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Study of Some Immunological Parameters in Iraqi Patients with Multiple Sclerosis

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يوسف (٧٦)

Supervisor Declaration

We declare that this thesis was prepared under our supervision at the Department of Biology / College of Education for Pure Science (Ibn Al-Haitham) / University of Baghdad, in partial fulfillment of the requirement for the master degree in Science of Biology / Immunology.

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Dedication

I'd like to dedicate my humble work to whom:

who make me love to learn and lighten my ways and do his best to keep me forward, stand steady, stay strong, my power and courage source and my pride my dear father.

Who taught me to chase my dreams, be ambitious, dear to challenge, never give up and give the best I can, my Source of tenderness and love, my late mother.

Who share all my days, my sister, soul mate and my best sweety friend Hala and my lovely sweet brother Faisal.

My soul mate, my love, who encourage and support me, help me, stand by me in darkest days my dear housband Hasan Abdullah Murad.

My little sunshine, sweet heart, lovely angel and my beautiful dear daughter Dana.

My friends Zubaida Zyad, Ayat Hazim, Hind Muhamed, Zahraa Khalid, Nazhat Qasim, Rana Sabah, Mays Ahmed and Hanadi Adil.

To whom seek knowledge and finally to my homeland Iraq, I dedicate my work.

Hind

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Summary

Multiple sclerosis (MS) is an inflammatory condition that affects central nervous system (CNS) causing neurological dysfunction. The current study aim was the evaluation of the role of Methylprednisolone (MP), Interferon Beta (IFN-β) as disease treatments, and to investigate their influence on $CD25^{+}$ FoxP3 T regulatory cells (it was counted by flow cytometry technique), IL-8, IL-17 (pro-inflammatory cytokines), IL-12 (inflammatory cytokine (T cells differentiation Factor)), IL-10, TGF-β (anti-Inflammatory cytokines) it estimated by ELISA technique, also the total and differential WBC count, ESR, CRP (inflammatory indicators), patient characteristics, clinical manifestation and life style characteristics.

Forty-five Iraqi MS patients (15 untreated as early onset patients, 15 MP treated patients and 15 IFN-β treated patients), in addition to 15 apparently healthy individuals as control. For patients treated with MP, samples collected after treatment period, while patients treated with IFN-β samples were collected in remission status.

The study revealed the following results:

1. Patient general characteristics showed that according to gender distribution female:male ratio was 2:1 (66.67% : 33.33%), according to the age of onset, (< 30 years) group of patients were the highest frequency in study sample (37.9%), 57 years was the maximum age of onset and 13 years was the minimum, with mean (34.93), Baghdad was the highest governorate in MS onset (73.33%).
2. Total WBC count showed that patients treated with MP ($10.38*10^{3}$µl) increases significantly (P≤0.002), (P≤0.001) as compared with both patients treated with IFN-β ($7.45*10^{3}$µl) and control ($7.33*10^{3}$µl) respectively. Lymphocytes were decrease

significantly (P≤0.013), (P≤0.030) patients treated with MP and INF-β respectively (1.82), (1.92) as compared to control (2.58) and there were a significant decrease (P≤0.03) in patients treated with MP ($1.82*10^3$µl) as compared to untreated patients ($2.47*10^3$µl). Neutrophils was increase significantly (P≤0.016) and (P≤0.000) in patients treated with MP and untreated patients respectively (7.81), (5.99) as compared to control (3.82), there were also a significant increase in patients treated with MP (P≤0.04), (P≤0.001) as compared with untreated patients ($5.99*10^3$µl) and patients treated with IFN-β ($4.73*10^3$µl) respectively. Basophils decrease significantly (P≤0.001), (P≤0.014) in both patients treated with MP and INF-β respectively (0.05), (0.06) as compared to control (0.08).

3. $CD25^+$ $FoxP3^+$ Tregs counting showed a significant increase (P≤0.002) in untreated patients (28.01%) as compared to control (14.06%), while there were a significant decrease (P≤0.016), (P≤0.007) in both patients treated with MP and IFN-β respectively (3.70%), (2.44%) as compared to control, also there were a significant increase (P≤0.000), (P≤0.000) in untreated patients (28.01%) as compared with patients treated with MP (3.70%) and patients treated with IFN-β (2.44%) respectively.
4. Serum cytokines estimation showed that IL-8 decreased significantly (P≤0.026) in untreated patients (0.003pg/ml) as compared to control (0.016), while IL-10, IL-12, IL-17 showed no significances and TGF-β increased significantly (P≤0.028) in patients treated with MP (700.27) as compared to control (392.13), while there were a significant increasing (P≤0.001), (P≤0.001) in patients treated with MP (700.27pg/ml) as compared with untreated patients (235pg/ml) and patients treated with IFN-β (484.67pg/ml) respectively.

Saliva cytokines estimation showed that IL-8 was increased significantly (P≤0.023) in patients treated with IFN-β (0.578) as compared to control (0.223), IL-10 increased significantly (P≤0.017) in patients treated with MP (0.023) as compared to control (0.00), IL-12 decreased significantly (P≤0.003) in patients treated with IFN-β (4.93) as compared to control (24.07), IL-17 increased significantly (P≤0.018) in patients treated with MP (19.17) as compared to control (0.00).

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List of Abbreviations

Abbreviation	Term
ABTS	azino-bis(3-ethylnenzothiazoline-6-sulphonic acid)6
ACTH	Adrenocorticotropic Hormone
ANOVA	Analysis of Varianses
APC	Antigen Presenting Cell
BBB	Blood Brain Barrier
BSA	Bovine Serum Albumin
CD	Cluster of Differentiation (classification determinant)
CNS	Central Nervous System
CRH	Corticotropin-Releasing Hormone
CSF	Cerebrospinal Fluid
DMD	Disease Modifying Drug
EAE	Experimental Autoimmune Encephalomyelitis
ELISA	Enzyme-Linked Immunosorbent Assay
FAS	Appoptotic antigen-1
FC	Fragment, Crystallizable
FOXP 3	Forkhead Box P3
HRP	Horseradish Peroxidase
IFN-β	Interferon-Beta
IFN-γ	Interferon-Gamma
IgG	Immunoglobulin G
IL	Interleukin
IVIG	Intravenous Immunoglobulin
KDa	Kilodalton
LFA-1	Lymphocyte Function-associated Antigen 1

LSD	Last Significant Difference
MCP-1	Monocyte Chemotactic Protein 1
MHC	Major Histocompatibility Complex
MS	Multiple Sclerosis
MP	Methylprednisolone
MRI	Magnetic Resonance Imaging
NK	Natural killer
OCBS	Oligoclonal Band
OPD	o-phenylenediamine Benzidine
PBMC	Peripheral Mononuclear Blood Cells
PGE 2	Prostaglandin E2
PPMS	Primary Progressive Multiple Sclerosis
RRMS	Relapsing-Remitting Multiple Sclerosis
PBS	Phosphate Buffer Saline
S.E.	Standard Error
SLE	Systemic Lupus Erythmatosus
SPMS	Secondary Progressive Multiple Sclerosis
TLRs	Toll Like Receptor
Treg	Regulayory T Cell
Th	T-Helper
TGF-β	Transforming Growth Factor Beta
TNF	Tumor Necrosis Factor
VEP	Visual Evoked Potential
WBC	White Blood Cell

CHAPTER ONE
INTRODUCTION

Chapter One

Introduction

1.1 Introduction

Multiple sclerosis (MS) is an autoimmune disorder hits the central nervous system (CNS) (brain and spinal cord) causing a chronic inflammatory condition in a genetically susceptible individual.

Nerve cells surrounded by a protective sheath (Myelin). MS gradually destroys this sheath in patches (lesions) and that called demyelination; without myelin, brain and spinal cord nerve cells do not communicate probably with nerve cells in the rest of the body (Ebers, 2000). MS symptoms include motor, sensory, visual, balance, behavioral, thinking, urinary, sexual, pain and digestive. It depends on which parts of the CNS damaged, and how bad the damage was.

Since auto immune disease result from different immune cell types interaction leads to an attack to self-antigen that's organized or orchestrate by cytokines and chemokines so it has a very important role in autoimmunity and observing the immune system status, and also monitoring Treg functions. $CD4^+$ $CD25^+$ $FoxP3^+$ Treg lymphocyte it's a subset of $CD4^+$T lymphocyte that regulate the immune response and maintain self-tolerance, so this cell had a key role in autoimmune disease like MS. because the immune system attacks itself.

Multiple Sclerosis varies from patient to another. Patient may live with minor disability, or progressive disability, most patients are somewhere between.

What causes MS exactly still unknown, but there is an agreement that it happened due to genetic and environmental factors interaction, since

MS runs in certain families, and grew up region also play a role. It became more common in cold regions near to poles and less common near the equator also it more common in Caucasian race (MSIF, 2013).

In 2013, the global estimated number of patients increased from 2.1 million to 2.3 million in the last five years. (MSIF, 2013). In Iraq the patients number increased significantly over recent decades, MS most often hit people at time when they are forgoing their career, finding a long-term partner or having children therefore it affects the social and economic well-being of the individuals, as well as their families. That is why this study was necessary in Iraq.

The current study considered a unique study because for the first time we investigate Tregs in Iraqi MS patients by using the flow cytometry (a technique also used for first time in studying MS locally).

This study investigated about certain salivary cytokines in MS patients for the first time in Iraq, at the global level, and according to the available data sources, that certain salivary cytokines investigation in MS patients was the first of its kind (unique).

1.2 Aims of Study

The study aimed to investigate the role of the common therapeutic strategies in MS clinical center and evaluated them as disease treatments, by investigate their influence on $CD25^+$ FoxP3 T regulatory cells, IL-8, IL-17 (pro-inflammatory cytokines), IL-12 (inflammatory cytokine (T cells differentiation Factor)), IL-10, TGF-β (anti-Inflammatory cytokines); also the total and differential WBC count, in Iraqi MS patients,

to do so, the following method was used:

1. Total and differential WBC count using automated blood analyzer.
2. $CD25^+$ $FoxP3^+$ Treg counting using flow cytometry technique.
3. Interleukins level estimation using sandwich ELISA technique.

CHAPTER TWO
REVIEW OF LITERATURE

Chapter Two

Review of Literature

2.1 Historical Background

Like many other diseases, multiple sclerosis (MS) existed long before it had a name. Back to Middle Ages there was writings described symptoms of patients, similar to MS symptoms. Dutch Lidwina was a saint he died in 1433, he might be first well known MS patients (Soto, 2013), which king George III grandson write in his diary symptoms, these symptoms reveal that he had MS (Internet 1, 2014). In 1868, Dr. Jean-Martin a scientist wrote for the first time MS whole characteristics with its brain features (lesions), and by which he credited for identifying MS as a disease. (Soto, 2013). Ten years later Dr. Ranvier discover the myelin sheath surrounding the nerve cell axon (Ebers, 2000). In 1916, Dr. James Dawson was able to describe the inflammation and demyelination, at that time, MS thought that it caused by toxin or virus and they have no proven for that (Soto, 2013). In 1925, Lord Edgar Douglas Adrian recorded the first nerve electrical transition; he also proved that demyelinated nerve can bears the electrical stream of the nervous transition. After few years, scientist Dr. Thomas Rivers put the animal model of MS; it named Experimental-Allergic Encephalomyelitis (EAE) that lead to our current autoimmune theory and showed that the body could attack itself (Ebers, 2000). In 1930s, the involvement of the immunity suggested. In 1947 unusual protein founded in MS patient cerebrospinal fluid (CSF), (Internet 1, 2014). In 1965 the white blood cells (WBC) that attack the myelin sheath and lead to having MS disease was discovered which lade to discover that MS probably an auto immune disease (Ebers, 2000). Steroids used to treat

attacks in the next decade; disease-modifying agent was develop for the first time, in 1980s and 1990s potential treatments, diagnostic took place in some clinical trials. (Soto, 2013). MRI (Magnetic Raciness Image) become a standard in diagnostics and testing the disease modifying agents effectiveness in showing down disease progress, followed by improvements in drug use to treat symptoms in addition to development more effective rehabilitation and other types of therapies.

In 2000, Frank , Mc Farland and Kreman Chatzky pointed that MS is one of the most important common inflammatory disease that attack the CSF and there is a little information about MS development and a noun origin (Ebers, 2000; KremanChatzky *et al.*, 2000;).

2.2: Immunologic Tolerance and Autoimmunity

Immunologic tolerance defined as the adaptive immune system Unresponsiveness to self-antigens, largely because of inactivation or death of self-reactive lymphocytes induced by exposure to these antigens. Self-tolerance (self-antigen tolerance), it is cardinal normal feature of immune system (Abbas *et al*., 2012).

Tolerance classified into:

- Central tolerance, it is a Self-tolerance induced in immature self-reactive lymphocytes by deletion auto reactive lymphocyte before their development inside the generative lymphoid organs (thymus, bone marrow) for both T and B-lymphocytes, respectively (Sprent, 2001).
- Peripheral tolerance, a Self-tolerance induced in mature T, B-lymphocytes in the peripheral tissues and lymph nodes (Murphy, 2012), this tolerance established by process called Clonal Anerg which T, and B auto reactive lymphocytes become dysfunctional cells. (Peakman and Rergani, 1977;

Cruse and Lewis, 2000).The importance of self-tolerance for the health of individuals appreciated from the early days of immunology; it is an essential Feature of the immune system normally, when self-tolerance failed, immune reactions happened against self (autologous) antigens. Such reactions called autoimmunity, the diseases that result from an immune tolerance failure named autoimmune diseases. (Abbas *et al*., 2012).

2.2.1: Pathogenesis of Autoimmunity

The possibility that an individual's immune system may activated against self-antigens, causing tissue damage injury appreciated by immunologists when they recognized the specificity for foreign antigen in the immune system (Abbas *et al*., 2012). In the early 1900s, Paul Ehrlich coined the rather melodramatic phrase "horror auto toxics" for harmful ("toxic") immune reactions against self (Cohen, 1999). Autoimmunity is an important cause of disease in humans. Term autoimmunity is often erroneously used for any disease in which immune reactions accompany tissue injury, even though it may be difficult or impossible to establish a role for immune responses against self-antigens in causing these disorders. Because inflammation is a prominent component of these disorders, they sometimes grouped under immune-mediated inflammatory diseases, which does not imply that the pathologic response directed against self-antigens. (Abbas *et al*., 2012). Autoimmunity results from a failure of the mechanisms of self-tolerance in T or B cells, which may lead to an imbalance between lymphocyte activation and control mechanisms (Bellanti, 1985, Abbas *et al*., 2012). The potential for autoimmunity founded in all humans body, the cause due to some randomly generated specificities of clones of developing lymphocytes may be for many of self-antigens, and lymphocytes can be accessible for these antigens. As

discussed before, the body maintain self-tolerance by process called selection which do not allowed or prevent the maturation of some self-antigen specific lymphocytes by mechanisms that inactivated or deleted these lymphocytes that do mature, self-tolerance failed if these self-reactive lymphocytes are not deleted or inactivated during or after their maturation and if APCs activated so that self-antigens presented to the immune system in an immunogenic manner (Abbas *et al*., 2012).

T cell role in autoimmunity recently has been under great attention, for two main reasons:

1. T helper (Th) cells are key regulators for all immune responses to proteins, and most self-antigens implicated in autoimmune diseases are proteins.
2. Several autoimmune diseases are genetically linked to the MHC (the HLA complex in humans), and the function of MHC molecules is to present peptide antigens to T cells. Failure of self-tolerance in T lymphocytes may result in autoimmune diseases in which tissue damage caused by cell-mediated immune reactions (Abbas *et al*., 2012). Helper T cell abnormalities may also lead to autoantibody production because helper T cells are necessary for the production of high-affinity antibodies against protein antigens.

Autoimmune diseases tend to be chronic, progressive, and self-perpetuating. The reasons for these features are that the self-antigens that trigger these reactions are persistent, and once an immune response starts, many amplification mechanisms are activated that perpetuate the response.

Autoimmune disease causing tissue injury in different mechanisms immune complexes, autoantibodies, self-reactive T lymphocytes.

The clinical and pathologic features of the disease usually determined by the nature of the dominant autoimmune response. In addition, a

response initiated against oneself leads to tissue damage, which may cause the releasing of the tissue antigens, which activate the lymphocytes that specific for those other antigens. This phenomenon called epitope spreading, and it may explain why once an autoimmune disease has developed, it may become prolonged and self-perpetuating. (Abbas *et al.*, 2012).

2.2.2: Progression to Autoimmune Disease Occurs In Stages

These findings allow us to conceptualize autoimmune disease as a multistep process. The first stage is predisposition of an individual to autoimmunity by his or her genes, and other factors such as female hormones (gender) and ethnic origin. The second phase initiated by an event, probably stochastic or perhaps caused by an environmental trigger such as infection, ultraviolet radiation, smoking; leading to loss of self-tolerance and autoantibody production (Whitacre, 2001; Ray *et al.*, 2012; male *et al.*, 2013) This however is not alone sufficient to cause disease. A further step is required before progression to a third phase involving tissue damage by the autoimmune attack. This autoimmune attack leads to further release of self-antigens, (Figure 2-1) which are not remove in the normal efficient manner, and propagation of the autoimmune response, resulting in the clinical manifestations of disease (Ray *et al.*, 2012; male *et al.*, 2013). The earliest clinical features of systemic autoimmune diseases such as SLE or RA are usually non-specific such as fatigue or constitutional symptoms. This prodromal typically precedes the development of the classic disease phenotype by weeks to months.

During the propagation phase, not only is there an autoimmune response to an increasing number of autoantigens (demonstrated by the sequential development of multiple autoantibodies in patients with SLE), but also to

more epitopes within each antigen – a phenomenon termed epitope spread. Epitope spread can be involve multiple epitopes on the same molecule (intramolecular spread), or epitopes on different molecules associated as part of a macromolecular complex (intermolecular spread). The latter provides a mechanism for how antibodies to non-protein self-antigens such as DNA and phospholipid can occur. (Male *et al*., 2013).

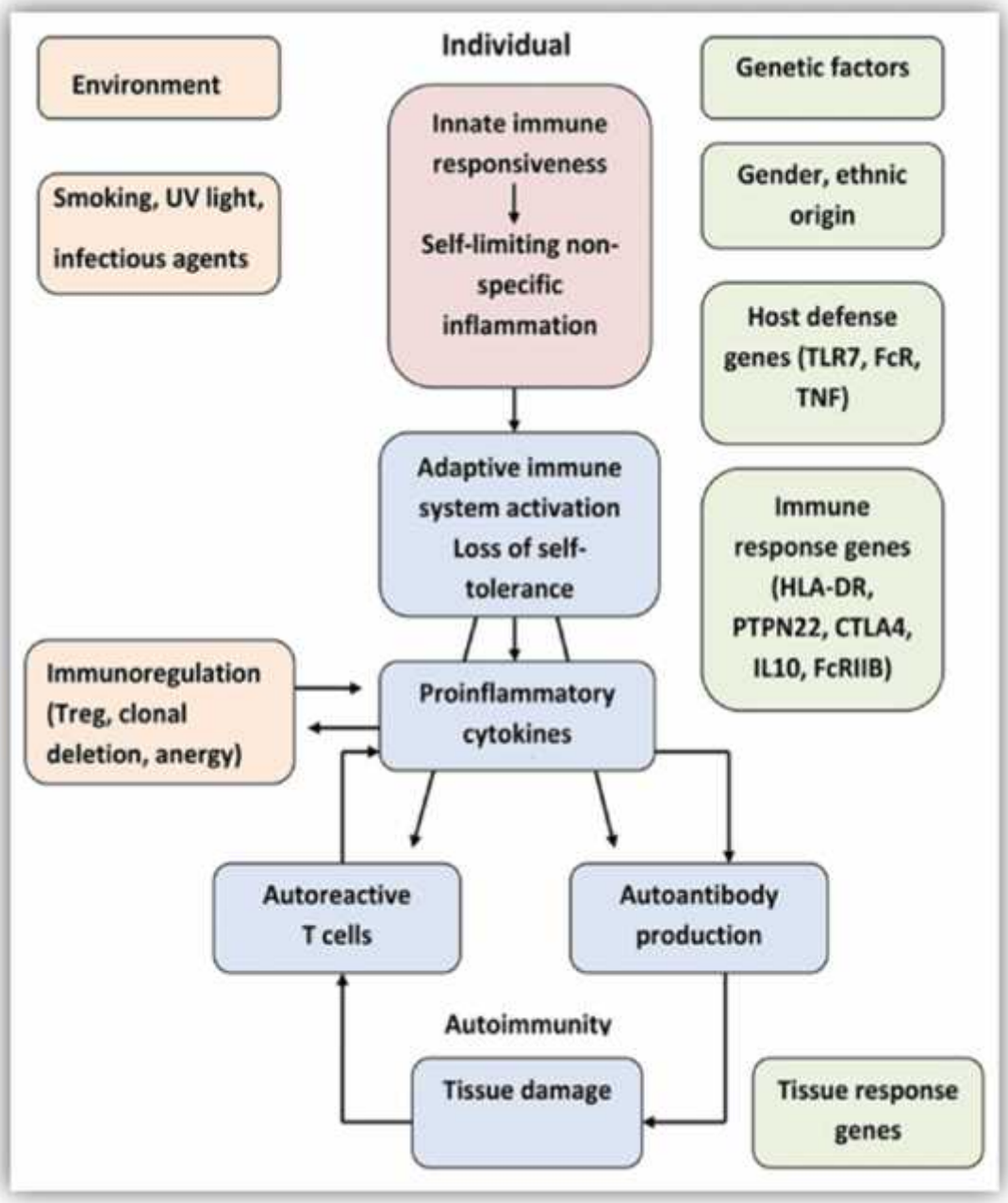

Figure 2- 1 Pathways influencing the development and perpetuation of autoimmune diseases. (Ray *et al.*, 2012)

2.2.3: Classification of Autoimmune Diseases

Depending on the distribution of the autoantigens that recognized by the immune system, autoimmune diseases could be classify into:

1- Organ specific autoimmune diseases: include autoimmune diseases that affect one organ usually like Hashimoto's Thyroiditis, Pernicious Anemia, Adison's Disease, Hemolytic Anemia and Multiple sclerosis.
2- Systematic autoimmune diseases: includes autoimmune diseases that affect more than one organ like Systemic Lupus Erythematosis, Rheumatoid Arthritis and Dermatomysitis.(Chapel *et al*.,1999)

2.3 Multiple Sclerosis (MS)

Is the most important, common neurological disease, it is an autoimmune chronic inflammatory, demyelinated disease; it is the first cause of non-traumatic disabilities in youth age. (Ebers, 2000; Goldsby *et al*., 2000; Braunwald *et al*., 2003; Stephen and Douglas, 2013; Internet 1). The (CNS): include brain, optic nerves and spinal cord. Nerve cells have a protective sheath called (myelin), it is very important for the nerve cells impulses transform. The immune system attack myelin and that lead to damages areas in myelin called scares (plaque or lesion) (Figure 2-2) from here MS had its name. (Ebers, 2000; Braunwald *et al*., 2003). That could leading to damage the nerve fiber itself when attacks repeated in the progressive stages of the disease, by damaging the nerve fiber the communication between the damaged cell and other nerve cells broken leading to, un reversible neurological dysfunctions, the disease exact cause still unknown experts prefer to call it immune mediated instead of autoimmune disease because the exact antigen or target that the immune system initiate an attack onto it still unknown (Christopher *et al*., 2000;

Stuve and Zamvil, 2001; Braunwald *et al*., 2003; Stephen and Douglas, 2013; Internet 1, 2014).

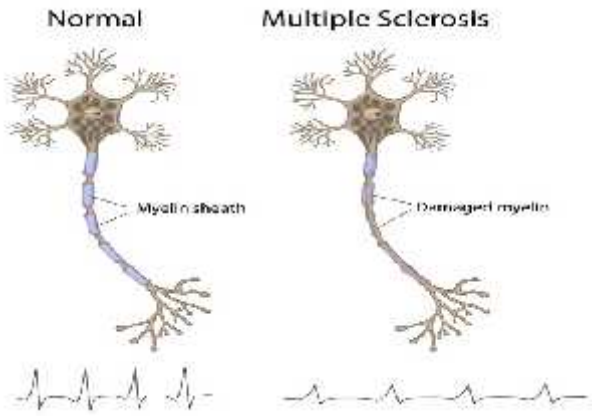

Figure 2- 2 How MS demyelinated nerve cell. (Internet 2, 2015)

2.3.1: Disease Courses

There are four described MS clinical types:

The stages of MS is highly variable and difficult to predict in a particular individual. The main clinical patterns are illustrate in Figure (2-3) and discussed further below.

1. Relapsing-remitting MS: Including (85% of cases). In this classic form of the disease, long periods of stability (remissions) are punctuated by discrete episodes of neurological dysfunction (relapses) followed by partial or complete recovery. New neurological symptoms or signs that are present for at least 24 hours and are not associated with a fever define a relapse. One in five patients will experience this form of the disease for at least 20 years (benign MS) but most will eventually convert to a phase of gradual functional decline (secondary progressive MS).
2. Secondary progressive MS: The percentage patients converting from relapsing-remitting to secondary progressive disease is around 50%

at 10 years and more than 90% at 30 years. This characterized by gradual accumulation of permanent neurological deficits.

3. Primary progressive MS: (which occurs in 10–15% of patients) there is steady functional decline from the start of the illness, with gradual accumulation of irreversible neurological deficits. Males and females are equally affected and age at onset is about ten years later than in relapsing-remitting disease, which coincides with the typical age of conversion from relapsing to secondary progressive MS.
4. Progressive-relapsing MS: Includes 5% of cases in this type, patients have steady functional decline, in addition to an acute exacerbations or 'flare ups' (attacks) may also occur occasionally.
5. Marburg variant MS: it's the most severe subtype (acute multiple sclerosis), this is a rare, hyper acute form of MS that usually leads to death within six months (sometimes after only a few weeks). (Kremenchutzky *et al*., 2000; Stephen and Douglas, 2013; Johns, 2014).

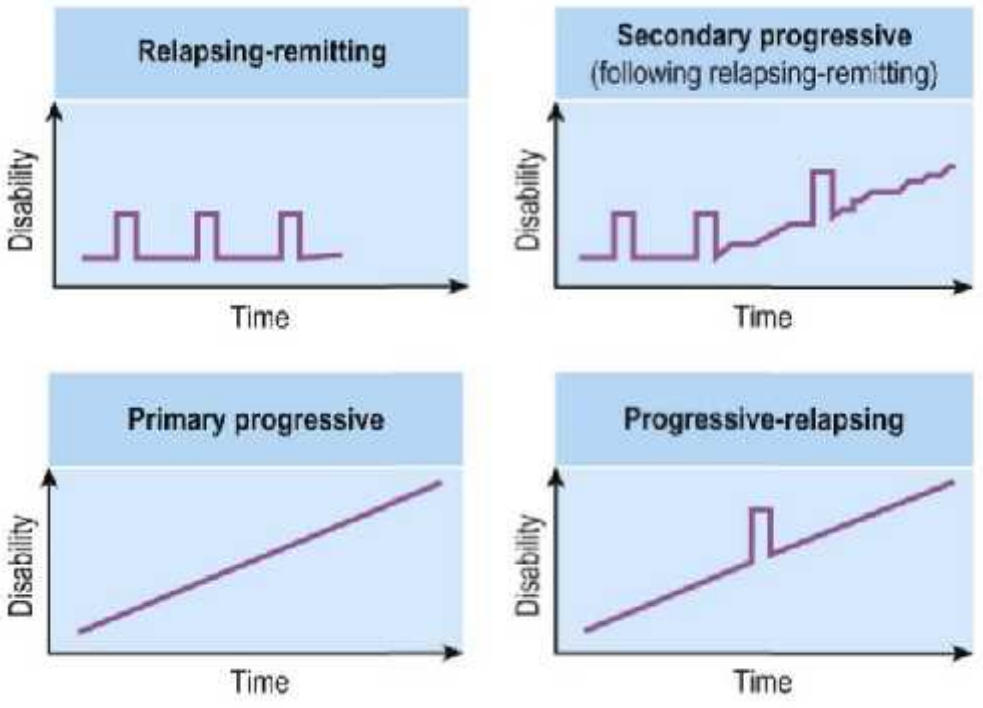

Figure 2- 3 Disease stages. (Johns, 2014)

2.3.2: Demyelination

The term demyelination refers to the loss of normally formed myelin and can classified as primary or secondary:

1. **Primary demyelination** is selective loss of myelin with relative preservation of axons. Multiple sclerosis is the most common and important cause.
2. **Secondary demyelination** is degeneration of the myelin sheath following axonal loss. (Johns, 2014).

2.3.3: Clinical Features of MS

Multiple sclerosis is usually a relapsing-remitting disorder. Each clinical episode (or relapse) caused by a focus of demyelination in the brain or spinal cord, which is referred to as a plaque. When a relapse occurs, symptoms typically develop over a few days and gradually resolve over a number of weeks, as the inflammation subsides and the plaques remyelinate to a greater or lesser degree (Internet 3, 2014; Johns, 2014).

2.3.4: Common Symptoms

Although plaques can occur anywhere in the brain or spinal cord, including the central visual pathways, some sites are more likely to affect than others are. This means that certain symptoms and signs are more common. (Johns, 2014). Common symptoms in multiple sclerosis:

- **Visual Symptoms (25% of cases)**

  Inflammatory demyelination of the optic nerve (termed optic neuritis) is common in MS. This causes focal-sensory deficit, unilateral or bilateral visual loss, and diplopia. Symptoms usually resolve completely within a few weeks.

- **Pain and fatigue**

  Up to 90% of people with MS suffer from chronic fatigue, which characterized by overwhelming mental and physical exhaustion.

- **Motor and sensory symptoms**

  They are common in MS, such as weakness in one or more limbs (40% of cases), temporary episodes of slurred speech, incoordination, muscle spasms or painful stabbing sensations, imbalance, vertigo.

- **Cognitive and emotional changes**

  They occur in at least 40% of patients with MS, such as attention, working memory, decision-making, but depression is more common (seen in up to 50% of patients) and the risk of suicide is also increased. Psychotic features (delusions and hallucinations) are rare.

- **Bladder, bowel and sexual dysfunction**

  Bladder problems such as urinary retention and frequency are common in multiple sclerosis, but faucal incontinence is rare. Sexual function may compromised in longstanding disease and up to 40% of male patients experience some degree of erectile dysfunction.

- **Cerebellar features**

  Involvement of the cerebellum or its connections with the brain stem may cause dysarthria (slurred speech), ataxia (incoordination) or nystagmus (a rhythmic abnormality of gaze fixation, with a frequency of 1–4 Hz, consisting of a slow drift phase and a brisk corrective 'snap'). There may also be a cerebellar intention tremor. This is worse towards the end of deliberate or precise movements. (Bagnato *et al*., 2000; Christopher *et al*., 2000; Johns, 2014).

- **Temperature sensitivity known as Uhthoff's phenomenon**

  Some MS symptoms are clinically silent lesions unmasked by an increase in body temperature, increases the chance of conduction failure

in partially myelinated or incompletely demyelinated axons. (Johns, 2014).

2.3.5: Diagnosis

1. Clinical history and examination MS clinical diagnosis needs two clinical relapses or remissions with one clinical evidence on two distinct damages in the CNS or two clinical remissions with one clinical evidence on one damage and another semi clinical evidence for another distinct damage (Poser *et al*., 1983). The two relapses should be in different parts of the CNS and it should be separated with a period of month and more and do not continue for more than 24 hours as maximum. (McDonald *et al*., 2001).

   The diagnosis of multiple sclerosis is primarily clinical, but is confirmed and supported by neuroimaging, serological testing and electrophysiology.

2. **Neuroimaging**

   The most sensitive method for demonstrating MS lesions is magnetic resonance imaging (MRI), which shows ten times more plaques than clinical episodes (since most lesions are clinically silent). MRI shows multifocal white matter abnormalities in 95% of cases. Administration of the MRI contrast agent gadolinium is useful for demonstrating acute (active) lesions. This correlates with breakdown of the blood–brain barrier in areas of active inflammation and demyelination. McDonald developed a criteria-using MRI for earlier diagnosis, it became the most common criteria used in MS diagnosis, but it cannot recognize between MS and cerebral vasculitis. (Mc Farland *et al*., 1998; Abdulamir, 2009; MSIF, 2013; Johns, 2014).

3. **Cerebro Spinal Fluid (CSF)**

   The CNS inflammatory response in multiple sclerosis is associated with synthesis of antibodies (immunoglobulins) in the brain and spinal cord. It is therefore possible to detect antibodies in the CSF that are not present in peripheral blood. A sample of CSF obtained by lumbar puncture and a specimen of venous blood taken at the same time, for comparison. The two specimens are run on an electrophoretic gel to look for bands indicating the presence of type G immunoglobulins (IgG) that are only present in the CSF (which is indicative of CNS inflammation). These known as oligoclonal bands (OCBs) and found in 90% of people with MS. (Abdulamir, 2009, 2004; Johns, 2014).

4. **Evoked Responses**

   Decreased conduction speed in the central visual pathways can demonstrated in the majority of patients with MS by obtaining Visual Evoked Potentials (VEPs). Scalp electrodes record electrical activity in the occipital cortex in response to a changing visual stimulus such as an alternating checker board pattern. The stimulus-response sequence repeated many times and averaged (to increase the signal-to-noise ratio). This reveals a characteristic positive wave in the visual cortex at 100 milliseconds (the P100 wave) which delayed by 30–40 milliseconds in 95% of people with MS. (Braunwald *et al*., 2003; Johns, 2014).

5. **Differential Diagnosis**

   This diagnose used to recognize MS from other disease share similar symptoms. (Lance D., 2004).

2.3.6: Management (Treatments)

There is no cure for MS and the treatment:

1. **Acute relapse suppression is mainly supportive**.

   Acute relapses usually managed with a 3–5-day course of high-dose intravenous corticosteroids (e.g. methylprednisolone) (Figure 2-4) or sometimes-oral prednisolone. This has an immunosuppressive effect that shortens relapses and provides symptomatic relief, but does not improve long-term outcome. (Lance D., 2004; Guzel *et al*., 2006; Stephen and Douglas, 2013).

Methylprednisolone mechanism of actions and effect on cellular immune system function and inflammation:

1. Redistribution of T cells with transient alteration in T cell counts
2. Decreased T cell responses to antigen and mitogen .
3. Decreased synthesis and release of pro-inflammatory cytokines and growth factors (IL-8).
4. Decreased in constitutive HLA-DR expression UP-regulation of TGF-β and IL-10 expression.
5. Increased number of monocytes, neutrophils and T and B-lymphocytes.
6. Increased proportion of fas-expressing $CD4^+$ T lymphocytes and decreased proportion of Fas-expressing $CD8^+$ T lymphocytes.
7. Decreased memory ($CD45RO^+$) $CD4^+$ T lymphocytes and increased CCR5 expression on $CD4^+$ lymphocytes, the latter lasting over 1 month.
8. Increased leukocyte apoptosis inhibitition of IFN-γ up-regulation of class II expression by macrophages and microglia
9. Decreased eicosanoid production by monocytes.
10. Decreased Fc receptor expression by macrophages.

11. Decreased immunoglobulin levels 2-4 weeks post-treatment.
12. Increased synthesis of lipocortin 1 and reduced transcription of cyclooxygenase II gene. (Kinkel, 1999).

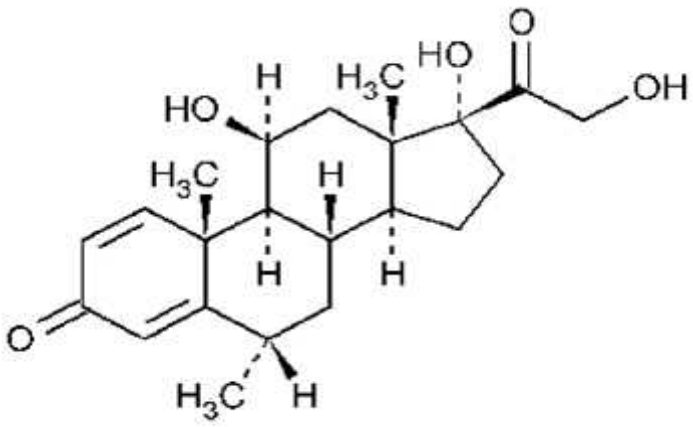

Figure 2- 4 Methylprednisolone chemical structure. (Internet 4, 2015)

2. **Disease Modifying Drugs (DMDs)**

   Several disease-modifying agents licensed for use in MS, but mainly suitable for relapsing-remitting disease, with little effect once the patient has entered the progressive phase. Although disease-modifying agents are not curative, they do reduce relapse frequency and severity by up to two thirds. First-line treatment in MS includes (i) interferon beta and (ii) glatiramer acetate. (John, 2014).

- **Interferon beta**

  Interferons (Figure 2-5) are cytokines (inflammatory mediators) that influence immune responses and interfere with viral replication. Its action mechanism not certain MS, but interferons known to have immune modulating and anti-inflammatory properties. Neuroimaging studies show that they reduce the number of inflammatory CNS lesions by more than 50%. (Stephen and Douglas, 2013; Johns, 2014).

Drug mechanism of actions and effect on immune system function and inflammation:

1. Down regulating of MHC expression on antigen-presenting cells surfaces.
2. Inhibiting pro-inflammatory cytokines level and elevating regulatory cytokine levels.
3. Inhibition the proliferation of T lymphocytes.
4. Limiting the trafficking of inflammatory cells in the Central Nervous System. IFN-β reduces the attack rate and ameliorates disease severity measurements like EDSS progression in addition to the MRI-documented disease lesions. (Stephen and Douglas, 2013).

Two forms of interferon beta used in the treatment of MS: 1. (Avonex, Rebif) contain Interferon beta-1a (administered by intramuscular or subcutaneous injection) (Figure 2-5). 2. Betaseron contain Interferon beta-1b (administered subcutaneously). Flu-like symptoms appear as a side effect in additional to (muscle aches, fever, chills and malaise) for 24–48 hours after injection. In the longer term, there is a risk of liver function abnormalities and immunosuppression (reduced white blood cell count). Interferons not recommended for children or for women who are pregnant or breast-feeding. (Weinbenker and Keegan, 2007; Johns, 2014).

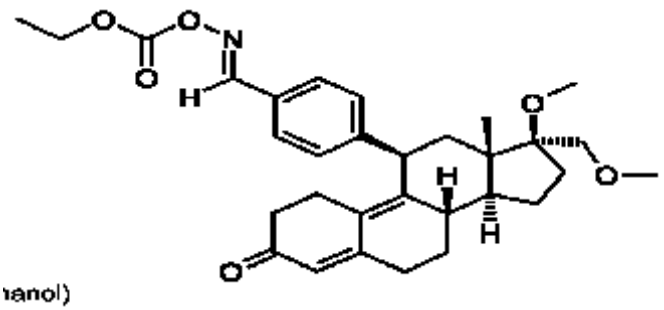

Figure 2- 5 Avonex, Rebif (Interferon-β1a) chemical structure. (Internet 5, 2006)

- **Glatiramer acetate**

  It is four synthetically mixed amino acids exist in myelin basic protein. It administered by daily subcutaneous injection. The original rationale for its use was to compete with (or mimic) myelin basic protein but its actual mechanism of action is not certain. It appears to reduce antigen presentation and to promote secretion of anti-inflammatory cytokines from activated immune cells. There are usually no major side effects, in contrast to interferon beta, but this drug also not recommended for children or for women who are pregnant or breast-feeding. (Mc Farland *et al*., 1998; Johns, 2014).

- **Natalizumab**

  This monoclonal antibody (immunoglobulin G, IgG) given by intravenous injection every 28 days. Clinical trials show that it reduces the number of relapses by about two-thirds. This designed to prevent leukocytes from binding to blood vessels, reducing the number of chronic inflammatory cells entering the CNS from the bloodstream. Side effects include skin rash, headache, nausea, and vomiting. (Rice *et al*., 2005; Niino *et al*., 2006; Stuve and Bennett, 2007; Mellergård *et al*., 2010)

- **Fingolimod**

  This is the first oral agent that has licensed for MS treatment. It reduce relapses number by around 50%. It works by inhibition of sphingosine 1-phosphate receptors, which prevent lymphocyte migration from lymph nodes. However, a number of potentially serious side effects have described. These include bradycardia, immunosuppression, liver toxicity and allergic reactions. This drug therefore only used in patients with severe relapsing-remitting MS, particularly people who are not responding to first-line treatments. (Pelletier *et al*., 2012).

- **Unlicensed drugs**

  Other drugs that may be useful in the management of MS are the immunosuppressive agent azathioprine and the chemotherapy drugmitoxantrone, but neither of these licensed in the UK.

  Mitoxantrone, in particular, may be beneficial in patients with secondary progressive MS and might delay the transition from relapsing-remitting to progressive disease. It appears to work by suppressing activity in lymphocytes and macrophages, which are responsible for the immune-mediated attack on myelin (discussed below). In keeping with other anti-cancer drugs, the side effects include nausea, hair loss, and vomiting; more serious adverse effects sometimes occur, such as cardio toxicity and bone marrow suppression (carrying a significant infection risk). (Johns, 2014).

- **Intravenous immunoglobulin (IVIG)**

  Is pooled human immunoglobulin G that may use as a second line therapy for RRMS patients who are unable to tolerate standard disease-modifying treatments. The mechanism of action in MS is complex and incompletely understood, but presumed to be immunomodulatory. It can give to women who are unable to take their normal disease-modifying agents due to pregnancy or breast-feeding. This is important, since one in three women experience a relapse in the post-partum period. (Johns, 2014; Stachowiak, 2014).

2.3.7: Epidemiology of MS

Globally MS had a widespread presence; it mean prevalence has elevated from 30/100,000 to 33/100,000 in five years period (from 2008 to 2013) with average age of onset 30 years old. This increasing in MS prevalence is not clear if it due to better diagnostic, reporting or other

causes (MSIF, 2013), the highest prevalence was reported in North America and Europe (140 and 108/100,000 respectively) and the lowest in Sub-Saharan Africa and East Asia, at 2.1 and 2.2/100,000 respectively.

MS incidence also varies in the same regions, the highest prevalence in Europe is 189/100,000 in Sweden, and the lowest is 22/100,000 in Albania. in addition, it has been reported that the disease incidence varies according to latitude. MS atlas 2013 found that the prevalence in South America, the i.e Argentina considered a medium risk country for this disease, is estimated to be 18/100,000, which is six times higher than Ecuador 3.2/100,000, MS low risk country (MSIF,2013). Global survey study founded that female to male ratio was 2:1 and it still the same since 2008, the cause behind the ratio gap not fully understood but it may due to sex hormone. In the last decades MS increased in many countries, the cause behind it still unknown. (Whitacre *et al.*, 1999; Ebers, 2000; MSIF, 2013).

2.3.8 Etiology

MS specific cause is unknown, but it is widely thought that it result from an interaction between genetic and environmental factors:

a) Genetic and immunologic factors

MS cannot inherited in a simple Mendelian fashion and there are no familial forms. Nevertheless, concordance is around 25% in identical twins, compared to around 5% for fraternal twins and siblings – and the risk is up to 20 times higher in first-degree relatives of people with MS. (Sadovnick, 1994; Xu, *et al*., 2001; MSIF, 2013).

Familial clustering is likely to be due to a number of unknown susceptibility genes, but candidates have been difficult to identify. This is probably because the genetic effects are small and involve multiple

genes, each making a modest contribution to overall risk. Robust associations have only found with certain human leukocyte-associated antigen (HLA) genes, particularly within the class II region of the major histocompatibility complex (MHC) of antigens on chromosome 6. The most consistently implicated subtype in Caucasians (the population at greatest risk) is HLA-DRB1*15 (HLA-DR15 haplotype). In other populations, different HLA types may be more important and the estimated contribution to overall genetic susceptibility varies from 20–50%. (Dyment *et al*., 2004; MSIF 2013).

b) Environmental Factors

The prevalence of multiple sclerosis varies with distance from the equator (Figure 2-6). Equatorial regions tend to have comparatively low prevalence rates, whereas more temperate areas to the north and south have a progressively greater incidence. Some of the highest recorded rates of MS have identified in the northern part of Scotland and in North America. (Forbes and Swngler, 1999; Lance D., 2004).

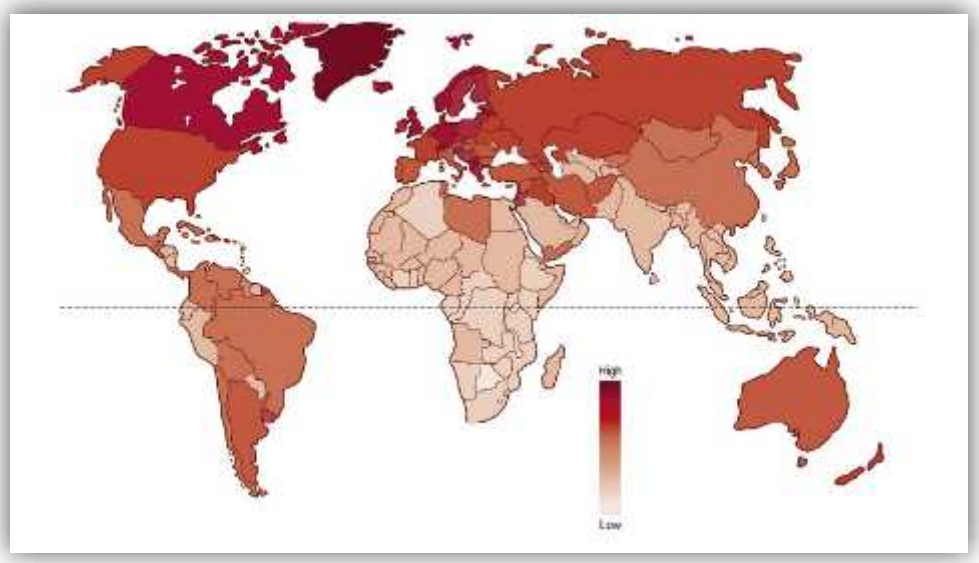

Figure 2- 6 Geographical distribution of multiple sclerosis risk. (MSIF, 2013)

Individuals who are closer to the equator below the age of 15 years are at a lower risk of MS in adulthood, regardless of subsequent migration. Migration studies showed that geographic risk in MS relates to location before puberty (under the age of 15) and individuals who migrate after this age carry the risk of their original location. This may reflect exposure to an environmental agent (such as a virus) during a critical time-window prior to puberty. The period coincides with maximal development and involution of the thymus gland. This is the site of T-cell (thymus cell) maturation and the deletion of potentially auto-aggressive cells by apoptosis. (Christopher *et al*., 2000; MSIF, 2013).

- **Sunlight and vitamin D**

Proximity to the equator may reduce MS risk because of increased sunlight exposure. This could reflect a lower chance of encountering a particular pathogenic virus (since viruses destroyed by ultraviolet radiation in sunlight) or higher levels of vitamin D (in keeping with research showing that supplementation may reduce relapse frequency). There is also a month of birth effect: in the Northern Hemisphere, more people with MS are born in the spring than the autumn. This phenomenon may related to maternal sunlight exposure or vitamin D status.

- **Viral and other infections**

A number of infectious agents have been proposed as the cause of multiple sclerosis including Epstein–Barr virus (EBV), human herpes virus 6 (HHV-6) and many of the immunological features of MS are suggestive of a virally mediated process. Which by viruses molecular mimic myelin or they activated pathogenic T cell by their super

antigen. The bacterial agent *Chlamydia pneumonia* has also implicated – and bacterial infection known to be associated with some cases of demyelination in the peripheral nervous system. Nevertheless, no causative agent has unequivocally implicated and no microorganisms have been isolated from human tissues. (Stephen and Douglas, 2013; Johns, 2014).

2.3.9: Pathogenesis of MS

MS pathogenesis is trigger is unknown, but for sure, it starts with activated T cell. When a self-antigen like myelin in this case binds to an antigen presenting cell (APC) through it toll-like receptors (TLRs) and phagocytized and expressed on (APC) surfaces this lead to activation of T cells with association with self-tolerance failure, this activated T cell become an auto reactive T cell (Gandhi *et al.*, 2009; Abbas *et al*., 2012). Then it will differentiate in to Th1 or Th17 or both (both of T cells subsets proved to be involved in the pathology of MS). Auto reactive T cell release a pro-inflammatory cytokines such as IFN-γ and interleukins. These cytokines binds to other T cells, B cells, Monocytes (macrophages) to augments the immune response, these cytokines induce epithelial cells (lining the blood vessels) to express adhesion molecules such Vcam on epithelial cells that, auto reactive T cell expressed VLA4 molecule which bind to Vcam which allow this auto reactive T lymphocyte to cross the blood brain barrier (BBB), to the CNS. (Weber *et al.*, 2007; Kasper and Shoemaker, 2010; MSIF, 2013; Johns, 2014;). Once the auto reactive T cell inter CNS it continues to release the pro-inflammatory cytokines and this affect the BBB by increase it permeability. Another types of immune cell included B cells and macrophages inter the CNS via BBB to enhance the response against myelin and oligodendrocytes. B cell produce antibody that directly attack myelin and oligodendrocytes, while macrophages

phagocytosis and destroyed myelin by their cytokines, which give them a foamy appearance because myelin debris accumulated within activated macrophages. (Loma and Heyman, 2011; MSIF, 2013; Johns, 2014). The involvement of innate and adaptive immune responses in MS pathology is clear from what we mention above (Figure 2-7).

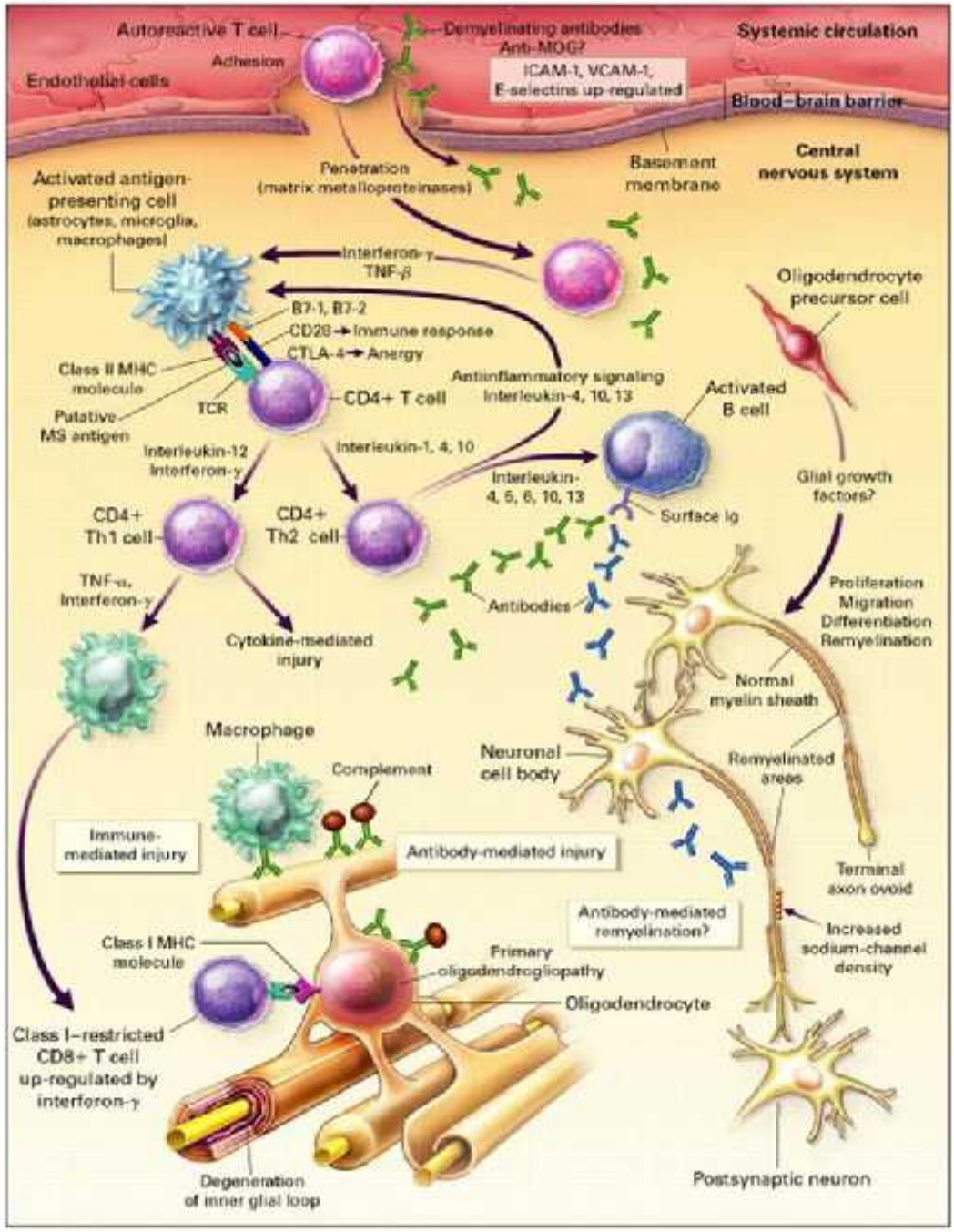

Figure 2 – 7 Multiple sclerosis pathogenesis (Internet 6, 2014)

2.4: T regulatory Cells

Since MS caused by autoimmune response to self-antigens and T Regulatory (Treg) cells are key regulators of immune homeostasis and self-tolerance, (Yomamura,T. and Gran,B. 2013).

Treg cells have defined as CD4+CD25+FoxP3+ Tcells that are capable of modulating the immune function of various effector cells. The population of CD4+CD25+FoxP3+Treg cells comprises two subpopulations: naturally occurring and induced Treg cells (Curotto de Lafaille and Lafaille 2009). Naturally, occurring Treg cells sub population that differ from induced Treg cells in their specialized for suppressive function that has been determined during its development in the thymus. Studies investigating immunological dysfunctions in autoimmune diseases need to consider the complex composition of the human Treg cell repertoire. Human Treg cells have described as CD4+CD25high T cells populationthat exist in thymus and peripheral blood (Sakaguchi *et al*., 1995; Baecher-Allan *et al*., 2001; Dieckmann *et al*., 2001; Jonuleit *et al*., 2001; Levings *et al*., 2001; Ng *et al*., 2001; Taams *et al*., 2002).

High CD25 expression Tregs isolation would lead to the exclusion of the naive, CD4+FoxP3lowCD25mid Treg cell population. (Roncador *et al*., 2001; Fontenot *et al*., 2003; Hori *et al*., 2003). It Is Essential for Immune Homeostasis, the Definitive proof on that is the IPEX syndrome. (Fatal immunodysregulation, polyendocrinopathy, enteropathy, X-linked) it is a multiple auto immune disorders resulted from a mutation in human FoxP3 (Bennett *et al*., 2001; Wildin *et al*., 2001; R. Wildin, 2002; Internet 7, 2014). CD4+CD25+ Non-regulatory T cells expressing low levels of FoxP3 and CD127 exist, that fact impairs the significance of a staining with CD25 and CD127 in order to isolate Treg cells (Miyara *et al*., 2009). These non-regulatory Treg cells secrete pro-inflammatory cytokines like IFN-γ and IL-2, but do not show suppressive ability in vitro. Thus, FoxP3 gene

methylation status can linked to the difference between FoxP3+ regulatory and non-regulatory T cells, with FoxP3+ regulatory T cells being completely and FoxP3+CD4+ non-regulatory T cells being incompletely demethylated (Miyara *et al*., 2009).

2.4.1 Treg Cells Activity

- **Mechanisms of Treg Cell Suppression**

It have shown by Laboratory experiments that Treg cells does not depended on one particular mechanism in there suppressive function ,so here is some suggested mechanisms :

a) Cytokine secretion. In vivo, Treg cells secrete the suppressive cytokines TGF-β and IL-10 (Powrie *et al*., 1996; Asseman *et al*., 1999).

   TGF-β has shown to be essential for Treg cell-mediated suppression of effector CD4+ T cells.

b) Cell–cell contact as one possible mechanism of suppression used by Treg cells. (Nakamura *et al*., 2001) have shown that membrane-bound TGF-β contributes to cell–cell contact-mediated suppression. Furthermore, the cell surface molecules Fas, Granzyme B, LAG3, and CTLA-4 have implicated in suppression (Read *et al*., 2000; Janssens *et al*., 2003; Huang *et al*., 2004; Cao *et al*., 2007;).

c) Competition for growth factors like IL-2 might contribute to the suppressive capacity of Treg cells (De la Rosa *et al*., 2004; Barthlott *et al*., 2005;).

- **Immune Functions Regulated by Treg Cells**

  Besides modulating the function of CD4+ T cells, Treg cells also regulate a broad variety of immune cells like CD4+ and CD8+ T cells, B cells, natural killer (NK) cells, natural killer T (NKT) cells, and APCs, through the suppression of activation, proliferation, and

cytokine production (Azuma *et al*., 2003; Misra *et al*., 2004; Taams *et al*., 2005; Zhao *et al*., 2006; Ralainirina *et al*., 2007).

2.4.2: Treg Cells in MS

The role of Treg cells in the development and in the course of MS has been in the focus of intensive clinical and basic research in the past years. These studies have investigated the frequency as well as the immune-modulating function of Treg cells, thereby considering disease activity and therapy status.

2.4.3 Flow Cytometry

Is a specialized instrument for detecting cell marked with fluorescence in cell suspension to count the cells that carrying target molecule in which fluorescence probe bound to it, after incubating the cell suspension with the desirable fluorescently labeled probe, the amount of this probe measured to detect the number of cells bound to it, it measured by passing one cell stream through a fluorimeter with laser beam in order to detect the fluorescent signals. Modern flow cytometry can detect three or more different colors, this allow to detect different molecules in the cell in the same time. It measures the forward and side scattering in addition to fluorescent signal detection, these light scatters used to distinguish different cell types, for example, compared with lymphocytes, neutrophils cause greater side scatter because of their cytoplasmic granules, and monocytes cause greater forward scatter because of their size (Abbas *et al*., 2012).

2.5: Present Study Cytokines

2.5.1: Interleukin 8

IL-8 (CXCL 8) is a pro inflammatory chemokine that identified originally as neutrophils chemoattractant after the observation of its role in inducing innate immunity, which is neutrophils chemotaxis and activation (Zeilhofer and Schorr, 2000). There is arrange of cell types that secrete IL-8 including leukocytes, endothelial cells, fibroblasts and malignant cancer cells, (Nazzal, 2014). IL-8 can exist in two forms, monomer or dimer differentially activates and regulate, it's two cell surface receptors, it is synthesized as an inactive 99 amino acid precursor protein, which N terminal cleavage producing a 77 amino acid protein from non-immune cells or 72 amino acid protein from immune cells (leukocytes), with a molecular weight of approximately 8 KDa (Nasser *et al*., 2009;).

2.5.2: Interleukin 10

IL-10 is an immune regulatory cytokine; many cell types secrete IL-10. Its function is inflammatory responses limitation, termination, and the regulation of differentiation and proliferation of T cells, B cells, natural killer cells, antigen-presenting cells (APCs), mast cells, and granulocytes. (Beebe *et al.*, 2002).

IL10 is a protein of 160 amino acids. It exists in non-covalent homodimer form. (Vieira *et al.*, 1991). Its molecular weight of approximately 18.5 KD. IL-10 immunomodulatory functions are various, like supporting B cell differentiation and Ig secretion in order to inducing a strong anti-inflammatory response (Kotiranta-Ainamo, 2006).

It was originally described as a murine Th2 cytokine, inhibiting Th1 cytokines. As later studies showed that in addition to the Th2 cells, there are different cell types (Th0 and Th1 cells, B cells and macrophages) that

produce IL-10. However many of IL-10 effects of are similar to, or overlap with, those of Th2 cytokines and that due apparently to the close correlation between IL-10 expression and the induction of Th2-like responses. (Özenci, 2002)

There is a strong relationship between IL-10 and autoimmune diseases especially MS due to its immune suppressive function.

There is a correlation between neurophathological lesions, MS symptoms severity and transferred auto reactive T cells amount. The balance between the Th1 and Th2 cytokine phenotypes may affect the disease activity. Since TH2 secreates IL-10, thus it is reasonable that inducing IL-10 may have a therapeutic effect in patient treatment (Ersoy *et al*., 2005).

2.5.3: Interleukin 12

Is an immune regulatory cytokine (inflammatory) made from two chains alpha and beta, it named because beta chain contain a beta sheath, it's the reactive port of IL-12, and alpha chain made of alpha helices. It secreted from APCs, its released caused the differentiation of naive T cells into Th1, Th1 secreted IFN-γ, which is a recruitment factor for macrophage, and monocytes, which results the accumulation of macrophages and monocyte in site of infection leading to inflammation. In addition, the continuing presence of IL-12 enhance Th1 function, which is a good thing during infection but very bad thing in autoimmune response. (O'Garra *et al.*, 1992; Magram *et al.*, 1996; Segal, 1998). There is an inverse relationship between IL-12 and IL-10; IL-10 controlled the production of IL-12, so IL-12 action controlled by IL-10. (Janeway, 2001; Segal, 1998). IL-12 action can start a new attack because this disease had

a sever nature MS, the immune system after the initial attack recognized myelin as invader, since the immune system had a memory, so all it takes to repeated the attack is to activate T cell, IL-12 can establish that. In addition, to the protective role of IL-12 to self-recognizing T cell, normally these cells signals a FAS binding protein to being a apoptosis, IL-12 inhibit this apoptosis pathway. (Cory and stone, 2002; Janeway, 2001).

2.5.4: Interleukin17

IL-17 is a pro-inflammatory cytokine (wright *et al*., 2008). It a glycoprotein of 155 amino acids with a molecular weight of 15 KDa. secreted as a disulfide-linked homodimer of 35 KDa (Vernal and Gacia-sanz, 2008). it increase the chemokine production like IL-8, the local production of chemokines such as IL-8 (wright *et al*., 2008), thereby promoting the recruitment of monocytes and neutrophils (Rachitskaya *et al*., 2008), it also can stimulate IL-6 and PGE2 production which enhance local inflammatory environment (Nurieva *et al.*, 2007), CD8+T cells, ɣ ST cells, NKT cells, Active neutrophils can produce IL-17. However, IL-17 considered as hallmark cytokine of Th17 (T-helper 17) cells. (Zhu, *et al*., 2012). Th 17 cells are a CD4+T helper cells subset, its mediate the defense against infections involved in auto immune disease development including MS. (Ouyang *et al*., 2008). IL-17 production can lead to excessive expression of pro-inflammatory cytokines and chronic inflammation leading to autoimmunity and tissue damage. (wei *et al.*, 2007).

2.5.5: Transforming Growth Factor Beta (TGF-β)

(TGF-β) family of proteins have attracted much attention because of their ability to control cellular functions that in turn and for their unique and potent immunoregulatory properties. Its Involved in cell growth,

differentiation, and immune modulation .TGF-β has contradictory effects on normal cells. Depending on cell type and the environment, it may stimulate or inhibit growth, regulate developmental fate in an instructive or a selective manner, and contribute to both the initiation and the resolution of processes involved in inflammation and tissue repair. It earned its name from inducing a transformed phenotype in cultured cells) . (Crawford *et al*., 1998; Letterio and Roberts, 1998; Herpin *et al*., 2003). It secreted by many cell types, including macrophages (Internet 8, 2015). TGF-β inhibit the adhesion molecules, provide a chemotactic gradient for leukocytes and other cells involving in an inflammatory response, and inhibit there activity was activation of latent TGF-β and activation was linked to immune defects associated with malignancy and autoimmune disorders, to susceptibility to opportunistic infection, and to the fibrotic complications associated with chronic inflammatory conditions. In addition to these roles in disease pathogenesis (Letterio and Roberts, 1998). It's so important for immune system regulation, since it is one of FOXP3 Treg cells regulation mechanisms is the secretion of TGF-β, it is also involved in FoxP3 T reg cells and Th17 differentiation from $CD4^+$ cells, it's block the activation of phagocytes derived by lymphocytes and monocytes. (Internet 8, 2015). EAE development can be suppressed by induced Tregs that stimulated with TGF-β and IL-12 iTreg suppress EAE development by $FoxP^+$, IL-10 mediated response that was a possible role of TGF-β and iTreg in MS regulation and treatment. (Selvaraj and Geiger, 2008).

2.5.6: Cytokines Estimation in Saliva:

Research involving salivary cytokines has grown over the last decade, and these studies have most often focused on periodontal disease or other oral diseases such as Sjögren's syndrome, oral lichen planus, and fungal infection (TSR, 2011). A major issue that has prevented salivary cytokines

from becoming more widely adopted in general research is that studies have generally found that salivary levels of these markers correlate only modestly to serum values, the apparent lack of correlation is not surprising, due to the multiple paths of entry into saliva that exist for these markers, In addition to being produced locally in the saliva glands, cytokines are produced in a variety of mucosal tissues and immune cells in the mouth, and they may also enter through micro-injuries or by transudation from blood, the permeability of membranes in oral tissues changes in response to infection and inflammation, hormone levels and short-term changes in the contributions from blood are therefore likely to occur, Because the salivary glands and other tissues in the mouth are also affected by the same outflow of autonomic nervous signals that govern systemic cytokine increases (TSR, 2011).

CHAPTER THREE

SUBJECTS, MATERIALS AND METHODS

Chapter Three
Subjects, Materials and Methods

3.1 Subject

Forty-five Iraqi patient with multiple sclerosis (MS) enrolled in this study. The patients attended the multiple sclerosis unit at consulting clinic of Baghdad teaching hospital at medicine city. During the period august – December 2014. The patients were clinically diagnosed by the consultant medical staff at the clinic, which was based on a clinical examination, magnetic resonance image (MRI), immunological tests, and under the supervision of this staff, information sheet (Appendix) was filled. The patients were divide into:

1. Untreated patients
2. Patients treated with MP.
3. Patients treated with IFN-β.

- For patients under treatment with suppression drug (MP), the collection of samples carried out after few days to one week from drug up take.
- For patients under treatment with (IFN-β), the collection of samples carried out in remission phase, the patients classified as relapsing remitting MS patients.
- For Untreated patients samples collected in attack period.

- **The Study Subject**

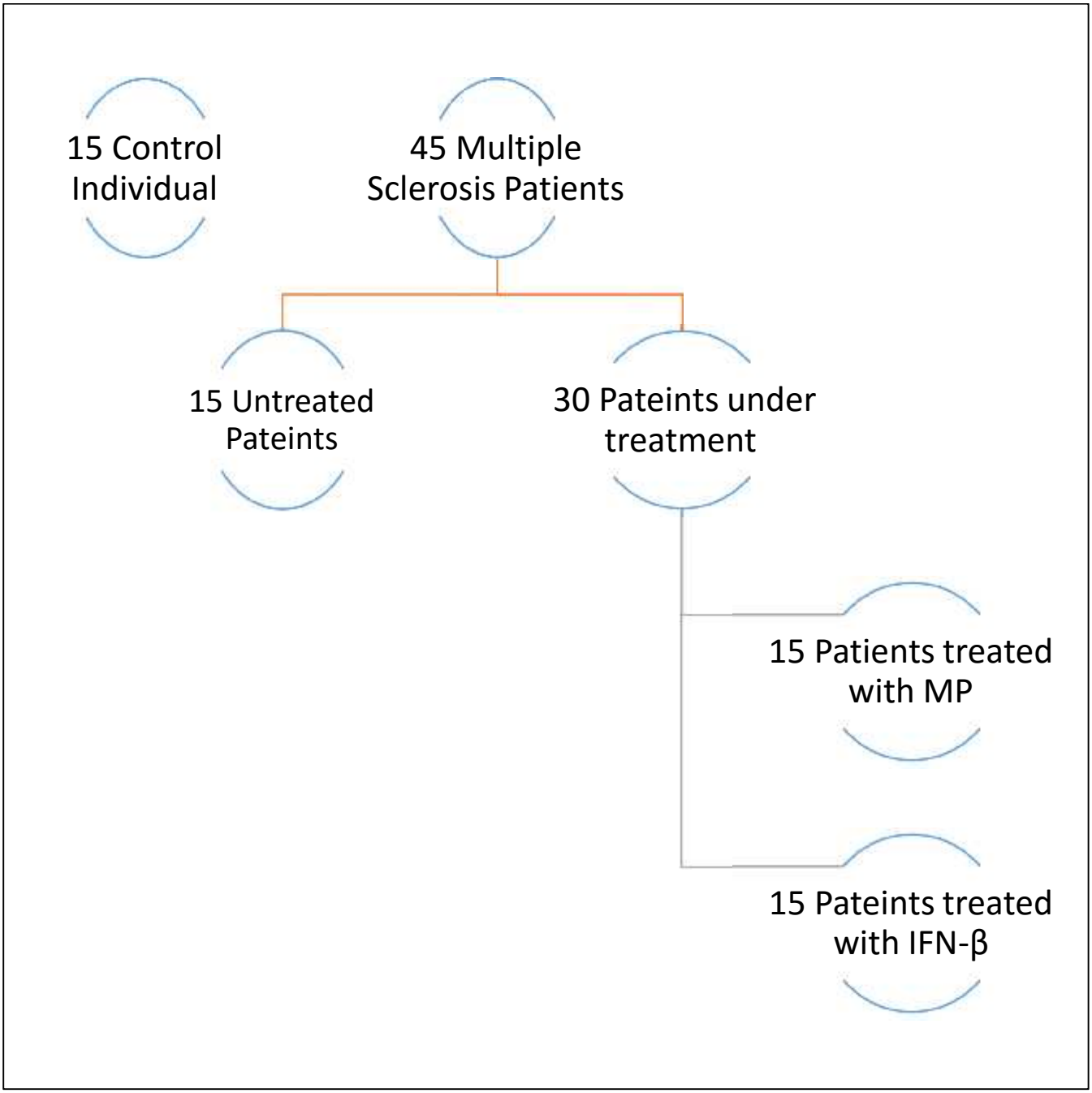

Figure 3- 1 Study Subject

- **The Study Plan**

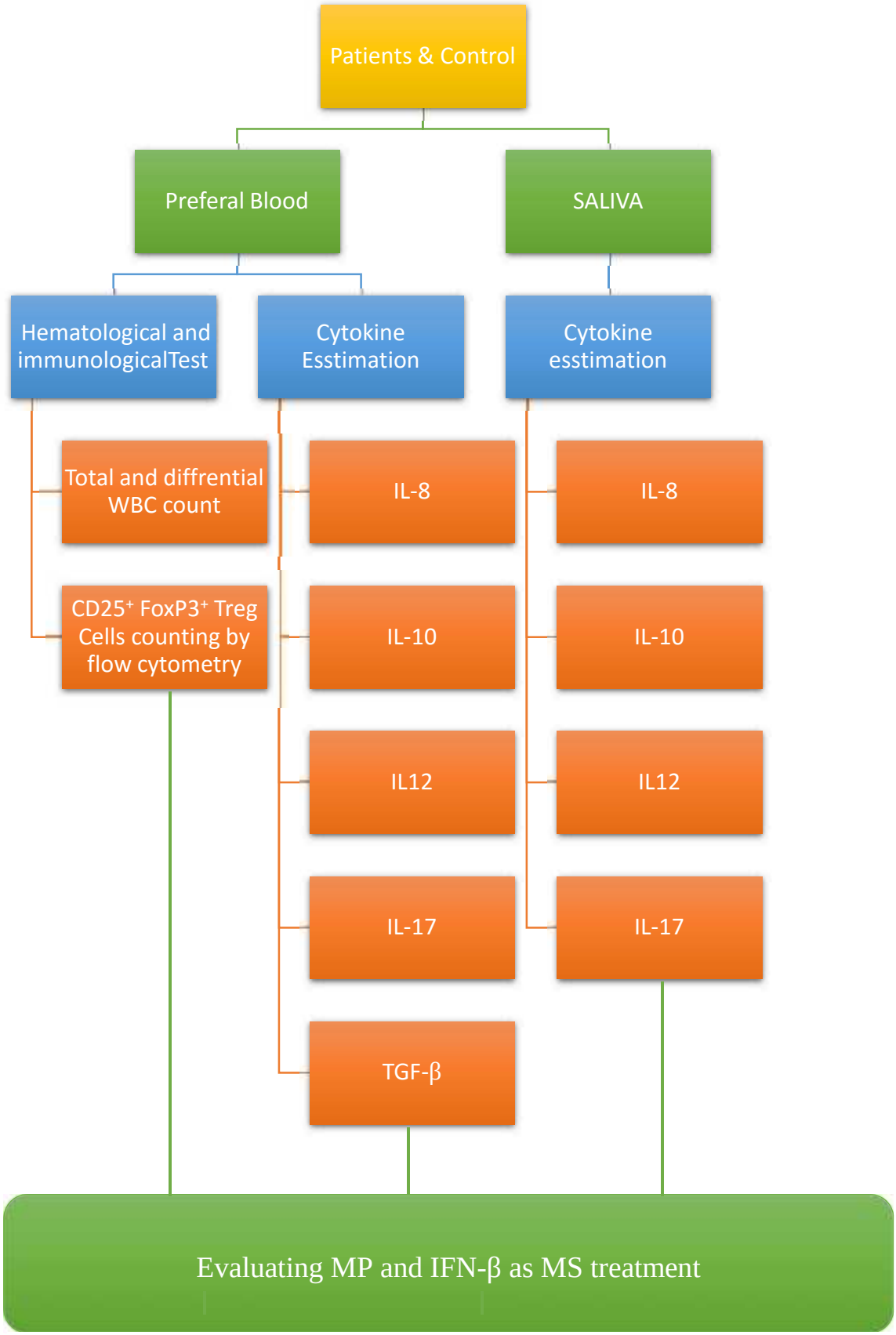

Figure 3- 2 The Study Plan

3.2 Materials

3.2.1 Equipment, Plastic and Glass Wares

The instruments and equipment used in this study with their remarks are list in table (3.1).

Table (3-1): Instruments and equipment.

Equipment & Instruments	Manufacturing Company	Origin
Alcohol pad		
Automated blood analyzer	a Ruby	
Centrifuge	Hettich	Germany
Cool box	VB	China
Deep freezer	Ishtar	Iraq
EDTA Tubes 2.5 ml		
ELISA printer	Epson	
ELISA Reader & washer	Bio tech	USA
Eppendorf Tube 0.5 ml		
Flow Cytometry	Cubic	Germany
Gel Tube 10 ml	AFCO	Jordan
Micropipette set	SLAMED	Germany
Multichannel micropipette set	SLAMED	Germany
Pipette tip		China
Plane Tube 10 ml	AFCO	Jordan
Plaster	PIC Solution	Italy
Refrigerator	Concord	
Saliva collecting tube		
Shaker	Karl Kolb	Germany
Syringe 10 ml	Q Ject	Qatar

3.2.2 Kits

Table (3-2): Kits and Solutions

Kit	Company	Country
H_2So_4		
HCl		
Humen Regulatory T Cell Multi-Color Flow Cytometry Kit	R&D Systems	USA
Interleukin-8 ELISA Development kit	PeproTech	USA
Interleukin-10 ELISA Development kit	PeproTech	USA
Interleukin-12 ELISA Development kit	PeproTech	USA
Interleukin-17A ELISA Development kit	PeproTech	USA
NAOH		
TGF-β ELISA Development kit	PeproTech	USA

3.3 Blood and saliva Collection

Ten milliliters of venous blood were collected using 10ml disposable syringe. The blood sample was immediately Distribute in two EDTA tubes 2.5ml in each one for flow cytometry and complete blood picture and 5ml of blood was transformed into jell plain tube and left to clot for 15 minutes in room temperature (20-25)°C. Then, it was centrifuge from 2500 to 3000 rpm for 10 min period to isolate serum.

(1.5 – 2.5 ml) saliva were collected from patients by saliva collecting plastic containers, we used unstimulated whole saliva (resting). Unstimulated saliva represents the usual, or baseline, it often correlates to

systemic clinical conditions more accurately than stimulated saliva, since materials use to stimulate flow may change salivary composition (Williamson, *et al*., 2012). Unstimulated saliva has traditionally been obtained by having the subject seated quietly with his or her head flexed forward and allowing the saliva to passively drip from the mouth to a collection container, or by having the subject gently spit into a collection contain for a specified amount of time (5 minutes in the present study), This method of collection is considered the "gold standard" for obtaining many components of saliva (Munro *et al.,* 2006).

After the collecting, it centrifuged from 2500 to 3000 rpm for 10 min period to isolate clear saliva, the isolated serum and saliva were distribute into aliquots (0.5 ml) in tightly closed eppendorf tubes, and by then the tubes were stored at -20ºC until assayed for CRP and cytokines level.

3.4 Laboratory Methods

3.4.1 Complete blood count

The blood was taken in EDTA tube and analyzed by automated blood analyzer devise.

3.4.2 Regulatory T cell Counting

Human Regulatory T cells counting assay by flow cytometry

3.4.2.1 Principle assay

The principle behind the flow cytometry technique is an antibody (marked) binds to target antigen in the sample after serial steps of washing and adding buffers and staining contain the marked antibody (detector), it resulted a cell suspension ready for flow cytometric analyzing. When the

cell or particles suspension inter the flow cytometer device a liquid sheath put a certain hydrostatic pressure on it leading to a single cell or particles stream. Laser pointed to this stream when a target cell or particle that binding to antibody marked cross the laser, it will counted or isolated according to the procedure or the purpose behind it (Figure 3-3).

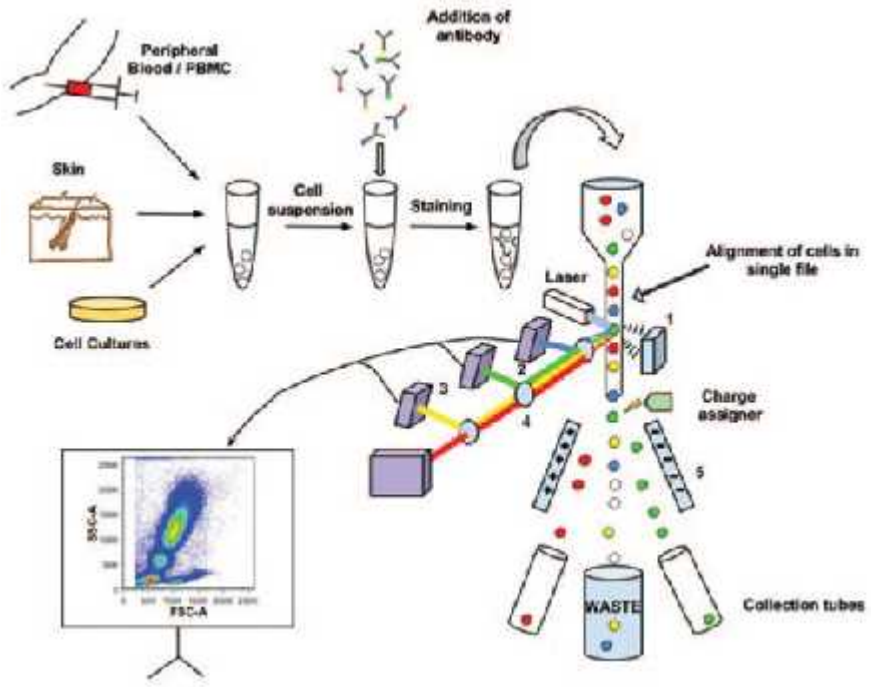

Figure 3 - 1 Schematic representation of a flow cytometer. (Internet 9, 2012)

3.4.2.2 Kit Contents

- CD25-PE (Clone 24212; mouse lgG_{2A})
- CD4-PerCP (Clone 11830; mouse lgG_{2A})
- FOXP3-APC (goat IgG)
- Flow Cytometry FOXP3 staining buffer (120 ml)
- Flow cytometry Staining Buffer (50 ml)

3.4.2.3 Assay Procedure

Before starting the procedure of $CD25^+$ $FOXP3^+$ T reg cells counting, kit components was left for 15 minutes at room temperature to equilibration. After that, procedure carried out by applying kit's instructions as following:-

1. The EDTA tube containing whole blood putted on the shaker for 5 minutes.
2. 100 µl of blood taken and put in 5 ml flow cytometry tube.
3. The blood washed by adding 2 ml of PBS (phosphate buffer solution).
4. Then it centrifuged at 300 rpm for 5 min.
5. All the remaining PBS removed and the sample re-suspended in 100 µl of flow cytometry staining buffer.
6. 10 µl of CD25-PE antibodies were added to the sample and shacked to mix well.
7. The mixture was leave for 30 minutes at 2-8 C in dark.
8. The excess antibodies removed by washing the sample with 1 ml of flow cytometry FOXP3 staining buffer.
9. Then it centrifuged at 300 rpm for 5 min.
10. Flow cytometry FOXP3 staining buffer was decanted and a small volume was left in the tube about 100 µl from FOXP3 staining buffer.
11. Then 10 µl of FOXP3-APC antibody was added to the sample and shacked to mix well.
12. The mixture incubated for 1 hour at 2-8 C in the dark.
13. The excess antibodies removed by washing the cells with 1 ml of flow cytometry FOXP3 staining buffer.

14. Then it was centrifuge at 300 rpm for 5 min and aspirated any excess flow cytometry to FOXP3 staining buffer.
15. The final cell pellet re-suspended in 200-400 µl of flow cytometry staining buffer.
16. Flow cytometric analysis.

3.4.2.4 Result Calculation

The results calculated by the device (cube 6 Partec) special computer program.

Five cytokines were assessed in sera of MS patients and four cytokines assessed in MS patients Saliva

3.4.3 Level of Cytokines in Serum and Saliva

Sera of MS patients were assessed for level of 5 cytokines (IL-8, IL-10, IL-12, IL-17 and TGF-β) and saliva levels of 4 cytokines (IL-8, IL-10, IL-12, IL-17A) because an error occurred while working in the kit, which led us to the exclusion of its results, to achieve the scientific integrity. The cytokines levels obtained by means of ELISA method that based on similar principles.

3.4.3.1 Principles of Assay

Kit is a sandwich enzyme-linked immune sorbent assay designed for quantitative measurement of natural or recombinant antigens in human serum, plasma and other biological fluids. In which the coating antibody (Capture Antibody) adsorbed onto wells of 96-well plate. The human target cytokine binds with the antibodies that presents in wells. Antibody placed biotinylated in wells and binds to the target cytokine that captured by first

antibody (Detection Antibody). Then an incubation period followed by washing process to remove the excess, unbound antibodies. Avidin (HRP) conjugate added to binds to biotinylated antibodies. Incubation period, followed by washing process to remove the excess unbound avidin-HRP conjugate. A solution (substrate) reactive with HRP added to wells. Then color formed in proportion to the amount of human cytokine. Color development monitored with ELISA plate reader and absorbance measured at a wavelength of 405 nm for IL-8, IL-10, IL-12 and IL-17A except for TGF-β the wavelength was 490. From the concentrations dilution and absorbency of standards, a standard was made to extract a curve fitting equation, in which by sample concentrations determine.

3.4.3.2 Kit Contents

- ELISA plate: Blank 96-well plate
- Capture antibody: Goat anti-human IL-8, IL-10, IL-12, IL-17A and TGF-b antibody.
- Detection antibody: Biotinylated anti-human IL-8, IL-10, IL-12, IL-17A, TGF-b antibody.
- Standards: Recombinant human IL-8, IL-10, IL-12, IL-17A, TGF-b.
- Avidin-HRP conjugate.
- ABTS liquid substrate solution.
- Washing buffer: 0.05% Tween-20 in phosphate buffer saline (PBS).
- Block buffer: 1% bovine serum albumin (BSA) in PBS.
- Diluent: 0.05% Tween-20 and 1% BSA in PBS.

3.4.3.3 Assay Procedure

Before starting the procedure of IL-8, IL-10, IL-12, IL-17A and TGF-β determination, kit components was left at room temperature for 15 minutes in order to equilibration. After that, procedure carried out by applying kit's instructions as following:-

1. The wells were coated with capture antibody of anti-human IL-8, IL10, IL-12, IL-17, TGFβ 100µl for each well, and the plates were sealed and incubated overnight at room temp (18-25) C.
2. The day after, the contents of wells discarded and the plate washed 300 µl of washing buffer for each well for four times and then the plates were inverted to remove residual buffer and blotted on a towel paper.
3. In each well, 300 µl of block buffer dispensed and the plates incubated at room temperature for 1 hour and then the washing step repeated.
4. 100 µl standard added of IL-8, IL10, IL-12, IL-17, TGFβ in serial concentration (0, 156.25, 312.5, 625, 1250, 2500, 5000) and 100 µl of serum and saliva samples added to the wells repeatedly except TGF-β kit, where TGF-β activated in the serum by taking 20µl of serum mixed with 20µl of HCL then after incubation for 10 minutes 20µl of NAOH was added to the mixture then it incubated 10 minutes then it diluted 9 times to activate TGF-β in serum then added to the wells the plates were incubate in room temperature for 2 hours, then the washing repeated.
5. 100 µl from detection antibody (biotinylated antihuman IL-8, IL10, IL-12, IL-17, TGF-β antibody), then dispensed 100 µl in

each well, the plates sealed and left in room temperature for 2 hours then the washing repeated.

6. 100 µl of Avidin-HRP conjugate dispensed in each well, the plates sealed, then incubated in room temperature for 30 min, then the wash repeated.
7. Finally, 100 µl of ABTS liquid substrate added in each well for IL-8, IL-10, IL-12, IL17 and TGF-β, and the color development was monitor with ELISA plate reader except TGF-β, where 100 µl of OPD substrate solution was dissolved in phosphate citrate buffer solution), then 50 µl of sulfuric acid (H_2SO_4) was added as a stop solution then the color development monitored with ELISA plate reader and absorbance measured at a wavelength of 405 nm. three reading were taken (5, 15, 25) minutes and the mean absorbance was considered for a calculation of sample results for IL-8, IL-10, IL-12, IL17 but the absorbance was measured at 490 nm, one reading was taken and considered for the calculation of sample result for TGF-β.

3.4.3.4 Results Calculation

The samples concentrations calculated by a standard curve fitting equation that performed in the same procedure for each cytokines, (Figures 3-4, 3-5, 3-6, 3-7 and 3-8)

IL-8 Formula Y = 0.09+1.266X-1.026X2+0.243X3

R^2 = 0.989

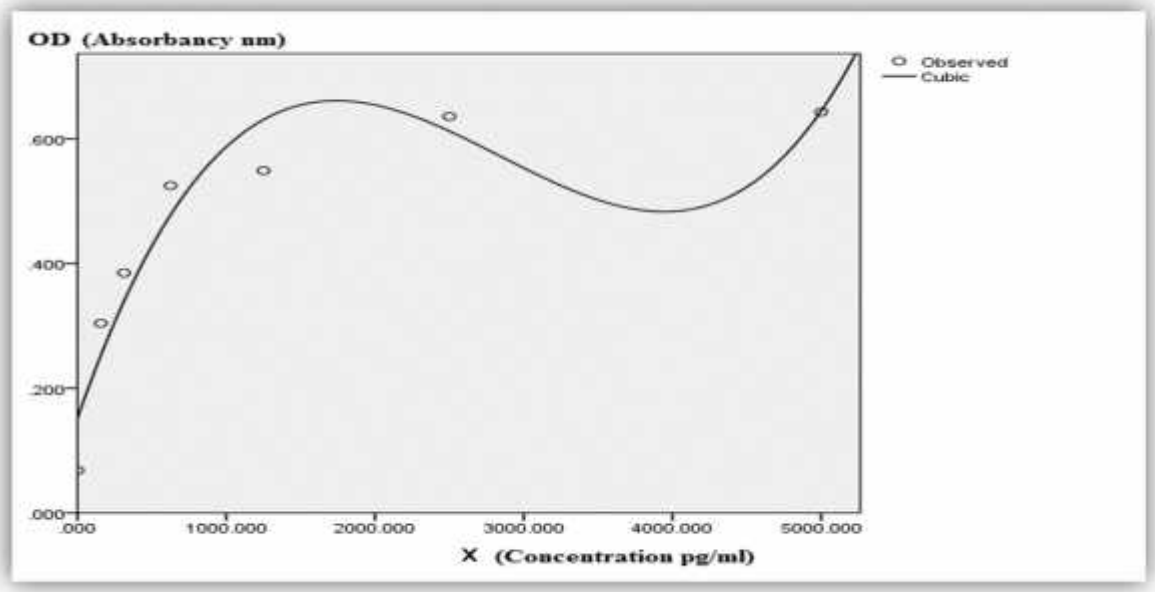

Figure 3- 4 Fitting equation, standard curve of IL-8

IL-10 Formula Y = 0.264+2.834X-2.154X2+0.505X3

R^2 = 0.996

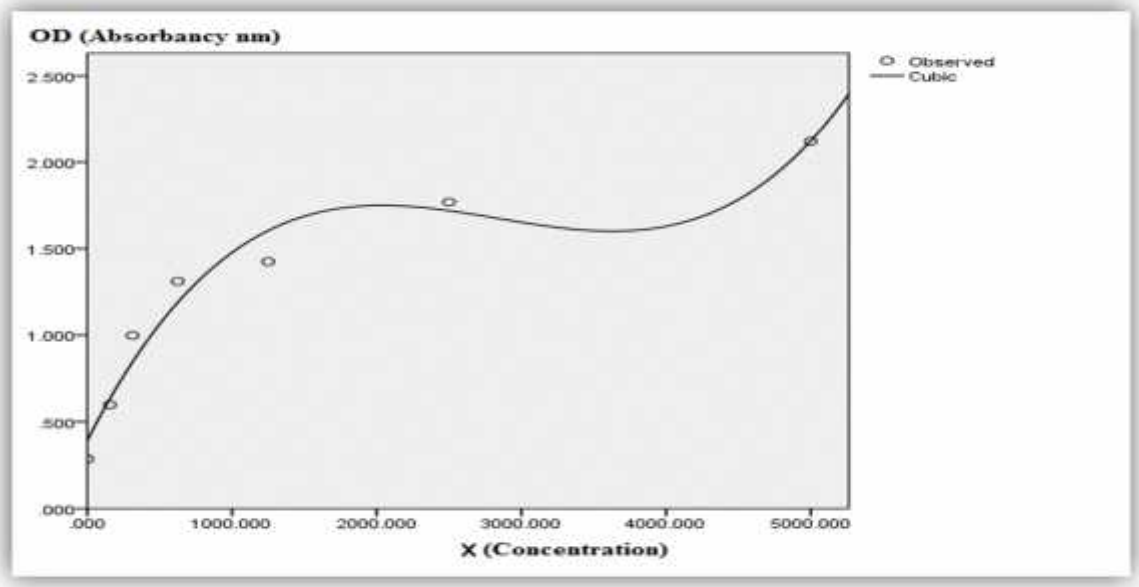

Figure 3- 5 standard curve of IL-10

IL-12 Formula $Y = 0.22 + 0.001X - 2.944*10^{-7}X2 + 3.315*10^{-11}X3$

$R^2 = 0.999$

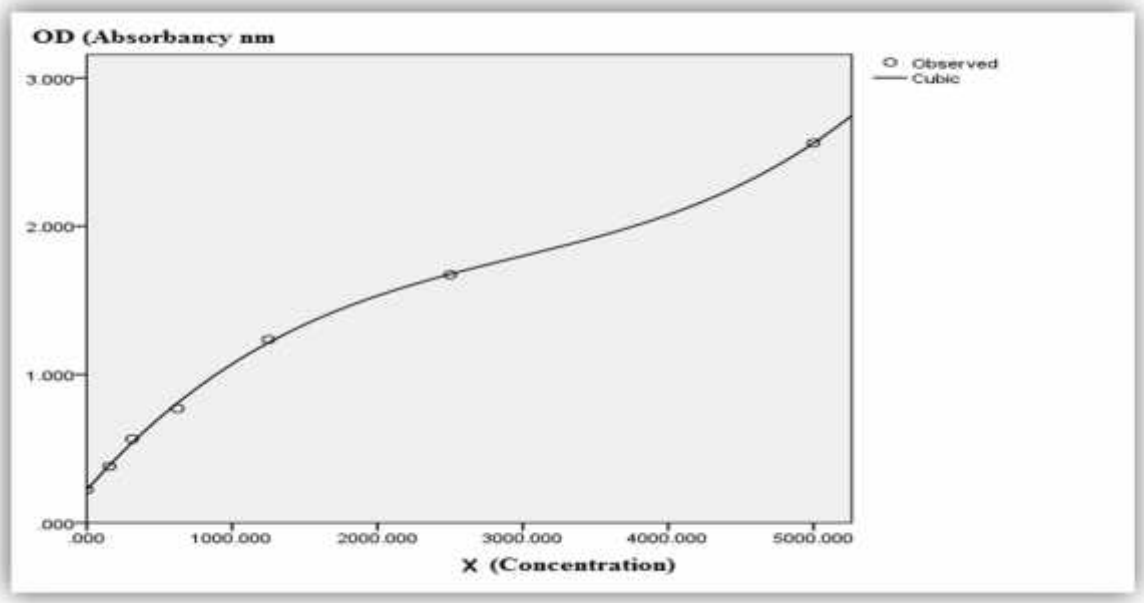

Figure 3- 6 standard curve of IL-12

IL-17 Formula $Y = 0.2+1.258X-0.29X2-0.001X3$

$R^2 = 0.996$

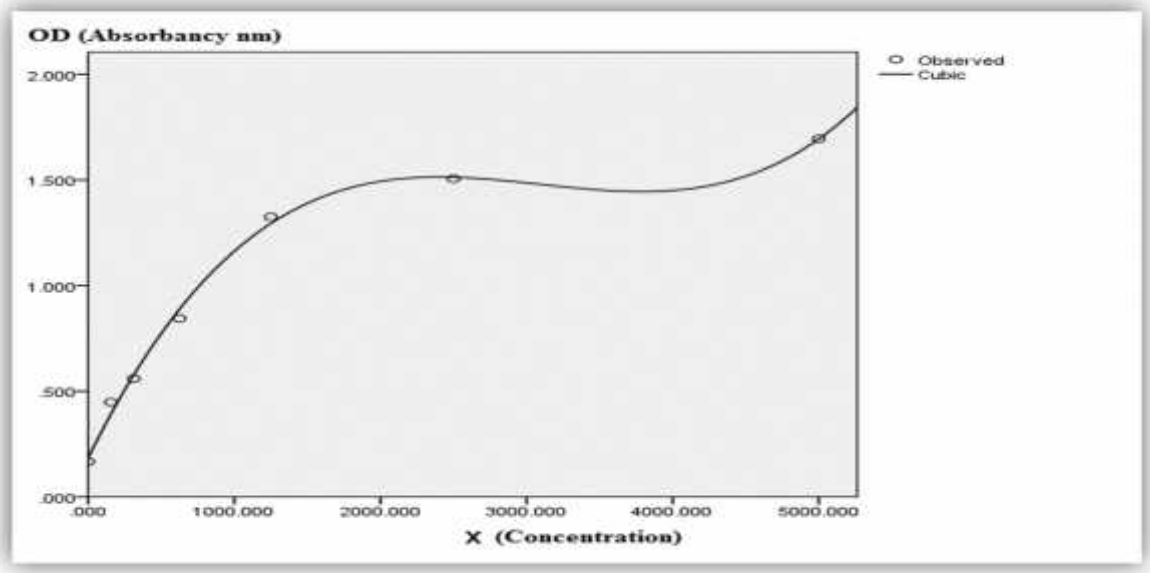

Figure 3- 7 standard curve of IL-17

TGF-β Formula $Y = 0.071 - 1.205*10^{-8}X2 + 1.253*10^{-12}X3$

$R^2 = 1.00$

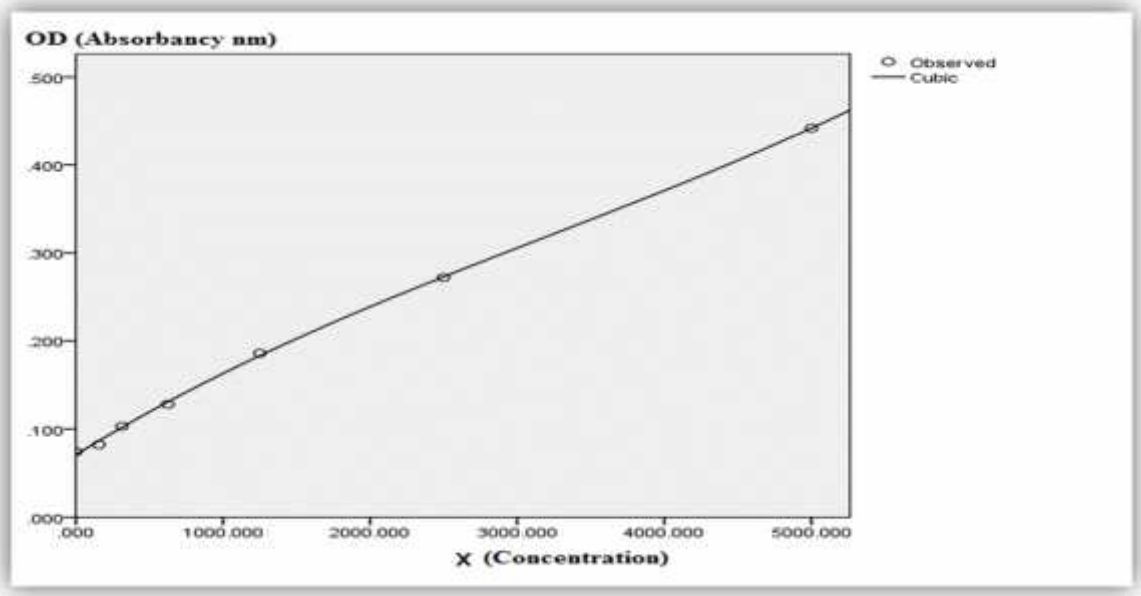

Figure 3- 8 standard curve of TGF-β

3.5 Statistical Analyses

SPSS program version 20 used to analyze the results. Data described as mean ± standard error (S.E.); differences between means were assessed by ANOVA (Analysis of Variance), followed by LSD (Least Significant Difference).

CHAPTER FOUR
RESULTS AND DISCUSSION

Chapter Four

Results and Discussion

4.1 General characteristics of study patients.

Forty-five Iraqi (MS) patients were distribute according to following:

4.1.1: Gender

The patients was distributed according to gender as it shown in figure (4-1), the present sample of patients reveals that female : male ratio was 2:1, where female (66.67%) presented two-third and male (33.33%) presented one third.

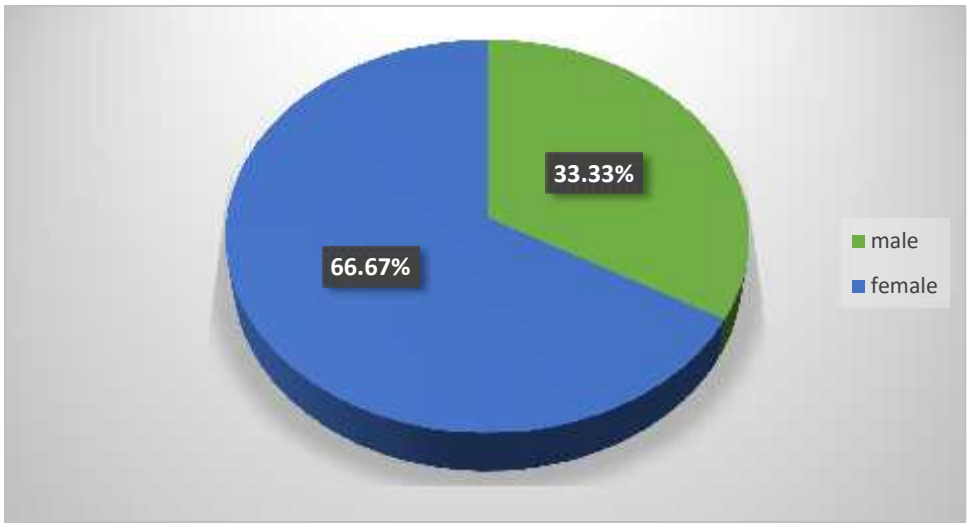

Figure 4 - 1 shows the male and female frequencies

4.1.2: Age of onset

The age of onset distribution reveals that the highest frequency (44.5%) occur in the group < 30 years old, followed by (22.2%) in the group between (30-39) years old, followed by (17.8%) in the group

between (40-49) years old, then the lowest frequency (15.6%) occur in the group (50-59) years old. Figure (4-2).

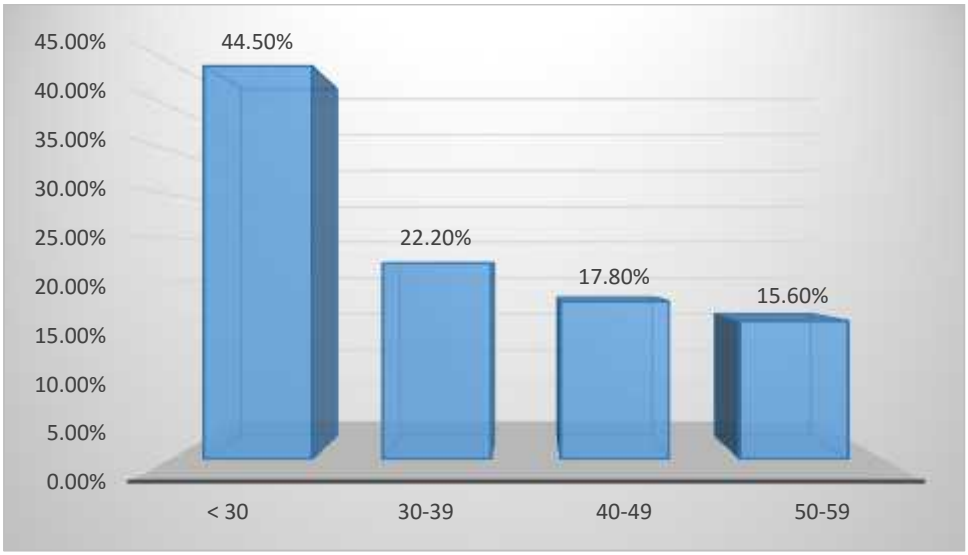

Figure 4 - 2 patients' distribution according to the age of onset

4.1.3 Demographic Features of patients:

As the table (4-1) shows the general demographics for MS patients which reveals that the maximum MS patients age was (57) years old and the minimum patients age was (17) years old with mean (37.47) years old, the maximum age for MS onset was (57) years old and the minimum age at onset was (13) years old with mean (34.93) years old, also the maximum disease duration was (16) years and the minimum disease duration was (0) year with mean (2.13).

Table 4 – 1 general demographics for MS patients

	Max.	Min.	Range	Mean	Standard Error	Median
Age (Year)	57.00	17.00	40.00	37.07	1.78	35.00
Age at onset	57.00	13.00	44.00	34.93	1.79	32.00
disease duration	16.00	.00	16.00	2.13	.56	.00

The present sample of MS patients reveals that there was an increasing in females to males' ratio and that came in agreement with previous studies (Alonso and Hernan, 2008; Holmberg *et al.,* 2013; Westerlind *et al.,* 2014). In this study the female: male ratio was 2:1 and that came typical with previous studies (MSIF, 2013; Johns, 2014), also it is worth to mention that the same sex disparity seen in MS and many autoimmune disorders, In patient population the percentage reached to over than 80% (Whitacre, 2001); and about 80% of autoimmune disease affect women (Gleicher and Barad, 2007). In children, the percentage of MS close to 1:1 and then number slope in a sharp manner in adults (Banwell *et al.,* 2007). That may explain by female sex hormone that stimulate inflammatory response, which cause autoimmune diseases consequently. Women have a more ability to defense against infection than man in addition to produce more CDW Lymphocytes and large amount of pro-inflammatory cytokines (Whitacre, 2001). This may related to the estrogen that skew cytokine signaling to activate Th1 and Th17 (Straub, 2007). That gives us a good but not fully explanation because this disparity between females and males has been increasing for at least 50 years (Nashold *et al.,* 2009; Chao *et al.,* 2011). For men it can explained also by their sex hormone (testosterone), it inhibits inflammatory cytokines production and that prevents them from owning an extreme immune response (Dalal *et al.,* 1997; Gold and Voskuhl, 2009) these findings support the result of this study.

There are other influence factors that may contribute beside the hormonal and immunological factors to explain the sex gap ratio like environmental and genetic factors, the environmental factor include the birth date (Sadovnick *et al.,* 2007). Thus, developing MS appear to originate from external stimuli that vary over the course of a year (Nashold

et al., 2009; Ramagopalan *et al.*, 2010), there are other environmental factors that their influence might appear during later stages of life.

Like the behavioral differences between boys and girls. "Hygiene hypothesis" (Clough, 2011; Kakalacheva *et al.*, 2011; Wingerchuk, 2011). In addition, there is smoking, in 2011 a study found that there were a tide correlation between the different gender ratios in smoking prevalence and MS disease (Palacios *et al.*, 2011).

Sun exposure, an environmental factor plays an important role, it's a source of vitamin D, which have a protective role against MS (Nashold *et al.*, 2009). The changing in lifestyle, like spending more time indoors or sunscreen over use ,all that could increase the women's ratio , thus altering MS gender ratio(Handunnetthi *et al.*, 2010). On the genetic level the *HLA-DRB1*1501* variant confer MS higher risk for women than men (Chao *et al.*, 2011; Irizar *et al.*, 2011).

MS resulted from an interaction between the previously discussed factors, it appear that some of these factors attend to affect women more than men. The present study shows that the patients age when they diagnose with MS was between (17-60) years and that came in agreement with (Ruthan, 2006), and the highest frequency was in (≥30) group and that agree with (Zivadinov, *et al.*, 2010), and disagree with (Mc Dowell *et al.*, 2010).

4.2 Total & Differential WBC Count

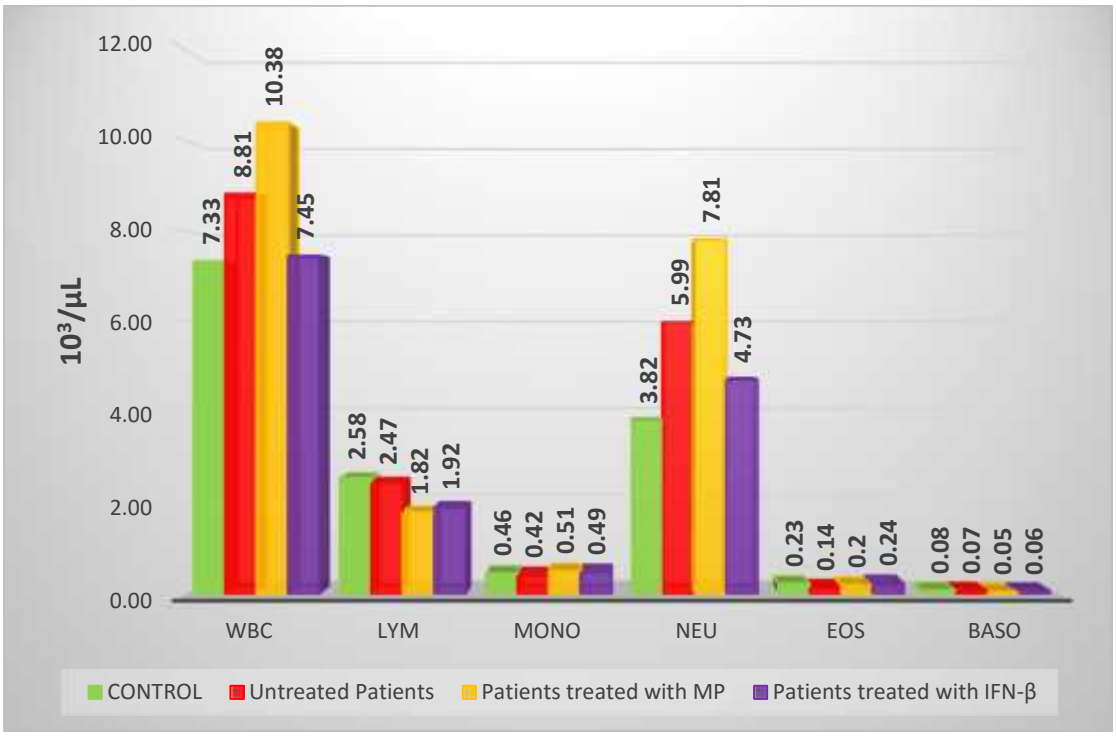

Figure (4-3) the Total and Differential WBC Count

Table (4-2) The Total and Differential WBC Count

Parameter 10^3/µL	Control	Untreated Patients	Patients treated with MP	Patients treated with IFN-β
WBC	7.33	8.81	10.38	7.45
LYM	2.58	2.47	1.82	1.92
MONO	0.46	0.42	0.51	0.49
NEU	3.82	5.99	7.81	4.73
EOS	0.23	0.14	0.2	0.24
BASO	0.08	0.07	0.05	0.06

4.2.1 Control and Untreated Patients

The mean for WBC (7.33 ± 0.41 & 8.81 ± 0.58) and NEU (3.82 ± 0.25 & 5.99 ± 0.59) showed an increasing in Untreated Patients as compare with control, the difference in WBC were not significant but in NEU was significant (P ≤ 0.016), LYM (2.58 ± 0.20 & 2.47 ± 0.21), MONO (0.46 ± 0.03 & 0.42 ± 0.03), EOS (0.23 ± 0.03 & 0.14 ± 0.02) and BASO (0.08 ± 0.01 & 0.07 ± 0.01) showed a decreasing in Untreated Patients compared to control and the differences was not significant. (Figure 4-3) and table (4-3).

Table 4-3 Total & Differential count in control and Untreated Patients

	Mean ± S.E.		**P≤ 0.05**	**Confidant Interval**	
$10^3/\mu L$	Control	Untreated Patients		Lower	Upper
WBC	7.33 ± 0.41	8.81 ± 0.58	N.S.	-3.2437	0.2757
LYM	2.58 ± 0.20	2.47 ± 0.21	N.S	-0.4766	0.7054
MONO	0.46 ± 0.03	0.42 ± 0.03	N.S	-0.0664	0.1519
NEU	3.82 ± 0.25	5.99 ± 0.59	0.016	-3.9085	-0.4248
EOS	0.23 ± 0.03	0.14 ± 0.02	N.S.	-0.0577	0.2387
BASO	0.08 ± 0.01	0.07 ± 0.01	N.S.	-0.0004	0.0363

4.2.2: Control and Patients Treated with MP

The WBC mean (7.33 ± 0.41 & 10.38 ± 0.78) and NEU (3.82 ± 0.25 & 7.81 ± 0.86) showed an increasing in patients Treated with MP compared to control, and the differences were significant WBC (P ≤ 0.001) and NEU (P ≤ 0.000), while LYM (2.58 ± 0.20 & 1.82 ± 0.23), MONO (0.46 ± 0.03 & 0.51 ± 0.06), EOS (0.23 ± 0.03 & 0.20 ± 0.07) and BASO (0.08 ± 0.01

& 0.05 ± 0.01) showed a decreasing in Patients Treated with MP compared to control, and the LYM and BASO differences were significant (P ≤ 0.013, 0.001 respectively), while MONO and EOS differences were not significant. (Figure 4-3) and table (4-4).

Table 4-4 Total & Differential count in control and Patients Treated with MP

	Mean ± S.E.		P≤ 0.05	Confidant Interval	
$10^3/\mu L$	Control	Patients Treated MP		Lower	Upper
WBC	7.33 ± 0.41	10.38 ± 0.78	0.001	-4.8137	-1.2943
LYM	2.58 ± 0.20	1.82 ± 0.23	0.013	0.1686	1.3507
MONO	0.46 ± 0.03	0.51 ± 0.06	N.S	-0.1583	0.0599
NEU	3.82 ± 0.25	7.81 ± 0.86	0.000	-5.7339	-2.2501
EOS	0.23 ± 0.03	0.20 ± 0.07	N.S.	-0.1172	0.1792
BASO	0.08 ± 0.01	0.05 ± 0.01	0.001	0.0140	0.0506

4.2.3: Control and Patients Treated with IFN-β

The WBC mean (7.33 ± 0.41 & 7.45 ± 0.65), MONO (0.46 ± 0.03 & 0.49 ± 0.04), NEU (3.82 ± 0.25 & 4.73 ± 0.60) and EOS (0.23 ± 0.03 & 0.24 ± 0.07) showed a differences in patients Treated with IFN-β compared to control, and the WBC, MONO, NEU and EOS differences were not significant, while LYM (2.58 ± 0.20 & 1.92 ± 0.19) and BASO (0.08 ± 0.01 & 0.06 ± 0.01) showed a decreasing in Patients Treated with IFN-β compared to control, and differences were significant (P ≤ 0.030 and 0.014 respectively). (Figure 4-3) and table (4-5).

Table 4-5 Total & Differential count in control and Patients Treated with IFN-β

	Mean ± S.E.		P≤ 0.05	Confidant interval	
$10^3/\mu L$	Control	Patients Treated IFN-β		Lower	Upper
WBC	7.33 ± 0.41	7.45 ± 0.65	N.S.	-1.8884	1.6310
LYM	2.58 ± 0.20	1.92 ± 0.19	0.030	0.654	1.2474
MONO	0.46 ± 0.03	0.49 ± 0.04	N.S.	-0.1445	0.0738
NEU	3.82 ± 0.25	4.73 ± 0.60	N.S.	-2.6552	0.8285
EOS	0.23 ± 0.03	0.24 ± 0.07	N.S.	-0.1607	0.1357
BASO	0.08 ± 0.01	0.06 ± 0.01	0.014	0.0048	0.0415

4.2.4: Untreated Patients and Patients Treated with MP

The WBC mean (8.81 ± 0.58 & 10.38 ± 0.78), MONO (0.42 ± 0.03 & 0.51 ± 0.06), NEU (5.99 ± 0.59 & 7.81 ± 0.86) and EOS (0.14 ± 0.02 & 0.20 ± 0.07) showed an increasing in patients Treated with MP compared to Untreated Patients, and the NEU difference was significant (P ≤ 0.040), while the WBC, MONO and EOS differences were not significant, while LYM (2.47 ± 0.21 & 1.82 ± 0.23) and BASO (0.07 ± 0.01 & 0.05 ± 0.01) showed a decreasing in Patients Treated with MP compared to Untreated Patients, and the LYM, difference was significant (P ≤ 0.033), while BASO difference was not significant. Fig (4-3) and table (4-6).

Table 4-6 Total & Differential count in Untreated Patients and Patients Treated with MP

	Mean ± S.E.		P≤ 0.05	Confidant interval	
$10^3/\mu L$	Untreated Patients	Patients Treated MP		Lower	Upper
WBC	8.81 ± 0.58	10.38 ± 0.78	N.S.	-3.3297	0.1897
LYM	2.47 ± 0.21	1.82 ± 0.23	0.033	0.0542	1.2363
MONO	0.42 ± 0.03	0.51 ± 0.06	N.S.	-0.2011	0.0172
NEU	5.99 ± 0.59	7.81 ± 0.86	0.040	-3.5672	-0.0835
EOS	0.14 ± 0.02	0.20 ± 0.07	N.S.	-0.2076	0.0888
BASO	0.07 ± 0.01	0.05 ± 0.01	N.S.	-0.0040	0.0324

4.2.5: Untreated Patients and Patients Treated with IFN-β

The mean of MONO (0.42 ± 0.03 & 0.49 ± 0.04) and EOS (0.14 ± 0.02 & 0.24 ± 0.07) showed an increasing in patients Treated with IFN-β compared to Untreated Patients, and the differences were not significant, while WBC (8.81 ± 0.58 & 7.45 ± 0.65), LYM (2.47 ± 0.21 & 1.92 ± 0.19), NEU (5.99 ± 0.59 & 4.73 ± 0.60) and BASO (0.07 ± 0.01 & 0.06 ± 0.01) showed a decreasing in Patients Treated with IFN-β compared to Untreated Patients, and differences were not significant. (Figure 4-3) and table (4-7).

Table 4-7 Total & Differential count in Untreated Patients and Patients Treated with IFN-β

	Mean ± S.E.		P≤ 0.05	Confidant interval	
$10^3/\mu L$	Untreated Patients	Patients Treated IFN-β		Lower	Upper
WBC	8.81 ± 0.58	7.45 ± 0.65	N.S.	-0.4044	3.1150

LYM	2.47 ± 0.21	1.92 ± 0.19	N.S.	-0.0490	1.1330
MONO	0.42 ± 0.03	0.49 ± 0.04	N.S.	-0.1872	0.0311
NEU	5.99 ± 0.59	4.73 ± 0.60	N.S.	-0.4885	2.9952
EOS	0.14 ± 0.02	0.24 ± 0.07	N.S.	-0.2511	0.0453
BASO	0.07 ± 0.01	0.06 ± 0.01	N.S.	-0.0131	0.0235

4.2.6: Patients Treated with MP and Patients Treated with IFN-β

The LYM mean (1.82 ± 0.23 & 1.92 ± 0.19), EOS (0.20 ± 0.07 & 0.24 ± 0.07) and BASO (0.05 ± 0.01 & 0.06 ± 0.01) showed an increasing in patients Treated with IFN-β compared to Patients Treated with MP, and the differences were not significant, while WBC (10.38 ± 0.78 & 7.45 ± 0.65), MONO (0.51 ± 0.06 & 0.49 ± 0.04) and NEU (7.81 ± 0.86 & 4.73 ± 0.60) showed a decreasing in Patients Treated with IFN-β compared to Patients Treated with MP, and the WBC and NEU differences were significant (P ≤ 0.002, 0.001 respectively), while MONO difference was not significant. (Figure 4-3) and table (4-8).

Table 4-8 Total & Differential count in Patients Treated with MP and Patients Treated with IFN-β

Mean ± S.E.			P≤ 0.05	Confidant interval	
$10^3/\mu L$	Patients Treated with MP	Patients Treated IFN-β		Lower	Upper
WBC	10.38 ± 0.78	7.45 ± 0.65	0.002	1.1656	4.6850
LYM	1.82 ± 0.23	1.92 ± 0.19	N.S.	-0.6943	0.4878
MONO	0.51 ± 0.06	0.49 ± 0.04	N.S.	-0.0953	0.1230
NEU	7.81 ± 0.86	4.73 ± 0.60	0.001	1.3368	4.8205

EOS	0.20 ± 0.07	0.24 ± 0.07	N.S.	-0.1917	0.1047
BASO	0.05 ± 0.01	0.06 ± 0.01	N.S.	-0.0275	0.0092

This study shows increase in WBC total count in all patients groups, it is significant increases in patient treated with (MP) compared to both control and patients treated with (IFN-β), and that disagree with (Hon *et al.*, 2012; Internet 10, 2015) that elevation may indicate to the inflammation (Pachner, 2012) the significant increasing in patients treated with (MP) may due to drug influence.

Lymphocytes show a significant decrease in both treated patients groups compared to control and untreated patients. This result may due to the disease modifying agents (DMAs) that targeting lymphocytes in order to up press clinical relapses by which their mechanism of action (Stuve, 2008; Kowarik *et al.*, 2011);

For monocytes, there were no significantly differences between patient's groups in compare with control and that do not agree with (Rumble *et al.*, 2015); it may be because monocyte can evoke damage in CNS (Epstein, 1983; Lin *et al.*, 1993; TofT-Hanson *et al.*, 2004; Mantovani *et al.*, 2011).

For neutrophils**,** there were a significantly increasing (untreated, treated with (MP)) as compared to control, in additional to a significantly increasing in patients treated with (MP) compared to untreated patients, patients treated with (IFN-β). This elevation in patient treated with (MP) may due to the mechanism of drug action (Kinkel, 1999), before the clinical onset will be occur, neutrophil and monocytes accumulate in the circulation after their expand in bone marrow before EAE clinical on set (Rumble *et al.*, 2015).Neutrophil elevation accelerated from normal to high level during the last decade in MS Patients, since this elevation found in

people with high blood pressure, stress, anxiety and depression (Steinbach *et al.*, 2013; Hon *et al.*, 2012; Internet 11, 2015). It might to physiological factors that come from environmental circumstances and life style rhythm.

This explanation agree with the present study results for neutrophil only. Since this elevation were found impale under physiological, physiological disorders (Internet 11, 2015)

4.3: CD25+ FOXP3+ T regulatory Counting

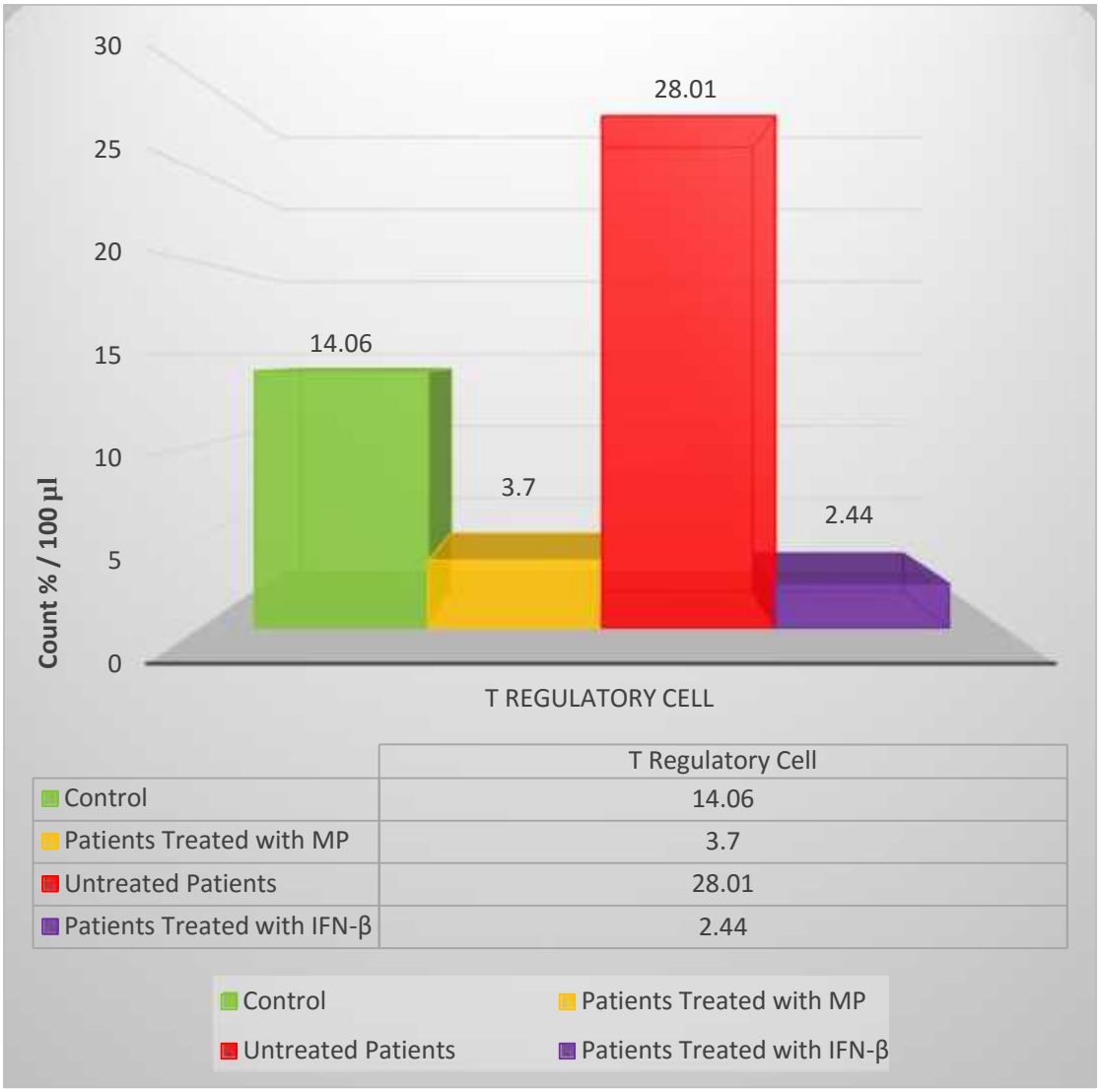

Figure 4 - 4 CD25+ FOXP3+ Tregs Count

4.3.1 Control and Untreated Patients

The CD25+ FOXP3+ Tregs mean (14.06 ± 3.70 & 28.01 ± 4.52) showed an increasing in untreated patients compared to control, and the difference was significant (P ≤ 0.002). (Figure 4-4) and table (4-9).

Table 4-9 CD25+ FOXP3+ Tregs count in control and Untreated Patients

	Mean ± S.E.		P≤ 0.05	Confidant interval	
Count % per 100µl	Control	Untreated Patients		Lower	Upper
CD25+ FOXP3+ Tregs	14.06 ± 3.70	28.01 ± 4.52	0.002	-22.3128	-5.5739

4.3.2 Control and Patients Treated with MP

The CD25+ FOXP3+ Tregs mean (14.06 ± 3.70 & 3.70 ± 0.67) showed a decreasing in patients treated with MP compared to control, and the difference was significant (P ≤ 0.016). (Figure 4-4) and table (4-10).

Table 4-10 CD25+ FOXP3+ Tregs count in control and Patients Treated with MP

	Mean ± S.E.		P≤ 0.05	Confidant interval	
Count % per 100µl	Control	Patients Treated MP		Lower	Upper
CD25+ FOXP3+ Tregs	14.06 ± 3.70	3.70 ± 0.67	0.016	1.9936	18.7324

4.3.3 Control and Patients Treated with IFN-β

The CD25+ FOXP3+ Tregs mean (14.06 ± 3.70 & 2.44 ± 0.62) showed a decreasing in patients treated with IFN-β compared to control, and the difference was significant (P ≤ 0.007). (Figure 4-4) and table (4-11).

Table 4-11 CD25+ FOXP3+ Tregs count in control and Patients Treated with IFN-β

	Mean ± S.E.		P≤ 0.05	Confidant interval	
Count % per 100µl	Control	Patients Treated IFN-β		Lower	Upper
CD25+ FOXP3+ Tregs	14.06 ± 3.70	2.44 ± 0.62	0.007	3.2539	19.9928

4.3.4 Untreated Patients and Patients Treated with MP

The CD25+ FOXP3+ Tregs mean (28.01 ± 4.52 & 3.70 ± 0.67) showed a high decreasing in patients treated with MP compared to untreated patients, and the difference was significant (P ≤ 0.000). (Figure 4-4) and table (4-12).

Table 4-12 CD25+ FOXP3+ Tregs count in Untreated Patients and Patients Treated with MP

	Mean ± S.E.		P≤ 0.05	Confidant interval	
Count % per 100µl	Untreated Patients	Patients Treated MP		Lower	Upper
CD25+ FOXP3+ Tregs	28.01 ± 4.52	3.70 ± 0.67	0.000	15.9369	32.6758

4.3.5 Untreated Patients and Patients Treated with IFN-β

The CD25+ FOXP3+ Tregs mean (28.01 ± 4.52 & 2.44 ± 0.62) showed a high decreasing in patients treated with IFN-β compared to untreated patients, and the difference was significant (P ≤ 0.000). (Figure 4-4) and table (4-13).

Table 4-13 CD25+ FOXP3+ Tregs count in Untreated Patients and Patients Treated with IFN-β

	Mean ± S.E.		P≤ 0.05	Confidant interval	
Count % per 100µl	Untreated Patients	Patients Treated IFN-β		Lower	Upper
CD25+ FOXP3+ Tregs	28.01 ± 4.52	2.44 ± 0.62	0.000	17.1972	33.9361

4.3.6 Patients Treated with MP and Patients Treated with IFN-β

The CD25+ FOXP3+ Tregs mean (3.70 ± 0.67 & 2.44 ± 0.62) showed a decreasing in patients treated with IFN-β compared to patients treated with MP, and the difference was not significant. (Figure 4-4) and table (4-14).

Table 4-14 CD25+ FOXP3+ Tregs count in Patients Treated with MP and Patients Treated with IFN-β

	Mean ± S.E.		P≤ 0.05	Confidant interval	
Count % per 100µl	Patients Treated MP	Patients Treated IFN-β		Lower	Upper
CD25+ FOXP3+ Tregs	3.70 ± 0.67	2.44 ± 0.62	N.S.	-7.1091	9.6298

CD25+ FOXP3+ Tregs: The result for CD25+ FOXP3+ Tregs counting in study patients showed that there were a highly significant differences as follow:

There were significantly elevation in untreated patients group compared to control, while there were a significantly decreasing in patients treated with both drugs ((MP), (IFN-β)) respectively compared to control and that came in contrast with (Libera *et al.*, 2011).

Since untreated patients suffering from an acute attack, so their Tregs elevated nembers may due the ongoing immune regulatory mechanisms in MS patients (Muls *et al.,* 2015), plus that Tregs considered as a bio marker for MS disease action (Libera *et al.*, 2011) and that supported our explanation. (MP) is glucocorticoid (Mirowska-Guzel *et al.*, 2006), such kind of hormones suppress the immune response by cytokine/ hypothalamic-Pituitary-adrenal feedback loop; Th1 and Macrophages release cytokines (IL-1, IL-6 – TNFα) which induces the parvocelluler neuroendocrine cells which is periventricular nucleus of hypothalamus to secrete CRH (Corticotrophin – Releasing Hormone) that carried by the hypothalamic - hypophseal system to anterior lop of pituitary and stimulate it corticotropes to secrete ACTH (Adrenocorticotropic Hormone) (Muls, 2015, Internet 12, 2015) leading to induce the adrenal gland to release glucocorticoid that suppress Th1, Macrophage which suppress the immune response (Muls,2015), plus that CRH can heighten inflammation, and that have been investigated in MS (Paul, 1993); therefore patients treated with (MP) have decrease number of $CD25^{+}$ $FOXP3^{+}$ Tregs. For patient treated with IFN-β, the significant decrease in CD25+ FOXP3+ Tregs numbers may due to drug effect (chen, et al., 2011).

Since Treg exist in acute immune response as we mention before, the extreme difference between acute attack (relapse) patients and treated patients may related to disease activity. In addition to, Tregs secondary increasing during MS inflammatory response this expansion may considered as reason in accordance with previous studies that described Treg as migrating to inflammatory sites along and together with other inflammatory cells (Vukmanoric-Stejic *et al.,* 2008).

4.4 Cytokines estimation

4.4.1 Serum Cytokines Estimation

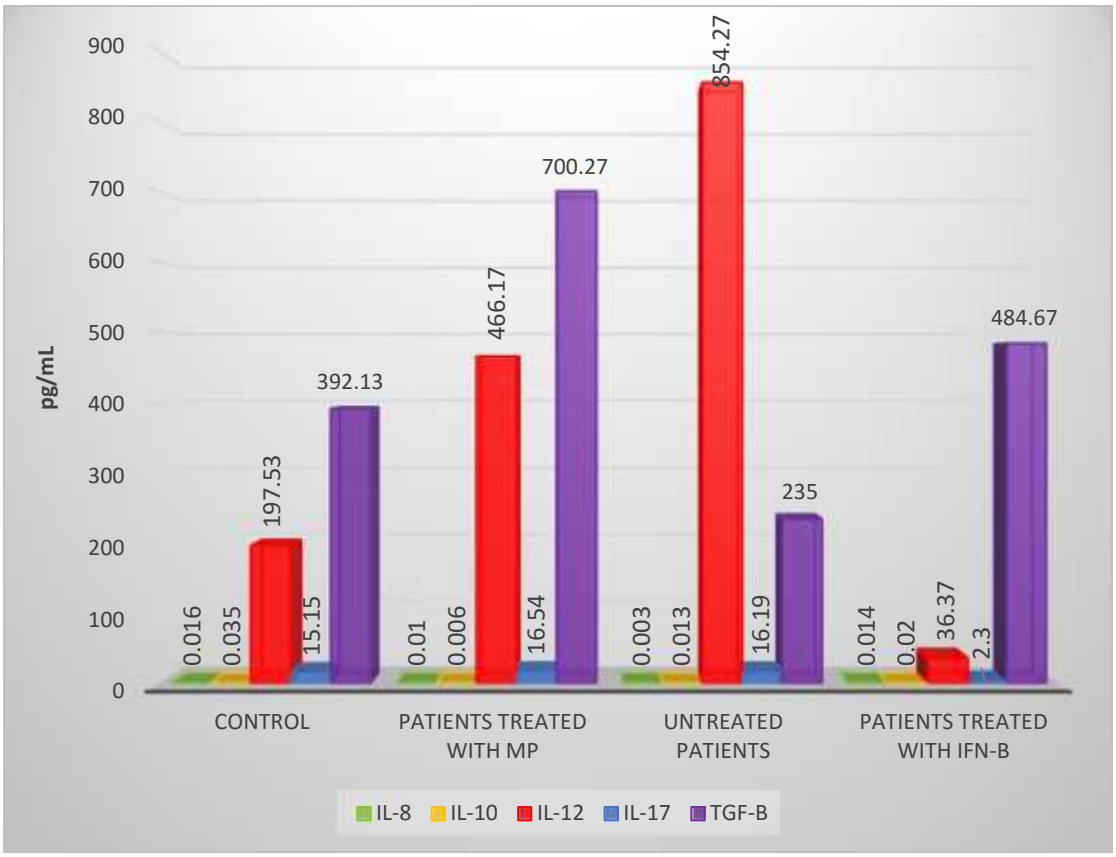

Figure 4 - 5 Serum Level of Study Cytokine

Table (4-15) Serum Level of Study Cytokine

pg/ml	Control	Untreated Patients	Patients treated with MP	Patients treated with IFN-β
IL-8	0.016	0.003	0.01	0.014
IL-10	0.035	0.013	0.006	0.02
IL-12	197.53	854.27	466.17	36.37
IL-17	15.15	16.19	16.54	2.3
TGF-β	392.13	235	700.27	484.67

4.4.1.1 Control and Untreated Patients

The serum level of IL-12 (197.53 ± 35.08 & 854.27 ± 433.98) and IL-17 (15.15 ± 5.11 & 16.19 ± 6.47 showed an increasing in untreated patients compared to control, and the differences were not significant, while IL-8 (0.016 ± 0.006 & 0.003 ± 0.001), IL-10 (0.035 ± 0.027 & 0.013 ± 0.006) and TGF-β (392.13 ± 61.82 & 235.00 ± 142.59) showed a decreasing in untreated patients compared to control, and the IL-8 difference was significant (P ≤0.026), while IL-10 and TGF- β differences were not significant. (Figure 4-5) and table (4-16).

Table 4-16 Serum level of study cytokine in control and Untreated Patients

	Mean ± S.E.		P≤ 0.05	Confidant interval	
pg/ml	Control	Untreated Patients		Lower	Upper
IL-8	0.016 ± 0.006	0.003 ± 0.001	0.026	0.00157	0.02357
IL-10	0.035 ± 0.027	0.013 ± 0.006	N.S.	-0.02110	0.06382
IL-12	197.53 ± 35.08	854.27 ± 433.98	N.S.	-1331.9978	18.5312
IL-17	15.15 ± 5.11	16.19 ± 6.47	N.S.	-18.9579	16.8912
TGF-β	392.13 ± 61.82	235.00 ± 142.59	N.S	-116.1033	430.37

4.4.1.2 Control and Patients Treated with MP

The serum level of IL-12 (197.53 ± 35.08 & 466.17 ± 145.84), IL-17 (15.15 ± 5.11 & 16.54 ± 6.48) and TGF-β (392.13 ± 61.82 & 700.27 ± 96.12) showed an increasing in patients treated with MP compared to control, and the TGF-β difference was significant (P ≤ 0.028), while IL-12 and IL-17 differences were not significant. IL-8 (0.016 ± 0.006 & 0.010 ± 0.003), IL-10 (0.035 ± 0.027 & 0.006 ± 0.003) showed a decreasing in

Patients Treated with MP compared to control, and the differences were not significant. (Figure 4-5) and table (4-17).

Table 4-17 Serum level of study cytokines in control and Patients Treated with MP

	Mean ± S.E.		P≤ 0.05	Confidant interval	
pg/ml	Control	Patients Treated MP		Lower	Upper
IL-8	0.016 ± 0.006	0.010 ± 0.003	N.S.	-0.00495	0.1704
IL-10	0.035 ± 0.027	0.006 ± 0.003	N.S.	-0.01417	0.07076
IL-12	197.53 ± 35.08	466.17 ± 145.84	N.S.	-943.897	406.6312
IL-17	15.15 ± 5.11	16.54 ± 6.48	N.S.	-19.3112	16.5379
TGF-β	392.13 ± 61.82	700.27 ± 96.12	0.028	-581.37	-34.8967

4.4.1.3 Control and Patients Treated with IFN-β

The serum level of TGF-β (392.13 ± 61.82 & 484.67 ± 100.89) showed an increasing in patients treated with IFN-β compared to control, and the difference was not significant, while IL-8 (0.016 ± 0.006 & 0.014 ± 0.004), IL-10 (0.035 ± 0.027 & 0.020 ± 0.007), IL-12 (197.53 ± 35.08 & 36.37 ± 15.97), IL-17 (15.15 ± 5.11 & 2.30 ± 2.30) showed a decreasing in patients treated with IFN-β compared to control, and the differences were not significant. (Figure 4-5) and table (4-18).

Table 4-18 Serum level of study cytokines in control and Patients Treated with IFN-β

	Mean ± S.E.		P≤ 0.05	Confidant interval	
pg/ml	Control	Patients Treated IFN-β		Lower	Upper
IL-8	0.016 ± 0.006	0.014 ± 0.004	N.S.	-0.0933	0.01267

IL-10	0.035 ± 0.027	0.020 ± 0.007	N.S.	-0.0348	0.05010
IL-12	197.53 ± 35.08	36.37 ± 15.97	N.S.	-1029.197	321.3312
IL-17	15.15 ± 5.11	2.30 ± 2.30	N.S.	-25.3912	10.4579
TGF-β	392.13 ± 61.82	484.67 ± 100.89	N.S.	-28.6366	517.8366

4.4.1.4 Untreated Patients and Patients Treated with MP

The serum level of IL-8 (0.003 ± 0.001 & 0.010 ± 0.003), IL-17 (16.19 ± 6.47 & 16.54 ± 6.48) and TGF-β (235.00 ± 142.59 & 700.27 ± 96.12) showed an increasing in patients treated with MP compared to untreated patients , and the TGF-β difference was significant (P ≤ 0.001), while IL-8 and IL-17 differences were not significant, IL-10 (0.013 ± 0.006 & 0.006 ± 0.003) and IL-12 (854.27 ± 433.98 & 466.17 ± 145.84), showed +a decreasing in patients treated with MP compared to untreated patients, and the differences were not significant. (Figure 4-5) and table (4-19).

Table 4-19 Serum level of study cytokines in Untreated Patients and Patients Treated with MP

	Mean ± S.E.		P≤ 0.05	Confidant interval	
pg/ml	Untreated Patients	Patients Treated MP		Lower	Upper
IL-8	0.003 ± 0.001	0.010 ± 0.003	N.S.	-0.01753	0.00447
IL-10	0.013 ± 0.006	0.006 ± 0.003	N.S.	-0.03553	0.04940
IL-12	854.27 ± 433.98	466.17 ± 145.84	N.S.	-287.1645	1063.3645
IL-17	16.19 ± 6.47	16.54 ± 6.48	N.S.	-18.2779	17.5712
TGF-β	235.00 ± 142.59	700.27 ± 96.12	0.001	-738.5033	-192.03

4.4.1.5 Untreated Patients and Patients Treated with IFN-β

The serum level of IL-8 (0.003 ± 0.001 & 0.014 ± 0.004), IL-10 (0.013 ± 0.006 & 0.020 ± 0.007) and TGF-β (235.00 ± 142.59 & 484.67 ± 100.89) showed an increasing in patients treated with IFN-β compared to untreated patients, and the differences were not significant, while IL-12 (854.27 ± 433.98 & 36.37 ± 15.97) and IL-17 (16.19 ± 6.47 & 2.30 ± 2.30) showed a decreasing in patients treated with IFN-β compared to untreated patients, and the differences were not significant. (Figure 4-5) and table (4-20).

Table 4-20 Serum level of study cytokine in Untreated Patients and Patients Treated with IFN-β

	Mean ± S.E.		P≤ 0.05	Confidant interval	
pg/ml	Untreated Patients	Patients Treated IFN-β		Lower	Upper
IL-8	0.003 ± 0.001	0.014 ± 0.004	N.S.	-0.02191	0.00009
IL-10	0.013 ± 0.006	0.020 ± 0.007	N.S.	-0.05618	0.02874
IL-12	854.27 ± 433.98	36.37 ± 15.97	N.S.	-372.4645	978.0645
IL-17	16.19 ± 6.47	2.30 ± 2.30	N.S.	-24.3579	11.4912
TGF-β	235.00 ± 142.59	484.67 ± 100.89	N.S.	-185.77	360.7033

4.4.1.6 Patients Treated with MP and Patients Treated with IFN-β

The serum level of IL-8 (0.010 ± 0.003 & 0.014 ± 0.004) and IL-10 (0.006 ± 0.003 & 0.020 ± 0.007) showed an increasing in patients treated with IFN-β compared to patients treated with MP , and the differences were not significant, while IL-12 (466.17 ± 145.84 & 36.37 ± 15.97), IL-17 (16.54 ± 6.48 & 2.30 ± 2.30) and TGF-β (700.27 ± 96.12 & 484.67 ± 100.89) showed a decreasing in patients treated with IFN-β compared to patients treated with MP, and TGF-β difference was significant

(P≤0.0001), while IL-12, IL-17 differences were not significant. (Figure 4-5) and table (4-21).

Table 4-21 Serum level of study cytokine in Patients Treated with MP and Patients Treated with IFN-β

	Mean ± S.E.		P≤ 0.05	Confidant interval	
pg/ml	Patients Treated MP	Patients Treated IFN-β		Lower	Upper
IL-8	0.010 ± 0.003	0.014 ± 0.004	N.S.	-0.01538	0.00662
IL-10	0.006 ± 0.003	0.020 ± 0.007	N.S.	-0.06312	0.02181
IL-12	466.17 ± 145.84	36.37 ± 15.97	N.S.	-760.5645	589.9645
IL-17	16.54 ± 6.48	2.30 ± 2.30	N.S.	-24.0046	11.8446
TGF-β	700.27 ± 96.12	484.67 ± 100.89	0.0001	279.4967	825.97

Level of IL-8 decreases significantly in untreated patient compare to control and that disagree with (Lund *et al.*, 2004), also serum levels of IL-8 decrease in patients treated by MP compared to control and that came in agreement with (Mirowska-Guzel, 2006) and that may due to drug (methyl -prednisolone) effect ,which decreasing synthesis or release of pro-inflamatory cytokines such as IL-8 as a part of its action mechanism (Kinkel,1999), and decrease monocytes producing IL-8 (Mirowska-Guzel *et al.*, 2006); also serum level of IL-8 decreased in patients treated with IFN-β, this decreasing may due to drug (beta feron) effect.). In untreated patients IL-8 should to be increasing because they are experience acute-phase response.

So why it decreased significantly? It may due to the neutrophil that phagocytosed by the macrophages, macrophages receptors recognizes antigens, bind to those antigens then phagocytosis them. When apoptotic

polymorphnuclear neutrophils phagocytosed by macrophages, the transforming growth factor-β and MCP-1 secretion increases and IL-8 production decrease, leading to a cytokine shift that favoring monocyte recruitment. As neutrophils are depleted from the inflammatory site, circulatory monocytes, in contrast, accumulate and differentiate into inflammatory macrophages, which complete phagocytosis and destruction of the injurious agents (Doherty *et al.*, 1988; Melnicoff *et al.*, 1989; Ryan and Majno, 1977). In addition to that, IL-8 is one of the factors that reduced in an inflammatory response, it's a chemokine. Chemokines are a group of cytokines with a hemotactic and other function. Some of IL-8 produced is held in the extracultular matrix on the endothelial surface and can bind to IL-5 receptor, on the neutrophil surface. The binding of IL-8 to neutrophil activate the neutrophil and LFA-1 change conformation and bind friendly to ICAM-1on the endothelium (wood, 2006). So this may explain the decreasing level of IL-8 in explanation. (there ware another explanation, in this disease, IL-8 might not produced by endothelic cells so it's levels decreased in circulation because most cytokines act close to where they are produced, so in this disease IL-8 may be increased in CSF (local of inflammation) (Abbas 2005).

We found that there was a decline in serum level of IL-10 in all patients groups as compared to control. Patients treated with INF-β, untreated patients then patients treated with MP. Data in the literature about IL-10 level are very different, the decrease of IL-10 in our study came in agreement with (Rieckmann *et al.*, 1994; Salmaggi *et al.*, 1996; Van Box El-Dezair *et al.*, 1999; Balashov *et al.*, 2000; Ozenci *et al.*, 2002;), but it doesn't agree with either (Nicoletti *et al.*, 1996; Bord *et al.*, 1997; Fassbender *et al.*, 1998; Hessen *et al.*, 1999; Losy *et al.*, 2002) who reported an elevation of IL-10 in MS patients, or (Moore *et al.*, 1993; Trabattoni *et al.*, 2000; Kvarastrom *et al.*, 2013) who reported that there

were no differences in IL-10 level between MS patients and control. The decrease in IL-10 level may related to genetic polymorphism in the IL-10 gen promoter (Corey and stone, 2002); the researcher even conclude that those who having the defect are more susceptible to MS onset (Karp, 2001), since IL-10 expression associated with disease activity and characterize the disease different stages (Losy and Zaremba, 2005). In addition, IL-10 production is correlated with spontaneous remition, also endogenous or transgenic delivery of IL-10 was generally protective (Karp, 1999; Legge *et al.*, 2000; Cua *et al.*, 2001); all that explain why patients treated with INF-β are the closer group to control because they are in remition phase of disease, what we mention above olso explain IL-10 level in untreated patients since they experience MS attack (relapse), and IL-10 level in patients treated with MP since they were suffering from a server attack, INF-β supposed to augmented IL-10 level (Ozenci, 1999) and MP supposed to control inflammatory response by up regulating IL-10 expression as one of its mechanisms of action (Gayo *et al.*, 1998; Rentzos *et al.*, 2008); but not one of both drugs acts like it supposed to act for unknown reasons.

The present study results found that serum level of IL-12 increased in untreated patients, patients treated with MP as compared to control, while it decreased in patients treated with INF-β copared to control. The increasing in untreated patients treated with MP agreed with (Matusevicius *et al.*, 1998; Losy *et al.*, 2002) disagreed with (Ferrante *et al.*, 1998).Since MS begins when a T cell is activated and recognize the protein of the myelin sheath, when T cell is activated it can pass through BBB; In the brain, T cells stimulates the response of monocytes / macrophages by releasing INF-α then the antigen presented by the T cell, the signaling between the two cells lead to an attack on the oligodendrocytes of myelin sheath (Corey and Stone, 2002). The production of IL-12 stimulated by the

contact of monocytes and dendritic cells with activated T cells through CD 40 – CD 40 ligand interaction. It stimulated also through the interaction of monocytes, macrophages with extra cellular matrix components that expressed selectively during inflammation (Karp, 2001). That may explain the high level of IL-12 in two groups of patient, but the decrease of IL-12 in patients treated with INF-β may due to the drug down regulation effect on IL-12; in addition, the production of IL-12 seems to track with MS activity in RR patients (Losy and Zaremba, 2005) and that may explain why it increased in untreated patients; since they an attack, and the increases in patients treated with MP because they suffer from server attack and MP have no influence on IL-12 (Rentzos *et al.,* 2008);

IL-12 is synthesized in peripheral immune system predominantly by monocytes, dendritic cells and subset of B cells, but also by neutrophil (Sartori *et al.,* 1997; Cassatella *et al.*, 1995), and that came in contrast with the elevation of neutrophils in our results, the mechanism of IL-12 in disease promotion suggested to be associated with direct effect on T cell (the induction of other Th1cytokines, suppression of Th2 cytokines and recruitment of macrophages and macroglia. (Sartori *et al.,* 1997). All that may explain the relationship between IL-10 and IL-12 by which appear they regulate each other expression and this unique immunorgulatory circuit play a critical role in controlling Th cell differentiation and provides a mechanism, by which microbial triggers of the innate immune system can modulate autoimmune disease (Segal *et al.,* 1998). That support our result for decreasing IL-10 and increasing of IL-12.

Our study showed an elevation in serum level of IL-17 in untreated patients and patients treated with MP, while it decreased in patients treated with INF-β. The elevation came in contrast with previous studies (Matusevicius *et al.,* 1999; Brucklacher-Walder *et al.,* 2009; Babaloo *et al.,* 2013; Kostic *et al.,* 2013; Huber *et al.,* 2014), while we disagree with

(Peelen *et al.,* 2013). We found that IL-17 level elevated in patient under disease attack and that may due to Th-17 important role in MS onset rather than MS progression and development, in addition to the inverse correlation between IL-17 and MS duration (Graber *et al.,* 2008; Kostic *et al.,* 2013). Since Th-17 immune responses via IL-17 secretion support the expansion, maturation and recruitment of innate immune system such as neutrophils to enhance inflammatory reaction (Fossiez *et al.,* 1996; yu and Gaffen, 2003). That explanation came fit with the elevation in neutrophil that we found in this study.

IL-17 decreases in INF-β treated patients may due to drug effect, production of IFN-β induces IL-17 either directly or indirectly by reducing osteopontin (dendritic cell cytokine amplifies IL-17 production). Or by inducing IL-27 (IL-17 supperser cytokine) (Murugaiyan *et al.,* 2008; Hong and Hutton, 2010; Kvarnstrom *et al.,* 2013).

We found that TGF-β increased significantly in patients treated with MP, it was increase in patients treated with INF-β, while it decreased in untreated patients, that results came in contrast with (Bertoletto *et al.,* 1999; Losy and Michalowska-Wender, 2002; Sellebjerg, 2004; Dobolyi *et al.,* 2012). The elevation disagree with (Rollnik *et al.,* 1997; Selvaraj and Geiger, 2008). The significant increase for patients treated with MP may due to drug effect, which is increasing TGF- β significantly (Sellebjerg, 2004). While the TGF- β increasing for patients treated with IFN- β also due to the long period treatment with IFN- β which upregulates TGF- β concentration level (Losy and Michalowska-Wender, 2002). For untreated patients, their decreased concentration may due to the role of TGF- β in disease onset and in the clearance of inflammation by apoptosis induction in T cells (Lassmann and Wekerle , 2006), when TGF- β decrease it cannot induce TH-17 apoptosis, then TH-17 will secrete TNFα which induces demylenation (Rollnik *et al.,* 1997).

The finding of an inverse correlation between TGF- β and disease activity that observed by MRI (Bertoloetto *et al.*, 1999) support our study results.

4.4.2. Correlation between Treg and Serum Cytokines

Correlation between Treg and Serum Cytokines in study patient groups showed that there was a strong positive relationship (r = 0.615, p = 0.015) between IL-12 and IL-17 in untreated patients. While there was a strong negative relationship ((r = - 0.636, p = 0.011) between $CD25^{+}$ $FoxP3^{+}$ Treg cells and IL-8 and another strong negative relationship (r= -0.765, P= 0.001) between $CD25^{+}$ $FoxP3^{+}$ Treg cells and IL-12 also there were a very strong positive relationship (r= 0.966, P= 0.00) between IL-8 and IL-12 in patients treated with MP. we haven't found related studies in this aspect support our results

4.4.2.1 Correlation Between Tregs, Serum IL-8, IL-10, IL-12, IL-17 and TGF-β for Untreated Patients

Table 4-22 Correlation Between CD25+ FOXP3+ Tregs, serum IL-8, IL-10, IL-12, IL-17 and TGF-β for Untreated Patients

		CD25+ FOXP3+ Tregs	IL-8	IL-10	IL-12	IL-17	TGF-β
CD25+ FOXP3+ Tregs	r	1	.241	-.005	0.734	.487	.316
	p		.387	.987	.002	.065	.251
IL-8	r		1	0.845	.117	0.733	0.728
	p			.000	.678	.002	.002
IL-10	r			1	.037	.502	.511
	p				.896	.057	.052
IL-12	r				1	.615*	.225
	p					.015	.419
IL-17	r					1	0.663
	p						.007
TGF-β	r						1
	p						

*. Correlation is significant at the 0.05 level (2-tailed).
r= Pearson Correlation
p= P value ≤ 0.05

4.4.2.2 Correlation Between Treg, Serum IL-8, IL-10, IL-12, IL-17 and TGF-β for Patients treated with MP

Table 4-23 Correlation Between CD25+ FOXP3+ Tregs, Serum IL-8, IL-10, IL-12, IL-17 and TGF-β for Patients treated with MP

		CD25+ FOXP3+ Tregs	IL-8	IL-10	IL-12	IL-17	TGF-β
CD25+ FOXP3+ Tregs	r	1	-.636*	-.288	-0.765	-.087	.023
	p		.011	.298	.001	.758	.935
IL-8	r		1	.191	0.966	.072	-.089
	p			.496	.000	.798	.752
IL-10	r			1	.142	-.169	-.160
	p				.614	.548	.569
IL-12	r				1	.039	-.043
	p					.889	.879
IL-17	r					1	-.400
	p						.139
TGF-β	r						1
	p						

*. Correlation is significant at the 0.05 level (2-tailed).
r= Pearson Correlation
p= P value ≤ 0.05

4.4.2.3 Correlation Between CD25+ FOXP3+ Tregs, Serum IL-8, IL-10, IL-12, IL-17 and TGF-β for Patients treated with IFN-β

Table 4-24 Correlation Between CD25+ FOXP3+ Tregs, Serum IL-8, IL-10, IL-12, IL-17 and TGF-β for Patients treated with IFN-β

		CD25+ FOXP3+ Tregs	IL-8	IL-10	IL-12	IL-17	TGF-β
CD25+ FOXP3+ Tregs	r	1	.124	.371	.128	.054	-.186
	p		.660	.174	.649	.850	.507
IL-8	r		1	0.717	.385	0.821	.500
	p			.003	.157	.000	.057

IL-10	r			1	.093	0.725	.162
	p				.742	.002	.565
IL-12	r				1	.359	.098
	p					.188	.729
IL-17	r					1	.324
	p						.238
TGF-β	r						1
	p						

r = Pearson Correlation
p= P value ≤ 0.05

4.4.3 Saliva Level of Study Cytokine

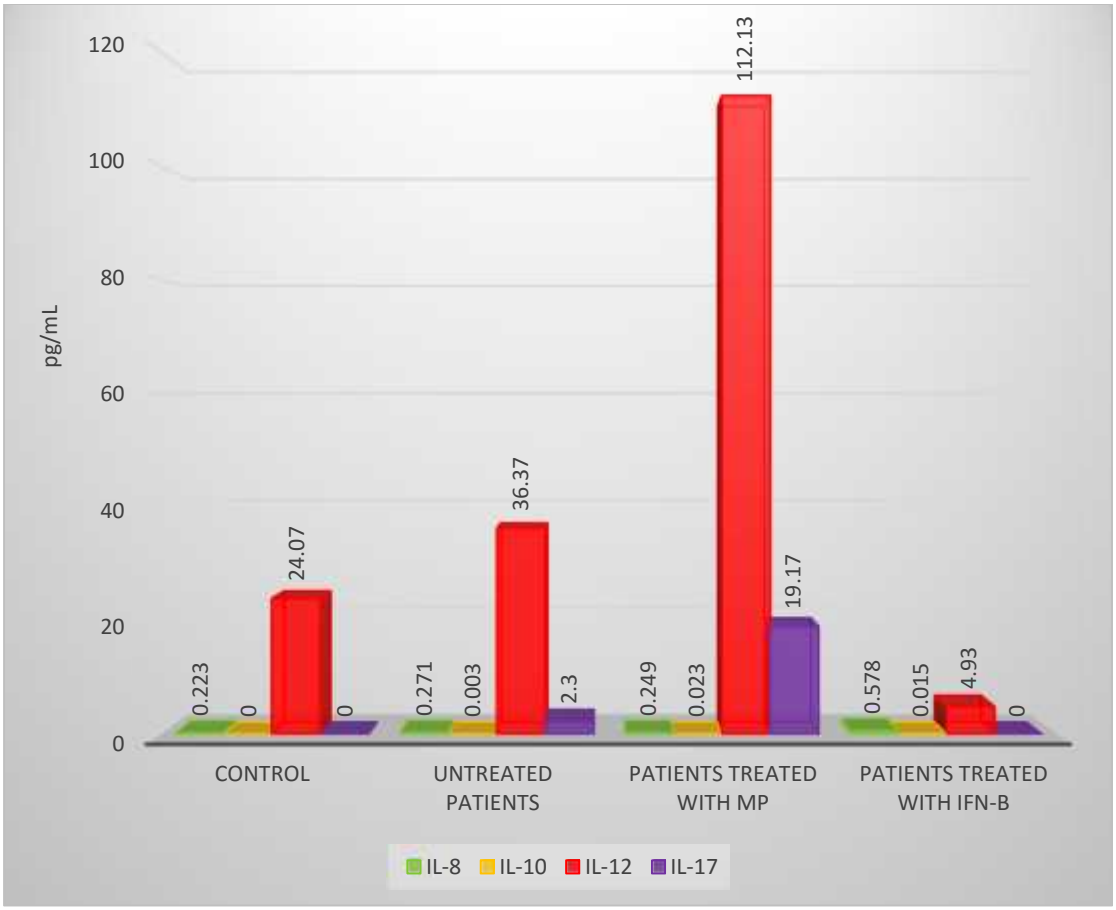

Figure 4 - 6 Saliva Level of Study Cytokine

Table (4-25) Saliva Level of Study Cytokine

pg/ml	Control	Untreated patients	Patients treated with MP	Patients treated with IFN-β
IL-8	0.223	0.271	0.249	0.578
IL-10	0	0.003	0.023	0.015
IL-12	24.07	36.37	112.13	4.93
IL-17	0	2.3	19.17	0

4.4.3.1 Control and Untreated Patients

The saliva level of IL-8 (0.223 ± 0.026 & 0.271 ± 0.051), IL-10 (0.00 ± 0.00 & 0.003 ± 0.002), IL-12 (24.07 ± 12.97 & 36.37 ± 15.97) and IL-17 (0.00 ± 0.00 & 2.30 ± 2.30) showed an increasing in untreated patients compared to control, and the differences were not significant. (Figure 4-6) and table (4-26).

Table 4-26 Saliva level of study cytokine in control and Untreated Patients

	Mean ± S.E.		P≤ 0.05	Confidant interval	
pg/ml	Control	Untreated Patients		Lower	Upper
IL-8	0.223 ± 0.026	0.271 ± 0.051	N.S.	-0.35381	0.25728
IL-10	0.00 ± 0.00	0.003 ± 0.002	N.S.	-0.02108	0.01584
IL-12	24.07 ± 12.97	36.37 ± 15.97	N.S.	-107.6488	83.0488
IL-17	0.00 ± 0.00	2.30 ± 2.30	N.S.	-18.0609	13.4609

4.4.3.2 Control and Patients Treated with MP

The saliva level of IL-8 (0.223 ± 0.026 & 0.249 ± 0.048), IL-10 (0.00 ± 0.00 & 0.023 ± 0.008), IL-12 (24.07 ± 12.97 & 112.13 ± 19.15) and IL-

17 (0.00 ± 0.00 & 19.17 ± 10.00) showed an increasing in patients treated with MP compared to control, the IL-10 difference was significant (P ≤ 0.017) and IL-17 difference was significant (P ≤ 0.018), while IL-8 and IL-12 differences were not significant. (Figure 4-6) and table (4-27).

Table 4-27 Saliva level of study cytokine in control and Patients Treated with MP

	Mean ± S.E.		P≤ 0.05	Confidant interval	
pg/ml	Control	Patients Treated MP		Lower	Upper
IL-8	0.223 ± 0.026	0.249 ± 0.048	N.S.	-0.33156	0.27952
IL-10	0.00 ± 0.00	0.023 ± 0.008	0.017	-0.04116	-0.00423
IL-12	24.07 ± 12.97	112.13 ± 19.15	N.S.	-183.4155	7.2821
IL-17	0.00 ± 0.00	19.17 ± 10.00	0.018	-34.9275	-3.4058

4.4.3.3 Control and Patients Treated with IFN-β

The saliva level of IL-8 (0.223 ± 0.026 & 0.578 ± 0.202) and IL-10 (0.00 ± 0.00 & 0.015 ± 0.010) showed an increasing in patients treated with IFN-β compared to control, the IL-8 difference was significant (P ≤ 0.023), while IL-10 difference was not significant. IL-12 (24.07 ± 12.97 & 4.93 ± 4.30) showed a significant (P≤0.003) decrease in patients treated with IFN-β compared to control. The IL-17 (0.00 ± 0.00 & 0.00 ± 0.00) showed equivalent value in patients treated with IFN-β and control and the difference was not significant. (Figure 3-6) and table (3-28).

Table 4-28 Saliva level of study cytokine in control and Patients Treated with IFN-β

	Mean ± S.E.		P≤ 0.05	Confidant interval	
pg/ml	Control	Patients Treated IFN-β		Lower	Upper
IL-8	0.223 ± 0.026	0.578 ± 0.202	0.023	-0.66131	-0.05022
IL-10	0.00 ± 0.00	0.015 ± 0.010	N.S.	-0.03344	0.00349
IL-12	24.07 ± 12.97	4.93 ± 4.30	0.003	-241.8155	-51.1179
IL-17	0.00 ± 0.00	0.00 ± 0.00	N.S.	-20.6942	10.8275

4.4.3.4 Untreated Patients and Patients Treated with MP

The saliva level of IL-8 (0.271 ± 0.051 & 0.249 ± 0.048), IL-10 (0.003 ± 0.002 & 0.023 ± 0.008), IL-12 (36.37 ± 15.97 & 112.13 ± 19.15) and IL-17 (2.30 ± 2.30 & 19.17 ± 10.00), showed an increasing in patients treated with MP compared to untreated patients, IL-10 difference was significant (P≤ 0.034) and IL-17 difference was significant (P≤ 0.036), while IL-8 and IL-12 differences were not significant. (Figure 4-6) and table (4-29).

Table 4-29 Saliva level of study cytokine in Untreated Patients and Patients Treated with MP

	Mean ± S.E.		P≤ 0.05	Confidant interval	
pg/ml	Untreated Patients	Patients Treated MP		Lower	Upper
IL-8	0.271 ± 0.051	0.249 ± 0.048	N.S.	-0.28330	0.32779
IL-10	0.003 ± 0.002	0.023 ± 0.008	0.034	-0.03854	-0.00161
IL-12	36.37 ± 15.97	112.13 ± 19.15	N.S.	-171.1155	19.5821
IL-17	2.30 ± 2.30	19.17 ± 10.00	0.036	-32.6275	-1.1058

4.4.3.5 Untreated Patients and Patients Treated with IFN-β

The saliva level of IL-8 (0.271 ± 0.051 & 0.578 ± 0.202) and IL-10 (0.003 ± 0.002 & 0.015 ± 0.010) showed an increasing in patients treated with IFN-β compared to untreated patients, the IL-8 difference was significant (P ≤ 0.049), while IL-10 difference was not significant. IL-12 (36.37 ± 15.97 & 4.93 ± 4.30) and IL-17 (2.30 ± 2.30 & 0.00 ± 0.00) showed a decrease in patients treated with IFN-β compared to untreated patients and the difference of IL-12 was significant (P ≤ 0.007), while IL-17 difference was not significant. (Figure 4-6) and table (4-30).

Table 4-30 Saliva level of study cytokine in Untreated Patients and Patients Treated with IFN-β

	Mean ± S.E.		P≤ 0.05	Confidant interval	
pg/ml	Untreated Patients	Patients Treated IFN-β		Lower	Upper
IL-8	0.271 ± 0.051	0.578 ± 0.202	0.049	-0.61304	-0.00196
IL-10	0.003 ± 0.002	0.015 ± 0.010	N.S.	-0.03082	0.00611
IL-12	36.37 ± 15.97	4.93 ± 4.30	0.007	-229.5155	-38.8179
IL-17	2.30 ± 2.30	0.00 ± 0.00	N.S.	-18.3942	13.1275

4.4.3.6 Patients Treated with MP and Patients Treated with IFN-β

The saliva level of IL-8 (0.249 ± 0.048 & 0.578 ± 0.202), showed an increasing in patients treated with IFN-β compared to patients treated with MP, and the difference was significant (P ≤ 0.035), while IL-10 (0.023 ± 0.008 & 0.015 ± 0.010), IL-12 (112.13 ± 19.15 & 4.93 ± 4.30) and IL-17 (19.17 ± 10.00 & 0.00 ± 0.00) showed a non-significant decrease in patients treated with IFN-β compared to patients treated with MP. (Figure 4-6) and table (4-31).

Table 4-31 Saliva level of study cytokine in Patients Treated with MP and Patients Treated with IFN-β

	Mean ± S.E.		P≤ 0.05	Confidant interval	
pg/ml	Patients Treated MP	Patients Treated IFN-β		Lower	Upper
IL-8	0.249 ± 0.048	0.578 ± 0.202	0.035	-0.63529	-0.02420
IL-10	0.023 ± 0.008	0.015 ± 0.010	N.S.	-0.01074	0.02618
IL-12	112.13 ± 19.15	4.93 ± 4.30	N.S.	-153.7488	36.9488
IL-17	19.17 ± 10.00	0.00 ± 0.00	N.S.	-1.5275	29.9942

there were no previous studies support our results according to the available resources. But we have some explanations as follow:

In both treated patient groups (MP and IFN-β) the results showed a significant increasing level of IL-10.

As we know, MP (Glucocorticoid drugs) which act as anti-inflammatory drug that reduce the function of Mϕ and T cells (Kinkel, 1999), but it may induce the function of Neutrophil to produce their mediator such as IL-10, this result supported by the increasing count of this cell in patients' samples.

IFN-β as we know have immune modulating and anti-inflammatory properties by inhabiting pro-inflammatory cytokines levels and elevating regulatory cytokines levels (Stephan and Douglas, 2013), which IL-10 concerned as one of them.

These cytokines behave in disturbance manner. But The main conclude suggest that PMNs may play a primarily anti-inflammatory role.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

The study established its aim by finding that:

- About MP drug:

1. Methylprednisolone drug caused increasing in (total WBC count, Neutrophils, serum TGF-β, saliva IL-10 and saliva IL-17), while it caused decreasing in (Lymphocytes, Basophils and T regs).
2. Methylprednisolone (acute attack treatment) act better than Interferon beta (long term treatment) basing on our current results.
3. MP elevated serum level of TGF-β.
4. In patients treated with MP there were:
   - A strong negative relationship between $CD25^+$ $FoxP3^+$ Treg cells and IL-8.
   - A very strong positive relationship between IL-8 and IL-12.

- About IFN-β drug:

1. Interferon Beta drug caused increasing in (saliva IL-8), while it caused decreasing in (Lymphocytes, Basophils, T regs and saliva IL-12).
2. IFN-β had no influence on serum level of study cytokines.

- About $CD25^+$ $FoxP3^+$ Treg cells:
  1. CD25 FOXP3 Treg cells increased in MS relapse phase as a result of inflammation, they are not responsible for maintain MS remission which means they decreases in remission phase.
  2. we concluded that Treg appear not to use IL-10 and TGF-β suppression mechanism.

3. and both drugs doesn't go long with the new tendency for disease treatment by increasing Tregs numbers, this type of treatment suggested by other studies based on their promising results.

- About cytokines:

1. There were contradictory results in cytokines level, which could be affected by patient's psychological health, Health status, Social circumstances and nutrition habits.
2. Both of IL_8 and IL-10 act in the same way (decreased) in MS patients groups, Both of IL-12 and IL-17 also act in the same way they elevated in relapse phase and decline in remition phase. While TGF-β increased in both treated patients groups and decreased in untreated group

Recommendations

The present study conclusions promote the researcher to suggest the following recommendations:

1. Using all T regs known markers to make a deep understanding for its important key role in autoimmune disease general and MS specially, and study it on molecular level.
2. Investigate autoantibodies of MS, Myelin basic protein and the role of other cytokines in CSF, Serum, and Saliva, to compare differences in order to locate the most sensitive on to the changings that caused by drugs, it will make better understanding to drugs effect.

3. Make a wide study by which investigate the role of involved cells like T regs, Ds cells, Th1, Th2, Th17 and B cells in this disease.
4. Widening the number of samples in order to be outside of bias and for longer time.
5. Make studies that follow up the same group of patients before and after certain time period of treatments.
6. Make genetic studies on families having history with auto immune diseases to locate the responsible genes.

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APPENDIX

الخلاصة

يعتبر التصلب المتعدد المنتشر (MS) حالة التهابية تصيب الجهاز العصبي المركزي (CNS) مما يؤدي الى حدوث خلل في الوظائف العصبية، تهدف الدراسة الى تقييم دور المثيل بريدنزولون (MP) والانترفيرون بيتا (INF-β) كعلاج للمرض والتحقق من تأثيرهما على الخلية التائية المنظمة (CD25$^+$ FoxP3 T regulatory cells)، IL-8, IL-17 (محركات خلوية قبل التهابية)، IL-12 (محرك خلوي التهابي (عامل محفز لتكاثر الخلية التائية))، IL-10، TGF-β (محركات خلوية مضادة للالتهاب)، العد الكلي والتفريقي لخلايا الدم البيضاء (WBC) وخصائص المرضى العامة.

شملت الدراسة 45 مريضا عراقيا مصاب بالتصلب المتعدد (15 مريض حديث الإصابة غير معالج، 15 مريض معالج بـ (MP) و15 مريض معالج بـ (INF-β)) بالإضافة الى 15 شخص يبدون اصحاء ظاهريا (كمجموعة سيطرة). اما بالنسبة للمرضى المعالجين بـ (MP) تم جمع عيناتهم بعد فترة العلاج اما بالنسبة للمرضى المعالجين بـ (INF-β) تم جمع عيناتهم في الطور الساكن من المرض.

كانت نتائج الدراسة كالتالي:

1. أظهرت الخصائص العامة للمرضى وفقا للجنس ان نسبة إصابة الاناث الى الذكور كانت 1:2 اما بالنسبة للعمر عند الإصابة فكانت مجموعة المرضى (<30) سنة هي الأكثر تكرارا بين عينة الدراسة، عمر 57 سنة هو اعلى عمر للإصابة في عينة الدراسة اما اقل عمر للإصابة فكان 13سنة وبمعدل (34.93%). اما بالنسبة لتوزيع المرضى وفقا للمحافظة، حصلت بغداد على اعلى نسبة إصابة بالتصلب (73.33%).
2. العد الكلي لكريات الدم البيضاء اظهر ارتفاعا معنويا ($P\leq 0.001$) لدى المرضى المتعالجين بـ (MP) (10.38) عند مقارنتها مع مجموعة السيطرة (7.33)، أظهرت الخلايا اللمفية انخفاضا معنويا ($P\leq 0.013$)، ($P\leq 0.003$) لدى كلا مجموعتي المرضى المتعالجين بالمثيل برتنزلون، الانترفيرون بيتا على التوالي (1.82)، (1.92) بالمقارنة مع مجموعة السيطرة (2.58)، ارتفعت الخلايا العدلة ارتفاعا معنويا ($P\leq 0.016$)، ($P\leq 0.000$) لدى المرضى غير المتعالجين ولدى المرضى المتعالجين بـ(MP) على التوالي (7.81)، (5.99) بالمقارنة مع مجموعة السيطرة (3.82)، انخفضت الخلايا القعدة معنويا ($P\leq 0.001$)، ($P\leq 0.014$)

في كلا مجموعتي المرضى المتعالجين بالمثيل بريدنزلون، الانترفيرون بيتا على التوالي (0.05)، (0.06) بالمقارنة مع مجموعة السيطرة (0.08).

3. اظهر عدد الخلايا التائية المنظمة $CD25^{+}$ FoxP3 T reg cells ارتفاعا معنويا (0.002 ≥P) لدى المرضى غير المعالجين (28.01) بالمقارنة مع مجموعة السيطرة (14.06) بينما اظهر انخفاضا معنويا (0.016 ≥P)، (0.007 ≥P) في كلا مجموعتي المرضى المعالجين بالمثيل برتزلون، الانترفيرون بيتا على التوالي (3.70)، (2.44) **بالمقارنة مع مجموعة** السيطرة.
4. تقدير مستوى المحركات الخلوية في مصل الدم اظهر **ان** IL-8 انخفض معنويا (0.026 ≥P) لدى المرضى غير المعالجين (0.003) بالمقارنة مع مجموعة السيطرة (0.016)، بينما IL-12،17 ،IL-10 **لم** يظهروا فروقا معنوية اما بالنسبة للمحرك الخلوي TGF-β **فلقد** اظهر ارتفاعا معنويا (0,028 ≥P) لدى المرضى المعالجين بـ(MP) (700.27) **بالمقارنة** مع مجموعة السيطرة (392.13).

بينما تقدير مستوى المحركات الخلوية في اللعاب اظهر ان IL-8 ارتفع معنويا (0.023 ≥P) لدى المرضى المعالجين بـ(INF-β) (0.578) بالمقارنة مع مجموعة السيطرة (0.223)، IL-10 ارتفع معنويا (0.017 ≥P) **لدى المرضى** المعالجين بـ (MP) (0.023) **بالمقارنة** مع مجموعة السيطرة (0.00)، IL-12 انخفض معنويا (0.003 ≥P) لدى المرضى المعالجين بـ(INF-β) (4.93) بالمقارنة مع مجموعة السيطرة (24.07) **اما بالنسبة الى** IL-17 **فلقد** ارتفع معنويا (0.018 ≥P) لدى المرضى المعالجين بـ(MP) (19.17) **بالمقارنة مع مجموعة** السيطرة (0.00).

وزارة التعليم العالي والبحث العلمي
جامعة بغداد
كلية التربية للعلوم الصرفة – ابن الهيثم
قسم علوم الحياة

دراسة بعض المعالم المناعية لمرضى التصلب المتعدد العراقيين

رسالة مقدمة الى مجلس كلية التربية للعلوم الصرفة (ابن الهيثم) / جامعة بغداد
وهي كجزء من متطلبات نيل درجة الماجستير في علوم الحياة / المناعة

من قبل
هند قصي إسماعيل الفرضي
بكالوريوس علوم حياة / كلية التربية / جامعة بغداد (2012)

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أ.م. د حازمة موسى خليل العباسي
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