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Clinical Evaluation of Disease Activity in Patients with Secondary Sjogren Syndrome Associated with Rheumatoid Arthritis in Relation to Certain Biochemical & Immunological Biomarkers

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In Oral Medicine

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

تَبَارَكَ الَّذِي بِيَدِهِ الْمُلْكُ

وَهُوَ عَلَى كُلِّ شَيْءٍ قَدِيرٌ

صدق اللَّه العظيم

سورة الملك (1)

Dedication

To:

My husband and my children,

To:

Dearest My Father and My Mother

To:

My Brothers and Sisters

I dedicate this work
Faithfully

Fatma

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Abstract

Background

Sjogren syndrome is a chronic autoimmune disorder characterized by lymphocytic infiltration of the exocrine glands, especially salivary & lachrymal glands. The characteristic features of the disease are xerostomia & xerophthalmia that appeared after impairment in the function of salivary & lachrymal glands respectively. Sjogren syndrome might be categorized either as primary or secondary Sjogren syndrome according to whether or not coupled with other connective tissue diseases, such as rheumatoid arthritis, systemic lupus erythromatous & scleroderma.

Objectives

The aims of the present study were to determine the (salivary & serum) melatonin & nitric oxide in secondary Sjogren syndrome patients, rheumatoid arthritis patients & healthy control subjects & correlate with increase susceptibility to secondary Sjogren syndrome. Evaluate salivary alpha-amylase & total salivary protein in secondary Sjogren syndrome patients, rheumatoid arthritis patients & healthy control subjects & correlate with the level of salivary melatonin

Study the effect of the salivary (melatonin, nitric oxide, alpha amylase & total protein) on the salivary flow rate. Evaluate the association of rheumatoid arthritis disease activity score (DAS-28 score) with occurrence of secondary Sjogren syndrome using: laboratory investigation to detect(Anticitrullinated Protein Antibody, Rheumatoid Factor, Anti-SS-A, Anti-SS-B, Erythrocyte Sedimentation Rate)in patients with secondary Sjogren Syndrome & Rheumatoid arthritis patients & clinical evaluation using salivary flow rate & Schirmer's test.

Subjects materials & methods

The study sample included ninety two patients, 61 rheumatoid arthritis patients of either gender with age range (20- 60) years, of them (31 patients with secondary sjogren syndrome diagnosed according to American-European Consensus Group criteria (AECC) & 30 Rheumatoid arthritis patients diagnosed clinically by rheumatology specialists) and both of them evaluated by disease activity depending on Disease Activity Score in 28 Joints (DAS-28) & 31 healthy control subjects. Schirmer's eye test & salivary flow rate estimated. Then Salivary &blood samples were collected to determine the immunological& biochemical analysis (Erythrocyte Sedimentation Rate, Anticitrullinated Protein Antibody, Rheumatoid Factor, Anti-SS-A & Anti-SS-B) serum & salivary melatonin & nitric oxide, salivary α-amylase & total salivary proteins, by using Enzyme Linked Immunosorbent Assay & Spectrophotometric assays.

Results

The results demonstrated that, the vast majority of diseased groups were reported between (40 – 49) years and between (60 – 70) years for the rheumatoid arthritis, and secondary Sjogren syndrome respectively, with mean and standard deviation of (48.30 ± 10.02) years and (52.65 ± 9.88) years.

The present study showed highly significant difference in disease duration between two diseased groups.

There is highly significant difference in the disease activity in each diseased group in relation to Erythrocyte Sedimentation Rate.

This study showed highly significant difference in serum nitric oxide in relation to DAS28-score between (remissions – Low Disease Activity, Low Disease Activity - Moderate Disease Activity, Low Disease Activity - High Disease Activity) in rheumatoid arthritis group,

so there was a decreasing in serum nitric oxide with increasing disease activity score 28 in rheumatoid arthritis group. Also, there was a decreasing in salivary melatonin with increasing disease activity score 28 in the sSS group. Also the current study showed highly significant difference in salivary flow rate between two diseased groups, with decreasing of salivary flow rate in secondary sjogren syndrome group.

The results of this study displayed a significant positive correlation between (salivary melatonin & hyposalivation) in rheumatoid arthritis group & between salivary alpha amylase & hyposalivation) in secondary Sjogren syndrome. Additionally, this study showed a highly significant difference in salivary nitric oxide between two diseased groups & control, so there was a decreasing in salivary nitric oxide in two diseased groups.

The result of the present study revealed significant difference in salivary alpha amylase between secondary Sjogren syndrome & control group, so there was a decreasing in salivary alpha amylase in secondary Sjogren syndrome group.

Conclusion

The incidence of secondary Sjogren syndrome in rheumatoid arthritis patients is dependent on the duration of the disease & independent on disease activity of rheumatoid arthritis. Disease activity is positively related to ESR value ,while negative relation between disease activity &(Serum nitric oxide ,salivary nitric oxide) in Rheumatoid arthritis group & with Salivary melatonin in secondary Sjogren syndrome. The occurrence of secondary Sjogren syndrome is affected by (serum melatonin, salivary α-amylase) & not affected by (salivary melatonin & serum nitric oxide, salivary nitric oxide). The state of hyposalivation directly related to the salivary α-amylase in secondary Sjogren syndrome & salivary melatonin in rheumatoid arthritis.

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List of abbreviations

ACR	American College of Rheumatology.
AECC	American-European Consensus Group criteria.
ACPA	Anticitrullinated Protein Antibody (ACPA)
SAMLT	Salivary melatonin
SMLT	Serum melatonin
(BAFF)	B-Cell Activating Factor.
(BCR)	B-cell receptor.
(Blk)	B-Lymphocyte Kinas.
(CCL11)	Chemokine (C-CMotif) Ligand 11.
(CXCR5)	Chemokine (C-X-C Motif) Receptor 5.
Cv	Coxsackie virus.
DMARDs	Disease Modifying Anti-Rheumatic Drugs.
(EBF1)	Early B-Cell Factor 1
(EBV)	Epstein–Barr virus.
ESR	Erythrocytes Sedimentation Rate.
(GTF2I)	General Transcription Factor 2I.
GWAS	Genome-wide association study
HCV	Hepatitis C virus.
HTL-1	Human T- cell Leukemia virus-1
HIV	Human immunodeficiency virus
IRF5	Interferon Regulatory Factor 5.
IFN	Interferon.
(IFN)	Interferon.
(IL12A)	Interleukin 12A.
(KCS)	Keratoconjunctivitis sicca.
(Lyp)	Lymphocyte tyrosine phosphatase.
(LTA)	Lymphotoxin Gene A.
MHC	Major Histocompatibility Complex.
MLT	Melatonin.
(NCR3).	Natural Cytotoxicity Triggering Receptor 3
NK	Natural killer.

NO	Nitric oxide.
NSAIDs	Non steroidal anti-inflammatory drugs.
NOD mice	Non-obese diabetic mice.
(Ox40L/ TNFSF4).	Ox40 Ligand/Tumor Necrosis Factor Superfamily 4
pSS	Primary Sjogren syndrome.
(PTPN22)	Protein Tyrosine Phosphates Non receptor 22
RA	Rheumatoid Arthritis.
RF	Rheumatoid Factor.
Sa – Tp	Salivary total protein.
Sa–AM	Salivary α-amylase.
sSS	Secondary Sjogren syndrome.
(STAT4)	Signal Transducer and Activator of Transcription 4.
SICCA	Sjorgen International Collaborative Clinical Alliance.
SLE	Systemic Lupus Erythematous.
(TCR)	T-cell receptor.
ICA96	The islet cell antigen 96.
(TNIP1)	TNFAIP3-Interacting Protein 1.
TLR	Toll- like receptors.
(TNFAIP3)	Tumor Necrosis Factor-Alpha Induced Protein 3.
(TNF)	Tumor necrosis factors.
ESR	Erythrocyte sedimentation rate
SNO	Serum nitric oxide
LDA	Low disease activity
MDA	Moderate disease activity
HDA	High disease activity

Introduction

Introduction:

Sjögren's syndrome is a chronic autoimmune disorder of the exocrine glands characterized by lymphocytic infiltrates of the affected gland & dryness of the eyes & mouth resulting from involvement of the lacrimal & salivary glands (Norheim *et al*., 2012). Sjogren's syndrome was named after the Swedish physician Henrik Sjogren. One of the most famous Swedish Ophthalmologists who was in 1933 strongly rooted the concept of Sjögren's syndrome when recited a set of ninety cases having Keratoconjunctivitis sicca two of them accompanied by major salivary glands swelling (Clair, 2013).Sjögren's syndrome may be manifestated as primary or secondary disease when it is coupled with other autoimmune disease such as rheumatoid arthritis (RA) & scleroderma (Antero *et al*., 2011; Gomes *et al*., 2012). The type I interferon & B cells has a role in primary SS (Dörner, 2006; Fox & Liu, 2006). In the other hand the pathophysiology of RA is multifactorial involving, B cells, T cells in addition to the complicated interaction of many pro-inflammatory cytokines, including IL-6 & TNF-a (Choy, 2012). So the pathophysiology of these two disordered was different, assumed that RA with secondary SS has a different physiopathology than the primary type or RA and/or secondary SS considered as two different diseases (Antero *et al*., 2011).

The American-European Consensus Group criteria (AECC) (Vitali *et al*., 2002) help in the diagnosis of Sjogren’s syndrome which based on autoimmunity & outlined to help in distinguishing Sjogren’s syndrome from other conditions. The AECC is progressively utilized as a direction or help for the clinical diagnosis of Sjogren's disorder. In the AECC four of six criteria are required for a diagnosis (Bijlsma *et al*., 2009).

Saliva is watery liquid found among the mouth composed mainly of (organic and inorganic products) as a complex mixture in the secretary products from the salivary glands in addition to the unique substances coming from (the oropharynx, upper airway, gastrointestinal reflux, gingival cravicular fluid, food deposits, and blood-derived compound) (Dodds *et al*., 2005; de Almeida *et al*., 2008; Lima *et al.,* 2010)

Melatonin (N-acetyl-5-methoxytryptamine) is a compound secreted mainly by the pineal gland, however synthesized additionally in several alternative tissues and cells, such as the retina, platelets, human and murine bone marrow cells, skin or lymphocytes, the gastrointestinal tract. Owing to the presence of melatonin receptors in various locations & the multiplicity of sites of formation, melatonin seems to be a versatile physiological signal that has been found concerned within the control of diverse physiologic process (De Almeida *et al.,* 2011).

Melatonin has a powerful antioxidant activity by scavenging the Reactive oxygen species, stimulating anti-oxidative enzymes & at the same time suppression inflammation of oral cavity (Cutando *et al*., 2007). Also Cevik-Aras *et al.*, (2011) proved evidence that melatonin elicited protein synthesis within the rat parotid salivary gland and thereby affects organ activity; hence suggest the clinical implication of melatonin in treatment of xerostomia.

Nitric Oxide has a vital role within the process of inflammation with increased levels were established within the exhaled breath of Sjögren's patients, the increasing levels of NO had a harmful effect upon the function of salivary glands (Konttinen et al,. 1997). Elevated nitrite was detected in SS patient serum and saliva compared to healthy controls (Czesnikiewicz-Guzik *et al.,* 2007; Pauley *et al.,* 2012). The impacts of this probable increased in concentration of NO has been investigated in an experiments that implicated on

(human & mouse) through acute exposure of NO to (submandibular gland acinar cells) which lead to transient (20-30 minutes) promotion of Ca2+ signaling, but in chronic exposure to NO lead to ultimate desensitization of these cells to stimulation (Caulfield *et al.*, 2007).

Alpha – amylase is considered as an important salivary digestive enzyme. There are two families of isoenzymes, had found one is glycosylated and the other contains no carbohydrate, however the salivary amylase deemed to be a valid marker of salivary gland serous cell function owing to the finding that the amylase concentration increase with increasing salivary flow rate (Rantonen ,2003).

Total salivary proteins; include those of salivary origin of which the most abundant are (amylase, proline rich proteins, secretory IgA (s-IgA) and carbonic anydrase). Other types of proteins albumin, transferrin, IgG were seepage from plasma (Chiappin *et al.*, 2007).

Many studies conducted on sjogren syndrome owing to the complexity of its pathophysiology, Delaleu *et al*.,(2008) studied the biomarker profiles in serum and saliva of experimental Sjögren's syndrome associations with specific autoimmune manifestations, while(Pauley *et al*., 2011) investigate expression & role of altered microRNA-146 in Sjogren's syndrome & its active part in innate immunity. On the other hand there were several Iraqi studies conducting on SS&/or RA: Fattah & Tahir, (2010) study the accuracy of the ultrasonographic changes in major salivary glands in the diagnosis of Sjogren's syndrome in Iraqi patients. While Al-Osami *et al*., (2013) study the association of smoking with the extra-articular manifestations in RA Patients. Hajer, (2013) stated that the hypo function of salivary glands in SS is associated with significant changes in the saliva constituents particularly the peptides. Also Khidhir & Al-Jubouri in (2013) study the tempromandibular joint disorders in relation to anticyclic

citrullinated peptide antibodies in both serum and saliva of patients with RA. Abdulla *et al*., (2016) suggested that the impairment of salivary gland function in RA associated with change in salivary biomarkers and disease activity**.** Recently Alahmed *et al*., (2016) study the correlation between the oral manifestations of RA Patients on different treatments with the clinical disease activity

Few studies have been conducted on sSS so this study designed to investigate several immunological parameters in salivary & serum in association with secondary Sjogren's syndrome.

Aims of the study

The present study aims:

1-To evaluate salivary & serum (melatonin, nitric oxide) in secondary Sjogren's syndrome patients & compare with rheumatoid arthritis patients & with healthy control subjects & correlate with increase susceptibility to secondary Sjogren's syndrome.

2- To Evaluate salivary alpha-amylase & total salivary protein in secondary Sjogren's syndrome patients & compare with rheumatoid arthritis patients & with healthy control subjects & correlate with the level of salivary melatonin.

3-Study the effect of the salivary (melatonin, nitric oxide, alpha amylase & total protein) on the salivary flow rate.

4-Study the association of Rheumatoid arthritis disease activity score according to with occurrence of secondary Sjogren's syndrome using:

a-Lab. Investigation by detecting (Anticitrullinated Protein Antibody (ACPA), Rheumatoid Factor, Anti-Ro/ SSA, Anti-La/SSB, Erythrocyte sedimentation rate) in serum & blood of patients with secondary Sjogren's Syndrome & Rheumatoid arthritis patients.

b- Clinical evaluation using salivary flow rate and Schirmer's test.

Chapter One

Review of Literature

Review of Literature

1. Sjogren Syndrome

1.1 Historical Aspect:

Sjogren's syndrome (SS) is a chronic autoimmune disease firstly described by Johan von Mikulicz Radecki in 1888 when mentioned a case of bilateral painless swelling of the lachrymal glands ,parotid & submandibular salivary glands(Mikulicz,1892).

Later on, Henri Gougerot ,well-known successful French dermatologist, was linked salivary gland disease with dryness of the eyes & oral mucosa, when he presented in 1925 a three cases of salivary gland atrophy associated with dryness of the mouth ,eye & vagina. In 1933 the modern concept of SS strongly rooted by Swedish Ophthalmologists who reported a series of 19 cases of Keratoconjunctivitis sicca of them two cases with swelling of the major salivary glands(Sjögren, 1935). Before that Mulock Houwer in 1927 explained the association of filamentary keratitis; the major ocular manifestation of the Sjogren's syndrome with chronic arthritis. Moreover, in 1953, Morgan and Castleman suggested that Sjogren's syndrome and Mikulicz disease were the same entity. Subsequently Talal and Bunim, 1964 linked between Sjogren's syndrome and malignant lymphoma. However the distinction between primary and secondary Sjogren's syndrome was established in 1965 by Bloch & his colleques and the auto antibodies associated with Sjogren's syndrome (Ro/SSA) in sera were reported in 1969. As a results, the first histological grading described the infiltration of labial glands was explained by Chisholm and Mason in 1968. Then a preliminary classification criteria was identified by a European Concerted Action in 1993, which has been widely accepted and modified by Vitali *et al*., 2002. (Govindjee *et al.,* 2010)

1.2 Definition

Sjögren syndrome (SS) is a chronic autoimmune inflammatory disease that initially involves the exocrine glands, resulted in their functional defacement. Sjögren syndrome can present either alone (primary Sjögren's syndrome (pSS)) or in association of an underlying connective tissue disease, most commonly rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE) (secondary Sjögren's syndrome (sSS)) (Peri *et al.,* 2012)

Moreover sSS may be associated with other connective tissue disease such as systemic sclerosis (scleroderma), or granulomatosis with polyangitis (Wegener's granulomatosis) clinically the hallmarks of Sjogren's syndrome are keratoconjunctivitis sicca and xerostomia, or named sicca complex. The term (keratoconjunctivitis sicca) came from Latin, and its mean dryness of the conjunctiva & cornea. While xerostomia represented the subjective symptoms of dry mouth (Clair, 2013). Sjogren's syndrome is a chronic autoimmune disease that characterized by symptoms of ocular and oral dryness, exocrine dysfunction, destruction of the exocrine glands & lymphocytic infiltration. Sjogren syndrome fundamentally affects women in the fourth and fifth decade with female to male ratio 9:1 (Glick, 2015).

Sjogren's syndrome occasionally coexists with other systemic autoimmune diseases, such that SLE and RA. Since 1965, several studies have been focusing on RA associated with SS (He *et al.,* 2013).

Pryczynicz *et al.,* (2013) suggested that Sjögren's syndrome is an autoimmune disease related to the group of collagenases that is manifistated by lymphocytic infiltration of the exocrine glands leading to their functional impairment. The inflammatory process involves cells of the lachrymal or salivary glands. However, other organs and systems can be affected.

Mignogna *et al*., (2005**)** studied the role of astute clinicians in estimation the possible development of SS in patients before the apparent of xerostomia and displaying bizarre early oral features such as, immature dental loss,

sialochemical alterations and sialorrhea have been distinguished before the onset of peculiar signs and symptoms namely xerostomia, which absolutely lead to clinical presentation and diagnosis of SS.

A study done by Bayetto & Logan *et al*., (2010) discussed the oral as well as the systemic manifestations of SS. They tried to understand factors that have a role in its pathogenesis & etiology. In addition, they evaluate the difficulties in diagnosis of SS & the role of the dental practitioner in the management of the oral complications.

Several studies conducted on SS trying to interpretate the complexity of this autoimmune disease (Antero *et al*., 2011) and evaluated the relationship between sSS& disease activity, disease duration of RA.

Hayashi, (2011) studied several factors that related to morphologic changes & glandular dysfunction by non-immunologic injury during the pre-inflammatory phase using mouse model. While Brown *et al*., (2013) studied the clinical characteristics of RA patients with sSS and their association with joint damage. Also, Nezo & Mavragani, in (2015) found that the genetic factors play a role in development of SS & SS associated lymphoma.

1.3 Epidemiology:

The incidence & prevalence of PSS suggested by (Clair, 2013) are higher in women than men (nearly 20:1), so the peak incidence in 5^{th} & 6^{th} decades of life. Sometimes there is ill defined prevalence and incidence of SS because dependable measure of the disease for epidemiologic studies are not accessible and the different currently used diagnostic criteria gives widely dissimilar results. Depending on the widely used 1993 Preliminary European Community (EC) diagnostic criteria, the estimated prevalence of pSS was as high as 1- 2% of the general population. While, information based on the 2002 American–European Consensus criteria for pSS the prevalence estimation of pSS range from 0.1- 0.6%(Bowman *et al.,* 2004).

Classically about 50% of patients with SS have the secondary form of the disease (sSS) and, in rheumatology clinics, nearly 25% of patients with rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE) have objective indication of sSS (Klippel *et al.,* 2008).The prevalence of secondary Sjogren's syndrome associated with rheumatoid arthritis, systemic lupus erythematosus, and systemic sclerosis has been estimated to be 17.1%, (Turesson *et al.,* 2002) ,8- 20%,(Baer *et al.,* 2010) and 14%(Avouac *et al.,* 2006), respectively.

1.4 Etiology, Risk Factors & Pathogenesis:

1.4.1 Etiology

The main etiology of SS is not well understood till now, however it has been proved that the following events occur in all the patients with SS; Initiation by an exogenous factor, followed by disruption of salivary gland epithelial cells, after that T-lymphocyte migration and lymphocytic infiltration of exogenous glands, then B-lymphocytic hyper reactivity and production of rheumatoid factor and antibodies to Ro (SS-A) and La (SS-B) (Konttinen & Kasna-Ronkainen , (2002); Bayetto & Logan, 2010).

1.4.2 Risk Factors

The occurrence of the disease can be attributed to the interaction between environmental, genetic and hormonal factors. The chronic stimulation of the immune system is thought to play a major role in the pathogenesis of the disorder, as explained by several pictures of immunological hyperactivity, including various auto antibodies, in particular anti-Ro/SS-A (anti-Ro) and anti-La/SS-B (anti-La) (Peri *et al.,* 2012)

1.4.2.1 Genetic Predisposition

Ice *et al.,* (2012) suggested that there is a genetic predisposition of SS. After that (Peri *et al.,* 2012) reported a familial clumping of different autoimmune diseases and gathering of many autoimmune diseases in same individuals, also 30% of SS patient have relatives that has other autoimmune

diseases. Recently a study conducted in Taiwanese population proved previous observations suggesting that first-degree relatives of SS patients had a higher chance of SS development as well as of other autoimmune disorders, mainly (SLE), RA, systemic sclerosis, and type 1 diabetes, compared to the normal population. Also, siblings of affected individuals show increasing risk for SS development compared to other first-degree relatives (parents and offspring), gathering both genetic effects and shared environmental exposures as contributory factors to SS development (Kuo *et al.,* 2015).

1.4.2.1 A: Genetic factors associated with Major Histocompatibility Complex

The polymorphic major Histocompatibility Complex (MHC) genes are the best attested genetic risk factors for the development of autoimmune diseases; in consideration to SS, DRB1*0301-DQB1*0201-DQA1*0501 haplotypes are the best documented risk factors for the formation of an anti-Ro/La response and to the development of the disease(Cruz-Tapias *et al.,* 2012).

There is a link between PSS & MHC that encode component of Human Leukocyte Antigen system (Den Berg *et al.,* 1998; Davies *et al.,* 2002; Cruz-Tapias *et al.,* 2012) including HLA class I (A, B, and C) and class II (DR, DQ, and DP), which is responsible for presenting endogenous and exogenous antigens to T lymphocytes, respectively.

Generally accepted from genetic studies of human autoimmune diseases that there are multiple genes contribute to disease risk and that specially each gene imply only simplest effects on disease susceptibility (Clair, 2013). Exception to this statute there is a strong signal associated with the human leukocyte antigen (HLA) locus on human chromosome 6p21.3, in a study conducted in populations of European descent, established HLA associations with primary Sjogren's syndrome include DRB1*0301 (DR3), DRB1*1501 (DR2), DQA1*0103, DQA1*0501, DQB1*0201, and DQB1*0601(Williams *et al.,* 2007 & Cobb *et al.,* 2008).

There was a major role of HLA locus in the pathogenesis of autoantibody responses associated with primary Sjogren's syndrome. So in patients with primary Sjogren's syndrome, found that higher titers of anti-Ro/SS-A and anti-La/SS-B antibodies have been associated to heterozygosity for the DQA1 and DQB1 alleles so there is a restricted relationship between HLA &theses autoantibody (Harley *et al.,* 1986; Gottenberg *et al.,* 2003).

1.4.2.1 B: Genetic Factors outside Major Histocompatibility Complex

Burbelo *et al.,* (2014) clarified that a novel SS-associated genetic variants (outside the MHC locus) generally classified into three main groups depending on suggeted signaling pathway. The first group composed of variants in genes imply in the activation of the interferon (IFN) signaling pathway. The second group involves important genes that affect B-cell function and production of autoantibody. Finally, the third group has apoptotic and inflammatory genes that play a role in the NF-κB signaling pathway.

1-The first group:

Gene associated with interferon pathways. In a study of gene expression in SS patients by (Mavragani & Crow, 2010) showed that up regulation of IFN-inducible genes (the so-called IFN signature) at the level of peripheral blood and affected salivary gland tissues of these patients.

a- Interferon Regulatory Factor 5: is a transcription factor involved in type I IFN induction following TLR ligation (Cham CM *et al.,* 2012).

b- Signal Transducer and Activator of Transcription 4: STAT4 is primarily involved in the signal transduction induced by the cytokines interleukin- (IL-) 12 and IL-23 leading to differentiation of T helper (Th) naive cells towards a Th1 phenotype and subsequent production of IFNγ (Kariuki *et al.,* 2009).

c- Interleukin 12A: is a cytokine that forms a heterodimer with the IL12B subunit inducing through STAT4 the differentiation of naïve T-cells in T

helper 1 cells which promotes immune response through IFNγ production by T helper 1 cells **(**Chan *et al*., 1991).

d- Natural Cytotoxicity Triggering Receptor 3 (NCR3): NCR3/ NKp30 is a natural killer (NK) specific receptor regulating the cross talk between NK and dendritic cells as well as type II IFN secretion (Wehner *et al.,* 2011).

e- Protein Tyrosine Phosphatase Nonreceptor 22 (PTPN22): PTPN22 gene encodes the protein lymphocyte tyrosine phosphatase (Lyp) previously shown to be implicated in both adaptive (inhibition of T-cell receptor (TCR) and Bcell receptor (BCR) signaling) and innate immune responses (type I interferon (IFN) production by myeloid cells through TLR ligation) (Rawlings *et al.,* 2015).

2-The second group: Genes Involved in B-Cell Function

a- B-Lymphocyte Kinase (Blk): is member of the family of the src tyrosine kinase, which is involved in signaling and differentiation of B lymphocytes (Tretter *et al.,* 2003).

b- B-Cell Activating Factor (BAFF). BAFF is an important cytokine that has a role in promoting survival and proliferation of B cells.(Nossent *et al.,* 2008)

c- Chemokine (C-X-C Motif) Receptor 5 (CXCR5). In a GWAS Caucasian study, (Lessard *et al.,* 2013) clarified that Chemokine receptor CXCR5 gene variants were found to have protection against SS development.

d- Early B-Cell Factor 1 (EBF1): In a large candidate gene association study in patients of SS from Scandinavian origin (Nordmark *et al.,* 2011) found that genetic variants of the *EBF1* play a role to confer increasing risk for SS.

e- Ox40 Ligand/Tumor Necrosis Factor Superfamily 4 (Ox40L/ TNFSF4). Ox40L (or TNFSF4), a TNF family Ligand member,

expressed on, endothelial cells, activated dendritic cells and the B-cell surface, has been previously found to be involved in B-cell activation by interaction with Ox40- positive T-cells(Stüber & Strober 1996; Walker *et al.,* 2000).

f- General Transcription Factor 2I (GTF2I) In a study (GWAS) in Han-Chinese done by (Y. Li *et al.,* 2013) clarified that a polymorphism in the GTF2I gene (namely, rs117026326) {that encodes a transcription factor involved in both T-cell signaling (Sacristán *et al.,* 2009) and activation of immunoglobulin heavy-chain transcription upon B-lymphocyte activation (Rajaiya *et al.,* 2006) }is strongly associated with SS development.

3-The third group: Genes Involved in the NF-*κ*B Pathway

a- Tumor Necrosis Factor-Alpha Induced Protein 3 (TNFAIP3). TNFAIP3 gene encodes the A20 protein, an enzyme with ubiquitination activity that appears to share an important role in the regulation of inflammation through the NF-*κ*B pathway. A20 protein is expressed at low levels on most of the cells but is rapidly induced after activation of NF-*κ*B, acting as a negative feedback regulating both inflammation and apoptosis (Wertz *et al.,* 2004)

b- TNFAIP3-Interacting Protein 1 (TNIP1).

Of interest, polymorphisms of the TNIP1 gene, a molecule which interacts with the TNFAIP3 gene regulating the NF-*κ*B activation, recently have been found to confer increasing risk to SS (Nordmark *et al.,* 2011; Nordmark *et al.,* 2013).

c- Lymphotoxin Gene A (LTA). Recently Polymorphisms of the lymphotoxin gene A (LTA), located on locus LTA/LTB/TNF and related to the activation of the NF-*κ*B pathway as well as inflammation, have been proved to increase the risk of SS (Bolstad *et al.,* 2012).

d- Chemokine (C-CMotif) Ligand 11 (CCL11). Finally, the CCL11 (eotaxin) is a chemokine which has an important role in SS(Reksten *et al.,* 2014).

The interest aspect of genetics that is of epigenetics, i.e. the effects of processes such as DNA methylation or histone acetylation on gene expression, there is some data is emerging in pSS in this field(Gonzalez *et al*., 2011)

In the last few years there were an attempts to improve the diagnostic methods for PSS using the salivary proteomic as a promising method to distinguish between PSS & healthy controls(Giusti *et al.*, 2007; Hu *et al.*, 2010) More over microarrays assay conducted in the minor salivary glands of healthy controls showed that microRNA expression profiles can be used to distinguish the glands of SS patients from those of controls and also used to distinguish subsets of SS patients with low- or high-grade inflammation. Of interest, the microRNA-17-92 cluster profile of PSS patients, which has been involved in specific types of lymphocytes and lymphocytic disorder, was down regulated (Alevizos *et al.,* 2009).

1.4.2.2 Viral Initiating Factors

The possible role of the virus in the pathogenesis of SS syndrome has been discussed for many years, either in the form of an infectious agent inducing chronic inflammation, or as a source of exogenous antigen triggering autoimmune response, or a molecular mimicry of the host autoantigen. (Klippel *et al.,* 2008). Viruses such as Epstein–Barr virus (EBV), Coxsackie virus, human immunodeficiency virus (HIV), human T- cell Lukaemia virus-1, and hepatitis C virus (HCV) have been suggested to be involved in the pathogenesis of SS. Till now, no definitive role for any virus has been established, cytomegalovirus infection in mice using animals models led to the development of SS-like symptoms and it has been eventually concluded that this may occur in humans as well (Al-Hashimi, 2005; Delaleu *et al.,* 2005).

Itescu & Winchester, (1992) proposed that HIV infection cause swollen salivary & other glands & also cause Diffuse Infiltrative Lymphocytosis Syndrome (DILS) mainly CD8+ cells phenotype. Then after that(Al-Hashimi, 2001) indicated that infection with (HIV) may lead to damage and inflammation of the salivary glands, leading to SS-like symptoms such as xerostomia and reduced salivary flow, but absence of characteristic autoantibodies & infiltrating cell is CD8+ lymphocyte unlike SS which is CD4+ T-cells & B cells(Agarwal ,2003).

Subsequently the DNA of EBV which is highly prevalent latent virus in large number of human has been recognized in minor & major salivary glands. This latent virus may act as a cofactor in SS by i role in chronic inflammation of salivary glands, but his etiologic job has not been established (Klippel *et al.,* 2008). Of interest, Coxsackie virus B4 RNA was identified in pSS minor salivary gland but not from sSS patient & controls consequently the environmental trigger role for this virus not been certified. (Triantafyllopoulou *et al.,* 2004)

Delaleu *et al.,*(2005) &Ramos-Casals *et al.,* (2005) founded that Hepatitis C virus causes SS- like symptoms & this led to include HCV in the list of ‘‘exclusion criteria’’ developed by the American-European Consensus Group, so there is a controversy whether , HCV actually cause SS, or may just display similar signs and symptoms.

1.4.2.3 Hormonal Influence

The high percentages of female in compared to male in SS lead to suggestion that immune regulatory properties of sex hormone may involved in its development. (Talal , 2000)

Taiym *et al.,* (2004) appreciated the effect of androgen to estrogen ratio in modulation the cellular immune response that may be involved in destruction of exocrine glands, also there is a declines in estrogen during menopause and this mean that this is the time when women are most susceptible to developing

SS, referring that either the decline in estrogen or the difference in the estrogen: androgen ratio is associated with disease onset, also high level of estrogen may be concerned with the development and proliferation of Ro⁄SSA and La⁄SSB antibodies, additionally Taiym *et al.,* 2004 appreciated the role of prolactin as a pro-inflammatory hormone that stimulates estrogen activity but at a high level, inhibits estrogen production, also prolactin is an immune stimulator this lead to a suggestion that estrogen-prolactin relationship has a role in immune response and may be involved in SS development. On other hand Pillemer *et al.,* (2004) estimated in their clinical trial that, treatment with the hormone dehydroepiandrosterone that acts on the androgen receptor has no clinical efficacy in women with PSS.

1.4.3 Pathogenesis

The main pathogenesis of primary Sjogren's syndrome is the disruption of T cells and B cells regulation, with central contributors from innate pathways of inflammation. So that many of these same concepts may be relevant to the pathogenesis of secondary Sjogren's syndrome; although, most researching into the mechanisms of the disease was focusing on patients with the primer form of the disease, & may be many of the immunologic features of secondary Sjogren's syndrome are determined by the pathogenic mechanisms involving the associated disorders(Clair, 2013).

1.4.3.1 Autoantigens & Autoantibodies

In PSS many types of auto antibodies & several autoantigens have been concerned in its pathogenesis.

A- Autoantigens :

Involved two types of proteins (intracellular proteins and plasma membrane proteins) which is normally tolerated by immune system ,but in the other hand apoptotic cell death as well as disturbances in cellular membrane trafficking has been suggested to expose normally sequestered autoantigens and novel surface epitopes, in response to immunogenetic & environmental factors

(Hansen *et.al*., 2003) Additionally, the islet cell antigen 69 [ICA 69] was considered as a new candidate autoantigen in PSS as it expressed in brain, pancreas, salivary and lacrimal glands and appears to have an important role in disease progression, also disfigurement of the genomic ICA 69 locus in NOD mice lead to prevention of lacrimal gland disease and reducing salivary gland disease, so ICA 69 can be considered as a promising new target for gene or immunotherapy(Winer *et al.,* 2002)

B-Autoantibodies:

Large numbers of SS patients showed increased polyclonal immunoglobulins and autoantibodies ,which include the highly nonspecific rheumatoid factor and antinuclear antibodies & and the more specific anti-Ro (SS-A) and anti-La (SS-B) antibodies, that more related with pSS and SLE, although its role in pathogenesis of PSS remain unclear (Klippel *et al.,* 2008).On the other hand the presence of these autoantibodies might be associated with an earlier onset of disease, longer duration of disease, increased disease severity, extraglandular manifestations & recurrent parotid gland enlargement so that, it may be possible to use the presence of these autoantibodies as a prognosticator of disease severity in newly diagnosed cases (Jonsson *et al.,* 2002; Al-Hashimi ,2005; Hammi *et al.,* 2005).

Klippel *et al.,* (2008) estimated that there is antibodies against alpha-fodrin,(which is a protein in the cytoskeleton of most eukaryotic cells), he noticed that anti-fodrin antibodies are available in almost all PSS patients that diagnosed by the San Diego diagnostic criteria (the most restrictive set), but patients diagnosed by the EC criteria have fewer level of this antibodies, of interest, the suggestion of usage anti-fodrin antibodies as specific marker in PSS remains controversial. In a murine model done by Hayashi *et al.,* (2003) found that anti-fodrin antibody is related to organ specific autoimmune lesion in salivary & lacrimal glands. While in another study Nordmark *et al.,* (2002) said

that anti-fodrin antibodies have similar frequency in PSS & SLE with or without sSS so it was not related to organ specific autoimmunity.

Tretter *et al.*,(2003) suggested that in SS there are autoantibodies to lachrymal & salivary gland muscarinic M3 acetylcholine receptors that prevents the synapse between the efferent nerves and the gland cells as a result there is a decreased in saliva production. (Fox & Stern, 2002)

Li *et al.,* (2004) presented the possible mechanism of PSS serum anti-M3R in decreasing lacrimal and salivary secretions which was supported by experiments showing that pSS sera inhibit aquaporin AQP-5 (a transmembrane protein affecting water transport in acinar cells) but the exact mechanism remain unclear.

1.4.3.2 Role of the lymphocytes

In SS the characteristic histological feature is the focal lymphocytic infiltration of the salivary glands (Kasper *et al.,* 2006; Stewart *et al.,* 2008). This focal infiltrate diagnostically is classified as a ‘‘focus score’’, that mean ‘‘one focus’’ defined as a collection of 50 or more lymphocytes within 4 mm2 of tissue. So that, exocrine glands display ductal hyperplasia, acinar atrophy and subsequent replacement of acinar cells with fibrosis and ⁄ or fatty infiltration, over time this lead to nonfunctioning areas of these glands (Jonsson *et al.*, 2002; Al-Hashimi, 2005). The ratio of lymphocytic infiltrate is 4:1 T and B cells, as well as plasma cells (Jonsson *et al.,* 2002; Fox, 2007).

Of interest, T cells produce tumor necrosis factors (TNF), interleukins and interferon (IFN). TNF could increase the antigen presenting nature of epithelial cells, and also TNF along with interleukin-1, can affecting exocrine gland secretion by inhibit the release of acetylcholine from cholinergic efferent nerves (Fox & Michelson, 2000; Fox & Stern, 2002). While IFN may stimulate apoptosis of the salivary gland epithelial cells; this may explain a mechanism for gland destruction as the ductal cells undergo apoptosis (Kassan & Moutsopoulos , 2004)

Kasper *et al.,* (2006) suggested that the acinar and ductal epithelial cells have a role in the autoimmune destruction by attraction of immune cells via producing pro-inflammatory cytokines and chemokines.

Also B cells produce immunoglobulins that lead to autoantibody activity (Jonsson *et al.,* 2002). Furthermore,(Kassan & Moutsopoulos, 2004) suggested that in SS there are (T& B) cells activation, so this suggestion support the hypothesis that consider B cells play a role in pathogenesis of SS.

1.4.3.3 Up- Regulated Expression of Human Leukocyte Antigen molecules:

Human Leukocyte Antigen molecules appear to play a role in the pathogenesis of SS & this role discussed by (Jonsson *et al.,* 2002) when he found that up-regulated expression of (HLA) molecules in the salivary glands epithelial cells is encompass with antigen-presentation, leading to destruction of exocrine glands by CD4+ T cells, also this up-regulated expression leads to stimulation of B cell proliferation & differentiation & increased cytokine production so increases the immune response. In the other hand, (Jonsson *et al.,* 2002) discuss the role of HLA molecules with the activation of cytotoxic T cells that induce cell apoptosis when presented with antigen.

1.4.3.4 Role of apoptosis

Apoptosis considere to play an important role in the pathogenesis of pSS (Manganelli *et al.,* 1997). It has been suggested that the pathogenesis of SS that result in glandular hypo function is via two-step mechanism ; firstly a primary immune attack by lymphocytic infiltration and secondly cytotoxic cell death (necrosis) and apoptosis, which considered one of factors relating to the dysfunction of salivary and lacrimal glands, in genetically predisposed individuals(Voulgarelis & Tzioufas ,2010). In SS (Bolstad & Jonsson ,1998) explained that that inflammatory mononuclear cells can escape apoptosis; so this lead to accumulation of lymphocytes and subsequent displacement of functioning acinar cells. Supporting to the role of apoptosis (Bolstad *et al.,* 2003) found that there was an increase in the expression of Fas and FasL "which

has a central role in the cascade of apoptotic signal Molecules" in patients with primary SS as compared to controls.

1.5 Clinical Features of Sjogren Syndrome

1.5.1 Oral Features of Sjogren Syndrome

Xerostomia (signs & symptoms)

Xerostomia considered as a constant oral symptoms in SS (Orellana *et al.,* 2006) the signs & symptoms of xerostomia resulted from changes in the quantity & quality of saliva (Clair, 2013) These symptoms includes difficulty in chewing or swallowing food, reduced taste perception, difficulty in speech, poor denture retention, increased rate of dental decay, bad breath, oral burning sensation (may associated with chronic candidiasis) & generalized oral irritation (Klippel *et al.*, 2008; Simon *et al.*, 2013).

Extra oral signs associated with xerostomia may include dry flaky lips, angular cheilitis, and salivary gland swelling, which involve all or any of the oral major salivary glands (Simon *et al.*, 2013).About 60% of primary Sjogren's syndrome patients have Parotid or major salivary gland enlargement, The enlargement of parotid gland may be chronic or episodic, unilateral or bilateral in nature (Pesaramelli *et al.,* 2010).

Consequently intraoral signs of xerostomia made up those of a sticky dry mucosa, that sticking to the gloved fingers due to poorly lubricated mucosa, also there is absence of salivary pool in the floor of the mouth and may be experience a difficulty in expressing saliva from the major duct orifice (Simon *et al.,* 2013) & there may be a persistence of food debris around the mouth owing to prolonged oral clearance times(Yurtseven & Gakalp, 2007) About 1/3 of SS patients show signs of chronic erythematous candidiasis i.e. depapillation of filiform papillae from the dorsal of the tongue and mucosal erythema as a symmetrical areas accompanied or not by angular cheilitis (Klippel *et al.,* 2008).

1.5.2 Ocular Feature of Sjogren Syndrome

Keratoconjunctivitis Sicca (signs & symptoms)

Keratoconjunctivitis sicca (KCS), represented the ocular component of SS that first described by Henrik Sjögren in 1933 (Klippel *et al.,* 2008**)**. In SS the Chronic inflammation of the lachrymal glands lead to diminish aqueous tears secretion,which if severe, leading to destroy conjunctival and bulbar epithelium.The deficiency of aqueous tear give rise to dry eye, & causing symptoms of foreign body sensation or grittiness, photophobia, burning, and eye fatigue (Clair, 2013).The clinical signs of KCS, most appropriate to observe at the slit lamp, include, decreased tear breakup time, scanty or absent tear meniscus and characteristic staining of the conjunctiva with lissamine green & cornea with fluorescein (Klippel *et al.,* 2008).

1.6 Extraglandular Manifestation of Sjogren Syndrome

About 50% of patients with SS have systemic manifestations that may involve general constitutional symptoms such as low-grade fever, & easy fatigability as well as specific organ involvement (Pesaramelli *et al.,* 2010).

1-Fatigue:

Approximately 75% of patients with pSS account physical and mental fatigue that higher than healthy controls and have similar picture to those patients affecting with other rheumatic diseases. (Bowman *et al.,* 2004)

2- Musculoskeletal

Arthralgia or arthritis considered as a presenting feature in about one - third of cases and can occur during the course of the disease in around more than one-half of cases (Fauchais *et al.*, 2010).

3-Cutaneous:

Cutaneous vasculitis usually takes the form of palpable purpura, erythematous maculopapules or urticarial lesions that occurs in 9% to 32% of pSS patients (Ramos-Casals *et al*., 2004).

4-Raynaud's phenomenon

Raynaud's syndrome occurs nearly in 13- 33% of Pss patients, & may be preced the sicca symptoms by several years (García-Carrasco *et al*., 2002).

5-Pulmonary involvement:

Persistent cough and/or dyspnea accompanied by "chronic diffuse interstitial infiltrates" a restrictive pattern on pulmonary function studies, and there may be evidence of "pulmonary alveolitis or fibrosis" Pulmonary disease, registered in 7% to 35% of patients with pSS (Klippel *et al.,* 2008).

6-Neurological Complications:

Klipple *et al.,* (2008) reported that symptoms of peripheral nerve dysfunction occur in 2- 38% of pSS patients & may include numbness, paresthesias, or motor defects of the upper or lower limbs.

7- Thyroid disease:

Hypothyroidism comprised about 20% among SS patients (Kang & Lin, 2010).Consequently autoimmune thyroiditis represented 10-24% of pSS patients, commonly in the form of Hashimoto's thyroiditis, that characterized by presence of goiter and the antithyroglobulin antibodies (Klippel *et al.,* 2008).

8-Haematological:

Manganelli *et al.,* (2006) explained that SS patients may have mild normochromic in 20%, leucopenia in 15% and thrombocytopenia may occur in 11% of those patients. Also neutropenia & autoimmune haemolytic anemia may be reported.

9-Gastrointestinal & Hepatobiliary features:

Dysphagia has been reported in SS patients due to either dryness of the pharynx and esophagus or abnormal esophageal motility, epigastric pain or nausea may commonly seen as clinical symptoms (Pesaramelli *et al.*, 2010)

Hepatic disease, occasionally take the form of primary biliary cirrhosis, or autoimmune hepatitis has been identified in 2- 4% of pSS patients (Klippel *et al.,* 2008). In a study conducted by (Matsumoto *et al.,* 2005) on pSS patients

selected for liver biopsy, found that 47% of them had autoimmune hepatitis, 35% had primary biliary cirrhosis, and 18% had nonspecific acute or chronic hepatitis.

10-Lymphoma:

Primary Sjogrens Syndrome patients have a significantly higher rate for developing non-Hodgkin's lymphoma than the normal population (Klippel *et al.,* 2008).

Solans-Laqué *et al.,* (2011) explained that the prevalence of lymphoma in PSS increases from 3.4% in the first 5 years to 9.8% at 15 years. The risk is increased in Ro/La-positive patients & the majority of cases are of the Mucosa Associated Lymphoid Tissue Lymphoma of B-cell type (Simon *et al.,* 2013).

11-Renal involvement:

In pSS significant renal disease is rare & reported renal disease that associated with pSS include type I renal tubular acidosis (RTA),tubular interstitial nephritis, glomerulonephritis, and nephrogenic diabetes insipidus. (Clair, 2013).

1.7 Diagnosis of Sjogren Syndrome

The diagnosis of SS is ambiguous owing to the fact that many of SS symptoms are subjective and misty and can be misdiagnosed initially as other conditions or the effect of different types of medications (von Bültzingslöwen *et al.,* 2007) This difficulty in diagnosis leads to increase the estimated phase between the initial symptoms and the diagnosis of the disease which is approximately 6- 10 years(Talal, 2000 ; Al-Hashimi , 2005) Unfortunately the diagnosing of pSS is more complicated than sSS because patients occasionally present with the three most conventional symptoms dry eyes, dry mouth, and musculoskeletal pain. Moreover, patients developing pSS unlikely to receive regular medical care during their progressive symptom onset, in compared to those patients with connective tissue disorders that are normally in periodic follow up with a rheumatologist when SS symptoms develop (Klippel *et al.,*

2008).The usual method of diagnosis is a set of criteria, the fulfillment of this criteria beside the assessment of skilled clinician is occasionally the gold standard for diagnosis of SS. (Al-Hashimi , 2005)

1.8 Classification Criteria

During the early 1980s, several criteria sets had been suggested for the classification of Sjogren's syndrome such as the Copenhagen criteria (Manthorpe *et al.,* 1985), the Japanese criteria (Skopouli *et al.,* 1986) the Greek criteria (Homma *et al.,* 1986) and the California criteria (Fox *et al.,* 1986).Each of these criteria sets has the same requirement for objective evidence of salivary gland involvement& Keratoconjunctivitis sicca. However ,they differed, in content of their item and weighting, as well as the method for evaluating salivary gland involvement, for example, (Copenhagen) criteria relied on whole salivary flow, whereas(Greek, California) relied on parotid flow rate ,only two sets of the criteria(Copenhagen and Greek) discriminate between primary and secondary Sjogren's syndrome. (Clair, 2013).

After that the American European Consensus Group Criteria (AECC) was introduced in 2002 & used as clinical guidance for diagnosis of sjogren syndrome (Vitali *et al.,* 2002). The latest criteria of Sjögren's syndrome was presented in 2012 by Sjögren's International Collaborative Clinical Alliance (SICCA) (Shiboski *et al*., 2012).

The (AECC) were revised in 2002 and modified for more sensitivity and specificity of testing, which is illustrated in appendix I.

1.9 Clinical Assessment of the Oral Component of Sjogren Syndrome

Salivary gland involvement can be assessed objectively by:

1-Measuring Salivary Flow Rates (Sialometry):

The functional impairment in salivary flow is measured by sialometry and used to measure salivary flow from individual glands "parotid, submandibular, or sublingual" or from the mouth as a whole. Usually an unstimulated whole

salivary flow rate ≤ to 1.5 mL/ 15 min meets the principle for xerostomia according to the classification criteria developed by AECC (Clair, 2013).

2-**Salivary gland biopsy**:

Usually of the minor glands within the lower lip histopathological examination reveal focal lymphocytic sialadenitis "lymphocytic infiltration of the salivary glands "with a focus score of ≥1 within4 mm2 of tissue meet the AECC. As a focus is considered to be a cluster of 50 or more lymphocytes (Vitali *et al*., 2002).

3- Various imaging techniques:

A-Sialography: technique that permits radiographic visualization of the submandibular & parotid salivary glands and ducts following retrograde instillation of soluble contrast material into Stensen's or Wharton's ducts. Not suitable for the ducts of the sublingual glands due to the small orifice size for injection of contrast medium (Abdullah *et al.,* 2013). Sialography considered the best diagnostic imaging technique with regard to SS. The characteristic changes sialectasis may be useful in the evaluation of SS (Jensen & Vissink, 2014). Sialography not usually indicative clinically due to its invasiveness & complications including, pain, duct rupture and infection (Clair, 2013).

B- Scintigraphy: helpfull in measuring salivary gland function by injecting sodium radio labeled pertechnetate technetium into the blood then absorbed into the salivary gland after that secreted into the mouth, & allowing for verification of the salivary flow rate. Its diagnostic value in evaluation of pSS estimated by its diagnostic sensitivity and specificity which is 75% and 78%, respectively (Vinagre *et al*. 2009).

C-Magnetic Resonance Imaging, Computed Tomography,Ultrasonography all these imaging techniques can be helpful in assessment of (anatomy of major salivary gland ,enlarged gland & presence of lymphadenopathies) (Wernicke *et. al.,* 2008; Jensen & Vissink, 2014).

1.10 Clinical Assessment of the Ocular Component of Sjogren Syndrome:

Dry eye can be assessed objectively by:

1-Using of the Schirmer's I test which represented a quantitative measurement of tear flow over a specific period of time (Al-Hashimi , 2001).

2-Vital Staining:

A- Lissamine green or Fluorescein dye

Used by instillation of these dyes onto the eye surface then examined the integrity of the conjunctival and corneal surface by slit lamp, the fluorescein dye focus on areas of cellular disruption on the ocular surface while lissamine green stains epithelial surfaces which lacking mucin(Clair ,2013).

B- Rose Bengal Dye

This type of dye stains dead &/or degenerated cells, now this stain no longer used for evaluation of the ocular surface owing to its toxic influence on the cornea(Clair ,2013).

3-Tear Film Break –Uptime

This procedure used to measure the tear film stability, & occasionally defined as the interval between the last complete blink and the first appearance of a dry spot or disruption in the tear film (Simon *et al.,* 2013).

4-Tear Film Osmolarity

Osmolarity considered to functioning as a non-invasive, objective, easily performed, continuously variable, clinical biomarker for assessment of dry eye severity ,the value s greater than 308 mOsm/L are occasionally indicative as dry eye disease mild 308 mOsm/L; moderate 320 mOsm/L; severe >355 mOsm/L (Lemp *et al*., 2011).

1.11Treatment of Sjogren Syndrome:

Occasionally, the effectiveness of treatment in modifying SS doesn't depend on single treatment strategy but depends on firstly: symptomatic

treatment of glandular manifestations and secondly: on the use of disease-modifying drugs for systemic involvement (Tincani *et al.,* 2011).

By other words (Klippel *et al,.* 2008) estimated that the treatment of SS need independently managing the oral and ocular secretary dysfunction, treating or preventing their sequelae, and effective treatments of extra glandular conditions as they occur.

1.11.1 Symptomatic Treatment of Glandular Manifestations

A- Managements of Oral Manifestations of Sjogren Syndrome

Preventive measures

-Regular dental visits (every 3–4 months, alternate between dentists and hygienists)

-Optimal oral hygiene (guidance from oral health professionals)

-Topical fl uorides and remineralizing solutions

• Fluoride mouth rinse (0.1%, weekly)

• Neutral sodium fl uoride gel (depending on the level of oral hygiene and residual level of salivary fl ow: from weekly to every second day; the gel is preferably applied with a custom- made tray)

-Avoid professional or over-the-counter fl uoride gels as many of them are acidifi ed which reduces patients' compliance (sore oral mucosa) and may damage the teeth (no saliva present to remineralize teeth)

- Diet modifi cations

• Avoid cariogenic food and beverages

• Minimize chronic use of alcohol and caff eine

• Use non-fermentable sweeteners (xylitol, sorbitol, aspartame, or saccharine) whenever possible

-If possible, avoid drugs that may worsen sicca symptoms (e.g. anti-depressants, anti-histamines, anti-cholinergics, anti-hypertensives,neuroleptics)

- Optimize treatment of other medical conditions that result in xerostomia (e.g. endocrine disorders, metabolic diseases, viral infections)
- Avoid other exacerbating factors
• Low-humidity atmospheres (e.g. air-conditioned buildings, windy locations)
• Irritants such as dust and cigarette smoke

Salivary gland stimulation

- Masticatory stimulation (e.g. sugar-free gums, lozenges, or pastilles)
- Combined gustatory and masticatory stimulatory
• Lozenges, mints, candies
• Water, with or without a slice of lemon
- Parasympathomimetic secretogogues
• Pilocarpine (5–7.5 mg, 3–4 times/day)
• Cevimeline (30 mg, 3 times/day)

Saliva replacement and other symptomatic treatments

- Relief of oral dryness (non-responders to systemic salivary stimulation)
• Increase humidifi cation (e.g. air moisturizers, humidifiers)
• Frequent sips of water
• Oral rinses, gels, and mouthwashes
• Saliva substitutes (Wan-Fai *et al.,* 2013).

B-Managements of Ocular Manifestations of Sjogren Syndrome.

Preventive measures

- Avoidance of exacerbating factors:
• Avoid/restrict low humidity, dusty or windy environment
• Avoid irritants such as dust and cigarette smoke
• Avoid restrict and take regular breaks during activities that reduce blinking or provoke tear fi lm instability (e.g. prolonged reading or computer use)
- Avoidance of drugs that may worsen sicca symptoms (e.g. antidepressants, anti-histamines, anti-cholinergics, anti-hypertensives, neuroleptics)

- Optimized treatment of other conditions (e.g. ectropion, meibomian gland disease, lagophthalmos) that result in/aggravate dry eyes.

- Use of air moisturizers or moisture glasses

Improved ocular surface lubrication

- Tear substitution therapy (preservative-free preparations if frequent use is needed)

• Low-viscosity eye-drops (e.g. cellulose derivatives) as frequently as needed

• Medium- to high-viscosity eye-drops (e.g. carbomer gel 940) 3-4 times/day in more severe cases

• Ophthalmic gels and ointments (at night)

- Mucolytic agents for sticky eyes or mucous threads/corneal filaments on eye examination

• *N* -acetylcysteine 5% eye-drops (preservative-free, 2–3 times daily)

- Autologous serum eye-drops

- Systemic parasympathomimetic secretogogues

• Pilocarpine (5–7.5 mg, 3–4 times/day) or cevimeline (30 mg, 3 times/day)

- Tear retention measures

• Lacrimal punctum plugs/occlusion (moderate to severe dry eyes)

Reduction of ocular surface inflammation

- Topical immunomodulatory agents

• Pulsed topical non-preserved corticosteroids (e.g. dexamethasone 0.1% eye-drops, 4 times daily twice daily for up to 8 weeks, taper dose or discontinue drops based on clinical findings and eye pressure)

• Ciclosporin eye-drops

• Dietary supplements: essential fatty acids (omega-3 and linoleic acid)

Other measures

-Treatment of blepharitis

• Daily eyelid rubs with warm water and diluted baby shampoo, eyelid cleaning solutions, or wipes

• Warm water compresses 2 times/day for 5 min

• Topical antibiotics for anterior blepharitis if indicated (e.g. chloramphenicol ointment) (Wan-Fai *et al.,* 2013).

1.11.2 Disease -Modifying Drugs for Systemic Involvement

Several clinical trials have been implicated for evaluation of systemic agents in treating SS associated exocrinopathy through immune system manipulation.

1. Corticosteroids:

In spite of the fact that the frequent use of glucocorticoids in the treatment strategy of primary SS is not supported by dependable scientific data. Corticosteroid may use in patients with SS is especially in patients with extraglandular manifestations or in patients with parotid swelling (Tincani *et al*., 2013).

2. Antimalarials :

Antimalarial agents can be used to ameliorate sicca & constitutional symptoms like fatigue and arthromyalgia (Rihl *et al.,* 2009; Yavuz *et al.,* 2011).AlsO, hydroxychloroquine has been estimated to increase salivary flow rate (Dawson *et al.,* 2005) and lower some inflammatory indices such as C-reactive protein & ESR , and also lead to improvement in immunologic profile involving anti-SSA , anti-SSB,& RF(Yavuz *et al.,* 2011).Additionally relating to its antineoplastic properties, hydroxychloroquine may have potential role to decrease the risk of lymphoma development in SS patients(Doria *et al.,* 2008).

3- Immunosuppressant Agents.

Immunosuppressant agents such as, azathioprine .cyclosporine A, mycophenolic acid, methotrexate, and leflunomide have been used to improve some extra glandular manifestations of SS; although, currently there is insufficient evidence to show efficacy (Ramos-Casals *et al.,* 2012).

4- Biologic Agents.

In spite of the fact that no biologic agents are till now approved for use in SS, but there are a number of clinical trials showed the therapeutic potential of some these agents such as tumor necrosis factor (TNF) alpha antagonists (e.g.,etanercept and infliximab) and interferon-α. Additionally, recent researches related to direct B-cell-targeted therapies such anti-CD20 (e.g., rituximab) and anti-CD22 monoclonal antibodies (mAbs) (e.g.epratuzumap). Subsequently indirect B-Cell-Targeted Therapies (e.g.belimumab) which is a newer targets proposed to involve major cytokines of B-cell homeostasis (e.g., B-cell-activating factor [BAFF], interleukin [IL]-6, and lymphotoxin-b) (Leah.*et al.,* 2015).

1.12 Rheumatoid Arthritis

Is a chronic autoimmune disease recognized by symmetric, inflammatory arthritis affecting small & large body joints (Harris *et al.,* 2005). It considered one of the popular autoimmune diseases, evaluated to be affected up to 2% of the general population in the United States near the age of 60 years, (Rasch *et al.,* 2003) with a higher predominance in women. When comparing with osteoarthritis degenerative joint disease, RA is a systemic disease characterized by constitutional symptoms, including weight loss, fatigue, low grade fevers, morning stiffness and anemia (Harris *et al.,* 2005).

Rheumatoid arthritis has been suggested to be the most common connective tissue disease associated with Sjogren syndrome, & may have some extra-articular manifestations that called sicca symptoms which include xerostomia or dry mouth &Keratoconjunctivitis sicca or dry eyes (Hajiabbasi *et al*., 2016).

1.12.1 Etiology and Pathogenesis

Even though the etiology of RA is still unknown, but there was fundamental evidence that RA is a complicated genetic disease. While, other

contributing factors appear either to initiate or sustain RA (Helmick *et al.,* 2008).

1.12.1 A Genetic Factors

There is solid evidence from a hard work over the last two decades that certain types of genes increase the risk for RA. Up to100 risk loci had been identified to date, there is confirmation that specific genetic markers predict best responses to recent, targeted therapies for RA (Okada *et al.,* 2014**)**

Twin studies are used to evaluate the contribution of Environment & genetics to the development of diseases (Oliver & Silman, 2006). The finding of concordance in only 16% of monozygotic twins with RA suggests that the environment plays a significant role in the etiology of this disease. Smoking, diet, environmental factors and infectious agents have all been proposed as triggers for RA (Oliver& Silman, 2006).

A study done to examine the interactions between environment and genetics factors in RA, found patients with certain HLA–DRB1 genotypes that entailed a genetic susceptibility to seropositive RA had a greater chance to develop RA if they are smoked. This study explained a gene–environment interaction between HLA– DRB1 genotypes and smoking. The hormonal influences supported by the finding that disease is much more common in women (2:1 - 4:1 women: men ratio) (Padyukov *et al.,* 2004).

1.12.3 B Immune Factors

Rheumatoid arthritis target organ is the synovial tissue and the cartilage of the affected joint. As a result of synovial infiltration by inflammatory cells, including T cells, leading to tissue expansion and formation of a "*pannus"* that covers the articular surface of the cartilage and pervade the bone (Walsh *et al.,* 2004; Harris *et al.,* 2005).

Destruction of the cartilage and bone mediated by inflammatory mediators such as the cytokine TNF-α, which released by invading cells, also B

cells that can differentiate into plasma cells found within the synovial infiltrate (Walsh *et al.,* 2004).

All these cells might be participated in the pathophysiology of RA through the production of cytokines and auto antibodies. Rheumatoid factor considered to be the most commonly available autoantibody in RA, RFan antibody reactive against antigenic determinants on the Fc fragment of the IgG molecule (Panayi *et al.,* 2005). Nearly 80% of RA patients having circulating RF. Usually, either IgA RF or IgM RF is found in the circulation. Both activity and severity of RA is related with RF levels. A suggestive role of RF in the pathogenesis of RA is through formation large immune complexes inside the synovium thereafter binding and activating other inflammatory molecules. Anticyclic citrullinated antibodies is another autoantibodies that described in RA & have aided in the diagnosis, also these antibodies found in other autoimmune diseases such as SLE(Zendman *et al.,* 2006).

1.12.3 C Infectious Agents

Many infections have clinical symptoms similar to RA so this finding generated interest in the role of infectious agents in this disease. These include parvovirus, Epstein–Barr virus, rubella virus, *Borrelia burgdorferi*, and others. However, epidemiological studies could not support the role for any one agent (Alamanos & Drosos, 2005).

1.12.4 Clinical Manifestations of Rheumatoid Arthritis

RA is a symmetric polyarthritis often involving the metacarpophalangeal joints of the hands & proximal interphalangeal joints of the fingers,the wrists, elbows, knees, and ankles also can be affected (Harris *et al.,* 2005).

In part of the patients, all joints may be involved, including the cricoarytenoid joint of the larynx & the TMJ. The affected joints develop swelling, redness, and warmth, with ultimate atrophy of the muscle around the affected area. Rheumatoid arthritis has clinical course and severity that varies greatly between patients (Harris *et al.,* 2005). Some RA patients may have a

short course of nondisabling disease, while others have progressive disease that poorly responds to therapy. Those with progressive active disease develop sever joint destruction and subsequent deformity with time, including swan-neck deformities & subcutaneous nodules. Also cervical spine disease may lead to C1–C2 subluxation and compression of spinal cord. Rheumatoid nodules may develop in the, pleura, lungs, pericardium, sclera, and rarely the eyes, heart or the brain. Life expectancy in RA patients could be decrease by as much as 5–10 years. Increase in cardiovascular disease considered one of the long-term complications of RA (Kaplan, 2010)

1.12.5 Laboratory Evaluation and Diagnosis

The primary diagnosis of RA is made firstly by observing clinical features. Like other autoimmune diseases, there was a list of diagnostic criteria developed by the ACR is used to evaluate patients Table (1-1) (Aletaha *et al.,* 2010) that take in consideration the type and extent of involved joint, the existence of rheumatoid factor, and other related immunological findings. Although RF & anti-CCP not specific to RA & found in other autoimmune disease like systemic lupus erythromatosus & scleroderma but a positive test for anti-CCP and RF antibodies give considerable weight to the diagnosis of RA. More destructive disease and a worse prognosis RA usually associated with high RF titers. Other related laboratory findings including normochromic normocytic anemia & an elevated erythrocyte sedimentation rate (Glick, 2015).

1.12.6 Treatment of Rheumatoid Arthritis

The emphasis on early diagnosis and intensive treatment of RA is due to the realization that damage and disability rapidly occur during the first years of the disease.

Table (1-1) American College of Rheumatology/European League Against Rheumatism Classification Criteria for Rheumatoid Arthritis

Rheumatoid arthritis: add score A through D; a score of ≥6 of 10 is needed for classification of a patient as having definite rheumatoid arthritis	
Classification	**Score**
A. Joint involvement*a*	
• 1 large joint (shoulders, elbows, hips, knees, ankles)	0
• 2–10 large joints	1
• 1–3 small joints*b* (with or without large joints)	2
• 4–10 small joints (with or without large joints)	3
• >10 joints (at least one small joint)	5
B. Serology (at least 1 test result is needed for classification)	
• Negative rheumatoid factor (RF) and negative anticitrullinated protein antibody (ACPA)	0
• Low-positive RF or low-positive ACPA	2
• High-positive RF or high-positive ACPA	3
C. Acute-phase reactants (at least 1 test result is needed for classification)	
• Normal C-reactive protein (CRP) and normal erythrocyte sedimentation rate (ESR)	0
• Abnormal CRP or abnormal ESR	1
D. Duration of symptoms	
• <6 weeks 0	0
• ≥6 weeks 1	1

The classification system is aimed at classifying newly presenting patients and is applied to those with at least one joint with synovitis that is not better explained by another disease. See full reference for complete details.
a -Joint involvement refers to any swollen or tender joint on examination. Distal interphalangeal joints, first carpometacarpal joints, and first metatarsophalangeal joints are excluded from assessment.
b-Small joints refers to the metacarpophalangeal joints, proximal interphalangeal joints, second through fifth metatarsophalangeal joints, thumb interphalangeal joints, and wrists
Source: Adapted from (Aletaha *et al.,* 2010)

The classes of drugs used for the treatment of Rheumatoid Arthritis include:

1-Nonsteroidal anti-inflammatory drugs (NSAIDs) and selective cyclooxygenase-2 (COX-2) inhibitors, NSAIDs and COX-2 inhibitors are used essentially for symptomatic relief of pain and are helpful co-therapies because of their analgesic & anti-inflammatory effects.

2-Disease modifying anti-rheumatic drugs (DMARDs) is diverse group of therapeutic agents that reduce the signs and symptoms of RA as well as retard radiographic progression of joint damage. This class of drugs is central to the control of RA, and is part of nearly every patient's treatment regimen. The ability of a drug to slow disease progression or produce a disease-modifying effect is that property which defines it as a DMARD, table (1-2) (Klippel *et al.*, 2005).

Table (1-2) Disease Modifying Drugs for the Treatment of Rheumatoid Arthritis

DMARD	CLINICALLY IMPORTANT SIDE EFFECTS	SCREENING/MONITORING	OTHER WARNINGS AND CONSIDERATIONS
Methotrexate	Nausea, diarrhea, stomatitis, fatigue, alopecia, elevated liver enzymes, myelosuppression, pneumonitis, increased risk of infection	CBC, renal function, liver enzymes every 8–12 weeks	Viral hepatitis B and C screening; contraindicated in renal disease (creatinine ≥2 mg/dL), teratogenic
Leflunomide	Nausea, diarrhea, rash, alopecia, elevated liver enzymes	CBC, renal function, liver enzymes every 8–12 weeks	Screening for viral hepatitis B and C; teratogenic
Hydroxychloroquine	Nausea, rash, skin hyperpigmentation, retinopathy (rare)	Yearly ophthalmologic exam[a]	Adjusted dose in renal insufficiency
Sulfasalazine	Nausea, abdominal bloating, rash, granulocytopenia	CBC, liver enzymes every 8–12 weeks	Screen for G6PD deficiency; reduce dose in renal or hepatic insufficiency
Injectable gold	Stomatitis, proteinuria, myelosuppression	CBC, urinalysis prior to each dose	
Oral auranofin	GI upset, stomatitis, proteinuria, myelosuppression	CBC, urinalysis every 8–12 weeks	
Minocycline	Skin hyperpigmentation, rash, nausea, drug-induced lupus		Avoid sun exposure
Cyclosporine	Nausea, abdominal pain, nephrotoxicity, hypertension, hypertrichosis, paresthesias, tremor, gum hyperplasia, increased risk of infection	CBC, renal function	Cyclosporine levels increase with concomitant use of ketoconazole, calcium antagonists, and H2 blockers; decreased levels with use of anticonvulsants and rifampicin; contraindicated in renal insufficiency

ABBREVIATIONS: CBC, complete blood count; G6PD, glucose 6-phosphate dehydrogenase.
[a]American Academy of Ophthalmology recommends annual eye exams for individuals older than 50 years.

3-The biologics are structurally engineered versions of natural molecules (e.g., monoclonal antibodies) designed to specifically target pathogenic mediators of joint inflammation and damage. In general, biologics are also considered to be DMARDs when they have been shown in large clinical trials to significantly inhibit the progression of joint damage, table (1-3) (Klippel *et al.*, 2005).

Table (1-3) Biologics for the Treatment of Rheumatoid Arthritis

BIOLOGIC	CLINICALLY IMPORTANT SIDE EFFECTS	MONITORING	OTHER WARNINGS AND CONSIDERATIONS
TNF antagonists Etanercept Infliximab Adalimumab	Injection site reaction, infusion reaction, reactivation of latent TB, increased risk of serious bacterial and opportunistic infection, possible increased risk of lymphoma, rare occurrence of demyelinating disorders and lupuslike syndromes	Periodic CBC	Question about prior history of TB exposure and screen with tuberculin skin testing; avoid in NYHA class III–IV heart failure
Kineret	Injection site reaction, neutropenia, increased risk of serious bacterial infection		Screen with tuberculin skin testing
Abatacept	Infusion reaction, increased risk of serious bacterial infection		Screen with tuberculin skin testing; use with caution in individuals with COPD because of an increased risk of adverse events and serious infections in this group; avoid live vaccines
Rituximab	Infusion reaction, increased risk of infection	Periodic CBC	Screen for viral hepatitis B infection

ABBREVIATIONS: CBC, complete blood count; COPD, chronic obstructive pulmonary disease; NYHA, New York Heart Association; TB, tuberculosis.

4-Corticosteroids are versatile agents with potent anti-inflammatory effects that represent yet another class of drugs .They are prescribed in a variety of clinical situations to control disease activity, but their use is limited by significant long-term toxicity (Klippel *et al.*, 2005).

All these different classes of drugs are usually combined in a multidrug regimen to give best suppression of disease activity.

1.13 Study Parameters

1.13.1 Rheumatoid Factor

Rheumatoid factor (RF) is an antibody directed against the Fc fragment of human immunoglobulin. Routinely in clinical practice usually measured IgM rheumatoid factor, although there are different methods allowing measuring of IgG & IgA RF too (Walker & Colledge, 2013).

Renaudineau *et al.*, (2005) considered that RF is a specific autoantibody against the C-terminal domain of the constant region of the heavy chain in human IgG. Suggesting that chronic inflammation & oxidative stress have a key role in altering the structure of IgG making the constant region of IgG" more

available which enhance the development of RF, also RF may have an important role in host defence against bacterial & viral pathogen .RF positive may occur in a wide range of diseases & little of normal adult especially with increasing age (Walker & Colledge, 2013**)**.Rheumatoid Factor considered to be important autoantibody in RA patients (Christopher*et al.,* 2008).

1.13.2 Anticitrullinated Protein Antibody (ACPA)

Anticitrullinated protein antibody is found in the sera of many patients with RA and is directed against the citrullinated residues of proteins. Citrulline is a non-naturally occurring amino acid generated by deimination of arginine residues on proteins by enzymes called *peptidylarginine deiminases*. Deiminated recombinant fillagrin protein in cyclic form is a particularly useful substrate to detect these auto antibodies. The sensitivity of the anti-CCP antibody test for RA is similar (70%), but specificity is superior (95%) to RF. Moreover, 35% of patients with a negative RF at presentation will test positively for ACPA (Schellekens *et al.*, 2000; Christopher *et al.*, 2008).

1.13.3 Erythrocyte Sedimentation Rate (ESR)

The erythrocyte sedimentation rate suggested to be an indirect measure for inflammation."It measures how fast erythrocyte fall through anticoagulated blood & determined by a combination of composition of plasma proteins & morphology of circulating erythrocytes", all above mentioned factors determine the tendency of the red cells to aggregate which is in turn determine ESR value(Marshall, 2013). ESR considered to be the most common depends inflammatory biomarker in clinical practice (in addition to CRP) regarding RA, so these markers may be elevated in RA patients with active disease & decline with treatment.Hence these inflammatory markers can be evaluate along with patients' symptoms & examination of the joints to monitor the disease activity over time (Christopher *et al.,* 2008).

1.13.4 Anti-Ro/SS-A & Anti-La/SS-B

These two autoantibody firstly described in 1961 as two precipitating antibodies reacting with antigens contained in extracts from salivary and lacrimal glands of patients with Sjogren syndrome (Yoshimi *et al.*, 2012).

Sisto *et al.,* (2007) clarified that in despite the presence of these serum autoantibodies which is directed against the ribonucleoprotein antigens SS-A(Ro) & SS-B(La) in Sjogren syndrome patients & considered in the classification criteria used to diagnosed SS ,the exact role of these antibodies in the pathogenesis of the disease is still ambiguous.

Usually SS patients with seropositive anti-SSA/Ro and anti-SSB/La antibodies show higher rate of extra glandular manifestations and more active immunological status. Also Anti-SSA/B-positive SS patients may show severe hypergammaglobulinemia and cryoglobulinemia, and appear to have higher risk for developing lymphoma (Tincani *et al.,* 2012). Anti-Ro antibodies may be presence alone in many sera, opposite to Anti-La antibodies which are commonly coupled by Anti-Ro antibodies (Yoshimi *et al.*, 2012).

1.13.5 Melatonin (MLT)

Melatonin (N-acetyl-5-methoxytryptamine) was firstly discovered in 1958 in the bovine pineal gland by Lerner and co-workers (Lerner *et al.,* 1958). Melatonin (N-acetyl-5-methoxytryptamine), mostly released from the pineal gland especially in the dark, melatonin considered as the disintegration product of serotonin & can play a role in various physiological processes such as, immune function, circadian rhythm and sexual behavior (Rudra *et al.*,2013). The rhythmic production of melatonin may be result from neural impulses from the Suprachiasmatic nucleus, from the biologic clock and hypothalamus (Kalsbeek & Buijs, 2002) (Cutolo & Maestroni ,2005) peak level of serum melatonin in healthy subjects between 12.00 a.m–2.00 a.m. and 2.00–4.00 a.m, with minimum level during the day 12.00 p.m–2.00 p.m (Buijs & Kalsbeek ,2001) The circadian nocturnal release of MLT encompasses a profound

influence on the interior environment of the organism, with numerous physiological effects(Simonneaux & Ribelayga, 2003).

Melatonin found in bacteria, algae, unicellular eukaryotic organisms, invertebrates and vertebrates, fungi, and plants and also present in a Variety of edibles, such as, fruit, vegetables, seeds, and herbs (Hardeland *et al.,* 2003; Tan *et al.,* 2012). In mammals melatonin is synthesized in various organs& tissues, such as, respiratory, gastrointestinal, genitourinary, skin, and immune systems (Bubenik 2008; Slominski *et al.,* 2008).

Also, melatonin shows an amazing functions in exhibiting oncostatic (Lissoni *et al.,* 1993; Sánchez-Hidalgo *et al.,* 2012) antioxidant (Galano *et. al* 2013),immunomodulatory(Carrillo-Vico *et al.,* 2013; Calvo *et al.,* 2013),& antigenic effects(Poeggeler,2005).

Regarding the molecular mechanisms responsible for the multiple effects of melatonin involve mechanisms of action as receptor-independents (Maldonado *et al.,* 2013; Korkmaz *et al.,* 2009) as well receptor-dependents (Dubocovich ,1995; Maldonado *et. al*. 2013).

Melatonin is also secreted within the saliva, though its role within the mouth isn't notable well. The imperative role of melatonin seems to come about because of its powerful "antioxidant, immune-modulator, protecting and anti-neoplastic properties. It has a role in stimulation the synthesis of ype I collagen fibers" and take part in bone formation. Therefore, melatonin could be used regionally and therapeutically, in different fields' such as in the oral cavity due to (mechanical, fungal, bacterial or viral origin) damaging, also in surgical field regarding wounds caused by various oral surgeries & tooth extractions, & serving in formation of bone in different disorders like periodontal problems & in sjogren syndrome(Czesnikiewicz-Guzik *et al.,* 2011; Hagh *et al.,* 2011) The antioxidant, oncostatic, immunomodulatory & other functions of melatonin is said to take a significant role in conserving the health of oral cavity(Malone, 2012).

1.13.6 Nitric Oxide (NO)

Nitric oxide (NO) is a widely distributed intercellular messenger molecule which has important neurological, cardiovascular, and immune functions. Nitric oxide, a free radical gas, is a noxious chemical in the atmosphere, but in small controlled concentrations in the body, it acts as a physiological and pathophysiological mediator and it plays an important role in the biological systems, the NO synthesis (NOS) defined as group of isoenzymes which are responsible for NO production from L-arginine in many tissues and cell types of mammalian which consist of three isoform of enzyme: endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible (iNOS)(Sundar *et al.,* 2013).

Li H, (2013) suggested that Nitric oxide is an endogenously produced tiny molecule that has crucial roles in cellular signaling and is involved in a diversity of physiological processes. NO can act in an opposite biological effects, rely on different pathophysiological & environmental conditions.

In plasma NO is highly labile molecule and has a extremely short half-life of only a few seconds so the plasma concentration of its stable metabolites, nitrate (NO-3) and nitrite (NO-2) are usually used as a marker of NO production and NOS enzyme activity, also the effects of NO depend on its concentration. (Dervisevic *et al.,* 2012).

1.13.7 Alpha –Amylase (Sa – AM)

Salivary α-Amylase is one of the most plentiful components in saliva, accounting for 10–20% of the total protein content.Salivary α-amylase is produced locally by the specialized epithelial acinar cells of the exocrine salivary glands mainly of the parotid glands; also it contributes in food digestion via the hydrolysis of starch to glucose and to maltose(Arhakis *et al.,* 2013) Consequently, salivary α-Amylase has been proposed to prevent the attachment of bacteria to oral surfaces and to permit the clearing of bacterial from the mouth(Bosch *et al.,* 2003).

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Miller *et al.*, 2010 showed that salivary alpha amylase has been rapidly increased during acute stress, and it has been stated that it may even be used as a marker of sympathetic nervous system activity, although this concept is still debated. Also, main function of salivary alpha amylase is to" break down high molecular weight carbohydrates to lower molecular weight sugars (i.e., glucose), in addition, alpha amylase may play a role in supporting mucosal immunity (Trueba *et al.*, 2012).

1.13.8 Salivary Total Protein (Sa – Tp)

Total salivary protein considered to be essential component of saliva, composing of mainly mucin, proline rich proteins, statherin, amylase, immunoglobulins, & antibacterial factors & these are responsible for most function of saliva (Panchbhai *et al.*, 2010) Salivary proteins may accomplish different functions like metabolic, transporting ,immune response, and several other cellular functions (Wong, 2006; Sathyapriya *et al.*, 2011).Salivary proteins also have protective effects against dental caries, these proteins act either directly &/or indirectly through different methods on plaque and bacteria via changing tooth susceptibility to dental caries (Vibhakar *et. al* .,2010).

Chapter Two

Materials & Methods

Subjects, Materials and Methods

2.1 The Subjects

A case control comparative study was performed in Baghdad Teaching Hospital /Department of Rheumatology from March 2015 to October 2016, after obtaining the approval from both the Scientific Committee of the oral diagnosis department & from the Ministry of health in Iraq for examining the patients (secondary Sjogren Syndrome & Rheumatoid Arthritis) and taking (blood & salivary) samples, then the principles of the study was explained to each participant & informed consent were taken from each patients participated in this study, the informed consent in appendix II. The study sample consist of ninety two subjects, sixty one Rheumatoid Arthritis patients of either gender with age range (20- 70) years, of them thirty one patients with secondary Sjogren Syndrome diagnosed according to American-European Consensus Group criteria (AECC) (Vitali *et al.*,2002) &thirty Rheumatoid Arthritis patients diagnosed clinically by rheumatology specialists and both of them evaluated by Disease activity depending on Disease Activity Score in 28 Joints (DAS-28) illustrated in appendix III"& thirty one Healthy control subjects with no signs & symptoms of systemic diseases.

The study sample was divided into three groups:

-Group I: (n=31) secondary Sjogren Syndrome patients with Rheumatoid Arthritis

-Group II: (n=30) Rheumatoid Arthritis Patients.

-Group III: (n=31) Healthy control subjects.

2.2 Exclusion criteria:

Past head and neck radiation treatment; hepatitis C infection; acquired immunodeficiency syndrome(AIDS); preexisting lymphoma; sarcoidosis; graft-vs.-host disease; use of anticholinergic drugs (since a time shorter than fourfold the half-life of the drug),smoker patients.

2.3 Study design

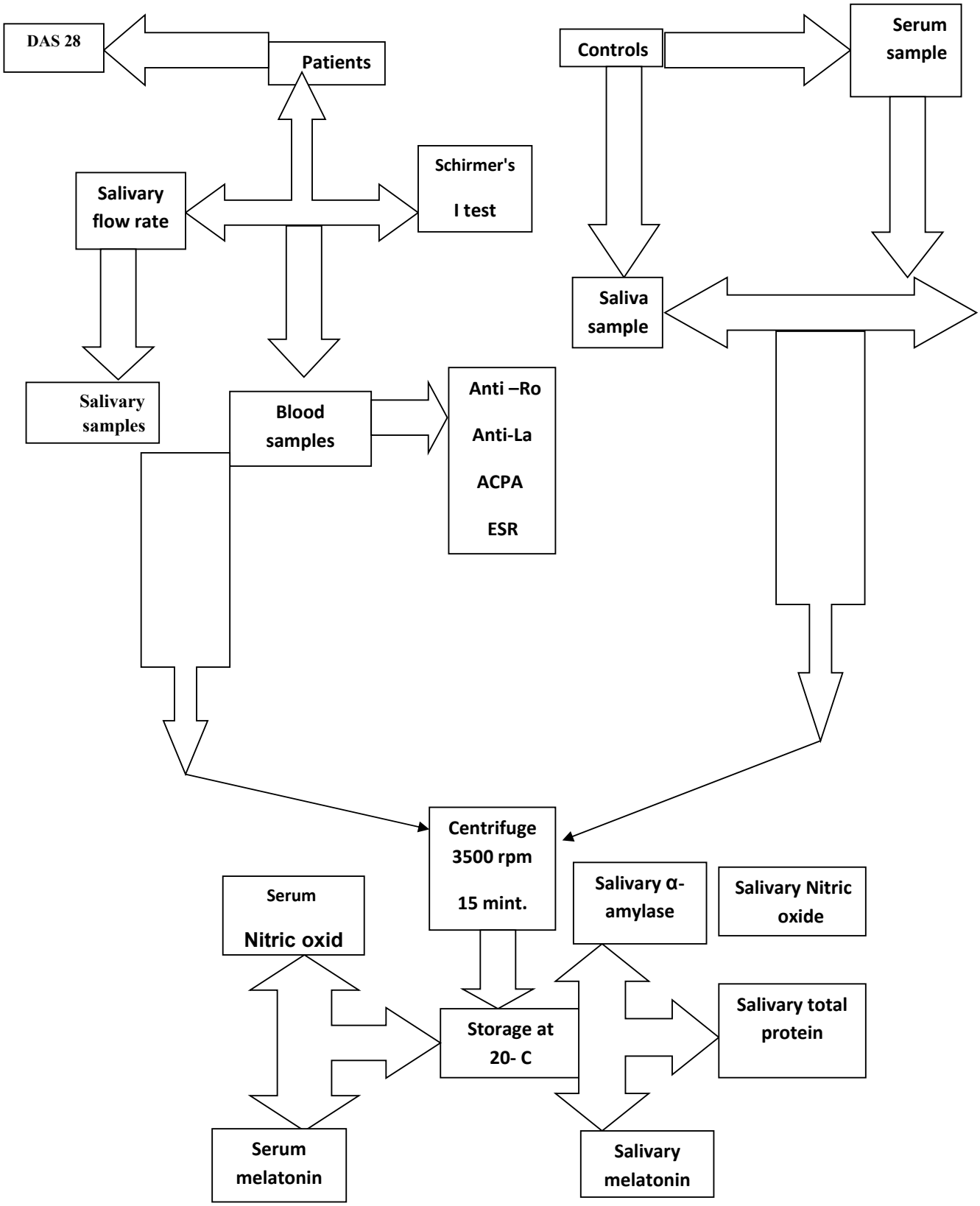

Figure: - (2-1) the study design

2.3 Materials

2.3.1Clinical Aspect

Examination used instruments:

- Disposable plastic gloves, facial mask.
- Disposable Dental Probes.
- Disposable Dental mirrors.
- Tweezers.
- Tongue depressor.
- Stop watch.
- Cotton.
- Schirmer's test papers. (figure 2-3)
- Graduated test tube (10 ml).{for saliva collection }
- Mobile camera.
- Alcohol for sterilization.
- Artificial light.
- Cooling box.

2.3.2 Materials Used for Immunological Study

2.3.2.1 Kits

- Anti-citrulinated peptide Antibody (ACPA), AESKU.DIAGNOSTICS, Germany.
- Anti-SS-B (La), ORGENTEC Diagnostika, Germany.
- Anti-SS-A (Ro), ORGENTEC Diagnostika, Germany.
- Rheumatoid Factor kit (slide agglutination)

2.3.2.2 Equipment's& Material

- Centrifuge,(Universal 320)
- Microplate reader of endpoint measurements at 450 nm,(Beckman Coulter).
- Multi-Channel Dispensers or repeatable pipette for 100µl.
- Vortex mixer, (Germany).
- Pipette for 10µl, 100 µl, & 1000 µl.
- Timer.
- Data analysis &graphing software.
- Incubator (37◦C), Memmert.
- Graduated cylinder for 100 & 1000ml.

- Plastic container for storage of the wash solution.
- Distilled or deionized water.

2.3.3 Materials used for Biochemical Study

2.3.3.1Kits

-Melatonin (MLT) ELISA Kits (Human), BioAssay, {USA}.

-Total Nitric Oxide (NO) ELISA Kits (Human), BioAssay, {USA}.

-Alpha -Amylase kit (Spinreact), Spain.

-Total protein kit (Spinreact), Spain.

2.3.3.2 Equipments & Materials

- Spectrophotometer or colorimeter.
- Thermostatic bath at (37°C).
- Matched cuvettes 1, 0 cm light path.
- General laboratory equipment.
- Precision pipettes & disposable tips to deliver 10-1000ul.
- A multi-channel pipette is desirable for assays.
- 100ml graduated cylinders.
- Tubes to prepare sample dilutions.
- Absorbent paper.
- Microplate reader capable of measuring absorbance at 450nm.
- Centrifuge capable of 3000xg.
- Microplate washer or washing bottle.
- Incubator (37°C)
- Data analysis & graphing software.

2.4 Methods

2.4.1Methods of examination

2.4.1.1Questionnaire

Demographic & clinical characteristic of patients & controls were recorded in questionnaire (appendix II).

2.4. 1.2 Oral Examination

Single examiner examined all patients under the same standardized conditions; using mouth mirror the oral cavity was examined by artificial light.

The examination procedure of the oral soft tissue accomplished according to **W.H.O.** (1987). The examination begun with the lips, upper and lower sulcus, retro-molar area, upper and lower labial mucosa, buccal mucosa, then hard and soft palate, dorsal margin and inferior surface of the tongue, & floor of the mouth were also examined.

In case of salivary gland & lymph node enlargement, the duration &consistency of enlargement was recorded.

2.4.1.3 Disease Activity Score in 28 Joints (DAS-28)

Disease Activity Score in 28 Joints is a weight multidimensional instrument that uses a physician's assessment of the joints, the patient's overall self-assessment of disease activity, and a laboratory marker of inflammation (C - reactive protein or Erythrocyte Sedimentation Rate (van der Heijde *et al*., 1993). Evaluation of the (DAS 28) carried out by specification of the number of swollen & tender joints in 28 joints encompassing the small joints in the hands, wrists, elbows, shoulders, and knees. Then the score is obtained from a complicated equation, illustrated in appendix III. DAS28 is carried on both study groups (secondary Sjogren syndrome & rheumatoid arthritis).

2.4.1.4 Schirmer's I test

According to Van Bijsterveld, 1969 the gold standard screening tool for dry eye is Schirmer's I test without anaesthesia.

The Schirmer's I test is considered as an easy method for evaluating aqueous tear flow, by inserting a sterile strips of filter paper in the conjunctival sac between the middle to lateral third of the lower eyelid and measuring the distance tears that appears on the filter paper over 5 minutes. Cut off for abnormally low tear production is 5 mm distance or less usually (Clair, 2013) (figure 2-2).

Figure (2-1):- Schirmer's test

2.4.1.5 Salivary Flow Rate (Dry Mouth Assessment)

Oral dryness is associated with the saliva production, so it can be measured by the unstimulated salivary flow rate. Patients were asked to collect saliva for 15 minutes into a collecting graduated tube. A less than1.5ml is considered as abnormal or xerostomia (Vitali *et al*., 2002).

2.4.1.6 Sample Collection

A-Saliva Collection:

The method of saliva collection was done according to the procedure suggested by (Wu-Wang *et al*., 1995). In order to avoid any circadian variation salivary sample were collected between 9 - 12 Am.

Before collecting saliva all subjects were instructed not to eat or drink (except water) for at least one hour, then asking the subjects to wash their mouth then sit down in comfortable position & collect saliva by spit into a sterile graduated tube .Then the salivary sample kept in ice until centrifuged at 3500 rpm for 15 minute; then the supernatants store at -20 $\overset{o}{C}$ freezer until analysis.

B-Blood Sample

Blood samples were obtained through vein puncture between 9-12 AM from both study subjects &control group, about (6-7ml) of blood taken from each patient. Then divided into two parts; one with anticoagulant (trisodium citrate solution), & the other part without. Then blood samples kept in ice until

centrifuged at 3500rpm for 15 minutes. Then serum samples store at -20°C freezer until analysis.

2.4.2 Laboratory Analysis

2.4.2.1 Immunological Analysis

1-Anti–citrulinated Protein Antibody (ACPA) test.

Principles of the test:

Serum samples diluted 1:101 are incubated in the microplates coated with the specific antigen. Patient´s antibodies, if present in the specimen, bind to the antigen. The unbound fraction is washed off in the following step. Afterwards anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) are incubated and react with the antigen-antibody complex of the samples in the microplates. Unbound conjugate is washed off in the following step. Addition of TMB-substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color changes to yellow).The intensity of color formation from the chromogen is a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of the respective antibodies in the patient sample. Appendix IV

2- Anti- La/SSB test.

Principles of the test:

Highly purified SS-B is bound to microwells. Antibodies against this antigen, if present in diluted serum or plasma, bind to the respective antigen. Washing of the microwells removes unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated antihuman IgG immunologically detects the bound patient antibodies forming a conjugate/antibody/ antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow color is measured photometrically at 450 nm. The

amount of colour is directly proportional to the concentration of IgG antibodies present in the original sample. Appendix V

3-Anti- Ro/ SS-A test.

Principles of the test

Highly purified SS-A is bound to microwells.Antibodies against this antigen, if present in diluted serum or plasma, bind to the respective antigen. Washing of the microwells removes unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated antihuman IgG immunologically detects the bound patient antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow color is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of IgG antibodies present in the original sample. Appendix VI

4-Rheumatoid Factor test (RF-Latex).

Principles of the method:

The RF-latex is a slide agglutination test for the qualitative and semi-quantitative detection of RF in human serum. Latex particles coated with human gammaglobulin are agglutinated when mixed with samples containing RF. Apendix VII

5-Erythrocyte Sedimentation Rate Evaluation (ESR)

Principle of the method:

When anticoagulated blood is allowed to stand in a narrow vertical glass tube, undisturbed for a period of time, the RBCs – under the influence of gravity- settle out from the plasma. The rate at which they settle is measured as the number of millimeters of clear plasma present at the top of the column after one hour (mm/hr). This mechanism involves three stages:

- Stage of aggregation: It is the initial stage in which piling up of RBCs takes place. The phenomenon is known as Rouleaux formation. It occurs in the first 10-15 minutes.
- Stage of sedimentation: It is the stage of actual falling of RBCs in which sedimentation occurs at constant rate. This occurs in 30-40 minutes out of 1 hour, depending upon the length of the tube used.
- Stage of packing: This is the final stage and is also known as stationary phase. In this, there is a slower rate of falling during which packing of sedimented RBCs in column occurs due to overcrowding. It occurs in final 10 minutes in 1 hour. Appendix VIII

2.4.2.2 A Biochemical Analysis

1-Salivary & serum Melatonin test (MLT).

Principle of the test

This ELISA Kit utilizes the quantitative Sandwich Enzyme Immunoassay technique. The microtiter plate has been pre-coated with a monoclonal antibody specific for MLT (Human). Standards or samples are then added to the microtiter plate wells and if the protein is present, will bind to the antibody pre-coated wells. In order to quantitatively determine the amount of protein present in the sample, a standardized preparation of horseradish peroxidase (HRP)-conjugated polyclonal antibody, specific for the protein are added to each well to "sandwich" the protein immobilized on the plate. The microtiter plate undergoes incubation, and then the wells are thoroughly washed to remove all unbound components. Next, substrate solutions are added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain the target protein and enzyme-conjugated antibody will exhibit a change in color. The enzyme-substrate reaction is terminated by addition of a sulfuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm. A standard curve is plotted relating the intensity of the color (O.D.) to the concentration of

standards. The protein concentration in each sample is interpolated from this standard curve. Appendix IX

2-Salivary & Serum Total Nitric Oxide test.

Principle of the test

This ELISA kit employs the quantitative competitive enzyme-linked immunoassay technique, utilizing a microtiter plate that has been pre-coated with a monoclonal anti-NO antibody. The assay samples and standards are incubated together with NO-HRP conjugate for one hour. A competition for limited antibody binding sites on the plate occurs between NO-HRP conjugate and NO in the samples and standards. After the incubation period, the wells are decanted and washed five times. The wells are then incubated with a substrate for HRP enzyme. The product of the enzyme-substrate reaction forms a blue colored complex. Finally, a stop solution is added to terminate the reaction, which will then turn the solution yellow. The intensity of color is measured spectrophotometrically at 450nm in a microplate reader. The intensity of the color is inversely proportional to the NO concentration in the sample or standard. A standard curve is plotted relating the intensity of the color (O.D.) to the concentrations of the standards. The NO concentration in each sample is interpolated from this standard curve. Appendix X

2.4.2.2 B Calculation of the Result

1-the standard curve is used to determine the amount of samples.

2-First, average the duplicate reading for each standard & sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.

3-construct a standard curve by plotting the average absorbance for each standard on the horizontal (x) against the concentration of the vertical (y) axis,& draw a best fit curve using graph paper or statistical software to generate a logistic (4-PL) curve fit or logit-log linear regression curve .An X-axis for the optical density & a Y-axis for the concentration is also choice . The data may be

linearized by plotting the log of the concentration versus the log of the absorbance & then constructing the best fit curve by regression analysis.

4-Calculate the concentration of samples corresponding to the mean absorbance from the standard curve.

5-Important, user must create their own standard curve. Appendix XI

3-Salivary α-amylase Assay

♦ **Principle**

Alpha amylase hydrolyzes the 2-chloro-4-nitrophenyle-α-D-maltotrioside (CNPG3) to release 2-chloro-4-nitrophenol (CNP) and form 2-chloro-4-nitrophenyl -α-D-maltoside (CNPG2), maltotriose (G3) y glucose (G) according to following reaction:

10 CNPG3 $\xrightarrow{\text{Amylase}}$ 9CNP + 1CNPG2 + G3 + G

The catalytic α-amylase concentration found in the sample is proportional to the rate of 2-chloro-4-nitrophenol generation which measured photometrically (Foo and Bais, 1998). Appendix XII

4-Salivary Total protein test.

Total Protein Assay

♦ **Principle**:

Proteins react in acid solution with pirogallol red and molybdate to form a colored complex, the intensity of the formed color is corresponding to the protein concentration in the sample (Orsonneau *et al*., 1989). Appendix XII**I**

2.5 Statistical Analysis

The following statistical data analysis approaches were used in order to analyze and assess the results of the study under application of the statistical package (SPSS) ver. (14.0):

I. Descriptive data analysis:

a. Observed frequencies and percents.

b. Mean value, Standard Deviation, Standard Error, (95%) Confidence interval for population Mean values, two Extreme values (min. and max.).

c. Pearson's Correlation Coefficients.

d. Spearman's Correlation Coefficients.

e. Odds Ratio of related rates : A measure of the strength of the association between the presence of a factor and the occurrence of an event, as well as 95% confidence interval to estimate the number of times (related rate).

f. Graphical presentation by using :

- Cluster Bar Charts.
- Bar Charts.
- Line Chart.

II. Inferential data analysis:

These were used to accept or reject the statistical hypotheses, which included the following:

a. Contingency Coefficient (CC) test for the cause's correlation ship of the association tables.

These were used to accept or reject the statistical hypotheses, which included the following:

a- The Independent-Samples T-Test procedure compares means for two groups of cases. Ideally, for this test, the subjects should be randomly assigned to two groups, so that any difference in response is due to the treatment (or lack of treatment) and not to other factors.

b- The One-Way ANOVA procedure produces a one-way analysis of variance for a quantitative dependent variable by a single factor (independent) variable. Analysis of variance is used to test the hypothesis that several means are equal. In addition to that we applied after rejecting the statistical hypotheses LSD test requiring equal variances are assumed, as well as Games-Howell test requiring equal variances are not assumed.

c- Homogeneity-of-variance: Calculates the Levene statistic to test for the equality of group variances. This test is not dependent on the assumption of normality.

d- Test Pearson's correlation coefficients, and spearman's correlation coefficients in two tailed alternative statistical hypotheses.

e- Kruskal-Wallis test: A nonparametric equivalent to one-way ANOVA. Tests whether several independent samples are from the same population. Assumes that the underlying variable has a continuous distribution, and requires an ordinal level of measurement.

f- Screening tests: Tests for mining data and estimating several indicators, such that (Sensitivity Rate, Specificity Rate, Accuracy Rate, Accuracy Rate), as well as receiver characteristic operation curve [ROC Curve] and estimating Area.

g- A contingency coefficient test: A measure of association based on chi-square. The value ranges between zero and 1, with zero indicating no

association between the row and column variables and values close to 1 indicating a high degree of association between the variables. The maximum value possible depends on the number of rows and columns in a table.

For the abbreviations of the comparison significant (C.S.), we used the followings:

NS: Non significant at $P>0.05$

S : Significant at $P<0.05$

HS: Highly significant at $P<0.01$

Chapter Three

Results

3. Results

3.1 The Sample

3.1.1 Demographic Parameters (Age & gender)

Results demonstrated that, the highest number for RA patients were in the age group between (40 – 49) years with a mean and standard deviation of (48.30 ± 10.02) years and for sSS patients were in the age group between (60–70) years with a mean and standard deviation of (52.65 ± 9.88) years. While control group were seen mostly between (30–59) years, with a mean and standard deviation of (41.87 ± 10.33) years. The highest percentages of the diseased groups were females, and they are accounted 28(93.3%) and 31(100%) in RA, and sSS groups respectively, and 21(67.74%) of male are included in control group. No significant differences at P>0.05 were found in (age & gender) between diseased groups, & control, as shown in table (3-1).

Table (3-1): Distribution of Demographical Characteristics variables (age & gender) of the studied groups with statistical significant

*DCv.	Groups	Groups						C.S. (*) P-value
		RA		sSS		Control		
		No.	%	No.	%	No.	%	
Age (years)	20 _	0	0.00	1	3.2	5	32.3	NS
	30 _	5	16.7	2	6.5	12	38.7	
	40 _	12	40	7	22.6	6	12.9	
	50 _	8	26.7	10	32.3	7	16.1	
	60 _ 70	5	16.7	11	35.5	1	0.00	
	Total	30	100	31	100	31	100	
Mean ± SD		48.30 ± 10.02		52.65 ± 9.88		41.87 ± 10.33		
C.S. (*) P-value		C.C.=0.301 P=0.195 (NS)						
Gender	Male	2	6.7	0	0.00	10	3	NS
	Female	28	93.3	31	100	21	67.74	
	Total	30	100	31	100	31	100	
C.S. (*) P-value		C.C.=0.184 P=0.144 (NS)						

(*) NS: Non Sig. at P>0.05; HS: Highly Sig. at P<0.01; C.C.; Contingency Coefficients* DCv.: Demographical Characteristics variables

3.1.2 Duration of the Disease

Results demonstrated that sSS mean disease duration and standard deviation was (145.97 ± 119.72) months. While RA disease duration mean and standard deviation was (71.97 ± 74.75) months, as shown in table (3-2).

Table (3--2): Duration of the Disease distribution per months

Groups	No. and Percent	Duration/m					Total	C.S. (*) P-value
		Up to 12 m.	Up to 48 m.	Up to 120 m.	Up to 240 m.	Up to 360 m.		
RA	No.	4	13	9	2	2	30	C.C.=0.346 P=0.080 NS
	% *Dur /m	57.1%	68.4%	52.9%	22.2%	22.2%	49.2%	
Mean ± SD		71.97 ± 74.75 (m.)						
sSS	No.	3	6	8	7	7	31	
	% Dur /m	42.9%	31.6%	47.1%	77.8%	77.8%	50.8%	
Mean ± SD		145.97 ± 119.72 (m.)						
Total	No.	7	19	17	9	9	61	
	% Dur /m	100%	100%	100%	100%	100%	100%	

(*) NS: Non Sig. at P>0.05; C.C.; Contingency Coefficients.
*Dur /m: duration per months.

3.2 Erythrocyte Sedimentation Rate

3.2.1 Descriptive statistics for the Erythrocyte Sedimentation Rate in the two Diseased Groups

Regarding ESR, results showed that RA, and sSS groups recorded high ESR value comparing with normal cutoff point, rather than sSS group were increased by one fifth percent compared with RA group, with a mean and standard deviation was (37.63±22.02) mm/hour for RA patients & was (46.13 ± 26.79) mm/hour for sSS, as shown in table (3-3) & figure (3-1).

Results showed that no significant differences at P>0.05 between RA, and sSS groups regarding Erythrocyte Sedimentation Rate, as shown in table (3-4).

Table (3-3): Descriptive Statistics of ESR in the two diseased groups

Parameters	Groups	No.	Mean	S.D.	S.E.	95% C.I. for Mean		Min.	Max.
						L.B.	U.B.		
ESR mm /hour	RA	30	37.63	22.02	4.02	29.41	45.85	3	98
	sSS	31	46.13	26.79	4.81	36.30	55.96	7	112

ESR: Erythrocyte Sedimentation Rate

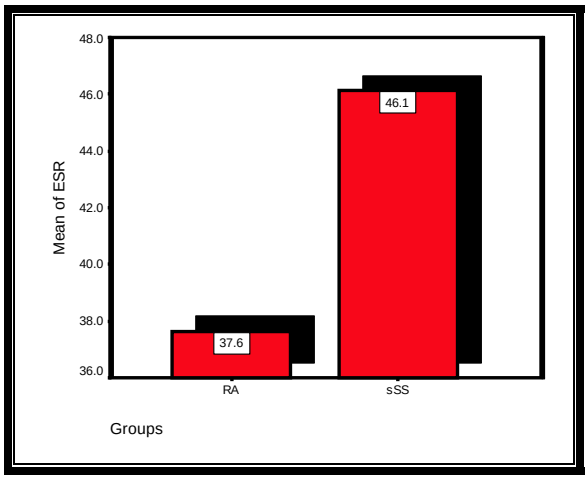

Figure (3- 1):- Mean values of ESR in the two diseased groups

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Table (3-4): Erythrocyte Sedimentation Rate according to equality of variances and equality of means

Parameters	Testing Homogeneity of Variances		T- Testing equality of means	
	Levene Statistic	Sig. (*)	T-test	Sig. (*)
ESR	0.828	0.367 (NS)	-1.351	0.182 (NS)

(*) **HS: Highly Significant at P< 0.01; NS: Non Sig. at P>0.05**

3.3 Anti-citrulinated peptide Antibody

3.3.1 Descriptive statistics for the Anti-citrulinated Peptide Antibody in the Two Diseased Groups

Regarding ACPA, results showed that RA, and sSS groups recorded high ACPA value comparing with normal cutoff point, rather than sSS group were increased by one third percent compared with RA group, with a mean and

standard deviation was (39.14±38.59) IU/ml for RA patients & was (51.04± 49.32) IU/ml for sSS, as shown in table (3-5) & figure (3-2).

Table (3-5): Descriptive Statistics of ACPA in the two diseased groups.

Parameters	Groups	No.	Mean	S.D.	S.E.	95% C.I. for Mean		Min.	Max.
						L.B.	U.B.		
ACPA (IU/ml)	RA	30	39.14	38.59	7.05	24.73	53.55	3.2	124.6
	sSS	31	51.04	49.32	8.86	32.95	69.13	2.1	206.1

ACPA: Anti-citrulinated peptide Antibody.
L.B: lower bound, U.B.: upper bound.

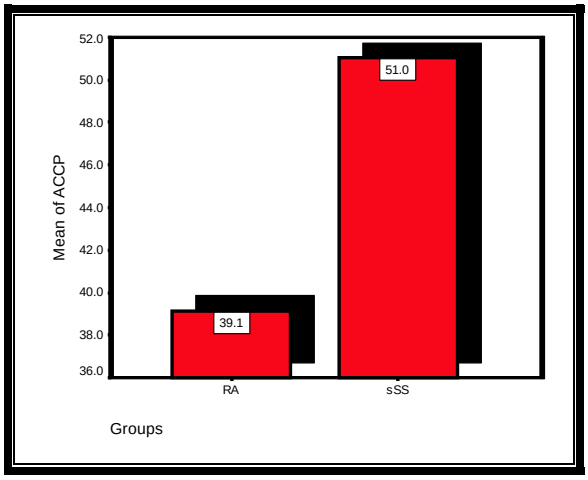

Figure (3-2):- Mean values of ACPA in the two diseased groups

Results showed that no significant differences at P>0.05 between RA, and sSS groups regarding Anticitrulinated Peptide Antibody, as shown in table (3-6).

Table (3-6): Anti-citrulinated peptide Antibody according to equality of variances and equality of means

Parameters	Testing Homogeneity of Variances		T- Testing equality of means	
	Levene Statistic	Sig. (*)	T-test	Sig. (*)
ACCP	1.704	0.197 (NS)	-1.048	0.299 (NS)

(*) **HS: Highly Significant at P< 0.01; NS: Non Sig. at P>0.05**

3.4 Anti-Ro/SSA

3.4.1 Descriptive statistics for the Anti-Ro/SSA in the two Diseased Groups

Regarding Anti-Ro/SSA, most of RA readings are recorded normal values, while sSS group are recorded in average abnormal (increased) value, with a mean and standard deviation was (8.94±5.71) IU/ml for RA patients & was (10.75± 9.21) IU/ml for sSS, as shown in table (3-7) & figure (3-3).

Table (3-7): Descriptive Statistics of Anti-Ro/SSA in the two diseased groups.

Parameters	Groups	No.	Mean	S.D.	S.E.	95% C.I. for Mean		Min.	Max.
						L.B.	U.B.		
Ati-Ro/SSA IU/ml	RA	30	8.94	5.71	1.04	6.81	11.07	2.9	27.2
	sSS	31	10.75	9.21	1.65	7.37	14.13	1	44.7

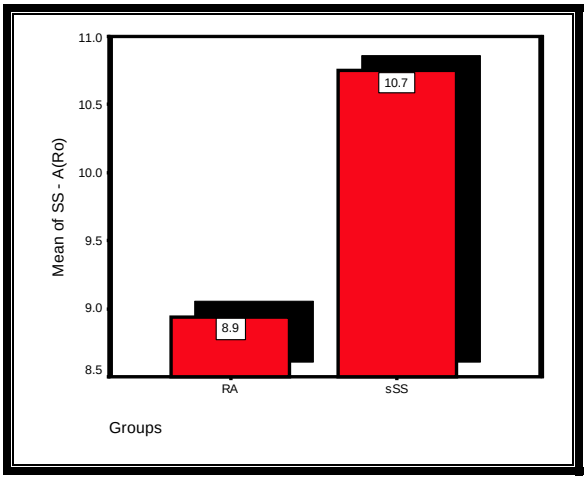

Figure (3-3):- Mean values of Anti-Ro/SSA in the two diseased groups

Results showed that no significant differences at P>0.05 between RA, and sSS groups regarding Anti-Ro/SSA, as shown in table (3-8).

Table (3-8): Anti-Ro/SSA according to equality of variances and equality of means

Parameters	Testing Homogeneity of Variances		T- Testing equality of means	
	Levene Statistic	Sig. (*)	T-test	Sig. (*)
Anti-Ro/SSA	2.688	0.106 (NS)	-0.918	0.362 (NS)

(*) HS: Highly Significant at P< 0.01; NS: Non Sig. at P>0.05

3.5 Anti-La/SSB

3.5.1 Descriptive statistics for the Anti-La/SSB in the two Diseased Groups

Regarding Anti-La/SSB, RA, and sSS of diseased groups are recorded normal values, since all readings are less than 10 IU/ml, with a mean and standard deviation was (7.50±4.54) IU/ml for RA patients & was (7.09± 4.69) IU/ml for sSS, as shown in table (3-9).

Table (3-9): Descriptive Statistics of Anti-La/SSB in the two diseased groups.

Parameters	Groups	No.	Mean	S.D.	S.E.	95% C.I. for Mean		Min.	Max.
						L.B.	U.B.		
Anti-La/SSB IU/ml	RA	30	7.50	4.54	0.83	5.81	9.19	2.8	26.6
	sSS	31	7.09	4.69	0.84	5.37	8.81	2.4	28.6

Results showed that no significant differences at P>0.05 between RA, and sSS groups regarding Anti-La/SSB, as shown in table (3-10).

Table (3-10): Anti-La/SSB, according to equality of variances and equality of means

Parameters	Testing Homogeneity of Variances		T- Testing equality of means	
	Levene Statistic	Sig. (*)	T-test	Sig. (*)
Anti-La/SSB	0.163	0.688 (NS)	0.349	0.728 (NS)

(*) HS: Highly Significant at P< 0.01; NS: Non Sig. at P>0.05

3.6 Rheumatoid Factor

3.6.1 Descriptive statistics for the Rheumatoid Factor in the two Diseased Groups with statistical significant

Results showed that no significant relationship at P>0.05 between RA, and sSS groups regarding Rheumatoid Factor, as shown in table (3-11).

Table (3-11): Descriptive statistics for the (RF) in the two diseased groups with statistical significant

Groups	Number & Percentage	RF		Total	C.S. (*) P-value
		Pos.	Neg.		
RA	No.	19	11	30	C.C.=0.021 P=0.869 NS (RA : sSS) (1 : 0.917) 95% CI (0.325 : 2.584)
	% RF	50.0%	47.8%	49.2%	
sSS	No.	19	12	31	
	% RF	50.0%	52.2%	50.8%	
Total	No.	38	23	61	
	% RF	100%	100%	100%	

(*) NS: Non Sig. at P>0.05; C.C.; Contingency Coefficients.
RF: Rheumatoid Factor
Pos.: positive RF test, Neg.: negative RF test.

3.7 Serum Melatonin

3.7.1Descriptive statistics for Serum Melatonin in the two Diseased Groups & Control Group

Regarding to Serum Melatonin, RA, and sSS of diseased groups were recorded low levels compared with controlled group, rather than sSS group increased by one third percent in light of RA group, with a mean and standard deviation was (55.7±92.6) pg/ml for RA patients; (72.3± 104.8) pg/ml for sSS, & was (113.2± 188.8) pg/ml for control group, as shown in table (3-12) & figure (3-4).

Results showed that no significant differences at P>0.05 of serum melatonin, as shown in table (3-13).

Table (3-12): Descriptive Statistics of Serum Melatonin in the two diseased groups and control group

Parameters	Groups	No.	Mean	S.D.	S.E.	95% C.I. for Mean		Min.	Max.
						L.B.	U.B.		
SMLT pg/ml	RA	30	55.7	92.6	16.9	21.1	90.3	0.57	495.9
	sSS	31	72.3	104.8	18.8	33.9	110.8	0.57	514.4
	Control	31	113.2	188.8	33.9	44.0	182.5	0.57	865.1

SMLT: serum melatonin

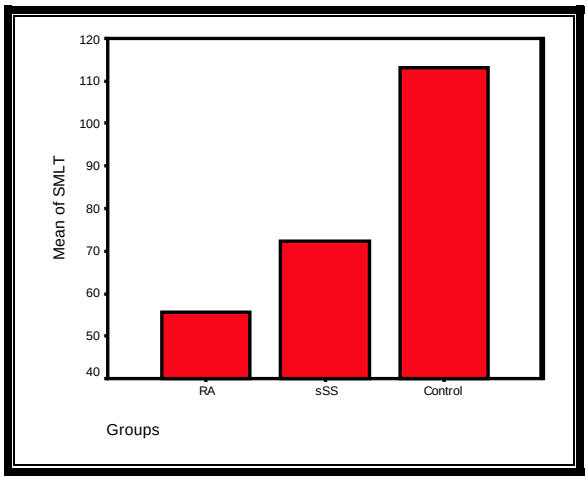

Figure (3-4):- Mean values of (SMLT) in the two diseased groups and control group

Table (3-13): Pair wise Comparisons by (GH, and LSD) tests concerning Serum Melatonin

Parameters	Group (I)	Group (J)	Mean Diff. (I-J)	Sig. (*)	95% C.I. for Diff.		GHD & LSD
					L.B.	U.B.	
SMLT	RA	sSS	-16.63	0.789	-77.48	44.22	60.85
		Control	-57.51	0.293	-149.43	34.41	91.92
	Sss	Control	-40.88	0.547	-134.76	53.00	93.88

(*) HS: Highly Sig. at P<0.01; NS: Non Sig. at P>0.05; Testing based on Games Howell Difference (GHD), and Least Significant Difference (LSD) test

3.8 Salivary Melatonin

3.8.1Descriptive statistics for Salivary Melatonin in the two Diseased Groups & Control Group

According to salivary melatonin, RA, and sSS of diseased groups were recorded low levels compared with controlled group, as well as simply different had recorded between diseased groups, with a mean and standard deviation was (11.0±8.8) pg/ml for RA patients; (10.9± 4.5) pg/ml for sSS, & was (30.3± 82.9) pg/ml for control, as shown in table (3-14) & figure (3-5).

Table (3-14): Descriptive Statistics of Salivary Melatonin in the two diseased groups and control group

Parameters	Groups	No.	Mean	S.D.	S.E.	95% C.I. for Mean		Min.	Max.
						L.B.	U.B.		
SAMLT pg/ml	RA	30	11.0	8.8	1.6	7.7	14.3	3.07	53.6
	sSS	31	10.9	4.5	0.8	9.2	12.5	6.04	23.3
	Control	31	30.3	82.9	14.9	0.00	60.7	0.57	460.8

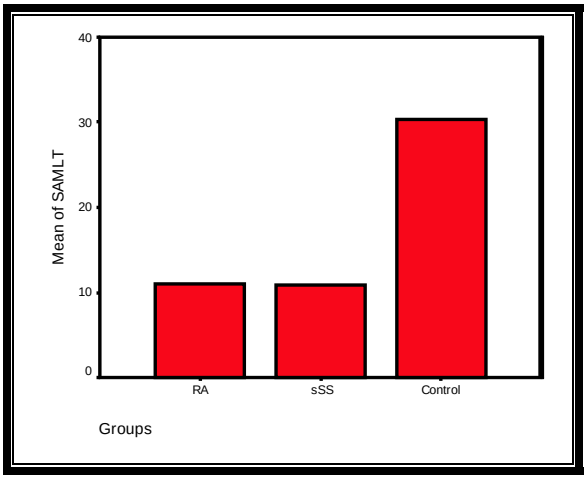

Figure (3-5):- Mean values of (SAMLT) in the two diseased groups and control group

Results showed that no significant differences at P>0.05 of salivary melatonin, as shown in table (3-15).

Table (3-15): Pair wise Comparisons by (GH, and LSD) tests concerning (SAMLT)

Parameters	Group (I)	Group (J)	Mean Diff. (I-J)	Sig. (*)	95% C.I. for Diff.		GHD & LSD
					L.B.	U.B.	
SAMLT	RA	sSS	0.11	0.998	-4.23	4.44	4.339
		Control	-19.32	0.411	-56.19	17.56	36.87
	sSS	Control	-19.42	0.405	-56.17	17.32	36.75

(*) **HS: Highly Sig. at P<0.01; NS: Non Sig. at P>0.05; Testing based on Games Howell Difference (GHD), and Least Significant Difference (LSD) test**

3.9 Serum Nitric Oxide

3.9.1Descriptive statistics for Serum Nitric Oxide in the two Diseased Groups and Control Group

Regarding to Salivary Melatonin, results showed that RA group showed simple decreased either compared with sSS, or controlled groups, with a mean and standard deviation was (115.7±134.3) umol/L for RA patients; (126.1± 136.9) umol/L for sSS, & was (124.4± 188.0) umol/L for control group, as shown in table (3-16) & figure (3-6).

Table (3-16): Descriptive Statistics of Serum Nitric Oxide in the two diseased groups and control group

Parameters	Groups	No.	Mean	S.D.	S.E.	95% C.I. for Mean		Min.	Max.
						L.B.	U.B.		
SNO umol/L	RA	30	115.7	134.3	24.5	65.5	165.8	16.69	565.9
	sSS	31	126.1	136.9	24.6	75.9	176.4	16.51	565.9
	Control	31	124.4	188.0	33.8	55.5	193.4	4.47	565.9

*__SNO__: serum nitric oxide.

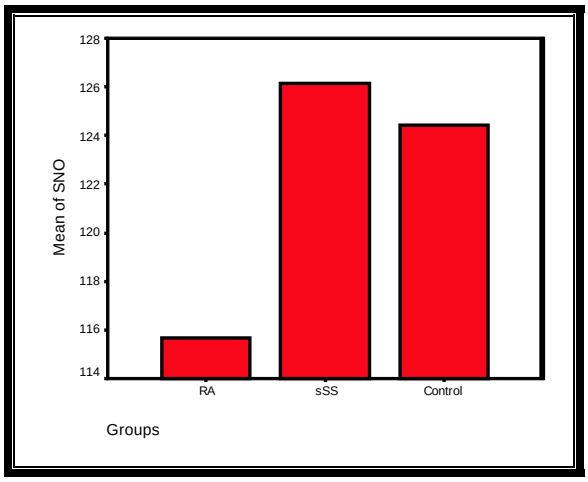

Figure (3-6):- Mean values of (SNO) in the two diseased groups and control group

Results showed that no significant differences at P>0.05 of serum nitric oxide, as shown in table (3-17)

Table (3-17): Pair wise Comparisons by (GH, and LSD) tests concerning (SNO)

Parameters	Group (I)	Group (J)	Mean Diff. (I-J)	Sig. (*)	95% C.I. for Diff.		GHD & LSD
					L.B.	U.B.	
SNO	RA	sSS	-10.45	0.793	-89.48	68.57	79.02
		Control	-8.77	0.826	-87.80	70.25	79.02
	sSS	Control	1.68	0.966	-76.69	80.05	78.37

(*) **HS: Highly Sig. at P<0.01; NS: Non Sig. at P>0.05; Testing based on Games Howell Difference (GHD), and Least Significant Difference (LSD) test**

3.10 Salivary Nitric Oxide

3.10.1 Descriptive statistics for Salivary Nitric Oxide in the two Diseased Groups and Control Group

Regarding Salivary Nitric Oxide, RA, and sSS of diseased groups were recorded low levels compared with controlled group, with a mean and standard deviation was (6.1±6.7) umol/L for RA patients; (10.4± 12.7) umol/L for sSS, & was (22.5± 8.9) umol/L for control group, as shown in table (3-18) & figure (3-7) .

Results showed that a highly significant differences at P<0.01 i.e. (between two diseased groups) in compared to controlled group, as shown in table (3-19).

Table (3-18): Descriptive Statistics of Salivary Nitric Oxide in the two diseased groups and control group

Parameters	Groups	No.	Mean	S.D.	S.E.	95% C.I. for Mean		Min.	Max.
						L.B.	U.B.		
SANO umol/L	RA	30	6.1	6.7	1.2	3.6	8.6	0.89	24.7
	sSS	31	10.4	12.7	2.3	5.7	15.0	0.89	47.4
	Control	31	22.5	8.9	1.6	19.2	25.7	3.60	42.3

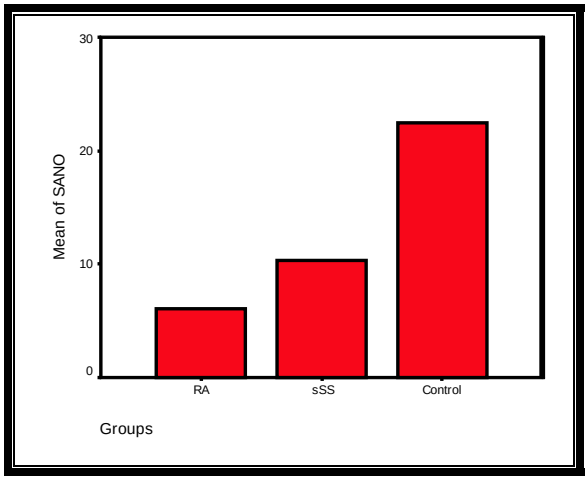

Figure (3-7):- Mean values of (SANO) in the two diseased groups and control group

Table (3-19): Pair wise Comparisons by (GH, and LSD) tests concerning Salivary Nitric Oxide

Parameters	Group (I)	Group (J)	Mean Diff. (I-J)	Sig. (*)	95% C.I. for Diff.		GHD & LSD
					L.B.	U.B.	
SANO	RA	sSS	-4.28	0.234	-10.55	1.99	6.271
		Control	-16.36	0.000*	-21.21	-11.52	4.846
	sSS	Control	-12.08	0.000*	-18.78	-5.38	6.700

(*) HS: Highly Sig. at P<0.01; NS: Non Sig. at P>0.05; Testing based on Games Howell Difference (GHD), and Least Significant Difference (LSD) test.

3.11 Salivary α-amylase

3.11.1 Descriptive Statistics for Salivary α-amylase in the two Diseased Groups & Control Group.

Regarding salivary α-amylase, RA, and sSS of diseased groups were recorded low levels compared with controlled group, rather than RA group are increased by one third percent in compared with sSS group, with a mean and standard deviation was (88273±113376) U/L for RA patients; (56135± 55602) U/L for sSS, & was (108778± 98920) U/L for control group, as shown in table (3-20) & figure (3-18).

Table (3-20): Descriptive Statistics of salivary α-amylase in the two diseased groups & control group

Parameters	Groups	No.	Mean	S.D.	S.E.	95% C.I. for Mean		Min.	Max.
						L.B.	U.B.		
Sa – AM U/L	RA	30	88273	113376	20699	45938	130608	3068	395600
	sSS	31	56135	55602	9986	35739	76530	8320	223200
	Control	31	108778	98920	17767	72494	145063	7810	448700

Sa – AM: salivary α- amylase.

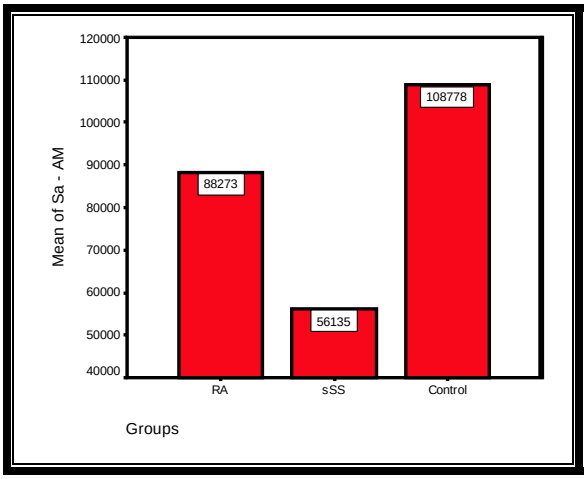

Figure (3-8):- Mean values of salivary α-amylase in the two diseased groups and control group

Regarding salivary α-amylase, results showed that no significant differences at P>0.05 of salivary α-amylas, except between sSS disordered group and controlled which showed significant differences at P<0.05, as shown in table (3-21).

Table (3-21): Pair wise Comparisons by (GH) tests concerning salivary α-amylase.

Parameters	Group (I)	Group (J)	Mean Diff. (I-J)	Sig. (*)	95% C.I. for Diff.		GHD Diff.
					L.B.	U.B.	
Sa – AM	RA	sSS	32138	0.351	-23704	87981	55842
		Control	-20505	0.734	-86138	45127	65632
	sSS	Control	-52644	0.034*	-101960	-3327	49316

(*) S: Sig. at P<0.05; NS: Non Sig. at P>0.05; Testing based on Games Howell Difference (GHD).

3.12 Salivary Total protein

3.12.1Descriptive statistics for Salivary Total protein in the two Diseased Groups & Control Group

Related to Salivary Total protein, Controlled and sSS group were recorded similar level according to mean values, while RA group had increased almost by ten percent in light others groups, with a mean and standard deviation was (154±74) mg/dl for RA patients; (143± 50) mg/dl for sSS, & was (144± 46) mg/dl for control group, as shown in table (3-22) & figure (3-9).

Table (3-22): Descriptive Statistics of Salivary Total protein in the studied groups

Parameters	Groups	No.	Mean	S.D.	S.E.	95% C.I. for Mean		Min.	Max.
						L.B.	U.B.		
Sa – Tp mg/dl	RA	30	154	74	14	126	181	72	361
	sSS	31	143	50	9	124	161	65	293
	Control	31	144	46	8	127	161	64	245

Sa – Tp: salivary total protein.

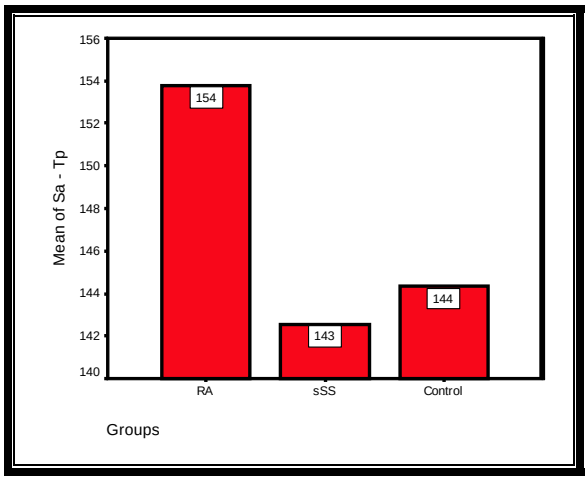

Figure (3-9):-Mean values of(Sa – Tp) in the two diseased groups and control group

Regarding to salivary total protein results showed that no significant differences at P>0.05, as shown in table (3-23).

Table (3-23): Pair wise Comparisons by (GH) tests concerning (Sa – Tp) parameters

Parameters	Group (I)	Group (J)	Mean Diff. (I-J)	Sig. (*)	95% C.I. for Diff.		GHD Diff.
					L.B.	U.B.	
Sa – Tp	RA	sSS	11.22	0.770	-28.04	50.48	39
		Control	9.44	0.824	-28.96	47.84	38
	sSS	Control	-1.77	0.988	-31.18	27.63	29

(*) S: Sig. at P<0.05; NS: Non Sig. at P>0.05; Testing based on Games Howell Difference (GHD).

3.13 Salivary flow rate

3.13.1 Descriptive statistics of Salivary flow rate in the two Diseased Groups

Regarding Salivary Flow Rate, RA group was in normal concerning average value, while sSS group had recorded abnormal value, with a mean and standard deviation was (2.70±1.66) ml/15min for RA patients; (0.92± 0.43) ml/15min for sSS, as shown in table (3-24).

Results showed highly significant different at P<0.01 between RA, and sSS groups regarding salivary flow rate as shown in table (3-25).

Table (3-24): Summary Statistics of Salivary Flow Rate in the two diseased groups

Parameters	Groups	No.	Mean	S.D.	S.E.	95% C.I. for Mean		Min.	Max.
						L.B.	U.B.		
SFR ml/15min.	RA	30	2.70	1.66	0.30	2.08	3.32	1	10
	sSS	31	0.92	0.43	0.08	0.76	1.08	0.13	1.5

SFR: salivary flow rate ml/minute.

Table (3-25): Test concerning Salivary Flow Rate according to equality of variances and equality of means

Parameters	Testing Homogeneity of Variances		T- Testing equality of means	
	Levene Statistic	Sig. (*)	T-test	Sig. (*)
SFR	7.299	0.009 (HS)	23.108	0.000 (HS)

(*) HS: Highly Significant at P< 0.01; NS: Non Sig. at P>0.05

3.14 Receiver Operating Characteristic Curve (ROC curve)

This test was used to rank the studied parameters according to the magnitude of their affection on the occurrence of secondary Sjogren Syndrome.

3.14.1 Receiver operating Characteristic curve and area under the curve in (sSS: RA) Groups according to (Serum Melatonin, Salivary Melatonin, Serum Nitric Oxide, Salivary Nitric Oxide, Salivary α-Amylase, and Salivary Total Protein)

Results regarding ROC curve assort (serum melatonin, salivary melatonin, serum nitric oxide, salivary nitric oxide, salivary α-amylase, and salivary total protein) according to their effect on the occurrence of the secondary Sjogren Syndrome, as shown in table (3-26), & figure (3-10).

Table (3-26): Statistics of ROC Curve in (sSS : RA) groups in light of studied variables (SMLT, SAMLT, SNO, SANO, Sa - AM, and Sa - Tp)

Area Under the Curve					
Test Result Variable(s)	Area	Std. Error	Asymptotic Sig.	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
SAMLT pg/ml	0.563	0.074	0.399	0.418	0.708
SMLT pg/ml	0.556	0.074	0.449	0.411	0.702
SANO umol/ml	0.548	0.075	0.521	0.401	0.695
SNO umol/ml	0.536	0.075	0.629	0.39	0.682
Sa - Tp mg/dl	0.488	0.076	0.868	0.339	0.636
Sa – AM U/L	0.443	0.075	0.445	0.297	0.589

3.14.2 Receiver operating Characteristic curve and area under the curve in (sSS:RA) Groups according (Erythrocytes Sedimentation Rate, Anticitrullinated Protein Antibody, Anti-Ro/SSA, Anti-La/SSB, Duration of the disease, and Salivary Flow Rate)

Results regarding ROC curve assort (ESR, ACPA, Anti-Ro/SSA, and Anti-La/SSB, Duration of the disease and SFR) according to their effect on the occurrence of the secondary Sjogren Syndrome, as shown in table (3-27), & figure (3-11).Additionally in table (3-27) results showed highly significant differences at P<0.01 in salivary flow rate & duration of the disease.

Table (3-27): Statistics of ROC in (sSS : RA) groups in light of studied variables (ESR, ACCP, SS-A(R0), SS-B(La), Duration, and SFL)

Area Under the Curve					
Test Result Variable(s)	Area	Std. Error	Asymptotic Sig.	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
SFR ml/15minute	0.067	0.033	0.000*	0.001	0.132
Duration of the disease /months	0.676	0.069	0.018*	0.54	0.812
ESR mm/hour	0.579	0.074	0.289	0.435	0.723
ACPA IU/ml	0.522	0.076	0.767	0.373	0.672
Anti-Ro/SSA IU/ml	0.525	0.075	0.735	0.379	0.672
Anti-La/SSB IU/ml	0.465	0.075	0.639	0.317	0.613

(*) HS: Highly sig. at P<0.01`; S: Sig. at P<0.0; NS: Non Sig. at P>0.05

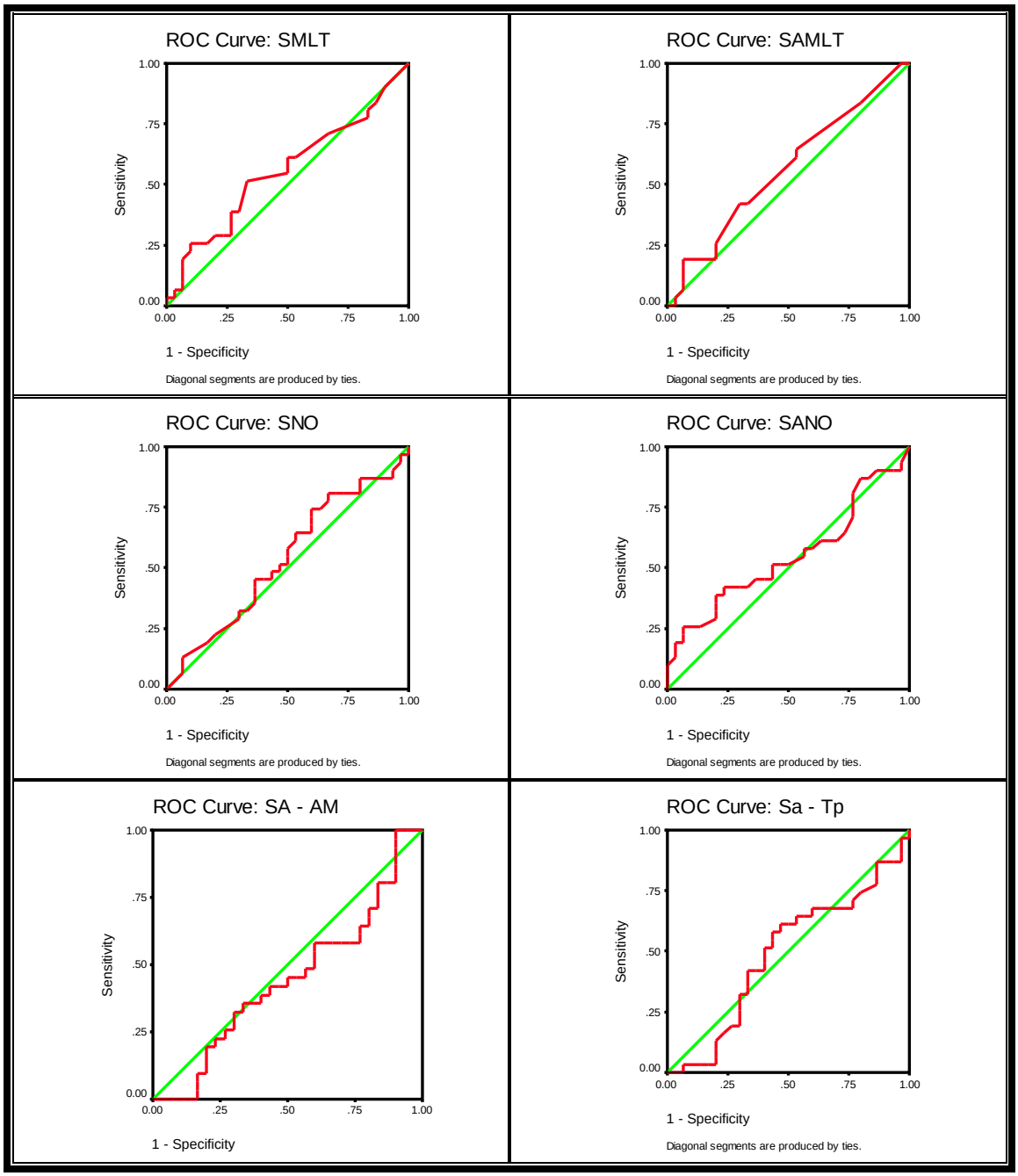

Figure (3-10): ROC Curve for the diseases (sSS : RA) groups in light of studied variables (SMLT, SAMLT, SNO, SANO, Sa - AM, and Sa - Tp)

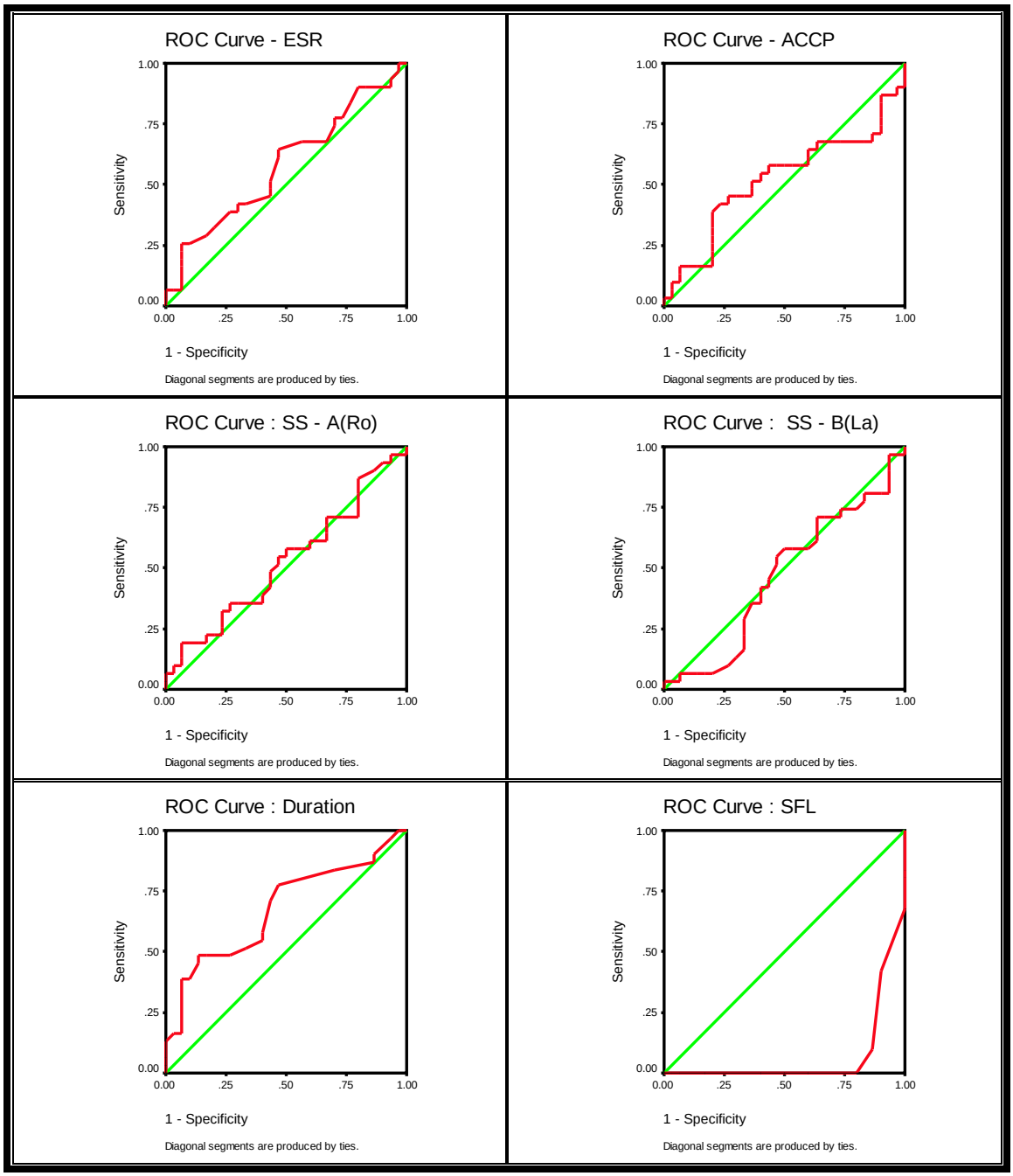

Figure (3-11): ROC Curve Plots for the diseases (sSS : RA) groups in light of studied variables (ESR, ACCP, SS-A(R0), SS-B(La), Duration of disease, and SFL)

3.15 Medications Used for the Treatment

The distribution of medications used for the treatment of two diseased groups RA & sSS with statistical significant. There were two types of responding (used, and unused), as well as a relationship throughout a contingency coefficients,

and an odds ratio to estimate the number of times (related rate) of using drugs in each group compared with the other group, as shown in table (3-28).

In this study, patients were found under five types of medication with differents combinations. Firstly Etanercept; results showed a strong correlation with (CC=0.327). A highly statistical significant at P<0.01was found in using this medication between, RA & sSS with an odds ratio, of more than four times.

With respect to Methotrixate, results showed that no significant correlation (CC=0.238, with P=0.055) at P>0.05, RA group showed increased uses of this medication compared with (sSS) group with an odds ratio, of almost three times.

Regarding Prednisolone, results showed that no significant correlation (CC=0.047) at P>0.05, which indicates that both disordered groups have used this medication similarly and at a rate of almost 40%.

Considering Hydroxichloroquine, results showed no significant correlation (CC=0.050) at P>0.05, which indicates that both disordered groups have used this medication similarly and at a rate of almost 25%, and 20% in (RA) and (sSS) groups respectively.

In relation to Rituximabe, results showed no significant correlation (CC=0.050) at P>0.05, which indicates that both disordered groups have used this medication similarly and at a rate of almost 10%.

Finally, regarding Azathioprine results showed no significant correlation (CC=0.050) at P>0.05, which indicates that both disordered groups have used this medication similarly and at a rate of almost 20%.

3.16 Disease Activity Score -28 (DAS-28)

3.16.1 Distribution of DAS-28 score in both Diseased Groups with statistical significant

Regarding DAS-28score, result showed no significant correlation (CC=0.279) at P>0.05, however the greats numbers of patients of DAS- 28 score

were found at MDA level in both groups, especially regarding sSS group, then followed by HDA level similarly, as shown in table (3-29).

Table (3-28): Distribution types of medication uses in the two disordered groups (RA & sSS) with statistical significant

Medication	Response	No. and Percent	Groups		Total	C.S. (*) P-value
			RA	sSS		
Enbrile	used	No.	12	23	35	C.C.=0.327
		% Group	40.0%	74.2%	57.4%	P=0.007
	unused	No.	18	8	26	HS
		% Group	60.0%	25.8%	42.6%	(1 : 0.232)
Methotrixate	used	No.	18	11	29	C.C.=0.238
		% Group	60.0%	35.5%	47.5%	P=0.055
	unused	No.	12	20	32	NS
		% Group	40.0%	64.5%	52.5%	(1 : 2.727)
Prednisolone	used	No.	9	8	17	C.C.=0.047
		% Group	30.0%	25.8%	27.9%	P=0.715
	unused	No.	21	23	44	NS
		% Group	70.0%	74.2%	72.1%	(1 : 1.232)
Hydroxichloroquine	used	No.	6	5	11	C.C.=0.050
		% Group	20.0%	16.1%	18.0%	P=0.694
	unused	No.	24	26	50	NS
		% Group	80.0%	83.9%	82.0%	(1 : 1.300)
Rituximabe	used	No.	2	3	5	C.C.=0.055
		% Group	6.7%	9.7%	8.2%	P=0.668
	unused	No.	28	28	56	NS
		% Group	93.3%	90.3%	91.8%	(1 : 0.667)
Azathioprine	used	No.	4	5	9	C.C.=0.039
		% Group	13.3%	16.1%	14.8%	P=0.758
	unused	No.	26	26	52	NS
		% Group	86.7%	83.9%	85.2%	(1 : 0.800)

(*) **HS: Highly Sig. at P< 0.05; NS: No Sig. at P> 0.05; Testing of random distribution are based on Contingency Coefficient test and an odds ratio.**

Table (3-29): Distribution of DAS-28 score in both diseased groups with statistical significant

Parameter	DAS-28	Groups	Groups		Total	C.S. (*) P-value
		No. & %	RA	sSS		
DAS28-Result levels	Remission	No.	4	0	4	C.C.=0.279 P=0.161 NS
		% Group	13.3%	0.0%	6.6%	
	*LDA	No.	2	2	4	
		% Group	6.7%	6.5%	6.6%	
	*MDA	No.	13	19	32	
		% Group	43.3%	61.3%	52.5%	
	*HDA	No.	11	10	21	
		% Group	36.7%	32.3%	34.4%	

(*) NS: Non Sig. at P>0.05; C.C.; Contingency Coefficients.
*LDA: Low Disease Activity; *MDA: Moderate Disease Activity; *HDA: High Disease Activity.

3.16.2 Disease Activity-28 Score in relation to Erythrocyte Sedimentation Rate & Anti–citrulinated Protein Antibody in both Diseased Groups with statistical significant

Relative to ESR, the results showed no significant correlation (CC=0.203) at P>0.05. In addition to that, ESR showed highly significant relationships at P<0.01in each of diseased groups by increases mainly of MDA of DAS-28 score levels, then followed by HAD level, as shown in table (3-30).

Regarding ACPA, result showed that no significant correlation (CC=0.323) at P>0.05. In addition to that, ACPA showed no significant relationships at P<0.01 in each of diseased groups by increases mainly of MDA of DAS-28 score levels, then followed by HDA level, as shown in table (3-30).

3.16.3 Disease Activity-28 Score in relation to Rheumatoid Factor in both Diseased Groups with statistical significant

Regarding to RF parameter, result showed that no significant correlation (CC=0.313) at P>0.05. In addition to that, RF showed no significant relationships at P<0.01 in each of diseased groups by increases mainly of MDA of DAS-28 score levels, then followed by HDA level, as shown in table (3-31).

Table (3-30): Distribution of ESR, ACPA result according to DAS-28 score in in both diseased groups with statistical significant

Parameters	DAS-28 Score	Groups	RA		sSS		C.S. (*)
		Responding	*Negative	*Positive	Negative	Positive	P-value
ESRmm/hr.	(Remission)	No.	4	0	0	0	C.C.=0.230 P=0.233 NS (No. of Pos. test)
		% ESR	66.7%	0.0%	0.0%	0.0%	
	(LDA)	No.	0	2	2	0	
		% ESR	0.0%	8.30%	66.7%	0.0%	
	(MDA)	No.	1	12	1	18	
		% ESR	16.7%	50.0%	33.3%	64.3%	
	(HDA)	No.	1	10	0	10	
		% ESR	100%	100%	0.0%	35.7%	
	C.S. (*) P-value		C.C.=0.618 P=0.000 (HS)		C.C.=0.628 P=0.000 (HS)		
ACPA IU/ml	(Remission)	No.	2	2	0	0	C.C.=0.323 P=0.278 NS (No. of Pos. test)
		% ACPA	13.3%	13.3%	0.0%	0.0%	
	(LDA)	No.	1	1	1	1	
		% ACPA	6.7%	6.7%	7.7%	5.6%	
	(MDA)	No.	7	6	7	12	
		% ACPA	46.7%	40.0%	53.8%	66.7%	
	(HDA)	No.	5	6	5	5	
		% ACPA	33.3%	40.0%	38.5%	27.8%	
	C.S. (*) P-value		C.C.=0.075 P=0.983 (NS)		C.C.=0.129 P=0.770 (NS)		

(*) NS: Non Sig. at P>0.05; HS: Highly Sig. at P<0.01; C.C.; Contingency Coefficients.
*Negative: normal ESR/ACPA according to cut off value. *Positive: abnormal ESR/ACPA according to cut off value

3.16.4 Relationship between Anti-Ro/SSA & Anti-La/ SSB and DAS-28 Score in both Diseased Groups with statistical significant

Regarding anti-Ro/SSA, results showed that no significant correlation (CC=0.431) at P>0.05.In addition to that, anti-Ro/SSA showed no significant relationships at P<0.01 in each of diseased groups by increases mainly of MDA of DAS-28 score levels, then followed by HDA level, regarding anti-La/ SSB, results showed that (CC=0.447) at P>0.05. In addition to that, anti-La/SSB showed no significant relationships at P<0.01 in each of diseased groups by increases mainly

of MDA of DAS-28 score levels, then followed by HDA level, as shown in table (3-32).

Table (3-31): Distribution of (RF) result according to DAS-28 score in both diseased groups with statistical significant.

Parameters	DAS-28 Score	Groups	RA		sSS		C.S. (*) P-value
		Resp.	Negative	Positive	Negative	Positive	
RF(-ve,+ve)	(Remission)	No.	1	3	0	0	C.C.=0.313 P=0.247 NS (No. of Pos. test)
		% RF	9.1%	15.8%	0.0%	0.0%	
	(LDA)	No.	0	2	1	1	
		% RF	0.0%	10.5%	8.3%	5.3%	
	(MDA)	No.	5	8	7	12	
		% RF	45.5%	42.1%	58.3%	63.2%	
	(HDA)	No.	5	6	4	6	
		% RF	45.5%	31.6%	33.3%	31.6%	
C.S. (*) P-value			C.C.=0.236 P=0.068 (NS)		C.C.=0.068 P=0.931 (NS)		

(*) NS: Non Sig. at P>0.05; HS: Highly Sig. at P<0.01; C.C.; Contingency Coefficients.

3.16.5 Distribution of Salivary Flow Rate and Schirmer's Test assessment according to DAS-28 Score in both diseased groups with statistical significant

According to Salivary Flow Rate and Schirmer's Test (normal, and abnormal), results showed that no relationship was found between RA & sSS according to DAS28-Result.In addition to that, salivary flow rate and schirmer's test showed no relationships between each disordered level by increases mainly by MDA of DAS-28 score level, then followed by HAD level, and that are found mostly in RA group with normal SFL, and S – test, while are found in sSS group with abnormal SFL, and S – test, as shown in table (3-33).

3.16.6 Distribution of Difficulty in Swallowing & DAS-28 Score in both Diseased Groups with statistical significant

Regarding difficulty in Swallowing, results showed that no significant correlation (CC=0.228) at P>0.05. In addition to that, Difficulty in swallowing showed no significant relationships at P<0.01 in each of diseased groups by

increases mainly of MDA of DAS-28 score levels, then followed by HDA level, as shown in table (3-34).

Table (3-32): Distribution of Anti-Ro/SSA & Anti-La/ SSB according to DAS-28 Score in both diseased groups with statistical significant

Parameters	DAS-28 Score	Groups	RA		Sss		C.S. (*)
		Resp.	Neg.	Pos.	Neg.	Pos.	P-value
Anti-Ro/SSA IU/ml	(Remission)	No.	3	1	0	0	C.C.=0.431 P=0.251 NS (No. of Pos. test)
		% SS - A(Ro)	13.6%	12.5%	0.0%	0.0%	
	(LDA)	No.	1	1	1	1	
		% SS - A(Ro)	4.5%	12.5%	4.8%	10.0%	
	(MDA)	No.	10	3	11	8	
		% SS - A(Ro)	45.5%	37.5%	52.4%	80.0%	
	(HDA)	No.	8	3	9	1	
		% SS - A(Ro)	36.4%	37.5%	42.9%	10.0%	
C.S. (*) P-value			C.C.=0.146 P=0.885 (NS)		C.C.=0.314 P=0.183 (NS)		
Anti-La/ SSB IU/ml	(Remission)	No.	4	0	0	0	C.C.=0.447 P=0.221 NS (No. of Pos. test)
		% SS - B(La)	15.4%	0.0%	0.0%	0.0%	
	(LDA)	No.	2	0	2	0	
		% SS - B(La)	7.7%	0.0%	6.9%	0.0%	
	(MDA)	No.	11	2	17	2	
		% SS - B(La)	42.3%	50%	58.6%	100%	
	(HDA)	No.	9	2	10	0	
		% SS - B(La)	34.6%	50.0%	34.5%	0.0%	
C.S. (*) P-value			C.C.=0.196 P=0.754 (NS)		C.C.=0.204 P=0.509 (NS)		

(*) NS: Non Sig. at P>0.05; C.C.; Contingency Coefficients.

3.16.7 Salivary α-Amylase & DAS-28 Score in both Diseased Groups with statistical significant

According to Salivary α-Amylase, results showed that no significant correlation (CC=0.280) at P>0.05. In addition to that, Salivary α-Amylase showed no significant relationships at P>0.05 in each of diseased groups by increases mainly of MDA of DAS-28 score levels, then followed by HDA level, as shown in table (3-35).

3.16.8 Salivary Total Protein & DAS-28 Score in both Diseased Groups with statistical significant

Regarding to salivary total protein, results showed that no significant correlation (CC=0.383) at P>0.05. In addition to that, salivary total protein showed no significant relationships at P>0.05 in each of diseased groups by increases mainly of MDA of DAS-28 score levels, then followed by HDA level, as shown in table (3-36).

Table (3-33): Distribution of Salivary Flow Rate & Schirmer's Test according to DAS-28 Score in both diseased groups with statistical significant

DAS-28 Score	Groups	RA				sSS				C.S. (*) P-value
	Probable Outcomes	*N. (SFR) – N. (S-test)	*A. (S-test) – N. (SFR)	N. (S-test) – A. (SFR)	A. (S-test) – A. (SFR)	N. (SFR) – N. (S-test)	A. (S-test) – N. (SFR)	N. (S-test) – A. (SFR)	A. (S-test) – A. (SFR)	
Remission)	No.	2	1	1	0	0	0	0	0	No Association
	% *Ass.	11.1%	14.3%	25.0%	0.0%	0.0%	0.0%	0.0%	0.0%	
(LDA)	No.	2	0	0	0	0	0	0	2	
	% Ass.	11.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	6.5%	
(MDA)	No.	8	5	0	0	0	0	0	19	
	% Ass.	44.4%	71.4%	0.0%	0.0%	0.0%	0.0%	0.0%	61.3%	
(HDA)	No.	6	1	3	1	0	0	0	10	
	% Ass.	33.3%	14.3%	75%	100%	0.0%	0.0%	0.0%	32.3%	
C.S. (*) P-value		C.C.=0.482 P=0.428 (NS)				No Association				

(*) NS: Non Sig. at P>0.05; C.C.; Contingency Coefficients. N: Normal responses; A: Abnormal responses
*N: normal, A: abnormal, SFR: salivary flow rate ml/15 minute, S-test: Schirmer's test, mm, Ass. : assessment.

3.16.9 Serum Melatonin & DAS-28 Score in both Diseased Groups with statistical significant

Regarding Serum Melatonin, the majority of the results were found within (MDA, HDA, and LDA) of DAS-28 Score in RA & sSS groups respectively, as shown in table (3-37).

Result showed that no significant differences at P>0.05 regarding Serum Melatonin & DAS-28 Score, as shown in table (3-38).

Table (3-34): Distribution of Difficulty in Swallowing according to DAS-28 Score in both diseased groups with statistical significant

Parameter	DAS-28 Score	Groups	RA		sSS		C.S. (*) P-value
		No. & %	Absent	Present	Absent	Present	
D in sw.	(Remission)	No.	2	2	0	0	C.C.=0.228 P=0.764 NS (No. of Absent test)
		% D in sw.	12.5%	14.3%	0.0%	0.0%	
	(LDA)	No.	2	0	1	1	
		% D in sw.	12.5%	0.0%	20.0%	3.8%	
	(MDA)	No.	7	6	3	16	
		% D in sw.	43.8%	42.9%	60.0%	61.5%	
	(HDA)	No.	5	6	1	9	
		% D in sw.	31.3%	42.9%	20.0%	34.6%	
C.S. (*) P-value			C.C.=0.253 P=0.563 (NS)		C.C.=0.245 P=0.372 (NS)		

(*) NS: Non Sig. at P>0.05; C.C.; Contingency Coefficients, D in sw.: difficulty in swallowing

Table (3-35): Distribution of Salivary α-Amylase according to DAS-28 Score in both diseased groups with statistical significant

Parameter	DAS-28 Score	Groups	RA		sSS		C.S. (*) P-value
		No. & %	Normal	Abnormal	Normal	Abnormal	
Sa – AM U/L	(Remission)	No.	1	3	0	0	C.C.=0.280 P=0.335 NS (No. of abnormal test)
		% Sa – AM	10.0%	15.0%	0.0%	0.0%	
	(LDA)	No.	1	1	1	1	
		% Sa – AM	10.0%	5.0%	9.1%	5.0%	
	(MDA)	No.	3	10	6	13	
		% Sa – AM	30.0%	50.0%	54.5%	65.0%	
	(HDA)	No.	5	6	4	6	
		% Sa – AM	50.0%	30.0%	36.4%	30.0%	
C.S. (*) P-value			C.C.=0.233 P=0.633 (NS)		C.C.=0.113 P=0.819 (NS)		

(*) NS: Non Sig. at P>0.05; C.C.; Contingency Coefficients.
(Normal, Abnormal): in relation to control value.
Sa – AM: salivary α-amylase.

Table (3-36): Distribution of Salivary Total Protein according to DAS-28 Score in both diseased groups with statistical significant

Parameter	DAS-28 Score	Groups	RA			sSS			C.S. (*) P-value
		Sa - Tp mg/dl	Normal	Abnormal -Lower Bound	Abnormal -Upper Bound	Normal	Abnormal -Lower Bound	Abnormal -Upper Bound	
		No. & %							
Sa – Tp mg/dl	Remission	No.	1	2	1	0	0	0	C.C.=0.383 P=0.173 NS (No. of Pos. test)
		% Sa-Tp	10%	14%	17%	0.0%	0.0%	0.0%	
	(LDA)	No.	0	1	1	2	0	0	
		% Sa-Tp	0.0%	7%	17%	13%	0.0%	0.0%	
	(MDA)	No.	4	7	2	8	9	2	
		% Sa-Tp	40%	50%	33%	50%	75%	67%	
	(HDA)	No.	5	4	2	6	3	1	
		% Sa-Tp	50%	29%	33%	38%	25%	33%	
C.S. (*) P-value			C.C.=0.291 P=0.836 (NS)			C.C.=0.294 P=0.571 (NS)			

(*) NS: Non Sig. at P>0.05; C.C.; Contingency Coefficients.
(Normal, and Abnormal -Lower Bound, and Abnormal -Upper Bound):in relation to control group value.
Sa-Tp: salivary total protein.

Table (3-37): Distribution of Serum Melatonin according to DAS-28 Score in both diseased groups with statistical significant

Groups	Parameters	DAS-28 Score.	No.	M pg/ml	S.D.	S.E.	95% C.I. for Mean		Min.	Max.
							L.B.	U.B.		
RA	SMLT pg/ml	(Remission)	4	18.2	10.2	5.1	2.0	34.4	6.9	31.6
		(LDA)	2	29.0	16.6	11.7	0.0	178	17.3	40.7
		(MDA)	13	80.6	135.1	37.5	0.0	162	0.6	496
		(HDA)	11	44.8	35.7	10.8	20.8	68.8	6.9	118
Sss	SMLT pg/ml	(LDA)	2	98.1	74.6	52.8	0.0	769	45.3	151
		(MDA)	19	68.0	118.7	27.2	10.8	125	6.9	514
		(HDA)	10	75.5	87.3	27.6	13.1	138	0.6	300

SMLT: serum melatonin

Table (3-38) Pair wise Comparisons by (GH, and LSD) tests concerning Serum Melatonin & DAS-28 Score.

Groups	Parameters	Group (I)	Group (J)	Mean Diff. (I-J)	Sig. (*)	95% C.I. for Diff.		GHD & LSD
						L.B.	U.B.	
RA	SMLT	(Remission)	(LDA)	-10.8	0.896	-179	157	168
			(MDA)	-62.4	0.259	-174	49	111
			(HDA)	-26.6	0.634	-140	87	113
		(LDA)	(MDA)	-51.6	0.479	-199	96	148
			(HDA)	-15.8	0.830	-165	134	149
		(MDA)	(HDA)	35.8	0.363	-43.8	115.4	79.6
sSS	SMLT	(LDA)	(MDA)	30.1	0.711	-135	195	165
			(HDA)	22.6	0.790	-149	194	172
		(MDA)	(HDA)	-7.5	0.860	-94.1	79.1	86.6

(*) **HS: Highly Sig. at P<0.01; NS: Non Sig. at P>0.05; Testing based on Games Howell Difference (GHD), and Least Significant Difference (LSD) test**

3.16.10 Salivary Melatonin & DAS-28 Score in both Diseased Groups with statistical significant

Regarding Salivary Melatonin, the majority of the results were found within (MDA, HDA, and LDA) of DAS-28 Score in RA & sSS groups respectively, as shown in table (3-39).

Result showed that no significant differences at P>0.05 regarding Salivary Melatonin & DAS-28 Score, as shown in table (3-40).

Table (3-39): Distribution of Salivary Melatonin according to DAS-28 Score in both diseased groups with statistical significant

Groups	Parameters	DAS-28 Score.	No.	M	S.D.	S.E.	95% C.I. for Mean		Min.	Max.
							L.B.	U.B.		
RA	SAMLT pg/ml	(Remission)	4	6.9	1.0	0.5	5.3	8.5	6.0	7.8
		(LDA)	2	9.7	2.7	1.9	0.0	34.4	7.8	11.7
		(MDA)	13	13.1	12.6	3.5	5.5	20.7	3.1	53.6
		(HDA)	11	10.2	4.1	1.2	7.4	13.0	6.0	20.9
sSS	SAMLT pg/ml	(LDA)	2	16.3	6.5	4.6	0.0	74.8	11.7	20.9
		(MDA)	19	10.8	4.5	1.0	8.6	13.0	6.0	23.3
		(HDA)	10	10.0	3.6	1.1	7.4	12.6	6.0	16.0

SAMLT:salivary melatoin.

Table (3-40) Pair wise Comparisons by (GH, and LSD) tests concerning Salivary Melatonin & DAS-28 Score.

Groups	Parameters	Group (I)	Group (J)	Mean Diff. (I-J)	Sig. (*)	95% C.I. for Diff.		GHD & LSD
						L.B.	U.B.	
RA	SAMLT	(Remission)	(LDA)	-2.8	0.720	-18.8	13.1	16.0
			(MDA)	-6.2	0.239	-16.7	4.4	10.5
			(HDA)	-3.3	0.532	-14.1	7.4	10.8
		(LDA)	(MDA)	-3.4	0.625	-17.4	10.6	14.0
			(HDA)	-0.5	0.942	-14.7	13.7	14.2
		(MDA)	(HDA)	2.9	0.443	-4.7	10.4	7.5
sSS	SAMLT	(LDA)	(MDA)	5.5	0.101	-1.1	12.1	6.6
			(HDA)	6.3	0.071	-0.6	13.2	6.9
		(MDA)	(HDA)	0.8	0.627	-2.6	4.3	3.5

(*) **HS: Highly Sig. at P<0.01; NS: Non Sig. at P>0.05; Testing based on Games Howell Difference (GHD), and Least Significant Difference (LSD) test**

3.16.11 Serum Nitric Oxide & DAS-28 Score in both Diseased Groups with statistical significant

Regarding Serum Nitric oxide, the great numbers of the results were found within (MDA, HDA, and LDA) of DAS-28 Score in RA & sSS groups respectively, as shown in table (3-41).

Result showed a significant differences at P<0.05 in RA group between (Remission – LDA), as well as between (LDA and MDA, HDA) regarding serum nitric oxide & DAS-28 Score, as shown in table (3-42), so there is decreasing in serum nitric oxide with increasing DAS-28 Score in RA group.

3.16.12 Salivary Nitric Oxide & DAS-28 Score with statistical significant

Regarding salivary nitric oxide, the great numbers of the results were found within (MDA, HDA, and LDA) of DAS-28 Score in RA & sSS groups respectively, as shown in table (3-43) & figure (3-12).

Result showed a no significant differences at P>0.05 regarding salivary nitric oxide & DAS-28 Score, despite that, there were meaningful results should be mentioned between (remission –HDA), (LDA-HAD), (remission –MDA) in

RA group & between (LDA-MDA), (MDA-HDA) in sSS group as shown in table (3-44), so there were increasing in salivary nitric oxide with increasing DAS-28 Score in RA group.

Table (3-41): Distribution of Serum Nitric oxide in in both diseased groups according to DAS-28 Score groups with statistical significant

Groups	Parameters	DAS-28 Score.	No.	M	S.D.	S.E.	95% C.I. for Mean		Min.	Max.
							L.B.	U.B.		
RA	SNO (umol/L)	(Remission)	4	113	74	36.9	0.0	231	39.9	206
		(LDA)	2	386	254	180	0.0	2671	206	566
		(MDA)	13	105	144	40.0	17.8	192	16.7	566
		(HDA)	11	80.1	55.4	16.7	42.9	117	30.0	206
sSS	SNO (umol/L)	(LDA)	2	73.5	15.7	11.1	0.0	215	62.4	84.6
		(MDA)	19	157	167	38.3	76.4	237	22.0	566
		(HDA)	10	78.4	41.3	13.1	48.9	108	16.5	159

SNO :serum nitric oxide .

Table (3-42) Pair wise Comparisons by (GH, and LSD) tests concerning Salivary Melatonin & DAS-28 Score

Groups	Parameters	Group (I)	Group (J)	Mean Diff. (I-J)	Sig. (*)	95% C.I. for Diff.		GHD & LSD
						L.B.	U.B.	
	SNO	(Remission)	(LDA)	-273	0.013	-483	-63	210
			(MDA)	8.3	0.903	-130	147	139
			(HDA)	33.1	0.634	-108	175	141
		(LDA)	(MDA)	281	0.004	97.2	465	184
			(HDA)	306	0.002	119.7	492	186
		(MDA)	(HDA)	24.8	0.612	-74.5	124	99
	SNO	(LDA)	(MDA)	-83.3	0.119	-185	18	101
			(HDA)	-4.9	0.956	-62.0	52.2	57.1
		(MDA)	(HDA)	78.3	0.152	-23.3	180	102

(*) HS: Highly Sig. at P<0.01; NS: Non Sig. at P>0.05; Testing based on Games Howell Difference (GHD), and Least Significant Difference (LSD) test

Table (3-43): Salivary Nitric oxide in the studied groups distributed according to DAS-28 Score level

Groups	Parameters	DAS-28 Score	No.	M	S.D.	S.E.	95% C.I. for Mean		Min.	Max.
							L.B.	U.B.		
RA	SANO (umol/L)	(Remission)	4	2.9	0.4	0.2	2.2	3.6	2.5	3.5
		(LDA)	2	3.3	0.0	0.0	3.3	3.3	3.3	3.3
		(MDA)	13	6.4	7.6	2.1	1.8	10.9	0.9	24.7
		(HDA)	11	7.4	7.4	2.2	2.5	12.4	2.0	21.8
sSS	SANO (umol/L)	(LDA)	2	19.6	24.3	17.2	0.0	238	2.4	36.7
		(MDA)	19	8.2	10.1	2.3	3.3	13.1	0.9	39.6
		(HDA)	10	12.7	15.2	4.8	1.8	23.5	0.9	47.4

SANO: salivary nitric oxide

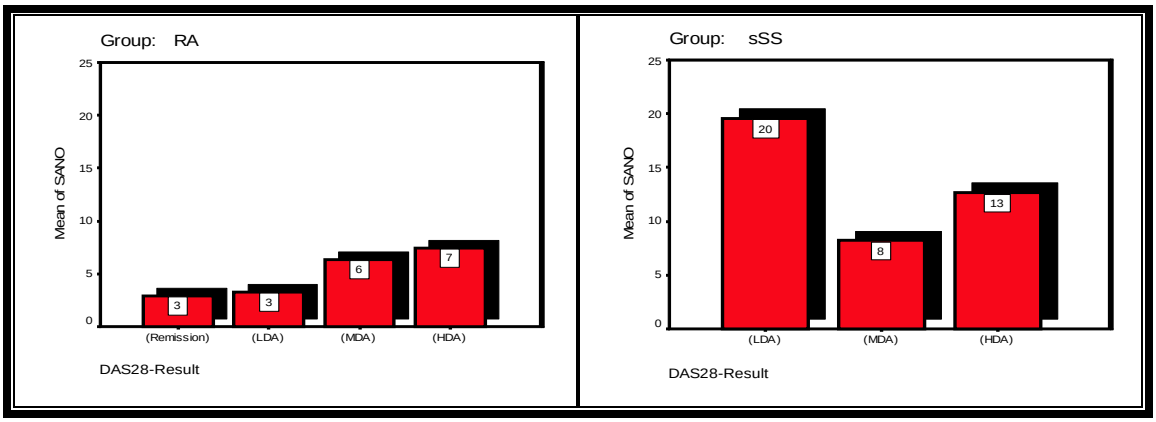

Figure (3-12):- Salivary Nitric oxide in both diseased groups distributed according to DAS- 28 Score level

Table (3-44): Pair wise Comparisons by (GH, and LSD) tests concerning Salivary Niric Oxide & DAS-28 Score

Groups	Parameters	Group (I)	Group (J)	Mean Diff. (I-J)	Sig. (*)	95% C.I. for Diff.		GHD & LSD
						L.B.	U.B.	
RA	SANO	(Remission)	(LDA)	-0.4	0.479	-1.4	0.7	1.1
			(MDA)	-3.4	0.398	-9.7	2.8	6.3
			(HDA)	-4.5	0.241	-11.4	2.3	6.8
		(LDA)	(MDA)	-3.1	0.483	-9.3	3.2	6.2
			(HDA)	-4.2	0.297	-11.0	2.6	6.8
		(MDA)	(HDA)	-1.1	0.984	-9.6	7.4	8.5
sSS	SANO	(LDA)	(MDA)	11.4	0.239	-8.0	30.7	19.3
			(HDA)	6.9	0.490	-13.3	27.0	20.2
		(MDA)	(HDA)	-4.5	0.376	-14.6	5.7	10.2

(*) **HS: Highly Sig. at P<0.01; NS: Non Sig. at P>0.05; Testing based on Games Howell Difference (GHD), and Least Significant Difference (LSD) test**

3.17 Person's Correlation Coefficients of DAS-28 Score in Relation to (Erythrocytes Sedimentation Rate, Anti-citrullinated Protein Antibody, Rheumatoid Factor, Anti-Ro/SSA, Anti-La/SSB, and Duration of the Disease), in RA & sSS Diseased Group and their Statistical significant.

Results showed a significant relation at P<0.01 between DAS-28 score & ESR in the sSS group, while no significant relation at P>0.05 were found between others parameters & DAS-28 score. In addition to that, a significant relation at P<0.05 were found among others parameters, except duration periods, which showed no relationships at P>0.05 in sSS group, as shown in table (3-45).

While in RA group results showed a significant relation at P<0.01 between DAS-28 score & ESR, also no significant relation at P>0.05 were found between others parameters & DAS-28 score. Additionally results showed a significant relation at P<0.05 were found among others parameters, except duration periods, which showed no relationships at P>0.05, as shown in table (3-45).

3.18Salivary Flow Rate in relation to (Serum Melatonin, Salivary Melatonin, Serum Nitric Oxide, and Salivary Nitric Oxide) in two Diseased Groups.

Regarding normal salivary flow rate, results showed significant positive relation at P<0.05 with salivary melatonin as well as results showed positive and highly significant relationship at P<0.01 between abnormal salivary flow rate and salivary melatonin, in RA group, as shown in table (3-46).

While in sSS diseased group, results showed significant positive relationship at P>0.05 between salivary α-amylase & abnormal salivary flow rate with, as shown in table (3-46).

Table (3-45): Pearson's Correlation Coefficients among DAS-28 Score in Relation to (ESR, ACPA, RF, Anti-Ro/SSA, Anti-La/SSB, and Duration of the Disease), in RA & sSS diseased group

Groups	Pearson Correlation Coefficient		ESR	ACCP	RF	SS - A(Ro)	SS - B(La)	Dur. /m
RA	DAS-28 Score	Corr. Coeff.	0.563	0.036	-0.212	-0.061	-0.035	-0.031
		Sig. (2-tailed)	0.001**	0.851	0.261	0.749	0.854	0.872
	ESR	Corr. Coeff.		-0.290	-0.100	-0.243	-0.175	0.159
		Sig. (2-tailed)		0.120	0.599	0.197	0.356	0.402
	ACPA	Corr. Coeff.			0.644**	0.781**	0.759**	-0.121
		Sig. (2-tailed)			0.000	0.000	0.000	0.525
	RF	Corr. Coeff.				0.704**	0.692**	-0.270
		Sig. (2-tailed)				0.000	0.000	0.149
	Anti-Ro/SSA	Corr. Coeff.					0.832**	-0.169
		Sig. (2-tailed)					0.000	0.372
	Anti-La/SSB	Corr. Coeff.						-0.003
		Sig. (2-tailed)						0.986
sSS	DAS 28-Result	Corr. Coeff.	0.401*	0.002	0.074	-0.248	-0.045	-0.142
		Sig. (2-tailed)	0.025*	0.990	0.692	0.178	0.809	0.445
	ESR	Corr. Coeff.		0.313	0.371*	0.177	0.230	-0.155
		Sig. (2-tailed)		0.086	0.040	0.340	0.213	0.404
	ACPA	Corr. Coeff.			0.667**	0.513**	0.543**	-0.044
		Sig. (2-tailed)			0.000	0.003	0.002	0.812
	RF	Corr. Coeff.				0.493**	0.497**	-0.163
		Sig. (2-tailed)				0.005	0.004	0.380
	Anti-Ro/SSA	Corr. Coeff.					0.692**	0.022
		Sig. (2-tailed)					0.000	0.904
	Anti-La/SSB	Corr. Coeff.						0.056
		Sig. (2-tailed)						0.765
** Correlation is significant at the .01 level (2-tailed) –Highly Sig. (HS)								
* Correlation is significant at the .05 level (2-tailed) – Sig. (S)								

Table (3-46): Pearson's Correlation Coefficients among SFR (normal and abnormal readings) with (SMLT, SAMLT, SNO, SANO, Sa–AM, and Sa–Tp) in two diseased groups with statistical significant

Parameters	Pearson Correlation Coefficient P-value	RA		sSS	
		Normal SFR ml/minute	Abnormal SFR ml/minute	Normal SFR ml/minute	Abnormal SFR ml/minute
SMLT	Pearson Correlation	0.484	0.181	-	0.165
	Sig. (2-tailed)	0.331	0.398		0.376
SAMLT	Pearson Correlation	0.908	0.782	-	-0.055
	Sig. (2-tailed)	0.012*	0.000**		0.769
SNO	Pearson Correlation	-0.675	-0.129	-	0.089
	Sig. (2-tailed)	0.141	0.549		0.636
SANO	Pearson Correlation	-0.338	-0.193	-	-0.090
	Sig. (2-tailed)	0.513	0.366		0.631
Sa – AM	Pearson Correlation	-0.314	-0.128	-	0.355
	Sig. (2-tailed)	0.545	0.551		0.0499*
Sa – Tp	Pearson Correlation	-0.621	0.002	-	-0.140
	Sig. (2-tailed)	0.188	0.991		0.452

*** Correlation is significant at the .05 level (2-tailed) – * Sig. (S); ** Sig. (HS)**

Chapter Four

Discussion

4. Discussion

Sjogren syndrome is a common autoimmune disease but till now it remains under diagnosed disorder that has a significant influence on oral health. Oral practitioners should be the first health care supplier recognizing the early stages. Therefore, oral practitioners must be aware about the manifestation of disease and be trained to have an important role in diagnosis, management and treatment of disease complication in the oral cavity related to the disease. Secondary Sjogren syndrome that associated with RA should be managed by a team consisting of rheumatologist, an ophthalmologist, and oral Medicine specialist (Sobhanam *et al*., 2015).

The current study evaluates the influence both RA & sSS on salivary (melatonin, nitric oxide, α-amylase, total protein) and salivary flow rate regarding composition & function. Also, this study analyze the level of serum melatonin& nitric oxide, disease duration, DAS-28 score in addition to Anti-Ro/SSA, Anti-La/SSB, ACPA, RF, ESR & study the possibility of the effect of these parameters on the occurrence of sSS. In addition, the study focused on probable relationships among these variables. Additionally, studying of these relationships may help to understand the mechanisms behind different oral pathologies associated with sSS. Finally, involving of oral and ocular exocrine gland in RA patients or any other connective tissue disease received a limited interest compared with primary SS, which may lead to miss-diagnosis of sSS. Many researchers evaluate the composition of saliva in patients suffering from xerostomia in ordered to ameliorate the xerostomic state of those patients.

4.1 Demographically

The results reveal that sSS patients & RA patients were mainly female with the majority at (40 – 49) years and at (60 – 70) years for RA, and sSS respectively. This result agreed with both (Glick, 2015 & dos Santos *et al*.,

2016) who found that sSS& RA patients were registered in old age groups with female predilection.

The increased prevalence of autoimmune disease in female explained by Whitacre, in (2001) that this difference is related to the differences in basic immune responses between males and females & most of these indexes obtained from studies accomplished in rodents. Female mice produce antibodies more than male mice and showed more vigorous activation of T-cell than male mice after immunization. Similarly, in humans, responses to vaccination were tested and then yielded mixed results; with either no differences shown or an increased antibody response in females. The higher absolute numbers of CD4+ lymphocytes in female relative to male might participate in increased responses. Also, under immunization conditions, higher production of T-helper 1 cytokines was seen in females compared to male. Finally, cytokine secretion may be enhanced in vitro in the presence of estrogen, observed most prominently with interferon-g (IFN-g), interleukin 1 (IL-1) and interleukin-10 and decreased in the presence of androgens interferon-g, interleukin-4 and interleukin-5 (Whitacre , 2001).

4.2 Duration of the Disease

The results showed a significant difference in disease duration between RA & sSS which is illustrated in ROC curve. This lead to the suggestion that secondary SS occurrence related to disease duration. This results in line with study done by He *et al.,* (2013); which proposed that patients with sSS have old age & long duration of the disease than those with RA only &/or pSS. On the other hand studies done by (Uhlig *et al.*, 1999; Antero *et al*., 2011& Abdelghani *et al*., 2015); found that no relation between the duration of the disease & sSS occurrence. The variations in the results of these studies may be related to the size of the sample, ethnic &/or genetic background.

4.3 Erythrocyte Sedimentation Rate between two Diseased Groups

The observations in this study reveal that there were no significant differences in ESR parameter between RA & sSS, even though in sSS group increased by one fifth percent compared with RA group.

Additionally, the observation seen in ROC curve reveals that sSS group showed area under the curve > RA group but not reach the significant level. So this need further studies to explain this relation. Also, Oliveira *et al*., (2015) found in his study, on sjogren syndrome secondary to RA that no differences between RA & sSS regarding ESR.

These findings may be due to the fact that all patients in both RA and sSS groups were receiving at least first-line treatment such as nonsteroidal anti-inflammatory, disease-modifying agents& corticosteroid, this could possibly have a role in the lack of difference.

Secondary Sjogren syndrome could be answerable for changes in immunoglobulin, especially the activation of polyclonal B-cell leading to chronic hypergammaglobulinemia and increased levels of β2-microglobulinemia which in turn affect the ESR be higher in those patients. Also, ESR value might also be influenced by various factors such as the levels of immunoglobulin, age, gender and abnormal size or shape of red blood cells Fox *et al.,* (2000). Finally, the present study diseased groups was predominated by females & ESR normal values for females are greater than males and increasing with age, these factors could be possibly have influenced the results of this study.

4.4 Anticitrulinated Peptide Antibody between two Diseased Groups

The present study showed that there were no significant differences observed between RA & sSS groups regarding ACPA, but most of sSS patients reveal higher ACPA value than RA patients, sSS group increased by one third percent compared with RA group, our findings are in line with Gonzalez-Lopez

et al., (2014) & Oliveira *et al*., (2015) who found that there were no association between ACPA & extra-articular manifestation of RA.

While Haye *et al*., 2013 suggested a significant association between ACPA &extra-articular manifestation of RA patients.

The variation in results of above studies regarding ACCP might be related to the treatment modality especially MTX & Anti-TNF therapy that may lead to decrease ACCP titer in diseased group's patients(Mikuls *et al*., 2004; Roland *et al*., 2008 & Hajiabbasi *et al*., 2016).

4.5 Anti-Ro/SSA between two Diseased Groups

The present study showed that no significant differences regarding Anti-Ro/SSA between RA & sSS groups. Additionally, regarding Anti-Ro/SSA parameter, most of RA readings are recorded normal values, while sSS group are recorded in average abnormal value, similar results were reported by Harley *et al*., 1986 who found that Anti-Ro/SSA and Anti-La/SSB autoantibody were relatively less sensitive test for sSS. Also, Hajiabbasi *et al*., (2016) did not found a significant difference in prevalence of positive autoantibodies (Anti-Ro/SSA & Anti-La/SSB) between RA patients with and without sSS.

In contrast to the finding of the present study He *et al*., (2013) found statistical differences in Anti-Ro/SSA between RA & sSS. While Abdelghani *et al*., (2015) studied the immunological pattern of sSS with regard to RA & found that there were unusual Anti-Ro/SSA / Anti-La/SSB absence in sSS .

According to this finding the (Anti-Ro/SSA) had no role in occurrence of sSS, opposite to their role in primary type of the disease as mentioned earlier.

4.6 Anti-La/SSB between two Diseased Groups

The observation of the present study revealed that no significant difference was found regarding Anti-La/SSB between RA & sSS groups. Occasionally, regarding Anti-La/SSB parameter, most of RA patients were with normal values, while sSS group are recorded in average abnormal value, similar

results were reported by Harley *et al*., (1986) found that Anti-Ro/SSA and Anti-La/SSB autoantibody were relatively less sensitive test for sSS. Also Hajiabbasi *et al*., (2016) did not found significant difference in prevalence of positive autoantibodies Anti-Ro/SSA and Anti-La/SSB between RA patients with and without sSS. On the other hand He *et al*., (2013) found statistical differences in Anti-La/SSB between RA & sSS.

While Abdelghani *et al*., (2015) studied the immunological pattern of RA related SS with regard to RA suggested that Anti-Ro/SSA and Anti-La/SSB were unusually absence in sSS .

According to the present findings (Anti-La/SSB) had no role in sSS, opposite to their role in primary type of the disease as mentioned earlier.

4.7 Rheumatoid Factor (RF) between two Diseased Groups

In accordance to RF the present study showed that there were no significant differences between RA & sSS groups. Also, there were no differences in the prevalence of positive RF in RA & sSS patients. Opposite to this finding, Oliveira *et al*., (2015) found that larger number of patients with RA/SS had RF positive than patients with RA but statistically non significant. So it has been concluded that RF is not relevant to evaluate the presence of sSS. On the other hand, Tincani *et al*., (2012) explained that RA patients with high titers of rheumatoid factor were more likely to developed secondary Sjogren syndrome. Additionally, He *et al*., (2013) showed that there were statistical differences in RF between RA & sSS.

Interestingly, there was a decrease in RF titers clearly during treatment with infliximab De Rycke *et al*., (2005) & the patients in the present study under different types of treatment including infliximab. In a study conducted on RA patients, Chen *et al.,*(2006) found that after three months of etanercept treatment there was a significant decrease in the levels of ACPA and RF in the sera of rheumatoid patients.

Also, variation in results of above studies regarding RF might be related to the treatment modality especially methotrixate & Anti-TNF therapy that may lead to decrease RF titer in diseased groups' patients Mikuls *et al*., (2004); Roland *et al*., (2008) & Hajiabbasi *et al*., (2016).

4.8 Serum Melatonin (SMLT) between two Diseased Groups

The present study showed no significant differences in serum melatonin between the two diseased groups & control, also RA, and sSS groups recorded low levels comparing with control group, rather than sSS group increased by one third percent in light of RA group. On the same line, a study done by West & Oosthuizen, (1992) found that melatonin levels were decreased in RA patients compared with controls. On the other hand, Maestroni *et al*., (2005) & Baykal *et al*., (2013) suggested that significantly higher concentrations of SMLT in RA patients than in controls. Additionally, according to ROC curve sSS group had receiver characteristic operation curve more than obtained in RA group, but not reach significant level in SMLT. This may create an idea about the effect of SMLT on occurrence of sSS but all potential effects, advantages, and risks of MLT have not been ascertained yet. Further studies are needed to determine the possible role of MLT in patients with RA & /or Sss.

4.9 Salivary Melatonin (SAMLT) between two Diseased Groups

Despite the finding in this study regarding salivary melatonin that no significant differences throughout probable pair wise comparisons of equal means, between RA, sSS & control groups, but RA, and sSS of diseased groups recorded low levels compared with control group, as well as simply different had recorded between two diseased groups. Up to our knowledge, there is no study considering salivary melatonin in patients with sSS in order to compare it with the results of the present study.

However, a study conducted by Hamdan *et al*., (2016) who found that SAMLT level in patients with lichen planus lower than controls, so these results

in addition to our results advocated by the previous findings of studies done by de la Rocha *et al*., (2007) & Tan *et al*., (2007) which revealed the protective function of melatonin via its anti-inflammatory or anti-oxidant and free radical scavenger & through immunomodulatory activity of this hormone.

4.10 Serum Nitric Oxide (SNO) between two Diseased Groups

The present study showed no significant differences in SNO & showed that RA group showed slight decreased either compared with sSS, or with controlled groups. In contrast, Miletic & co-workers (2012) found that there was an increased IL-17 and nitric oxide production in patients with pSS, especially, in those associated with elevated antinuclear antibody &rheumatoid factor values. Also, Malone, (2012) observed increased nitric oxide in the exhaled breath of Sjogren sufferer so he considered that nitric oxide has noxious effect upon the function of salivary & lacrimal glands. While {Mahmoud & Ismail, (2011); Ali & his colleagues (2014)} proved an increased serum nitric oxide level in RA patients when compared with controls.

These variations in the above mentioned studies regarding SNO may be related to the fact that patients on treatment may showed decrease in SNO level due to the effects of therapy which might be indirectly inhibited the production of endogenous nitric oxide, probably through suppression of the immune system. This comes in line with Nakamura *et al*., (2000) they believed that actarit "disease modifying anti-rheumatic drug" treatment ameliorate disease activity in early phase RA by repressing serum nitric oxide levels & they suggested that nitric oxide might considered as a valuable marker for monitoring improvement in the early stages of RA. Also steroid are able to inhibit nitric oxide production both inducible nitric oxide & macrophage producing nitric oxide {Di Rosa *et al*., (1990); Radomski *et al*., (1990)}.

4.11 Salivary Nitric Oxide (SANO) between two Diseased Groups

The results of this study showed that there was a highly significant differences & low level of SANO i.e (between two diseased groups) in contrasts to controlled group. Konttinen *et al.,* (1997) study is not in line with the current study; the study suggested that in patients with pSS there was higher concentration of nitric oxide and inducible nitric oxide synthesase in the salivary gland acini in these patients despite the hypo-function state of their salivary glands. While Xia *et al*., (2003) agreed with the present finding, as they found that SS patients showed decreased concentration in SANO in comparable with control group. The present findings of decreased nitric oxide concentration in the saliva of RA patients is not in line with the results of Weinberg *et al.,* (2006) who reported that basal and stimulated salivary nitrate concentrations were not different between healthy controls and patients with RA, also not in accordance with a study done by Dervisevic, (2012) & co-worker who evaluated that the concentration of nitric oxide in the saliva of patients with RA was statistically significant & higher than the concentration of nitric oxide in the saliva of healthy subjects.

These fluctuant in SANO concentration between this study & the above mentioned studies might reflect the state of immune suppression of those patients due to treatment strategy, or might be due to the state of salivary hypo function which could be associated with significant changes of nitrate and nitrite levels in the saliva.

4.12 Salivary α-amylase (Sa - AM) between two Diseased Groups

The current study revealed that there is a significant difference in concentration of Sa– AM between sSS disordered group and controlled, so the control group showed increase Sa – AM concentration than sSS group. while no significant differences in concentration of Sa – AM between neither (RA & control) group nor (RA & sSS), additionally RA group registered low

concentration compared to control. This study agreed with Kim *et al*., (2013) who found that there was no difference in salivary α-amylase levels between the RA and the control group. However, Abdulla *et al*., (2016) found a significant decrease in level of amylase in RA patients when compared to control. Subsequently, (Greabu *et al.*, (2009) & Malamud, (2011) suggested a decreased in levels of salivary amylase and carbonic anhydrase in SS patients. This finding perhaps suggests that in the initial inflammatory stage the secretary epithelium is stimulated by the cellular inflammation or by lymphokines to produce more substances. Alternatively, the increased levels in patients with mild inflammation can be attributed to the fact that the metaplastic epithelial cells that replace the normal ductal cells in these patients are not capable of reabsorbing of these substances effectively. In the late inflammatory stage, however, the decrease of the salivary constituents can be attributed to the significant decrease of the secretary epithelium or to the replacement of these cells by non-functional collagen tissue (Tsianos *et al*., 1985).This decrease in salivary α-amylase may reveal the low oral defense mechanism in sSS patients owing to decrease important salivary composition.

4.13 Salivary Total protein (Sa - TP) between two Diseased Groups

Data of the present study showed that no significant differences in **Sa- TP** between the two diseased groups & control group. However, controlled and sSS diseased group were recorded similar mean values, while RA group had increased almost by ten percent in light of others. On the other hand Helenius *et al.,* (2005) mentioned that there was an increase in salivary total protein concentration in patients with RA & sSS than control, while, Abdulla *et al*., (2016) found a decrease in level of salivary total protein in RA patients compared with control, and there was lower level of salivary total protein in patients with hypo-salivation. In contrast to these findings, Reijden *et al.,*

(1996) showed that absolute concentrations of total protein were increased significantly in both primary and secondary Sjögren's syndrome.

These differences in those finding might be due to different methodology in measuring SA-TP &/or deterioration in immune regulation.

This finding probably suggests that the secretary epithelium in the initial inflammatory stage is stimulated by the cellular inflammation or by Lymphokines so produce more substances. While in patients with mild inflammation the increased levels can be related to the fact that the metaplastic epithelial cells that replace the normal ductal cells in those patients not able to reabsorb those substances sufficiently. Alternatively, during the late inflammatory stage, though, decreasing of the salivary constituents can be related to the significant decrease of the secretary epithelium or to the replacement of these cells by non-functional collagen tissue (Tsianos *et al.*, 1985).

4.14 Salivary flow rate (SFR) between two Diseased Groups

The observation of our study reveals that there were highly significant differences in salivary flow rate between RA & sSS groups, since; RA group had normal SFR ml/15minute concerning average value, while sSS group had recorded abnormal value.

The low salivary flow rate in SS may be resulted from irreversible damage of the salivary acini by lymphocytic infiltrating leading to subsequent fibrosis & the low unstimulated salivary flow rate is well established in both pSS & sSS, although in the early state of the disease may not be obvious.

According to these observations whole SFR might be considered as a clinical predictor for occurrence of sSS, so this attribute can easily obtained by dentists in their clinics so the dentist should take a role in assisting those patients with undiagnosed xerostomia.

4.15 Medication Used for the Treatments

In the present study, regarding types of treatmet, etanercept reveal highly significant relationship between sSS & RA patients

Sankar *et al*., (2004) found that etanercept (enbrile) at a dosage of 25 mg twice weekly for 12 weeks not certified to be clinically efficacious in SS; at the same time, one of the patients with sSS with RA who was treated with etanercept, showed considerable improvement in her arthritis, while no significant change in the exocrine measure. Chen *et al*., (2006) showed regarding the therapeutic action of etanercept on RA patients, after three months of etanercept treatment, there was a significant decrease in the serum levels of ACPA and RF of rheumatoid patients.

Etanercept (enbrile) as a biological agent is one of the tumor necrosis factor TNF blocker considered to be clinically efficacious in treatment of RA.

The biologic agents radically changed the treatment of RA because of their essential benefits on the signs and symptoms, & also their capability to impede the radiographic alteration concerning joint damage (Alyce *et al*., 2008).

These differences may be due to different treatment strategies of each rheumatologist & the availability of each medication, the treatment in this study is primarily targeting RA patients despite their state of sSS.

Different types of medications are frequently used in combination as a multidrug regimen to get ideal remission in disease activity for the treated patient.

4.16 Disease Activity

Regarding disease activity using DAS-28 score the results of the present study showed that the occurrence of sSS was not affected by the disease activity. This agrees with previous studies done by Antero *et al*., (2011); Zakeri *et al*., (2013) & Abdelghani *et al*., (2015). On the other hand, Uhlig *e. al.*,

(1999); Wolfe *et al.*, (2008) & He *et al.*, (2013) found that the occurrences of sSS is higher who experienced higher disease activity.

There were many variations between studies regarding relationship between sSS & disease activity, however one of the suggestions is related to the variability in treatment modality such as, steroids or nonsteroidal anti-inflammatory medications, disease modifying Rheumatoid arthritis drugs that modified the disease severity, secondly even though DAS28-ESR equation is quantitative measure but some portion of it depending on how patient bearing pain so it considered as subjective criteria, this in turn affect the reliability of DAS-28 formula in deciding RA severity .

4.16.1Erythrocyte Sedimentation Rate & Anti-citrulinated Peptide Antibody in relation to DAS-28 score

Concerning Erythrocyte Sedimentation Rate the present study showed that even though no significant relationship had been reported in respect to positive outcomes of each DAS-28 score between the studied disordered groups, the ESR parameter shows highly significant relationships in each of disordered groups by increases mainly of moderate disease activity of DAS-28 score levels, and then followed by high disease activity level. So the Erythrocyte Sedimentation Rate – DAS-28 score relationship had no effect on the occurrence of sSS.

Up to our knowledge, this study is the first study that conducted on the occurrence of sSS in relation to (Erythrocyte Sedimentation Rate - DAS-28 score) relation.

However to understand how ESR increases in the two diseased groups Fox *et al.,* (2000) described that sSS could be answerable for immunoglobulin changing, particularly, in activation of polyclonal B-cell leading to chronic hypergammaglobulinemia and increased levels of β2-microglobulinemia which in turn modify the ESR being increased in those patients. Moreover, ESR can also be

modified by many factors as gender, age, immunoglobulin levels and abnormal shape or size of red blood cells.

Additionally, due to the fact that the study group was prevail by females & the ESR normal values for females are greater than males and increasing with age, these factors could possibly have influenced the results.

On comparison between RA & sSS groups regarding Anti-Citrulinated Peptide Antibody in relation to DAS-28 score between the studied diseased groups, the current results showed no significant relationship. In addition to that, no significant relationships at P>0.05 for each of the disordered groups by increases mainly of moderate disease activity of DAS-28 score levels, then followed by high disease activity level. So, no detected relationship between (DAS-28 score -ACPA) & the occurrence of sSS.

Up to our knowledge, this study is the first study that conducted on the occurrence of sSS in relation to (ACCP- DAS-28 score). On the other hand, Mancarella *et al*., (2007) demonstrated that in biologically treated patients (anti-TNF), there is a reduction in a significant way of ACPA, RF with remission of the disease. While Pereira *et al*., (2014) found indirect relation between DAS28, RF and ACPA in their research on biologically treated RA patients with anti-TNF.

4.16.2 Rheumatoid factor in relation to DAS28-score

The present study showed that no significant relationship at P>0.05 was found regarding RF in relation to DAS-28 score between the studied diseased groups. In addition to that, no significant relationships at P>0.05 for each of the diseased groups by increases mainly of moderate disease activity of DAS-28 score levels, and then followed by high disease activity level.

Up to our knowledge, this study is the first study that conducted on the occurrence of sSS in relation to (RF- DAS-28 score) relation.

In a study conducted on RA patients Chen *et al.,* (2006) found that after three months of etanercept treatment there was a decrease in the serum levels of ACPA and RF, significantly in the rheumatoid patients.

On the other hand, Mancarella *et al.,* (2007) found that in biologically treated patients with anti-TNF, there was a significant reduction in ACPA, RF with remission of the disease. While Pereira *et al.,* (2014) found indirect relation between DAS-28 score, RF and ACPA in their research on biologically treated RA patients with anti-TNF.

4.16.3 Anti-(Ro), Anti-(La) in relation to DAS-28 score

The present study showed that no significant relations between DAS-28 score & both Anti-Ro/SSA & Anti-La/ SSB in two diseased groups. In addition to that, no significant relationships in each of the disordered groups by increasing mainly of moderate disease activity of DAS-28 score levels, and then followed by high disease activity level. So according to these findings the Anti-Ro/SSA, Anti-La/SSB couldn't use as a marker of disease activity & there was no previous study to compare their results with this study.

4.16.4 Salivary flow rate & Schirmer's, test assessment according to DAS-28 score

The current study reveals that with respect to salivary flow rate, and Schirmer's test (normal and abnormal) no relationship was found concerning abnormal outcomes & DAS-28 score. In addition to that, no relationships between each disordered groups by increasing mainly in moderate disease activity of DAS-28 score levels, then followed by high disease activity level, and found mostly in RA group with normal SFR, and Schirmer's test, while in sSS group is found with abnormal SFR and Schirmer's test.

These finding agreed with Zakeri *et al.,* (2013) who found that there was no relationship between dry eye syndrome & severity of RA.

On the other hand, reduction in tear or saliva production is considered to be a frequent extra-articular manifestation in patients with RA & could be

related to disease activity and health status, but not to duration of the disease and number of deformed joints Uhlig *et al.*,(1999). Also, Gilboe *et al*., in (2001) suggested that RA patients with dry eye syndrome revealed high disease activity and there was a relation between ESR, dry eye and number of painful joints. While Wolfe & Michaud, (2008) showed that RA patients appearing with more common sicca symptoms & the sicca symptoms in those patients showed a higher disease activity score, disability & pain.

One of the possible reasons is utilizing various types of treatments such as steroids or nonsteroidal anti-inflammatory medications that can modify disease severity, which is considering one of the shortcomings in the present study. Secondly, although, DAS-28 score-ESR equation is quantitatively but portion of this equation depending on how individual patient bearing pain considering to be subjective criteria. This in turn decreases the reliability of this equation in deciding RA severity.

4.16.5 Difficulty in swallowing & DAS-28 score

The present study found that there is no significant relationship regarding difficulty in swallowing in each DAS-28 score, between two diseased groups. Also, difficulty in swallowing showed no significant relationships in each diseased group by increasing mainly in moderate disease activity of DAS-28 score level, and then followed by high disease activity level.

Up to our knowledge, there was no previous study to compare with it. But from practical point of view, saliva provided fundamental role in preserving oropharyngeal health and it has many functions in the "oral and gastrointestinal environment. Saliva helps in oral cleansing, swallowing speech, taste and digestion. At the point when salivary hypofunction and xerostomia happen, transient and long standing oral and extra oral upset can appear also saliva has essential role in preparing food for digestion and deglutition. The difficulty in mastication and swallowing, particularly dry foods, observed in patients with low

salivary flow, so these patients may need sips of fluid to swallow food. As a result this might lead to change in patients dietary habits (food and fluid selection) that may affect the nutritional status**,** so the oral health status should be evaluated in those patients to prevent further deterioration.

4.16.6 Salivary α-Amylase & DAS-28 score

The results of the present study showed that no significant relationship was found related to abnormal salivary α-Amylase in each DAS-28 score, which observed between diseased groups. In addition to that, redistribution of the salivary α-Amylase (normal and abnormal) value, showed no significant relationships in each diseased group by increasing mainly in moderate disease activity of DAS-28 score level, and then followed by high disease activity level. Matthews *et al*., (1985); Kim *et al*., (2013) noticed that salivary α-amylase concentration recorded no difference when comparing with RA patients, possibly due to the method of measurement of amylase. Pedersen *et al.,* (2005) also established that the amylase activity in the whole saliva or parotid saliva has no differences between other autoimmune disease as primary SS & healthy controls.

Up to our knowledge, there was no previous study to compare with it. But the salivary α-amylase is considered a stress measure marker & in the same time Kim *et al*., (2013) demonstrated in their study on Rheumatoid arthritis patients that stress measure is not related to disease activity.

4.16.7 Salivary Total Protein & DAS-28 score

Regarding Salivary total protein the results revealed that no significant relationship found in each DAS-28 score, between two diseased groups. In addition to that, Salivary total protein showed no significant relationships at P>0.05in each disordered groups by increasing mainly in moderate disease activity of DAS-28 score level, and then followed by high disease activity level.

Up to our knowledge, there was no previous study to compare with this study. However Abdulla *et al*., (2016) found a decrease in the level of total salivary protein among RA patients compared to control, and suggested that patients with hyposalivation were with lower level of total salivary protein.

4.16.8 Serum Melatonin & DAS28-Result

In the present study, there was no significant differences at P>0.05 found at each DAS-28 score between two diseased groups .In addition to that, the vast majority of results were found within (moderate disease activity, high disease activity & low disease activity) of DAS-28 score in RA & sSS groups respectively.

Up to our knowledge there was no previous study to compare with this study. Even though, Senel *et al*., (2011) stated that melatonin level related to Bath Ankylosing Spondolytis disease activity index (BASDAI) but not to ESR; suggesting the possible role for this immunoregulatory hormone in the disease activity in Ankylosing Spondolytis patients. Regarding autoimmune disease, till now the role of melatonin not certified & need further studies to confirm anti-inflammatory & atni-oxidant role or the proinflammatory & promoting role.

4.16.9 Salivary Melatonin & DAS-28 score

The present study showed that there was no significant differences at P>0.05 were found at each DAS-28 score between two diseased groups, in addition to that, the vast majority of results were found within (moderate disease activity, high disease activity & low disease activity) of DAS-28 score in RA & sSS groups, respectively.

Also the most meaningful results to be recorded in Salivary melatonin regarding RA group between (remission–moderate disease activity), (moderate disease activity - high disease activity) & between (low disease activity - high disease activity), (low disease activity - moderate disease activity) in sSS group.

Though non significant relationship was found, this result suggested that there was a decreasing in salivary melatonin with increasing DAS-28 score in the sSS group. So salivary melatonin had negative relation with DAS-28 score in the sSS group so could be used as a marker of DAS-28 score in future, but this needs further studies.

At the same time, up to our knowledge there was no previous study to compare this study. Alternatively, Reiter *et al*., (2015) explained that the salivary melatonin levels reached maximum values at night owing to its circadian rhythm. In oral cavity melatonin cells, exhibit both receptor-independent& receptor-mediated actions. The secretion of melatonin into saliva occurs through acinar cells of the major salivary glands and through the gingival fluid. Additionally, melatonin in the oral cavity has both antioxidant activities & anti-inflammatory action.

4.16.10 Serum Nitric Oxide & DAS-28 score

The present study showed that no significant differences at $P>0.05$ were found at each DAS-28 score in sSS group.

While in RA group the results showed a significant differences at $P<0.05$ between Remission- low disease activity, as well as between low disease activity and moderate disease activity, high disease activity of DAS-28 score level. So there were a negative relation between SNO & DAS-28 score at RA group, these negative relations between SNO & disease activity may reflect one of the abnormalities in immune regulation. Similarly, a significant relation between serum nitrate concentrations and, DAS-28 score has been also detected by Onur *et al*., (2001). While Khan & Siddiqui, (2006) suggested that reactive nitrogen species may be produced within an inflamed joints of RA patients and that their levels correlate directly with the disease activity.

These results are not in line with the studies done by Al-Nimer *et al*., (2010); Mahmud & Ismail, (2011) who found that there were non-significant

correlation between the duration of disease or ESR as a marker of disease activity and the serum levels of each of nitrogen species.

The differences between these studies may be related to the differences in patient's tolerance to pain which is considered as subjective criteria on DAS-ESR formula. So this again affects the accuracy of DAS-28 score formula in determining the severity of RA.

4.16.11 Salivary Nitric Oxide & DAS-28 score

The present study revealed that, there was no significant difference at $P>0.05$ were found at each DAS-28 score between two diseased groups. In addition to that, the vast majority of the results were found within (moderate disease activity, high disease activity, & low disease activity) of DAS28 results in RA, & sSS respectively.

Despite non-significant relationship, there were meaningful results that should be mentioned between remission–high disease activity, low disease activity - high disease activity, remission – moderate disease activity in RA group & between low disease activity - moderate disease activity, moderate disease activity - high disease activity in sSS group.

Interestingly, the observed differences in SANO between different stages of disease activity in RA group. Despite that, the above mentioned differences were not statistically significant. These results suggestively revealed increasing in saliva concentration of NO in patients with RA related to increase disease activity score in those patients. This comes in line with study done by (Dervisevic *et al*., 2012) who showed that the concentration of salivary NO may possibly be used as useful biochemical marker for estimation the disease activity in patients with RA. These changes in concentration of SANO may reveal one of the many oddity regulations of immunity.

4.17Correlations between (Erythrocytes Sedimentation Rate, Anti-citrullinated Protein Antibody, Rheumatoid Factor, Anti-Ro/SSA, Anti-La/SSB, and Duration of the Disease) & DAS-28 result in the two Diseased Groups

The present study showed that there was a significant correlation between DAS-28 score & ESR, ACPA & (RF, Anti-Ro, Anti-La), RF & (Anti-Ro , Anti-La), Anti-Ro & Anti-La in both RA & sSS groups, while there was a significant correlation between ESR & RF only in sSS group.

Harley *et al.*, (1986) suggested that in patients with primary Sjogren's syndrome there was an association of RF titer with concentration of anti-Ro (SS-A) and anti-La (SS-B) .

Up to our knowledge, no previous study included all of these parameters to compare with.

4.18 Salivary flow rate in relation to (Serum Melatonin, Salivary Melatonin, Serum Nitric Oxide, Salivary Nitric Oxide, salivary α-amylase & salivary total protein) in two Diseased Groups

The current study found that there was a positive significant correlation between salivary melatonin & normal salivary flow rate in RA group. In addition to that, there was highly significant correlation at $P<0.01$ between abnormal salivary flow rate and salivary melatonin. While regarding sSS group salivary α-amylase showed a significant positive correlation at $P>0.05$ in abnormal salivary flow rate.

Melatonin has a role in the improvement oral health. Since it affect the protein composition of the saliva in rat Aras & Ekström, (2008) and stimulates salivary gland protein synthesis, so it may be of benefit in the treatment of xerostomia. Also, a recent animals study on parotid rat suggested that exogenous melatonin evokes the *in vivo* secretion of protein and amylase from the rat parotid gland (Cevik-Aras *et al*., 2011).

So further studies are needed to explain the role of melatonin & alpha-amylase in improving salivary flow rate.

Chapter Five

Conclusions & Suggestions

Conclusions & Suggestions

5.1 Conclusions

1-The incidence of secondary Sjogren syndrome in Rheumatoid arthritis patients is dependent on the duration of the disease & independent on disease activity of Rheumatoid arthritis

2-Disease activity is positively related to ESR value, while negative relation between Disease activity & (Serum nitric oxide salivary nitric oxide) in Rheumatoid arthritis group & with Salivary melatonin in secondary Sjogren syndrome.

3-Disease activity does not related to (ACPA, Rheumatoid factor, Anti-(Ro), Anti-(La), salivary α-Amylase, and Salivary total protein Serum melatonin) value.

4- Sicca symptoms not related to disease activity in both Rheumatoid arthritis & secondary Sjogren syndrome patients.

5- The occurrence of secondary Sjogren syndrome in Rheumatoid arthritis patients is affected by (ESR & ACCP) & not affected by (Anti-Ro/SSA, Anti-La/SSB, Rheumatoid factor)

6- The occurrence of sSS is affected by (serum melatonin, salivary α-amylase) & not affected by (salivary melatonin, serum nitric oxide, salivary nitric oxide& total salivary protein).

7- Positive relation between (ACCP, Anti-Ro/SSA, Anti-La/SSB, RF) in the two diseased groups(RA,sSS) while positive relation between (ESR & RF) in sSS group only.

8- The whole salivary flow rate can utilized by the dentist as a clinical predictor for diagnosis of secondary Sjogren syndrome &the state of hyposalivation directly related to the salivary α-amylase in sSS & salivary melatonin in rheumatoid arthritis .

5.2 Suggestions

1- Evaluate the effect of treatment interruption on the occurrence of secondary Sjogren syndrome.

2-Study the effect of melatonin in treatment of xerostomia.

3-Study the hormonal effects on the occurrence of secondary Sjogren syndrome.

4-Compare the histopathological finding in salivary gland in secondary Sjogren syndrome & rheumatoidarthritis with hyposalivation.

5-Study the effects of micro-RNA on the occurrence of secondary Sjogren syndrome.

Recommendation

Dry mouth problems have a clinically significant deleterious impact on oropharyngeal health so the important role of dental practitioner to diagnose dry mouth disorders in their patients and provide preventive and interventional treatments to reduce the impact of these disorders on patients ’s quality of life.

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Appendices

Appendix

Appendix I
American European Consensus Group Criteria (AECC)

I. Ocular symptoms: a positive response to at least one of the following questions:
1. Have you had daily, persistent, troublesome dry eyes for more than 3 months?
2. Do you have a recurrent sensation of sand or gravel in the eyes?
3. Do you use tear substitutes more than 3 times a day?

II. Oral symptoms: a positive response to at least one of the following questions:
1. Have you had a daily feeling of dry mouth for more than3 months?
2. Have you had recurrently or persistently swollen salivary glands as an adult?
3. Do you frequently drink liquids to aid in swallowing dry food?

III. Ocular signs: a positive result for at least one of the following two tests:
1. Schirmer I test, performed without anesthesia ≤5 mm in 5 minutes)
2. Rose bengala score or other ocular dye score (≥4 on the van Bijstervled scale)

IV. Histopathology: In minor salivary glands (obtained through normal-appearing mucosa) focal lymphocytic sialadenitis, evaluated by an expert histopathologist, with a focus score ≥1, defi ned as a number of lymphocytic foci (which are adjacent to normal-appearing mucous acini and contain more than 50 lymphocytes) per 4 mm2 of glandular tissue.

V. Salivary gland involvement: a positive result for at least one
of the following tests:
1. Unstimulated whole salivary fl ow ≤1.5 mL in 15 minutes)
2. Parotid sialography showing the presence of diffuse sialectasis (punctate, cavitary, or destructive pattern), without evidence of major duct obstruction
3. Salivary scintigraphy showing delayed uptake, reduced concentration, and/or delayed excretion of tracer

VI. Autoantibodies: presence in the serum of the following:
1. Antibodies to Ro(SS-A) or La(SS-B) antigens, or both

Rules for Classifi cation
For *primary SS*: In patients without any potentially associateddisease:
a. Presence of any 4 of the 6 items indicates pSS as long aseither item IV (histopathology) or VI (serology) is positive
b. Presence of any 3 of the 4 objective criteria items (i.e.,items III, IV, V, VI)
c. The classifi cation tree procedure (best used in clinical–epidemiological surveys)

For *secondary SS*: patients with a potentially associated disease(e.g., another well-defi ned connective tissue disease), the presenceof item I or item II plus any 2 from among items III, IV and V.

Exclusion criteria: Past head and neck radiation treatment; hepatitis C infection; acquired immunodefi ciency syndrome(AIDS); preexisting lymphoma; sarcoidosis; graft-vs.-host disease; use of anticholinergic drugs (since a time shorter than fourfold the half-life of the drug)

SOURCE: From Vitali C, Bombardieri, Jonsson R, et al. Ann Rheum Dis2002;61:544–558, by permission of *Annals of the Rheumatic Diseases*.a Rose bengal has now been replaced by lissamine green for this test.

Appendix

Appendix II

Questionnaire

Patient name : date:

Age: occupation:

gender:

marital state:

phone no.:

address:

medical history: dental history:

Disease duration (yrs):

Salivary flow rate:

Schirmers test:

Salivary gland enlargement:

Difficult in swallowing:

DAS score:

Type of treatments:

Appendix

موافقة للإشتراك في البحث العلمي

اسم الباحث:
عنوان البحث:
مكان إجراء البحث:

أنت مدعو(ة) للمشاركة ببحث علمي سريري سيجرى في ----------.
الرجاء أن تأخذ(ي) الوقت الكافي لقراءة المعلومات التالية بتأن قبل أن تقرر(ي) إذا كنت تريد(ين) المشاركة أم لا. بإمكانك طلب إيضاحات أو معلومات إضافية عن أي شيء مذكور في هذه الاستمارة أو عن هذه الدراسة ككل من طبيبك.

في حال وافقت على المشاركة في هذه الدراسة، سيبقى اسمك طي الكتمان . لن يكون لأي شخص، ما لم ينص القانون على ذلك، حق الاطلاع على ملفك الطبي باستثناء الطبيب المسؤول عن الدراسة ومعاونيه.

موافقة المشترك:

لقد قرأت استمارة القبول هذه وفهمت مضمونها. تمت الإجابة على أسئلتي جميعها. وبناء عليه فأنني، حرا مختارا، أجيز إجراء هذا البحث و أوافق على الإشتراك فيه، وإني أعلم ان الباحث الدكتور _____ وزملاءه ومعاونيه او مساعديه سيكونون مستعدين للإجابة على أسئلتي، وأنه باستطاعتي الإتصال بهم على الهاتف _____ واذا شعرت لاحقا ان الأجوبة تحتاج الى مزيد من الإيضاح فسوف أتصل بأحد اعضاء لجنة الأخلاقيات. كما أعرف تمام المعرفة بانني حر في الإنسحاب من هذا البحث متى شئت حتى بعد التوقيع على الموافقة دون ان يؤثر ذلك على العناية الطبية المقدمة لي.

إسم المشترك:

توقيع المشترك:

Appendix III

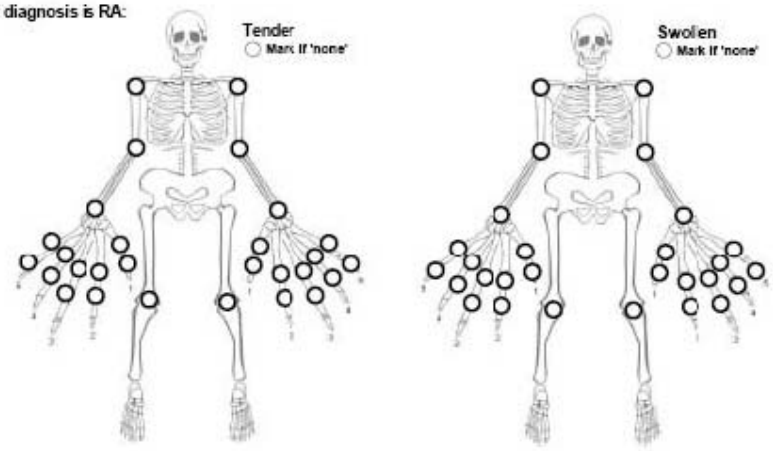
Disease Activity Score in 28 Joints (DAS28)

Patient global assessment
Considering all the ways in which illness and health may affect you at this time, please indicate below how you are doing:

VERY WELL ├──────────────────────────────────────┤ VERY POORLY

FOR PROVIDER USE ONLY

If diagnosis is RA:



VAS (0-100) ☐

28TJC ☐

28SJC ☐

ESR ☐

DAS28 ☐

DAS28=0.56*√(28TJC) + 0.28 * √(28SJC) + 0.70*Ln(ESR/CRP) + 0.014*VAS

How to calculate a DAS28 score:

1. Ask the patient to make a vertical mark on a 100 mm Visual Analog Scale (VAS) corresponding to their general health or global disease activity. Using a ruler, measure from the left-hand side in mm. Note: DAS28 calculations may be performed without a VAS measurement.
2. Perform a swollen and tender joint examination on your patient. Add all of the swollen and tender joints and record the totals in the appropriate boxes.
3. Erythrocyte Sedimentation Rate (ESR) should be measured (in mm/hour). Note: C-reactive protein (CRP) levels may be used as a substitute for an ESR.
4. Plug the appropriate values into the formula (many online calculators are available including http://www.das-score.nl/www.das-score.nl/dasculators.html).
5. If using CRP instead of ESR or calculating a score from only 3 variables please see http://www.reuma-nijmegen.nl/www.das-score.nl/ for the appropriate formula.

Interpretation:

- The DAS28 provides you with a number on a scale from 0 to 10 indicating current RA disease activity.
- Remission: DAS28 ≤ 2.6
- Low Disease activity: 2.6 < DAS28 ≤ 3.2
- Moderate Disease Activity: 3.2 < DAS28 ≤ 5.1
- High Disease Activity: DAS28 >5.1

Adapted from: DAS-Score.nl. Available at http://www.das-score.nl/www.das-score.nl/index.html. Accessed April 15, 2010.

Appendix

Appendix IV

ACPA test (AESKU.DIAGNOSTICS)

Product Ref.3166

Assay procedure:
Preparations prior to pipetting
Dilute concentrated reagents:
Dilute the concentrated sample buffer 1:5 with distilled water (e.g. 20 ml plus 80 ml).
Dilute the concentrated wash buffer 1:50 with distilled water (e.g. 20 ml plus 980 ml).

Samples:
Dilute serum samples 1:101 with sample buffer (1x)
e.g. 1000 µl sample buffer (1x) + 10 µl serum. Mix well!

Washing:
Prepare 20 ml of diluted wash buffer (1x) per 8 wells or 200 ml for 96 wells
e.g. 4 ml concentrate plus 196 ml distilled water.

Automated washing:
Consider excess volumes required for setting up the instrument and dead volume of robot pipette.

Manual washing:
Discard liquid from wells by inverting the plate. Knock the microwell frame with wells downside vigorously on clean adsorbent paper. Pipette 300 µl of diluted wash buffer into each well, waitfor 20 seconds. Repeat the whole procedure twice again.

Microplates:
Calculate the number of wells required for the test. Remove unused wells from the frame, replace and store in the provided plastic bag, together with desiccant, seal tightly (2-8°C/35-46°F).
-Pipette 100 µl of each patient‘s diluted serum into the designated microwells.
- Pipette 100 µl calibrators OR cut-off calibrator and negative and positive controls into the designated wells.
- Incubate for 30 minutes at 20-32°C/68-89.6°F.
- Wash 3x with 300 µl washing buffer (diluted 1:50).
- Pipette 100 µl conjugate into each well.
- Incubate for 30 minutes at 20-32°C/68-89.6°F.
- Wash 3x with 300 µl washing buffer (diluted 1:50).
- Pipette 100 µl TMB substrate into each well.
- Incubate for 30 minutes at 20-32°C/68-89.6°F, protected from intense light.
- Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.
- Incubate 5 minutes minimum.
- Agitate plate carefully for 5 sec.
- Read absorbance at 450 nm (optionally 450/620 nm) within 30 minutes.

Appendix

Appendix V

Anti –La/SSB test (www.orgentec.com)
ORG 509

Preparation of the reagent:

Preparation of sample buffer

Dilute the contents of each vial of the sample buffer concentrate (5x) with distilled or deionized water to a final volume of 100 ml prior to use. Store refrigerated: stable at 2-8 °C for at least 30 days after preparation or until the expiration date printed on the label.

Preparation of wash solution

Dilute the contents of each vial of the buffered wash solution concentrate (50x) with distilled or deionized water to a final volume of 1000 ml prior to use. Store refrigerated: stable at 2-8 °C for at least 30 days after preparation or until the expiration date printed on the label.

Sample preparation

Dilute all patient samples 1:100 with sample buffer before assay. Therefore combine 10 µl of sample with 990 µl of sample buffer in a polystyrene tube.Mix well. Controls are ready to use and need not be diluted.

Test procedure

1. Prepare a sufficient number of microplate modules to accommodate controls and prediluted patient samples.
2. Pipet **100 µl** of calibrators, controls and prediluted patient samples in duplicate into the wells.
3. Incubate for 30 minutes at room temperature (20-28 °C).
4. Discard the contents of the microwells and wash 3 times with **300 µl** of wash solution.
5. Dispense **100 µl** of enzyme conjugate into each well.
6. Incubate for 15 minutes at room temperature.
7. Discard the contents of the microwells and wash 3 times with **300 µl** of wash solution.
8. Dispense **100 µl** of TMB substrate solution into each well.
9. Incubate for 15 minutes at room temperature.
10. Add **100 µl** of stop solution to each well of the modules and incubate for 5 minutes at
11. Read the optical density at 450 nm and calculate the results. Bi-chromatic measurement with a reference at 600-690 nm is recommended.

The developed colour is stable for at least 30 minutes. Read optical densities during this time.

Appendix

Appendix VI
Anti – Ro/SSA test (www.orgentec.com)
ORG 508
Preparation of the reagents:
Preparation of sample buffer
Dilute the contents of each vial of the sample buffer concentrate (5x) with distilled or deionized water to a final volume of 100 ml prior to use. Store refrigerated: stable at 2-8 °C for at least 30 days after preparation or until the expiration date printed on the label.

Preparation of wash solution
Dilute the contents of each vial of the buffered wash solution concentrate (50x) with distilled or deionized water to a final volume of 1000 ml prior to use. Store refrigerated: stable at 2-8 °C for at least 30 days after preparation or until the expiration date printed on the label.

Sample preparation
Dilute all patient samples 1:100 with sample buffer before assay. Therefore combine 10 µl of sample with 990 µl of sample buffer in a polystyrene tube.Mix well. Controls are ready to use and need not be diluted.

Test procedure :
1. Prepare a sufficient number of microplate modules to accommodate controls and prediluted patient samples.
2. Pipet **100 µl** of calibrators, controls and prediluted patient samples in duplicate into the wells.
3. Incubate for 30 minutes at room temperature (20-28 °C).
4. Discard the contents of the microwells and wash 3 times with **300 µl** of wash solution.
5. Dispense **100 µl** of enzyme conjugate into each well.
6. Incubate for 15 minutes at room temperature.
7. Discard the contents of the microwells and wash 3 times with **300 µl** of wash solution.
8. Dispense **100 µl** of TMB substrate solution into each well.
9. Incubate for 15 minutes at room temperature.
10. Add **100 µl** of stop solution to each well of the modules and incubate for 5 minutes at room temperature.
11. Read the optical density at 450 nm and calculate the results. Bi-chromatic measurement with a reference at 600-690 nm is recommended.
The developed colour is stable for at least 30 minutes. Read optical densities during this time.

Appendix

Appendix VII

Reumatoid factor test (RF-Latex)

SPINREACT –SPAIN (Slide agglutination)

Samples

Fresh serum. Stable 7 days at 2-8ºC or 3 months at –20ºC.

Samples with presence of fibrin should be centrifuged before testing. Do not use highly haemolized or lipemic samples.

Procedure

Qualitative method

1. Allow the reagents and samples to reach room temperature. The sensitivity of the test may be reduced at low temperatures.

2. Place 50 µL of the sample and one drop of each Positive and Negative controls into separate circles on the slide test.

3. Mix the RF-latex reagent rigorously or on a vortex mixer before using and add one drop (50 µL) next to the sample to be tested.

4. Mix the drops with a stirrer, spreading them over the entire surface of the circle. Use different stirrers for each sample.

5. Place the slide on a mechanical rotator at 80-100 r.p.m. for 2 minutes. False positive results could appear if the test is read later than two minutes.

Readings & interpretation

Examine macroscopically the presence or absence of visible agglutination immediately after removing the slide from the rotator.

The presence of agglutination indicates a RF concentration equal or greater than 8 IU/mL .

Appendix VIII

ESR Evaluation (Laboratory .com)

Requirement

- Anticoagulated blood (0.4 ml of 3.13% trisodium citrate solution + 1.6 ml blood)
- Westergren tube
- Westergren stand
- Rubber bulb (sucker)

PROCEDURE:

1. Mix the anticoagulated blood thoroughly.
2. Draw the blood into the tube upto 0 mark with the help of rubber bulb.
3. Wipe out blood from bottom of the tube with cotton.
4. Set the tube upright in stand. Make sure the pipette fits snugly to eliminate possible leakage and that the pipette is in vertical position.
5. Leave the tube undisturbed for 1 hour.
6. At the end of 1 hour, read the result.

Appendix

Appendix IX

Salivary & Serum Melatonin (MLT) test (www.usbio.net)

Melatonin BioAssay ELISA Kit (Human) catalog No:196822

Reagent Preparation:

1-Bring all kit components & samples to room temp. before use.

2-Wash solution :dilute 10 ml. of wash solution concentrate ,100x with 990ml of deionized or distilled water to prepare 1000ml of wash solution 1x.

Test procedure:

1. Secure the desired numbers of coated wells in the holder then add 50µl of Standards or samples to the appropriate well in the antibody pre-coated microtiter plate .Add 50µl of PBS (pH 7.0-7.2) in the blank control well.

2. Dispense 5 µl of Balance solution into 50µl specimens, mix well.(This step only in case of saliva samples)

3. Add 100 µl of Conjugate to each well(Not blak control well).Mix well,(mixing well in this step is important)

Cover & incubate the plate for 1 hr. at 37°C.

4-Wash the microtiter plate using Automated washing :wash plate 5 times with diluted wash solution (350-400 µl/well/wash) using an autowasher.It is recommended that the washer be set for a soaking time of 10 sec.& shaking time of 5 sec. between each wash.

5- Dry the plate by holding the side of the plate frame firmly & blot the plate dry into absorbent paper until no moisture appears.

6-Add 50 µl Substrate A &50 µl Substrate B to each well including blank control well. Subsequently cover & incubate for (10-15) min. at room temp. (avoid sun light)

7-Add 50 µl of Stop solution to to each well including blank control well. Mix well.

8- Determine the Optical Density at 450 nm using a microplate reader immediately.

Appendix

Appendix X

Salivary & Serum Total Nitric Oxide test (www.usbio.net)
Total Nitric Oxide BioAssay ELISA Kit (Human) catalog No: 205262

Reagent Preparation :

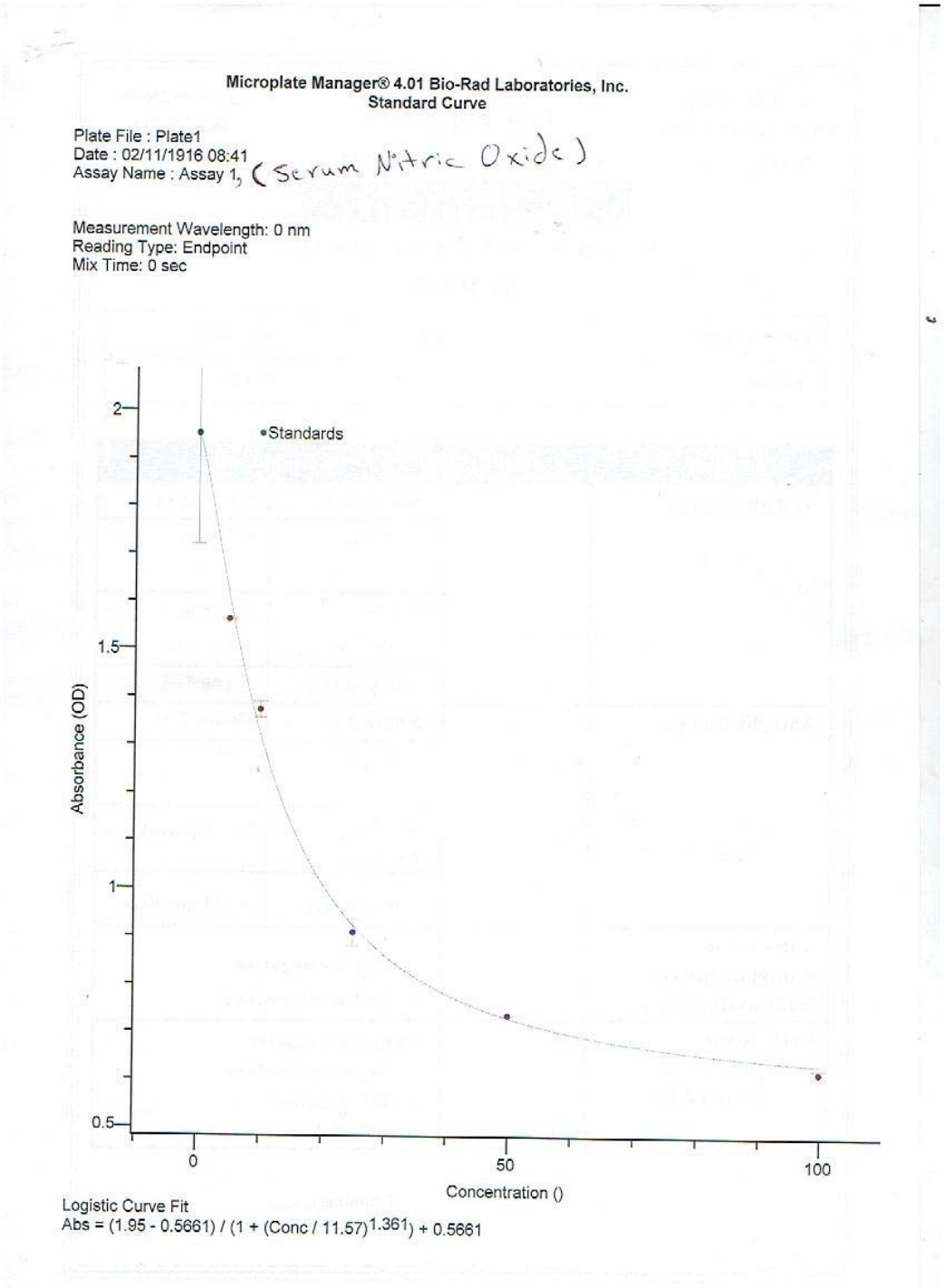
1-Bring all kit components & samples to room temp. before use.
2-Wash solution :dilute 10 ml. of wash solution concentrate ,100x with 990ml of deionized or distilled water to prepare 1000ml of wash solution 1x.

Test procedure:

1.Secure the desired numbers of coated wells in the holder then add 100µl of Standards or samples to the appropriate well in the antibody pre-coated microtiter plate .Add 100µl of PBS (pH 7.0-7.2) in the blank control well.
2. Dispense 10 µl of Balance solution into 100µl specimens, mix well.(This step only in case of saliva samples)
3.Add 50 µl of Conjugate to each well(Not blak control well).Mix well,(mixing well in this step is important)
Cover & incubate the plate for 1 hr. at 37°C.
4-Wash the microtiter plate using Automated washing :wash plate 5 times with diluted wash solution (350-400 µl/well/wash) using an autowasher.It is recommended that the washer be set for a soaking time of 10 sec.& shaking time of 5 sec. between each wash.
5- Dry the plate by holding the side of the plate frame firmly & blot the plate dry into absorbent paper until no moisture appears.
6-Add 50 µl Substrate A &50 µl Substrate B to each well including blank control well. Subsequently cover & incubate for (10-15) min. at room temp. (avoid sun light)
7-Add 50 µl of Stop solution to to each well including blank control well. Mix well.
8- Determine the Optical Density at 450 nm using a microplate reader immediately.

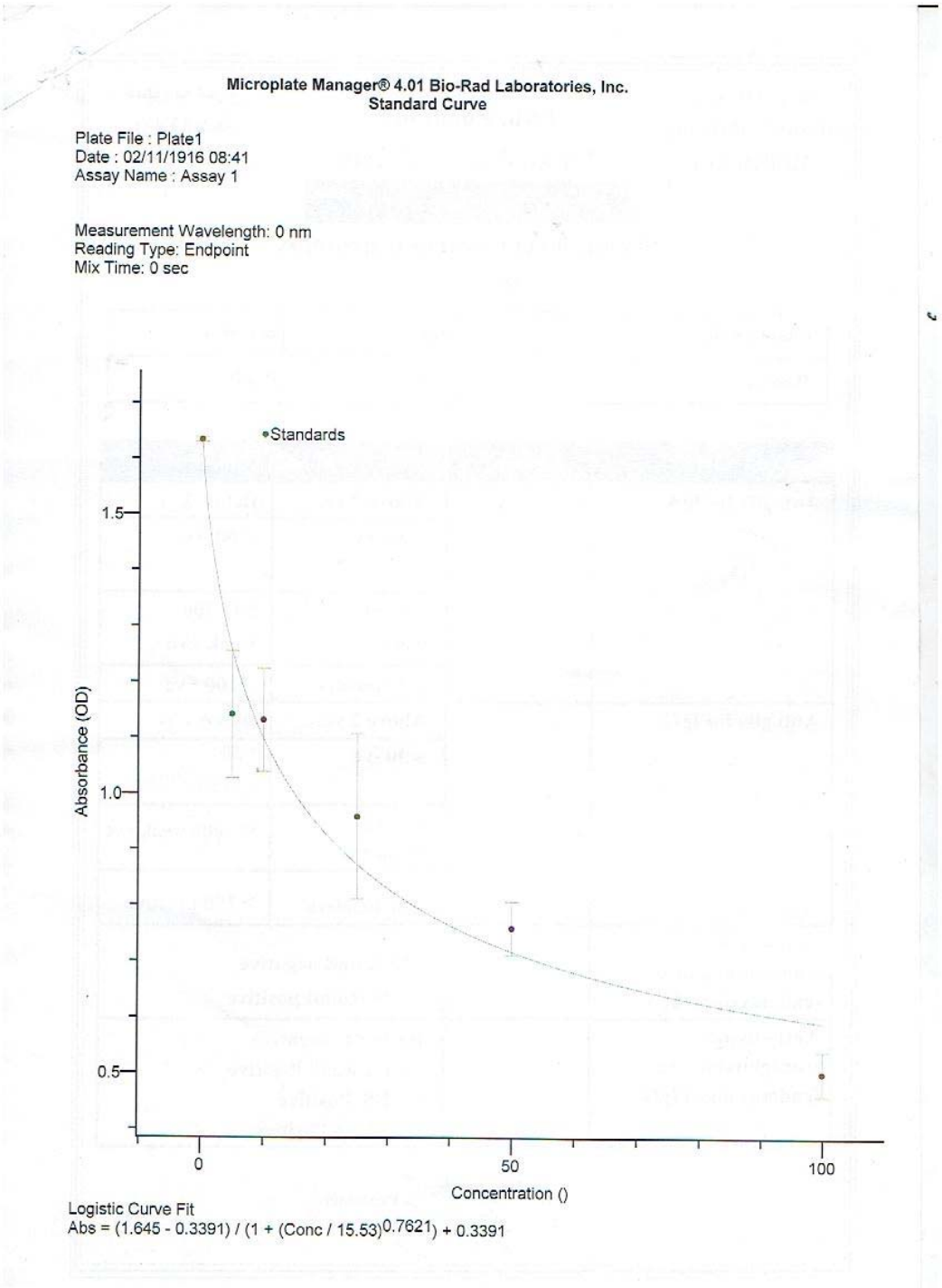
Appendix XI

Serum Nitric oxide graph



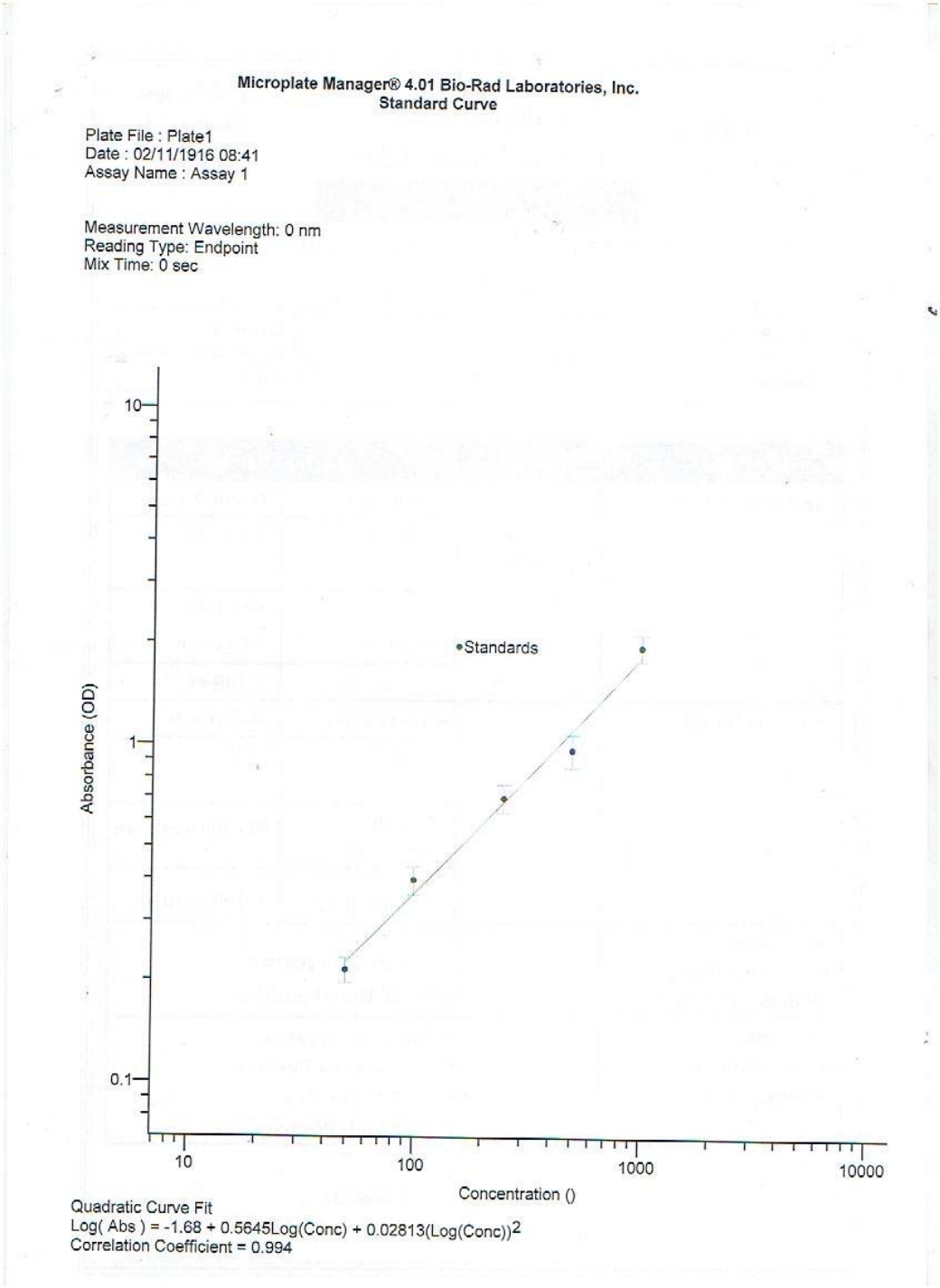
Appendix XI

Salivary Nitric oxide graph



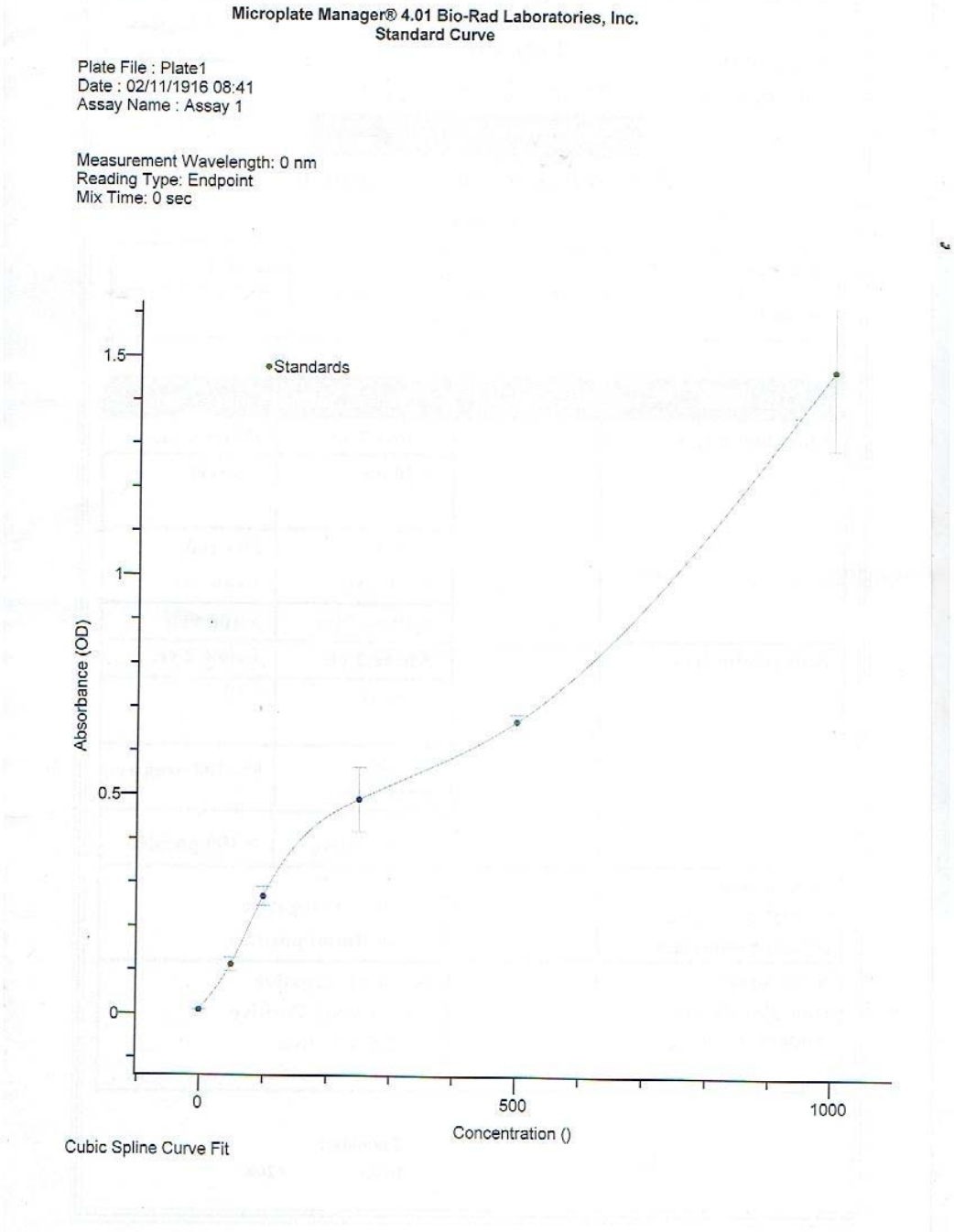
Appendix XI

Salivary Melatonin Graph



Appendix XI

Serum Melatonin Graph



Appendix XII

Salivary α-amylase Assay

♦ ***Sample preparation***

The salivary sample was diluted with distilled water as (1:100) by putting 10µL of saliva in plain tube and adding 1ml of distilled water and mixing well.

♦ ***Procedure***

1. Assay conditions were:
Wavelength: 405nm
Constant temperature: 370C
Cuvette: 1 cm light path
2. The instrument was adjusted to zero with distilled water.
3. Then it was Pipette into a cuvette; 1mL of reagent and 20 µL of diluted saliva.
4. After that it was mixed and incubated for 30 seconds.
5. After that the initial absorbance (A) of the sample was read, starting the stop watch and reading absorbance at 1 minute intervals thereafter for 3 minute.
6. Then the difference between absorbance and the average absorbance was calculated as differences per minute.

♦ ***Calculations***

$\Delta A/\text{ min} \times 7908 \times 100 = \text{U/L Amylase}$

Appendix

Appendix XIII

Salivary Total protein test

1. Total Protein Assay

♦ ***Principle***

Proteins react in acid solution with pirogallol red and molybdate to form a colored complex, the intensity of the formed color is corresponding to the protein concentration in the sample (Orsonneau *et al*., 1989; Koller, 1984).

♦ ***Procedure***

1. Assay conditions were:

Temperature: 370 C

Wavelength: 598 nm

Cuvette: 1 cm light path

2. The instrument was adjusted to zero with distilled water.

3. Then was Pipette into a cuvette:

	Blank	Standard	Sample
R(mL)	1.0	1.0	1.0
Standard (µl)		20	
Sample(µl)			20

4. After that it was mixed and incubated for 10 min at room temperature.

5. The absorbance (A) of the sample and standard was read against the blank. The color is stable for at least 30 min.

♦ ***Calculations***

(A) Sample

---------- X 1000 (Standard conc.) = mg / L protein in the sample

(A) Standard

الخلاصه

متلازمة شغرن هي اضطراب المناعة الذاتية المزمن الذي يتميز بالتسلل اللمفاوي للغدد الإفرازية، خاصة الغدد اللعابية والدمعية. السمة المميزة للمرض هي جفاف العين وجفاف الفم، التي ظهرت بعد اعتلال في وظيفة الغده اللعابية والدمعية على التوالي. ويمكن تصنيف متلازمة شغرن إما متلازمة شغرن الأولية أو الثانوية وفقا لما إذا كان مقترنا بأمراض النسيج الضام الأخرى، مثل التهاب المفاصل الروماتيدي ، الذئبة داء الذئبه الاحمراري، تصلب الجلد.

اهداف الدراسه

هدفت هذه الدراسة إلى_تحديد مستوى الميلاتونين وأكسيد النيتريك في(لعاب ومصل) مرضى متلازمة شغرن الثانويه ، ومقارنة مع مرضى التهاب المفاصل الروماتيدي ومع المجموعه الضابطه وتحديد ارتباطها مع زيادة قابلية حدوث متلازمة شغرن الثانوية. وارتباطها مع مستوى الميلاتونين اللعابي.

تقييم معدل الاميليزاللعابي وبروتين اللعاب الكلي في مرضى متلازمة شغرن الثانوية ومقارنتها مع مرضى التهاب المفاصل الروماتيدي ومع المجموعه الضابطه. دراسة تأثير (الميلاتونين، وأكسيد النيتريك، ألفا الأميليز والبروتين الكلي) اللعابي على معدل تدفق اللعاب.

تقييم ارتباط نشاط التهاب المفاصل الرثوي (DAS-28) مع حدوث متلازمة شغرن الثانوية باستخدام: الفحوصات المختبريه للكشف عن (سرعة تثفل الكريات الحمراء، العامل الروماتيدي ، المضادة سيتروليناتد الببتيد, المستضدات ريبونوكلوبروتين) في دم المرضى الذين يعانون من متلازمة شغرن الثانوية ومرضى التهاب المفاصل الروماتيدي. والتقييم السريري باستخدام معدل تدفق اللعابية، واختبار شيرمر.

المواد وطرائق العمل

شملت عينة الدراسة 92شخصا ، 61 مريضا من كلا الجنسين مع الفئة العمرية (20-60)، منهم (31 مريضا من متلازمة شغرن الثانوية تم تشخيصهم وفقا لمعايير مجموعة التوافق الاوربيه الأمريكية و 30 مريضا من مرضى التهاب المفاصل الروماتيدي تشخيصهم سريريا من قبل المتخصصين في الروماتزم) وكلاهما تم تقييمها من قبل تقييم ارتباط نشاط التهاب المفاصل الروماتيدي (DAS28),31 شخصا في المجموعه الضابطه. تقييم فحص شيرمر للعين ومعدل تدفق اللعاب . ومن ثم جمع العينات اللعابية والدمويه لتحديد التحليل المناعي والبيوكيميائي (سرعة تثفل الكريات الحمراء، العامل الروماتيدي ، المضادة سيتروليناتد الببتيد, المستضدات ريبونوكلوبروتين)، الميلاتونين وأكسيد النيتريك اللعابي،الأميليزاللعابي والبروتينات اللعابية الكلية، وذلك باستخدام فحص الانزيم المرتبط المناعي وفحص المقياس الطيفي.

النتائج

أظهرت النتائج أن الغالبية العظمى من المجموعات المريضة يبلغ عمرها (40-49) سنة، (60-70) سنة. لمجموعة مرضى التهاب المفاصل الروماتيدي و مجموعة متلازمة شغرن الثانويه على التوالي مع الانحراف المعياري والانحراف المعياري (48.30 ± 10.02) سنة، و (52.65 ± 9.88) سنة.

أظهرت الدراسة الحالية اختلافات كبيرة في مدة المرض بين المجموعتين المرضيه , هناك فرق كبير جدا في نشاط المرض في كل مجموعة مريضة فيما يتعلق بسرعة تنفل الكريات الحمراء.

أظهرت هذه الدراسة وجود اختلافات معنوية في أكسيد النيتريك في مصل الدم فيما يتعلق بارتباط نشاط التهاب المفاصل الروماتيدي (DAS28) في مجموعة التهاب المفاصل الروماتيدي,كما أظهرت الدراسة الحالية وجود فرق معنوي في معدل تدفق اللعاب بين المجموعتين المدروستين.

أظهرت هذه الدراسة انخفاض في الميلاتونين اللعابي مع زيادة نشاط المرض نشاط التهاب المفاصل الروماتيدي. أظهرت نتائج هذه الدراسة وجود علاقة ارتباط معنوية بين (الميلاتونين اللعابي والتهاب ونقص اللعاب) في مجموعة التهاب المفاصل الروماتيدي وبين(الأميليز ألفا اللعابي ونقص اللعاب) في متلازمة شغرن الثانوية.,بالإضافة إلى ذلك أظهرت هذه الدراسة فروق ذات دلالة إحصائية كبيرة في أكسيد النيتريك اللعابي بين المجموعتين مريضتين مقارنة بالمجموعه الضابطه.وقد أظهرت نتائج الدراسة الحالية وجود فرق معنوي في الأميليز ألفا اللعابي بين متلازمة شغرن الثانوية ومجموعة السيطرة.

الاستنتاج

حدوث متلازمة شغرن الثانوية في مرضى التهاب المفاصل الروماتيدي يعتمد على مدة المرض ولا تعتمد على نشاط المرض من التهاب المفاصل الرثوي. يرتبط نشاط التهاب المفاصل الروماتيدي ارتباطا إيجابيا بقيمة سرعة تنفل الكريات الحمراء، في حين أن العلاقة السلبية بين نشاط التهاب المفاصل الروماتيدي و (أكسيد النيتريك المصلي و اللعابي) في مجموعة التهاب المفاصل الروماتيدي ومع (الميلاتونين اللعابي) في متلازمة شغرن الثانوية. حدوث متلازمة شغرن الثانوية يتأثر (بالميلاتونين المصلي، و الأميليزاللعابي) ولا تتأثر (بالميلاتونين اللعابي وأكسيد النيتريك المصلي، وأكسيد النيتريك اللعابي). حالة نقص اللعاب ترتبط مباشرة بالأميليزاللعابي في (متلازمة شغرن الثانوية) والميلاتونين اللعابي في (التهاب المفاصل الروماتيدي).

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بغداد - كلية طب الاسنان

التقييم السريري لنشاط المرض في المرضى الذين يعانون من متلازمة شغرن الثانوية المرتبطة بالتهاب المفاصل الروماتيدي فيما يتعلق ببعض المؤشرات الحيوية الكيميائية والمناعية

اطروحه
مقدمه الى مجلس كلية طب الاسنان في جامعة بغداد وهي جزء
من متطلبات درجة الدكتوراه في علم طب الفم

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