hematologic recovery.

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a monomeric glycoprotein secreted by macrophages, T cells, mast cells, NK cells, endothelial cells and fibroblasts that functions as a cytokine. GM-CSF is a white blood cell growth factor (Francisco-Cruz et al., 2014). GM-CSF stimulates stem cells to produce granulocytes (neutrophils, eosinophils, and basophils) and monocytes (US National Library of Medicine, 2015).

Granulocyte-macrophage colony-stimulating factor (GM-CSF), a glycoprotein with hormonal properties, is produced by several cell types, most of which exist outside the CNS. GM-CSF, however, affects the CNS. If capable of crossing from blood to CNS, GM-CSF might be an important signaling molecule between the CNS and periphery. It was found that GM-CSF crossed the blood-brain barrier and blood-spinal cord barrier; it was demonstrated that a saturable mechanism transports of GM-CSF intact from blood to CNS (McLay et al., 1997).

Granulocytopenia and agranulocytosis are severe side effects of antipsychotic therapy. Patients suffering from agranulocytosis are extremely endangered by infectious diseases for up to 3 to 4 weeks until hematologic recovery. A patient in whom severe agranulocytosis developed after 7 weeks of antipsychotic treatment is presented.
After antipsychotic discontinuation, treatment with granulocyte-macrophage colony-stimulating factor (GM-CSF), the total granulocyte count rose from 63/cu mm to a value greater than 1500/cu mm within 5 days without complications or major side effects (Barnas et al., 1992). Granulocyte-macrophage colony-stimulating factor (GM-CSF) levels started to increase after 8 weeks, until hematologic recovery (Barnas et al., 1992).

It was suggested that Clozapine–mediated inhibition of release of GM-CSF is involved in clozapine-induced agranulocytosis (Carvajal et al., 2005). Neutrophil count, in the present study, was within the normal levels; this indicate that the decrease in the release of GM-CSF may affect the function and not the count number of neutrophil.

In the present study, GM-CSF levels or expression were decreased in the first 8 weeks after the beginning of treatment with antipsychotic drugs; GM-CSF, which is considered neurotropic cytokine, is consumed in the first few weeks in repairing CNS disorder in immune system. The decrease in the levels of GM-CSF were significant in the first eight weeks which is associated with the disappearance of positive results. It is concluded that antipsychotic drug decreases the

expression and the levels of GM-CSF protein, therefore it may produce agranulocytosis. Although the WBC counts in all different treatments were within the normal count, antipsychotic may decrease the function and not WBC count.

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a hematopoietic cytokine that has the potential for clinical application. The biological effects of GM-CSF have been well characterized, and include stimulation of bone marrow hematopoietic stem cell proliferation and inhibition of apoptosis of hematopoietic cells (Kim et al., 2009).

It was found that, If GM-CSF is given prior to MPTP (n 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) protected nigral dopaminergic neurons; GM-CSF modulation of immunity could be of clinical benefit for Parkinson's disease (Kosloski et al., 2013). In CNS disease states, GM-CSF is involved in recovery and control of cell death following spinal cord injury (Ha et al., 2005). It was demonstrated that GM-CSF protects dopaminergic neurons from 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced death (Kosloski et al., 2013). Importantly, GM-CSF has been shown to be neuroprotective in parkinson models, by decreasing the proteins associated with neuronal apoptosis, Bcl-2 and Bax, and inducing brain derived neurotrophic factor (BDNF) (Kim et al., 2009, Choudhury et al., 2011, Mangano et al., 2011). Administration of GM-CSF in vivo protected dopaminergic neurons in the substantia nigra and improved locomotor behavior in a mouse MPTP model of Parkinson's disease (Kim et al., 2009).

Antipsychotic drugs decrease the levels and expression of GM-CSF during the first 8 weeks, where the least levels were on the 8^{th} week. Since GM-CSF is responsible to protect the dopamine neurons, as a consequence, the decrease in GM-CSF levels will lead to a decrease in dopamine levels. This might be the explanation for the mechanism of action of antipsychotics in decreasing the dopamine levels; where the clinical response of antipsychotics may be through a decrease in GM-CSF levels and expression. Because the positive symptoms generally respond well to medication (American Psychiatric Association, 2000), it disappeared gradually through the first 8 weeks.

Liver function tests abnormalities were found in about 10% of schizophrenic patients treated with antipsychotics (Garcia-Unzueta et al., 2003). Antipsychotics elevate serum alkaline phosphatase activity (rang et al., 2012), which is usually arising within the first 8 weeks of

treatment (United states national library of medicine, 2014). Both pharmacological and clinical factors could be related with these alterations (Garcia-Unzueta et al., 2003). Alkaline phosphatases are present in many human tissues, including bone, intestine, kidney, liver, placenta and white blood cells (Kaplan 1972). Damage to these tissues causes the release of ALP into the bloodstream (Li-Fern and Rajasoorya 1999). In this study, in liver function tests, there was an increase in alkaline phosphate after two-month treatment which is due to antipsychotic therapy. While Kidney function investigation was within the normal range.

Patients treated with antipsychotics are at a higher risk for the development of lipid abnormalities than the general population (Roohafza et al., 2013). Antipsychotic-induced metabolic adverse effects in the clinical setting (Skrede et al., 2013). The molecular mechanisms mediating metabolic disturbances are incompletely understood (Skrede et al., 2013). In 1965, haloperidol was demonstrated to inhibit cholesterol biosynthesis (Summerly and Yardley 1965). It was demonstrated that haloperidol, clozapine, risperidone, and ziprasidone reduce de novo cholesterol biosynthesis. This occurs through inhibition of several enzymatic steps in the later part of the cholesterol biosynthesis pathway, leading to accumulation of various cholesterol precursors (Adams et al., 2003; Yang et al., 2007).

Patients with acute-phase schizophrenia had lower high-density lipoprotein (HDL) levels, higher low-density lipoprotein (LDL) levels (Huang and Chen 2005). The outcome of this research confirms the same results.

Conclusion

GM-CSF expression were decreased after antipsychotic treatment for one month and continue the decrease after two months' treatment. GM-CSF expression starts to increase after the two-month treatment and continue increasing to levels of healthy or newly diagnosed schizophrenic patients after chronic treatment.

Complete blood counts were no changed compared to the normal levels. **Liver function** showed transient increase in ALK after one and two months' treatment. All other parameters were not changed compared to normal levels. **Kidney function** showed transient decrease in urea levels after chronic treatment, although the levels were within the normal range; while

creatinine levels were within the normal range during the treatments. In **Lipid profile**, LDL level was increased after one and two months of treatment also after chronic administration of antipsychotics. While All other parameters were within the normal range

Chapter V

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Appendix

Appendix

BD pharmingen™	R-phycoerythrin-conjugated mouse anti-human CD116"GM-CSF RECEPTOR ∝ CHAIN"Monoclonal Antibody. BD,BD logo and all other trademarkes are property of Becton, Dickinson and company.-for research use only.not for use in diagnostic or therapeutic procedures.not for resale. (purchased from Germany).
Sheath fluid	Becton ,Dickinson and company(purchased from Germany). Composition**:** Sodium chloride, Disodium EDTA, Potassium chloride, Potassium phosphate, monobasic, Sodium phosphate, Dibasic.
Cell back.	NaCL(normal saline) as Diluent. sysmex europe GMBH. Bornbach 1,224848Norderstedt,Germany.
Stromatolyser®-WH	sysmex europe GMBH. Bornbach 1,224848Norderstedt,Germany
CELLCLEAN:	sysmex europe GMBH. Bornbach 1,224848Norderstedt,Germany
UreaReagents(workingsolutions).	Components: TRIS buffer,pH 7.8(100 mmol/L), 2-Oxoglutarate(5mmol/L),NADH(0.25mmol/L),ADP(0.6 mmol/L),GLDH(>1,500mmol/L).
Creatinine Reagents(working solution).	Components:TAPSa

Creatinine Reagents(working solutions).

R1= Buffer, enzymes and HTIB in vial A (liquid).

R2=SR Buffer, enzymes and 4-aminophenazone in vial C (liquid).

Active ingredients:

Components	Concentrations			
	R1	R2	Test	
TAPS[b]	30	50	34.5	mmol
creatinase ≥332	≥209	μkat/L(≥12.6 KU/L)		

			(microorganism)
Sarcosine oxidase	≥132	≥83	μkat/L(≥4.9 KU/L)
			(microorganism)
Ascorbate oxidase	≥33	≥21	μkat/L(≥1.2 KU/L)
			(microorganism)
HTIB	1.2	0.76	g/L
Creatininase	≥498	≥155	μkat/L(≥9.3 KU/L)
			(microorganism)
Peroxidase	≥16.6	≥5.2	μkat/L(≥9.3 KU/L)
			(horseradish)
4-Aminophenazone	0.4	0.13	g/L
Potassium hexacyanoferrat(II)	60	18.7	mg/L
PH	8.1	8.0	8.1

b) N-tris-(hydroxymethyl)methyl-3-aminopropanesulfonic acid

both reagents contain nonreactive detergents and preservatives.

Note: Reagent Handling:Ready for use.

6.8<u>Uric reagents(working solutions).</u>

R1= Buffer, enzymes and HTIB in vial A (liquid).

R2=SR Buffer, enzymes and 4-aminophenazone in vial C (liquid).

<u>Active ingredients:</u>

Components	Concentrations		
	R1	R2=SR	Test

phosphate	0.05	0.1	0.04	mol/L
TOOLS	7		3.76	mmol/L
Fatty alcohol polyglycol ether	4.8		2.58	%
Ascorbate oxidase (Zucchini)	≥83.5		≥44.9 (≥2.7	µkat/L KU/L)
POD(horseradish)		≥50	≥1.74 (≥0.1	µkat/L KU/L)
Uricase (microbial) (≥0.05 KU/L)		≥83.4	≥0.87	µkat/L
Potassium hexacyano-ferrate(II)	0,3		0.031	mmol/L
4-Aminophenazone	≥3		≥0.31	mmol
PH		7.8		7.8

Both reagents contain nonreactive stabilizers.

Note: Reagent Handling:Ready for use.

6.9 electrolytes reagent(sodium and potassium).

ISE calibrators-intended use is in vitro diadnostic calibrators intended for the quantitative determinations of sodium and potassium.

ISE Calibrator :150 mmol/L sodium,5 mmol/L potassium,115 mmol/L,115 mmol/L, 0.3 mmol/L lithium.

Note: Reagent Handling:Ready for use.

6.10-Cholesterol reagent(working solution).

R Mono reagent in vial A (liquid).

Active ingredients:

Components	Concentrations R	Test	
PIPES[a] buffer	225	75	mmol/L
Mg2+	10	3.3	mmol/L
Sodium cholate	0.6	0.2	mmol/L
4-Aminoantipyrine	≥0.45	0.15	mmol/L
Phenol	≥12.6	4.2	mmol/L
Fatty alcohol polyglycol ether	3	1	%
CE (Pseudomonas spec.)	≥25	≥8.3	µkat/L(≥0.5 KU/L)
CHOD (E.coli)	≥7.5	≥2.5	µkat/L(≥0.15 KU/L)
POD (horseradish)	≥12.5	≥4.1	µkat/L(≥0.15 KU/L)
PH	6.8	6.8	

a) PIPES= Piperazine-1,4-bis(2-ethanesulfonic acid).

The reagent contains nonreactive preservative and stabilizer.

Note: Reagent Handling:Ready for use.

6.11 -<u>Triglycerides(TG) reagent(working solutions)</u>

Components	Concentrations		
	R	Test	
PIPES	50	40	mmol
LPL(microbial)	≥83	≥66	μkat/L(≥0.15 KU/L)
GK(microbial)	≥3	≥2.4	μkat/L(≥0.14 KU/L)
GPO(microbial)	≥41	≥33	μkat/L(≥2 KU/L)
POD (horseradish)	≥1.6	≥1.3	μkat/L(≥0.08 KU/L)
ATP	1.4	1.1	mmol/L
Mg2+	40	32	mmol/L
4-Aminophenazone	0.13	0.1	mmol/L
4-Chlorophenol	4.7	3.8	mmol/L
Sodium cholate	0.2	0.16	mmol/L
PH	6.8	6.8	

The reagent contains non- reactive surfactants and stabilizers.

Note: Reagent Handling:Ready for use.

6.12 -<u>Low Density Lippoprotein(LDL) reagents.</u>

Components	Concentrations		
	R1	R2=SR	Test
mmol/L	19.2MOPS[b]	20.1	20.1
HSDA	0.958	0.688	mmol/L
AOD (recombinant)	≥50	≥36	µkat/L (≥2.2 KU/L)
POD (horseradish)	≥167	≥334	≥200 µkat/L (≥12KU/L)
Magnesium sulfate.7 H2O	8.11	1.94	mmol/L
4-aminoantipyrine	2.46	0.58	mmol/L
CE(microbial)	≥50	≥12	µkat/L(≥0.7KU/L)
CHOD(microbial)	≥33	≥8	µkat/L(≥0.5KU/L)
PH	6.5	6.8	6.4

a) 3-morpholino-propanesulfonic acid.

Both reagents contain stabilizers and preservative.

Note: Reagent Handling:Ready for use.

6.13 <u>High Density Lipoprotein(HDL) reagents.</u>

Components	Concentrations		
	R1	R2=SR	Test
mmol/L	14.1MOPS[a]		19.1
Dextran sulfate	0.001		0.0007 mmol/L
Magnesium sulfate.7H2O	≥8.1		≥6.0 mmol/L
HSDA[b]	0.958		0.709 mmol/L
AOD (recombinant)	≥50		≥37 µkat/L (≥2.2 KU/L)
POD (horseradish)	≥167	≥334	≥206 µkat/L (≥12KU/L)
PIPES[c]	9.9		2.44 mmol/L
CE(microbial)	≥3.3		≥0.8 µkat/L (≥0.05KU/L)
CHOD(microbial)	≥127		≥31 µkat/L(≥1.9KU/L)
4-aminoantipyrine	2.46		0.60 mmol/L
PH	7.0	7.0	7.1

a) 3-Morpholino-propanesulfonic acid.

b) Sodium N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline.

c) Piperazine-1,4-bis(2-ethanesulfonic)acid.

Both reagents contain stabilizers and a preservative.

Note: Reagent Handling:Ready for use.

6.14- Alkaline phosphatase(ALK) reagents.

Components	Concentrations			
	R1	R2=SR	Test	
2-Amino-2-methyl-1-propanol	1.724		0.92	mol/L
Magnesum acetate	3.83		2.0	mmol/L
Zinc sulfate	0.766		0.41	mmol/L
N-(2-hydroxyethyl)-ethylenediamine triacetic acid	3.83		2.0	mmol/L
P-nitrophenyl phosphate	132.8		16.0	mmol/L
PH(30˚C)	10.44			

Both reagents contain non-reactive preservatives and stabilizers.

Note: Reagent Handling:Ready for use.

6.15 Gamma-Glutamyl Transferase(GGT) reagent.

Components	Concentrations			
	R1	R2=SR	Test	
TRIS	492		100	mmol/L
Glycylglycine	492		100	mmol/L

Acetate	10	1.63	mmol/L
L-γ-glutamyl-3-carboxy -4-nitroanilide	22.5	3.7	mmol/L
PH (25˚C)	8.25	4.5	

Both reagents contain nonreactive stabilizers and preservatives.

Note: Reagent Handling:Ready for use.

6.16 -<u>Glutamate Pyurvate Transaminase(GPT or ALT).</u>

Components	Concentrations		
	R1	R2=SR	Test
TRIS	224	100	mmol/L
L-Alanine	1120	500	mmol/L
LDH(microbial)	≥45	≥20	μkat/L (≥1.2KU/L)
Albumin(bovine)	0.25	0.11	%
NADH	≥1.7	≥0.2	mmol/L
2-Oxoglutarate	94	12	mmol/L
Sodium azide	0.09	0.09	0.05 %
PH(37˚C)	7.3	7.3	

Reagent R1 contains nonreactive stabilizers, reagent R2 (SR) a non-reactive buffer.

Note: Reagent Handling:Ready for use.

6.17 -Bilirubin reagents (working solutions).

Components	Concentrations		
	R1	R2=SR	Test
Sulfanilic acid	35		13.5 mmol/L
Oxalic acid	40		15.4 mmol/L
HEDTA	4.0		1.5 mmol/L
Sodium nitrite		3.9	0.5 mmol/L
PH	1.2	6.0	1.4

Note: Reagent Handling:Ready for use.

6.18 -Glutamate Oxaloacetate Transaminase(GOT or AST) reagents.

Components	Concentrations		
	R1	R2=SR	Test
TRIS	224		100 mmol/L
L-Aspartate	792		240 mmol/L

MDH (microbial)	≥24		≥7	μkat/L (≥0.4KU/L)
LDH(microbial)	≥48		≥15	μkat/L (0.9KU/L)
Albumin(bovine)	0.25		0.08	%
NADH		≥1.7	≥0.2	mmol/L
2-Oxoglutarate		94	12	mmol/L
Sodium azide	0.09	0.09	0.05	%
PH(37˚C)	7.8		7.8	

Reagent R1 contains nonreactive stabilizers, reagent R2 (SR) a non-reactive buffer.

Note: Reagent Handling:Ready for use.

HAEMATOLOGY TESTS REAGENTS:

hematology reagents for sysmex KX-21N, including Diluents(CELLPACK) Approx.30ml per sample,WBC/NGB lyse(STROMATOLYSER-WH)Approx 1.0 ml per sample ,cell cleaner(detergent).with this instrument ,sample mixing ,cap removal, and tube setting are performed manually.

Cell back. NaCL(normal saline).for dilution.

Stromatolyser®-WH.composition:

Organic quaternary ammonium salt 8.5 g/L

Sodium chloride. 0.6g/L.

This reagent is colorless transparent and contains no cyanide or azide compound.it is a reagent that lyses RBC for accurate WBC count determination,WBC trimodal size distribution analysis and hemoglobin level measurement.

CELLCLEAN:is a strong alkaline detergent,