University of Mosul

College of Dentistry

Antioxidant and Anti-Inflammatory Effects of Folic Acid on Chronic Gingivitis

A Thesis Submitted

By

Rawia Fehr Fathil Al-Busrawi

B.Sc/Pharmacist

To

The Council of College of Dentistry / University of Mosul

In Partial Fulfillment of the Requirements for the Degree of

Master Science in

Pharmacology / Dental Pharmacology

Supervised by

Assistant Prof. Dr. Faehaa Azher Al-Mashhadane

2020 A.D **1442 A.H**

The Supervisor`s Certification

I certify that this thesis entitled (Antioxidant and Anti-inflammmatory Effects of Folic Acid on Chronic Gingivitis) was prepared under my supervision at the Department of Dental Basic Sciences, College of Dentistry, University of Mosul, in a partial fulfillment of the requirements for the Degree of Master of Sciences in Pharmacology / Dental Pharmacology.

Signature:

Assistant Prof. : *Dr. Faehaa Azher Al-Mashhadane*

Date: / / 202

Report of Linguistic Evaluator

I certify that the linguistic evaluation of this thesis was carried out by me and it is accepted linguistically and in expression.

Signature:

Assistant Prof. : *Dr.Mohammed Nihad Ahmed*

Date: / / 202

Report of The Head of The Department

I certify that this thesis was carried out in the Department of Dental Basic Sciences. I nominate it to be forwarded to discussion.

Assistant Prof. : *Dr. Ahmed Shihab Al-Taweel*

Signature:

Date: / / 202

Report of The Director of Postgraduate Studies Committee

According to the recommendations represented by the supervisor of this thesis and the linguistic evaluator, I nominate this thesis to be forwarded to discussion.

Signature:

Assistant Prof : *Dr. Ne'am Reyadh Saleem*

Director of Postgraduate Studies Committee

Date: / /202

The Committee Certification

We, the examining committee, certify that we have read the thesis which entitled (**Antioxidant and Anti-inflammmatory Effects of Folic Acid on Chronic Gingivitis**), prepared by (**Rawia Fehr Fathil Al-Busrawi**), and examined in its contents in 1 / 12 / 2020 and that in our opinion, it meets the standards of a thesis for the degree of Master science in Pharmacology/ Oral Pharmacology.

Signature:
Prof. Dr. Ghada Abdulrahman Taqa
(Chairman of the Examining Committee)

Signature:
Asst. Prof. Dr.
Maha Talal Fattah
(Member)

Signature:
Asst. Prof. Dr.
Abbas Fadhil Al- Taie
(Member)

Signature:
Asst. Prof. Dr.
Faehaa Azher Al-Mashhadane
(Member and Supervisor)

The Council Certification

Approved by the council of the Collage of Dentistry in its session No. …… in / / 202 . Accordingly it is decided to award the candidate with a master degree in Basic dental Science/Oral Pharmacology.

Signature:
Asst. Prof. Dr.
Ne'am Reyadh Saleem
(Council Registrar)

Signature:
Asst. Prof. Dr.
Rayyan Salim Hamid
(Dean of the Collage of Dentistry)

ACKNOWLEDGEMENTS

First and foremost, I express my thanks, obedience and gratitude to ***Allah*** from whom I received guidance and inspiration.

Blessing and peace of ***Allah*** *upon Prophet* ***Muhammed*** *and his family and companions* .

I owe my deep gratitude to the Dean of College of Dentistry; Asst. Prof.***"Dr. Rayyan Salim Hamid "*** for his great support and encouragement all over my study.

Many thanks for ***"Dr. Ahmed Shihab Al-Taweel"*** the Head Department of Basic Sciences for his generous cooperation.

Sincere thanks and appreciation to my supervisor Asst. Prof. ***"Dr. Faehaa Azher Mohe Aldeen Al-Mashhadane"***, for her patience, encouragement, guidance, endless support and unlimited help.

Special thanks to the University of Mosul and College of Dentistry

and all members of the Department of Dental Basic Sciences / College of Dentistry for giving me the study chance and providing the requirements of the research.

My heartfelt gratitude to my family; my father, mother, brother and sisters and special thanks to my husband ***Younus Mudhafar Younus*** for their great support and patience to overcome the hardship of the study.

ABSTRACT

Introduction: Folic acid is a water-soluble vitamin, its supplementation is able to scavenge free radicals very efficiently, and decreases the expression of cytokines. Periodontal disease especially gingivitis is one of the most prevalent oral diseases in the world. That high prevalence urged the researchers to study the effects of many drugs or supplements on the gingival inflammation that improves the case with least side effects, more availability in the markets and has a high bioavailability.

Aims: This thesis aims to study the effects of folic acid on chronic gingivitis and to evaluate its effect on the tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) in their saliva. Also, to investigate the antioxidant effects of folic acid on the oral health and to evaluate its effect on the total salivary proteins.

Materials and Methods: This study is a randomized clinical trial, carried out on 40 subjects diagnosed as chronic gingivitis patients, aged (20-40) years old. They were divided into two groups, twenty for each.Treatment group received 1 mg oral tablet of folic acid once daily for 42 days. Control group did not receive any drug. Scaling and polishing were accomplished for each participant before starting the study. At the next day (the 1st day of study); plaque, gingival, oral hygiene, and calculus indices scores for the two groups were measured, then at day 21st and 42nd of the study. At those three visits, saliva samples were collected for salivary IL-6, TNF-α, total antioxidant capacity (TAC) and total salivary proteins (TP) measurements. One-way Analysis of Variance test (ANOVA-test), Tukey's Pair-wise comparisons and Dependent t-test of two means (paired) was applied for statistic results. p value was < 0.05.

Results: The treatment group consisted of 11 females (55%) and 9 males (45%), the mean of age of patients was 28.2 ± 5.72 years, while the control group consisted of 9 females (45%) and 11 males (55%), the mean of age was 28.9 ± 7.05 years with no significant differences between their gender. The results showed that there is a significant difference between means of treatment and control group in PI at 21st day of the study. There is a significant difference in mean of CI between 1st day and the 21st day of the study, while there is no significant difference in means of the other oral health scores during all the study period. Moreover, there is a significant difference between means of treatment group and control group in TNF-α at 21st and 42nd day. In the treatment group there is a significant increase in mean of salivary TAC level during all study period. **Conclusions**: The use of folic acid supplements could improve the oral health and help in the treatment of gingivitis by increasing the salivary TAC and decreasing the salivary TNF-α.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Abbreviation	Definition
AMPs	Antimicrobial peptides
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
CI	Calculus index
DHFR	Dihydrofolate reductase
ELISA	Enzyme-linked immunosorbent assay
FAD	Folic acid deficiency
FDA	Food and Drug Administration
FRs	Free radicals
GCF	Gingival crevicular fluid
GI	Gingival index
GSH	Glutathione
Hcy	Homocysteine
HRP	Horseradish peroxidase
Ig	Immunoglobulin
IL	Interleukin
IOM	Institute of medicine
LPS	lipopolysaccharides
MCI	Mild cognitive impairment
MTHF	Methyltetrahydrofolate
MTX	Methotrexate
MW	Mouth wash
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells

NK cells	Natural killer cells
NTDs	Neural tube defects
Nrf2	Nuclear factor erythroid 2-related factor 2
OD	Optical density
OHI	Oral hygiene index
OS	Oxidative stress
PABA	Paraaminobenzoic acid
PAF	Platelet activating factor
PG	Prostaglandin
PI	Plaque index
PMNLs	Polymorphonuclear leucocytes
RCF	Relative Centrifugal Force
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RPM	Round per minute
SOD	Superoxide dismutase
TAC	Total antioxidant capacity
THF	tetrahydrofolate
TLR	Toll-like receptor
TMB	Tetramethylbenzidine
TNF-α	Tissue necrosis factor-alpha
TP	Total proteins
TUL	Tolerable upper level/limit
UA	Uric acid

Chapter One

INTRODUCTION

INTRODUCTION

Periodontal disease, including gingivitis and periodontitis, could be the most common disease of the world (Tonetti *et al*., 2017; Akram *et al*., 2020). Gingivitis is a reversible disease. In gingivitis, gum becomes red, swollen and bleed easily while touching, brushing or sometimes spontaneous gum bleeding happens and there is no bone or tissue loss, so it is important to treat gingivitis as early as possible to prevent the periodontitis (Akram *et al.,* 2020), which is an oral inflammatory disorder results in the periodontal tissue damage and loss as a result of the complex interactions between host's immune mediators and many oral pathogenic bacteria (Hartenbach *et al*., 2020). The early stages of periodontal disease are often without symptoms, and most affected patients do not visit a dentist.

The “silent” nature of the early stages of the disease, and the low awareness of periodontal health, leads many patients to seek a professional care for the advanced disease through periodontal therapy protocol when available (Jin, 2015). One of the suggested hypotheses to describe the potential mechanism responsible for periodontal disease could be oxidative stress-mediated inflammatory pathways (Kharaeva *et al*., 2020).

Oxidative stress is a phenomenon of increase in the production of free radicals (oxidants) and/or a decrease in the production of protective antioxidants in the tissue. Free radicals are classified as reactive oxygen species (ROS) and reactive nitrogen species (RNS) and both possess unpaired electrons. ROS include superoxide anion, hydroxyl radicals, peroxyl radicals, alkoxyl radicals, hydrogen peroxide and singlet oxygen, while RNS include nitric oxide, nitric dioxide and peroxynitrite (El-Bahr, 2013).

ROS are naturally produced oxidants involved in many cellular biochemical events that are essential to life, but they are capable of causing

harmful oxidative stress when overproduced or imbalanced with antioxidants (Zhang *et al.,* 2019).

Free radicals always cause damage to all essential bio-compounds such as DNA, proteins, and cell membrane lipids, thereby causing cell death. The antioxidants are exists in two main groups: enzymatic (glutathione peroxidase, catalase myeloperoxidase, and superoxide dismutase) and non-enzymatic (minerals, vitamins, polyphenols, and thiols) (Pisoschi and Pop, 2015; Inan, 2019). A loss of normal balance between oxidants and antioxidants can cause damage in the periodontal tissues by variety of damaging mechanisms including lipid membranes peroxidation, protein inactivation and stimulation of cytokines overproduction (Kharaeva *et al.,* 2020). So, the important function of the antioxidants is to serve as a guard for oral cavity against harmful effects of endogenous and exogenous ROS/RNS.

It also able to stimulate the infiltration of pro-inflammatory cytokines as interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) that participate as integral part of periodontal disease pathogenesis (Naiff *et al*., 2020). These inflammatory cytokines are detectable in the oral fluids (Miller *et al*., 2010 ; Belstrøm *et al*., 2017). Saliva is an easily collectable oral biologic fluid that contains number of essential proteins that are produced locally or emerged from the vascular beds in the gingival tissues (Hartenbach *et al*., 2020). Also, the use of saliva to measure oxidative stress (OS) is increasing (Buczko *et al*., 2015), since researchers have been proved that saliva contains antioxidative biomarkers (Ho *et al*., 2013; de Almeida *et al*., 2016 ; Madi *et al*., 2016; Ahmadi-Motamayel *et al*., 2017).

Elevated concentrations of salivary total proteins (TP), IL-6, IL-8 and TNF-α have been reported as associated with gingivitis (Wu *et al.,* 2007; Lee *et al*., 2012).

Folic acid is a water-soluble hemocytopoietic vitamin, it belongs to the vitamin B group (B9), it is oxidized form of folate (the naturally occurring form) and has a higher bioavailability and stability than folate (Batra *et al.,* 2020). It cannot be synthesized in the body, but can be obtained from food. Folic acid is itself inactive, it is converted in-vivo into the biologically active coenzyme, tetrahydrofolic acid (THF), which is essential factor in the biosynthesis of nucleic acid and methylation reactions as a methyl donor and therefore in cell division (Batra *et al.,* 2020).

Folic acid has been associated with a number of health benefits ranging from the reduction of neural tube defects to the prevention of several types of cancer, cardiovascular disease, and dementia (Qu *et al*., 2019). Folic acid supplementation is responsible for significant reduction in inflammation (Thomson and Pack, 1982; Pack, 1984) by scavenging free radicals very efficiently and serving as redox regulator (Joshi *et al*., 2001). It is able to decrease the expression of cytokines (Hayden *et al*., 2006). It has been reported that low folate level in serum is associated with the periodontal disease in non-institutionalized older adults (Yu *et al*., 2007).

This study focused on the effect of folic acid supplementation on the oral health and it hypothesized that folic acid supplementation might be associated with gingival inflammation improvement.

AIMS OF THE STUDY

The current study aims to :

1- Investigate the effects of systemic treatment with folic acid on the plaque index, gingival index oral health index, and calculus index of patients with chronic gingivitis compared to control group.

2- Evaluate the effects of systemic treatment with folic acid on the concentration of interleukin-6 and tumor necrosis factor-α in the saliva of patients with chronic gingivitis compared to the control group.

3- Assay the effect of systemic treatment with folic acid on total antioxidant capacity in saliva of patients with chronic gingivitis compared to control group.

4- Assay the effect of the systemic treatment with folic acid on the total salivary proteins of patients with chronic gingivitis compared to the control group.

5- Study correlations between all these parameters.

Chapter Two

REVIEW OF LITERATURES

CHAPTER TWO

REVIEW OF LITERATURES

2.1 : Vitamins Overview

Vitamins are organic molecules, they are considered as catalysts for all metabolic reactions, using proteins, fats and carbohydrates for energy, growth and cell maintenance. As only small amounts of these essential substances are obtained from food, vitamins are often administered though food supplements. Fat soluble vitamins such as A, D, E and K can be stored in the liver and fat tissues, while water-soluble vitamins as B and C are expelled from the body in a short period since the human body is not capable of storing these vitamins (Cagetti *et al*., 2020).

Vitamin B refers to eight different vitamins. The B vitamin group includes the B1 (Thiamine), B2 (Riboflavin), B3 (Niacin), B5 (Pantothenic acid), B6 (Pyridoxine), B7 (Biotin), B9 (Folate) and finally B12 (Cobalamin) (Khaneghah *et al.*, 2019).

2.2 : Folic Acid

Folic acid is a synthetic form or the oxidized form of the folate, also it is called pteroylglutamic acid or vitamin B9. Folic acid is the form primarily used as supplementation, due to its economical synthesis and good bioavailability (Tsuji and Shibata, 2019). It is formed by the some plants, microorganisms, it can also be found in human and animals body like bone marrow and liver (Ruengsitagoon and Hattanat, 2012).

Folic acid is more stable than folate, which is highly labile and sensitive to oxidation. Folic acid supplements may be administered in various forms, such as oral drops, tablets, intravenous injection. In addition,

folic acid may be added to the foods as fortification of grains and cereals (Wien *et al*., 2012; Wald *et al.,* 2020).

2.2.1 : History of Folic Acid

During 1920s, scientists thought that both folic acid deficiency (FAD) and anemia were similar disorder. Lucy Wills, a researcher who made an inspections that identify folate as a nutrient had a role in preventing anemia for the period of pregnancy. Wills, discovered that anemic patients could be returned to normal condition by using type of yeast named brewer's yeast (Hillman, 2010). Folate was isolated for the first time in 1941, through spinach leaves extraction by group of researchers. In 1943, the pure crystalline form was isolated by Stokstad B, who determined its chemical structure (Hoffbrand and Weir, 2001). This history of folic acid since 1945, was prepared by the teamwork named the "folic acid boys," (Augieb *et al*., 1945). Then, this led to the production of the antifolate drug called Aminopterin, which considered as first novel drug with approved clinical anticancer efficacy by Farber in 1948. Between 1950s and 1960s, researchers started to find out folate mechanisms of action. At 1960, scientists started for first time to interrelated FAD to the neural tube defects (Lanska, 2010).

In the late 1990s, scientists and experts understood, in spite of the accessibility of folate from food and supplements, a challenge that was still present for the population to reach their sufficient daily needs (Crandall *et al*., 1998; Malinow *et al*., 1998). Food fortification program with folate was considered firstly in 1996 in US then implemented in many countries worldwide (Wald *et al.,* 2020).

2.2.2 : Chemistry of Folic Acid

2.2.2.1 : Chemical Properties of Folic Acid

A yellow, yellow-brownish, or yellowish orange, almost odorless, tasteless, crystalline powder. It is very slightly soluble in water; insoluble in alcohol, acetone, chloroform, and ether. It rapidly dissolves in the dilute solutions of alkali hydroxides and carbonates (Sean *et al.,* 2009). Folic acid solutions should be protected from light because they decompose rapidly in the presence of light (Gerald, 2005). A suspension of 1 g of folic acid in 10 ml of water has a pH of 4.0 - 4.8 (O'Neil *et al.,* 2001).

2.2.2.2 : Chemical Structure of Folic Acid

The parent chemical structure of folic acid consists of an aromatic pteridine ring linked by a methylene bridge to paraaminobenzoic acid (PABA), which in turn is bound to glutamic acid by a peptide bond (Blom *et al*., 2006). The chemical formula of folic acid (pteroylglutamic acid) is $C_{19}H_{19}N_7O_6$ as illustrated in figure (2.1).

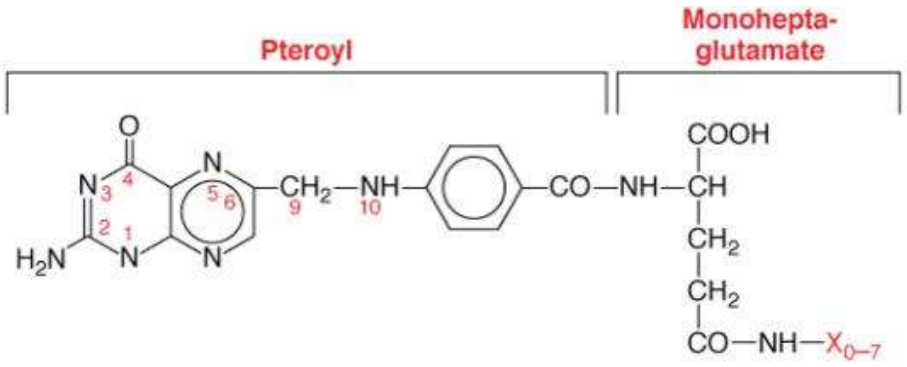


Figure (2.1): Chemical Structure of Folic Acid
(Laurence and Brunton, 2010).

2.2.3 : Mechanism of Action

Folic acid, is biochemically inactive. So it converted to tetrahydrofolate (THF) and methyltetrahydrofolate (MTHF) by dihydrofolate reductase enzyme (DHFR). These folic acid derivatives are transported across cells by receptor-mediated endocytosis process, where they are required to maintain the normal red blood cells generation, methylate tRNA, metabolism of amino acids such as glycine and methionine, synthesize purine and thymidylate nucleic acids which are required for mitochondrial and cytosolic adenosine triphosphate (ATP) generation (Crider *et al*., 2012; Iscan *et al*., 2019).

2.2.4 : Pharmacokinetics

Unaltered folic acid dissolved in gastric HCl readily and completely absorbed in proximal jejunum (Mason, 1990). Alterations in the intestinal pH by achlorhydria or pancreatic insufficiency can alter the rate and extent of folate absorption (Russell *et al*. 1979;1986). Folate-mediated one-carbon metabolism. Dietary folic acid is metabolized to tetrahydrofolate, which is then activated with a one-carbon unit to form 10-formyltetrahydrofolate, 5,10-methylenetetrahydrofolate, and 5-methyltetrahydrofolate (figure 2.2). Each of these folate cofactors supports a biosynthetic pathway for the synthesis of purines and thymidylate and the remethylation of homocysteine to methionine.

Synthesis of methionine requires 5-methyltetrahydrofolate and vitamin B12. Folic acid is converted to dihydrofolate and then tetrahydrofolate by dihydrofolate reductase, which is dependent on NADPH. (Field and Stover, 2018). Folic acid supplementation is almost 100% bioavailable especially when administered before meal because food in stomach decreases this percentage to about 85%. The naturally occurring

folate in food bioavailability is lower than synthetic form (Caudill, 2010; Alpers, 2016).

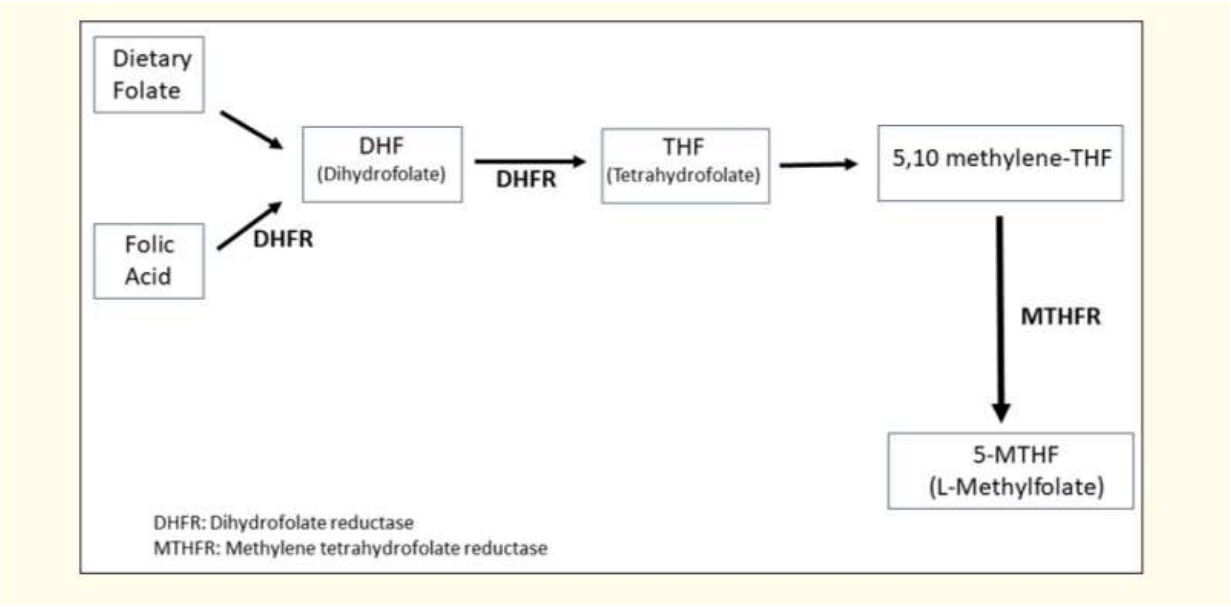


Figure (2.2) : Folic Acid Biotransformation (Von, 2019).

Approximately two-thirds of folate is bound to plasma protein. (Hendler and Rorvik, 2001). After 6 hours a majority of the folate metabolic products appeared in the urine and excretion is generally completed within 24 hours. Only Small amounts of folic acid have also been recovered in the feces. Folic acid is also found in the milk of nursing mothers (Hendler and Rorvik, 2001; Page *et al*., 2017).

2.2.5 : Clinical Uses

1. Neural Tube Defects (NTDs)

In pregnant women, the FAD could be associated with NTDs occurrences (Petersen *et al*., 2019). They include spina bifida encephalocele and anencephaly, usually due to the incomplete closure of neural tube during first trimester of pregnancy (Al-Mashhadane *et al*., 2018). NTDs are unique in that more than two-thirds of cases can be prevented by a regular ingestion of safe dose of folic acid prior to and during the first trimester of pregnancy (Bortolus *et al*., 2014).

2. Megaloblastic Anemia

Folate deficiency defined as a low level of folate and their derivatives in the body. It develops in weeks to months in persons with folate-deficient diets. It is characterized by the appearance of large-sized, abnormal red blood cells (megaloblasts). These cells proliferate rapidly. Most of the serum folate is present in 5-methyltetrahydrofolate form. Upon entering cells, 5-methyl THFA demethylates to THFA, the biologically active form involved in the folate-dependent enzymatic reactions.

B12 serves as a co-factor for this demethylation to occur, and in its absence, folate is trapped inside the cells as 5-methyl THFA. THFA is involved in the purine and pyrimidine synthesis, nucleoprotein synthesis and maintenance in erythropoiesis. Simple blood smear of an individual with a folate deficiency will reveal erythrocyte macrocytosis, decrease in hemoglobin and hyper-segmented polymorphonuclear cells (PMNs) (Merrell and McMurry, 2020).

This abnormal morphology is a result of impaired DNA synthesis, which causes precursor cells in the bone marrow to have nuclei that are immature relative to their cytoplasm. Patients being evaluated for the FAD should also be evaluated for vitamin B12 deficiency as both cause macrocytic anemia. All patients with folate deficiency should be offered supplemental folic acid in addition to vitamin B12 for the correction of the condition (Tamparo, 2011; Gupta, 2017; Khan and Jialal, 2019).

3. Fertility

Many studies had demonstrated that women receiving folic acid supplementation had a better quality oocytes and a higher degree of mature oocytes compared to women who did not receive folic acid supplementation (Ebisch *et al*., 2006). In immature super-ovulated rats, deficiency of folates partially inhibit ovulation (Willmott *et al*, 1968).

Studies highlight the importance of B-vitamin status in spermatogenesis in humans. They suggest that the low folate concentrations in the microenvironment of spermatozoa may affect on sperm DNA stability.

Folate may affect spermatogenesis through DNA methylation and subsequent regulation of DNA function, in addition to its contribution to DNA replication through purine biosynthesis (Boxmeer *et al*., 2009; Schisterman *et al*., 2020).

4. Cerebrovascular and Coronary Arterial Diseases

Evidence reported from numerous clinical studies over the past decade has revealed an association between increased plasma Hcy concentrations and cardiovascular disease (CVD), peripheral vascular, cerebrovascular and coronary arterial diseases. Folic acid supplementation can lower plasma Hcy concentrations (Fratoni *et al*., 2015).

Furthermore, folic acid can reverse endothelial dysfunction observed in patients with CVD (Daly *et al*., 2002). One study has been indicated that a 10% lower risk of stroke and a 4% lower risk of overall CVD patients which treated with folic acid supplementation (Li *et al*., 2016).

2.2.6 : Drug - Drug Interactions

There are number of drugs that affect with the biosynthesis of THF from folic acid. Among them are the dihydrofolate reductase inhibitors such as trimethoprim, pyrimethamine and methotrexate (MTX) (Gonen and Assaraf, 2012).

folic acid reduces phenytoin, carbamazepine and phenobarbital levels thus increasing the episodes of seizures in dose of 10 mg/day by increasing their metabolism (Steinweg and Bentley, 2005).

2.2.7 : Folic Acid Toxicity

Folic acid toxicity is rare, in case of ingestion of 15 mg once daily for 1 month the patient exhibit gastrointestinal disturbances, anorexia, nausea, abdominal distension and discomfort, flatulence, bad or bitter taste which impaired normal taste, altered sleep patterns, varying degrees of insomnia either waking intermittently throughout the night, malaise, irritability, overactive and excitable mild confusion, and increasing difficulty in concentrating.

After discontinuing the drug the majority of those symptoms improved rapidly, patient regaine normal sleep and dream patterns within a week, gastrointestinal symptoms subsided in the second week but normal mental function was not fully restored in all until the end of the third week (Hunter *et al.,* 1970).

2.3: Saliva

2.3.1: Definition

Saliva is the extracellular fluid produced and secreted by numerous salivary glands in the mouth. The presence of saliva is essential for the maintenance of both, oral healthy hard (teeth) and soft (mucosa) tissues. Saliva serves as a diagnostic fluid, and as an indicator of risk for diseases creating a close relation between oral and systemic health (François *et al*., 2019).

It has been used to reflect the body physiological and pathological changes as a mirror. It is easily available for non-invasive collection and analysis in compares with blood. It also can be used to observe the presence and levels of drugs, antibodies, hormones, microorganisms and ions (Edgar *et al.,* 2012).

2.3.2 : Saliva Secretion and Composition

In a healthy human, the daily secretion of mixed saliva (whole saliva) usually ranges from 0.5 to 1.5 L depending on diurenal variation and season. Approximately 90% of mixed saliva is produced by major salivary glands and the residual 10% is derived from minor salivary glands (labial, lingual, buccal and palatal mucosa) which dispersed in the oral mucosa (Zhang *et al*., 2016).

Salivary fluid is an exocrine secretion, it releases from both serous and mucous acinar cells. The parotid gland has serous type that produces watery thin saliva and loaded by amylase enzyme. The submandibular gland contains serous and mucous acinar cells and forms a more viscous saliva and rich in mucin. The sublingual gland has mucous acinar cells and also secrets a viscous and mucin-rich saliva. The minor salivary glands mainly are mixed glands. (Nizamuddin *et al*., 2018)

Salivary secretion is controlled by the sympathetic and parasympathetic autonomic nervous systems. Parasympathetic stimulation typically evokes the release of high-flow, low-protein serous secretions, while sympathetic stimulation leads to the release of low-flow, high-protein mucinous secretions (Kubala *et al*., 2018).

Saliva consists of water, variety of electrolytes (sodium, potassium, chloride, calcium, phosphate, magnesium, bicarbonate) and mucosal glycoproteins, traces of albumin and some polypeptides and oligopeptides that have many benefits to oral health. There are also glucose and nitrogenous substances, such as urea and ammonia (Zhang *et al*., 2016). Those constituents interact and are responsible for the various functions associated with saliva in the following areas: (1) bicarbonates, phosphates, and urea serve as saliva buffering capacity which maintain normal pH of

saliva between 6.6 to 7.2; (2) proteins and mucins act to clear, aggregate, and/or attach oral microorganisms; (3) calcium, phosphate, and proteins work together to control demineralization and remineralization; and (4) immunoglobulins, proteins, and enzymes produce antibacterial action (Humphrey and Williamson, 2001; Kubala *et al*., 2018).

Unstimulated whole saliva often correlates to the systemic clinical conditions more accurately than stimulated saliva, since materials use to stimulate flow may change salivary composition (Williamson *et al.,* 2012).

2.3.3 : Functions of Saliva

Saliva has numerous vital functions. Saliva, as a digestive fluid, is secreted in response to the stimulation by food. It also acts as a solvent for tasting materials, protection of oral hard and soft tissues from infections, and aids speech (Mese and Matsuo, 2007; Kubala *et al*., 2018).

2.3.4 : Types of Saliva

2.3.4.1 : Unstimulated Saliva

Unstimulated saliva or named mixed saliva is the mixture of secretions produced in the mouth in the absence of exogenous stimuli such as tastings, chewing, smelling and thinking.

It consists of secretions from major, and minor salivary glands, gingival crevicular fluid (GCF), bacteria, desquamated epithelial cells , and occasionally food residues, blood, and viruses. Unstimulated saliva is possibly collected with the person sitting quietly, the head down and mouth opened slightly to let the saliva to drool from the lower lip into a beaker or a tube, or the person can spit out the saliva at regular intervals (Edgar *et al.*, 2012). Unstimulated saliva in compare with stimulated type, contains inflammatory cells then cytokines are present in sufficient amount for measurements (George and Donald, 2012).

2.3.4.2: Stimulated Saliva

It is non concentrated saliva, released in response to the stimulation by mastication or by thinking in food, or to other stimuli such as certain drugs (e.g. pilocarpine) or to stimulation of the vomiting center. Participation percentage of the different salivary glands during unstimulated flow are; 20% from parotid gland, 65% from submandibular gland, 7-8% from sublingual gland, and less than 10% from numerous minor glands (Humphrey and Williamson, 2001; De Almeida, 2008).

2.3.5 : Salivary Proteins

Saliva has large group of constitutive proteins to maintain the homeostasis and oral health (Van, 2004). There is a great interest of proteins measurement in saliva to monitor general health (Si *et al*., 2015; Sun *et al*., 2016; Wang *et al*., 2018). Salivary protein component as a considerable group of up to 1166 proteins, includs 914 in parotid and 917 in submandibular/sublingual saliva (Denny *et al*., 2008). Acinar cells synthesizes and secretes the majority of these proteins into the oral cavity, which can be divided into families: proline rich proteins (PRPs), salivary mucins, salivary cystatins, salivary α-amylase, histatins and statherin (Zhang *et al*., 2013; Cabras *et al*., 2014).

2.3.5.1 : Functions of Salivary Proteins

Several mechanisms involved in maintaining the gingival health and tooth integrity by proteins (Gao *et al.,* 2016; Wang *et al.,* 2019).

1. Acquired enamel pellicle formation to defend against tooth wearing (by mucins and PRPs).
2. Inhibition of demineralization process of exposed tooth surfaces (mucins).

3. Encouragement of enamel remineralization by attracting calcium ions (PRPs and statherin).
4. Antimicrobial effects that include impediment of cariogenic bacterial species adherence onto enamel surface (histatins and cystatins), microorganism accumulation and clearance from the oral cavity (agglutinin and immunoglobulins), and the secretion of antimicrobial peptides (AMPs).

Many salivary proteins have been studied, and proteomic investigates have been shown that differences in the salivary protein profile are associated with systemic diseases as well as oral pathologies (Haigh *et al*., 2010; Gonçalves *et al*., 2010).

However, no studies have taken gingivitis alone, except one study by Goncalves *et al*. (2011) who made a comparison between salivary proteome of a healthy gingiva and that of an inflamed gingiva, they found that immunoglobulins (as IgA), hemoglobin, albumin, cystatin C, and Keratins, are elevated in whole saliva samples taken from gingivitis patients compared to healthy patients (Goncalves *et al*., 2011).

At the onset of gingivitis, the increase in cytoprotective proteins may inhibit periodontal tissue destruction and clinical attachment loss in patients at risk of developing periodontitis due to other host and environmental factors (Syndergaard *et al*., 2014; Aboodi *et al*., 2016).

Modification of the composition of salivary proteins is caused not only by pathological changes, but is also a result of numerous physiological states. It is supposed that such the change may be connected with the release of a range of pro-inflammatory cytokines such as IL-6 or TNF-α, which induce the release of antibacterial peptides. The change in the level of these proteins results from the inflammatory state caused by oxidative stress (Allgrove *et al*., 2009).

Ackermann *et al* have examined the ability of human α- and β-defensins to inhibit the pro-inflammatory cytokines of synthesis and to increase production of class IgG antibodies as a response to lipopolysaccharides (LPS) of bacterial cell membranes of *Porphyromonas gingivalis* which produce gingivitis (Ackermann *et al.*, 2010; Kościelniak *et al*., 2012). One study in 2016 has been found that the salivary antioxidants may not be the primary source of protection against oxidative damage in periodontal tissue inflammation also there are diverse salivary proteins defend against immunity harmful effects (Aboodi *et al*., 2016).

2.4 : Inflammation

Inflammation typically represents the response to the tissue injury. The clinical features of inflammation include edema, redness, heat, pain, plus loss of function. In the case of tissue injury from minor trauma or a surgical procedure, the inflammatory process results in a series of well-regulated humoral and cellular events leads to the localization of injury, removal of noxious agents, repair of physical damage, and restoration the function of the injured tissue. The immune system can be separated into the innate immunity and adaptive immunity. Innate and adaptive systems are frequently described as separate entities, in reality, components of the innate and adaptive responses overlap and both systems are required to work in conjunction to maintain homeostasis and defend against insult (Frank *et al*., 2017).

The inflammatory response is, of course, not always beneficial to the host. In the dental setting, acute inflammation can result in moderate to severe pain, edema, limited mouth opening, and diminished quality of life for four or six days following oral surgical procedures. If it becomes excessive or chronic, as is the case with periodontal disease. Inflammation can be divided into three stages: acute, subacute, and chronic

inflammation. In acute inflammation stage, inflammatory mediators as histamine are released, producing vasodilation and augmented capillary permeability. In the subacute stage, inflammatory cells migrate and occupy the site. Prostaglandins, leukotrienes, Platelet activating factor (PAF), and cytokines also play major roles. The chronic stage of inflammation contains the lymphocytic phase of injury cleansing and repair. Cytokines, particularly ILs and TNF-α, are essential in this stage (Ebersole *et al*., 2016; Frank *et al.*, 2017).

2.5 : Cytokines

Cytokines are small proteins released by cells have a specific effect on the interactions and communications between cells by cellular signaling. They are especially important in inflammation mediating the immune response following specific binding to their complementary receptors, and playing a role in chronic inflammation (Corbitt *et al*., 2019).

They are often produced in a cascade, as one cytokine stimulates its target cells to make additional cytokines. They can also act synergistically or antagonistically (Zhang and An, 2007). The main factors of systemic inflammation are inflammatory cytokines, such as TNF-α, and IL-6; chemokines and other mediators of inflammation (Nibali *et al*., 2012).

IL-6 (figure 2.3) plays a fundamental role in regulating the immune system, and directing the transition from innate to the acquired immunity, a process that can be attributed to recruitment, activation and survival of different leukocyte subsets. It is a multifunctional cytokine synthesized in response to the stimuli such as infection and trauma by a variety of cells such as macrophages, neutrophils, keratinocytes, fibroblasts, and endothelial cells (Nibali *et al*., 2012). Its role was clarified in the immune regulation, hematopoiesis, inflammation, and oncogenesis (Kishimoto, 2010).

Because of these multifaceted abilities, it is thought that individual variability in the ability to synthesize and release IL-6 may modulate the susceptibility, development, and progression of a number of autoimmune and inflammatory diseases (such as periodontal disease, atherosclerosis, and rheumatoid arthritis) and malignancies (myeloma and mesothelioma) (Park *et al*., 2007; Packard and Libby, 2008; Nishimoto, 2010). High circulating IL-6 concentrations are associated with many oral diseases including oral cancer, lichen planus, and periodontal diseases (Heikkilä *et al*., 2008; Nibali *et al*., 2012). Studies have shown a presence of IL-6 in endothelial cells, fibroblasts, and macrophages of subjects with periodontal disease (Shimada *et al*., 2010; Nibali *et al.,* 2012).

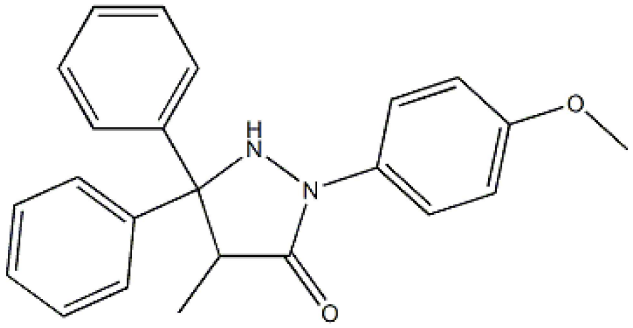
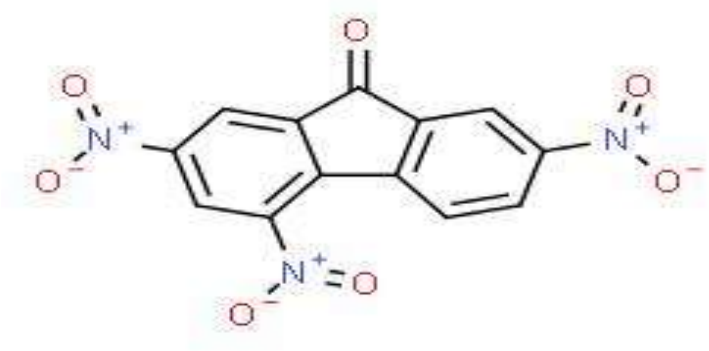


Figure (2.3): The chemical structure of IL-6 molecule ($C_{23}H_{22}N_2O_2$).

TNF-α (figure 2.4) is a pro-inflammatory cytokine that plays an important role in the inflammatory reaction. TNF-α is produced by the activated macrophages, neutrophils, keratinocytes, monocytes, and mast cells in response to LPSs (Hatem *et al.,* 2020). This cytokine is a cell-signaling protein that regulates diverse cell functions, leading to a necrosis or apoptosis (Idriss and Naismith, 2000; Zhang and An, 2007). TNF-α is a key regulator of other pro-inflammatory cytokines and of leukocyte adhesion molecules and it is a priming activator of immune cells.

A local increase in the concentration of TNF-α will cause the cardinal signs of Inflammation to occur; heat, swelling, redness, pain and loss of function, the common signs of gingivitis (Türer *et al*., 2017).



Figure(2.4) : Chemical structure of TNF-α ($C_{13}H_5N_3O_7$).

The systemic increased production of TNF-α has been related to the pathogenesis of various chronic disorders such as rheumatoid arthritis and Crohn's disease (Brogin *et al*., 2012 Alijotas *et al*., 2017). Also, many studies found that significant amounts of proinflammatory cytokines such as IL-1β, TNF-α and IL-6 are often secreted in response to these inflammatory and infectious stimuli (Saini *et al.,* 2011; Zhu *et al*., 2015).

High values of TNF-α have been found in patients with periodontal disease (Frodge *et al*., 2008; Garlet, 2010), So it is considered as one of the key periodontal pathogens-induced early inflammatory cytokines in destructive periodontal disease as gingivitis in comparison with healthy subjects (Türer *et al*., 2017; Keles *et al*., 2020).

The concept of modulating host destructive pathways as a strategy for treating periodontal diseases has come a long way since the 1970, blocking specific inflammatory mediators such as IL-1, IL-6 and TNF-α can be efficacious in slowing periodontal disease progression. (Williams, 2008; Al-Noori, 2013).

2.6 : Oral Health

Oral diseases are major public health problems with a high prevalence and incidence according to many epidemiological studies (Dye, 2012; Chi *et al*., 2014). Consequently, efforts to reduce the negative influences of oral diseases and their impacts on overall health, represent important priorities for dentists (Dye, 2012; Sreenivasan *et al*., 2016).

The most recurrent oral diseases in the world include caries and gingival inflammatory conditions that affect oral health and may progress to tooth loss (Marsh, 2012; Sreenivasan *et al*., 2016). Also, oral diseases may lead to systemic diseases as endocarditis, and ischemic arterial disease that impacts on human general health (Nazir, 2017).

2.7 : Characteristics of Healthy And Unhealthy Gingivae

- ✓ Healthy gingivae are usually coral pink in light skinned people, but may be naturally darker due to melanin pigmentation whereas unhealthy gingivae color is usually red.
- ✓ Healthy ginivae have a firm texture that is resistant to movement while unhealthy gingivae is often swollen and less firm.
- ✓ Healthy gingivae have a smooth curved or scalloped shape around each tooth. Healthy gingivae fill and fit each space between the teeth, unlike the swollen gingiva papilla seen in gingivitis.
- ✓ Healthy gingivae gives tightness to each tooth in that the gingival surface narrows to "knife-edge" thin at the free gingival margin whereas inflamed gingivae have a "puffy" or "rolled" margin.
- ✓ In healthy gingivae, no bleeding during daily plaque removal by brushing or flossing or during eating (Lang and Bartold, 2018).

Inflammation of marginal gingiva, without loss of attachment, is known as gingivitis (Togoo *et al*., 2019). With mild gingivitis, the patient's

gingiva bleed easily and become red and swollen with increased sensitivity. As symptoms worsen, the gums begin to regress and take on a red, inflamed color. The patient complains of generalized severe pain of the gingiva, often with a bad taste or smelly odor (Al-Noori, 2013).

Bleeding occurs due to multi micro-ulcerations in the sulcular epithelium or periodontal pocket. Gingival bleeding has been used as an essential parameter in the assessment of gingivitis because of its objectivity and ease of clinical access.

The fact that the gingival tissues can be sensitive to bleed just by touching the gingival margin with a blunt tool suggests that the epithelial changes and the vascular changes are well proven. These findings support the importance and applicability of using indices of visual and bleeding changes (Rebelo *et al*., 2011).

2.8 : Dental Plaque

Gingivitis mainly induced by dental plaque (Muhammed, 2017), that is a unique ecosystem composed of about to 800 bacterial species live in human oral cavity and these various gram-positive and gram-negative bacterial species form a community of dental plaque which is defines as a white to yellowish substance contain host saliva- and GCF-derived metabolites, microbial metabolites, that forms a strong bond with the surface of the teeth or other hard surfaces in the oral cavity (Masadeh *et al*., 2013; Velsko *et al*., 2017) which their metabolites causing inflammation of the gingival and periodontal structures, and dental caries (Pereira *et al*., 2013).

Most people may not remove the plaque mechanically, or if these are done, they are not enough protected against periodontal disease and plaque accumulation (Pereira *et al*., 2013; Tonetti *et al*., 2015). There is a large

surface area provided by teeth for colonization and maturation of plaque biofilm including *Streptococcus* and *Lactobacillus* species, and some combinations of anaerobic species such *as Prevotella, Veillonella* and *Porphyromonas* species are found (Xie *et al*., 2010; Peyyala and Ebersole, 2013).

An accumulation of dental plaque on the gingival margin triggers inflammatory effects that can become chronic (Kornman, 2008). Therefore, routinely oral washing with antimicrobial agents improves oral health and can act as an effective way to control and eliminate bacterial plaques and limit gingivitis and periodontitis.

It has been recurrently reported that dental plaque bacteria that forming biofilms can enter the bloodstream, causing bacteremia. The risk of bacterial endocarditis increases as the oral hygiene indice increase (Elshibly *et al*., 2014).

2.9 : Dental Calculus

The presence of calculus in oral cavity may limit the ability to perform optimal oral hygiene applies (Pradeep *et al*., 2011). Dental calculus develops when non-mineralized dental plaque biofilms become mineralized with calcium phosphate ($CaPO_4$) mineral salts.

These mineralized biofilms mainly form both supragingivally and subgingivally. The process of mineralization of plaque involves metabolic activities of the bacterial colonies and strengthens the adhering of plaque to the tooth surface. Therefore, it maintains close distance to the gingival tissues, as dental biofilms always cover the surface of the mineralized deposits (Akcalı and Lang, 2018).

Nonmineralized dental biofilm entraps particles from the oral cavity, (figure 2.5) including large amounts of oral bacteria, human proteins, viruses and food remnants (Velsko *et al*., 2017, 2019; Hendy *et al*., 2018).

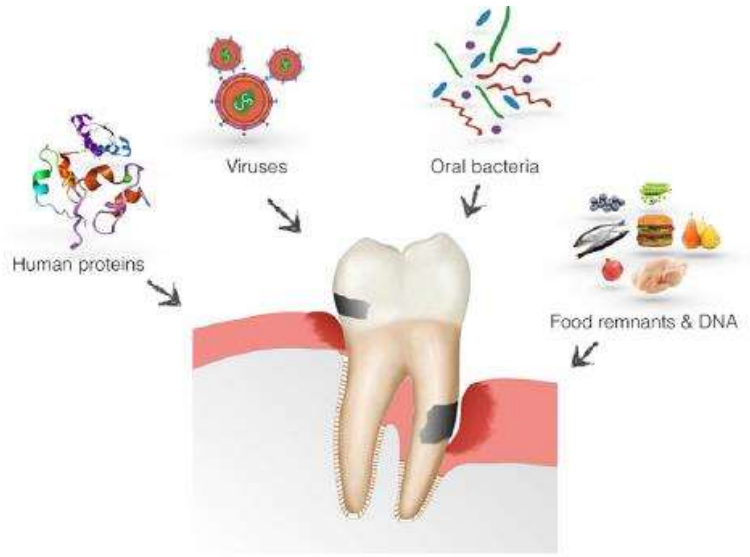


Figure (2.5): Dental Calculus (Mann *et al.,* 2018).

There are strong associations between calculus formation and chronic form of periodontal disease have been demonstrated in experimental and epidemiologic studies, and there is a positive correlation between the presence of calculus and the prevalence of gingivitis (Schätzle *et al*., 2004; Akcalı and Lang, 2018).

This study concerned with three essential indices for general oral health, those are;

1- Plaque Index (PI) (according to Silness and Löe, 1963)
2- Gingival Index (GI) (according to Silness and Löe, 1964)
3- Simplified Oral Hygiene Index (OHI) (according to Greene and Vermillion, 1960); which is comprised of two indices
    a- Simplified Oral Debris Index, and
    b- Simplified Calculus Index (CI)

Since periodontal diseases (gingivitis and periodontitis) are mainly inflammatory in the nature, the detection of inflammatory lesions in gingival tissues is vital for the diagnosis and monitoring of gingival status. Clinical indices provide a means of converting observed clinical data into numerical data for forming statistical analysis (Muthukumar *et al.,* 2014)

2.10 : Salivary Total Antioxidant Capacity

2.10.1 : Oxidative Stress

It is a regulatory biochemical process essential for human body in the production of the energy that human need for life. It is a normal phenomenon, presents in the healthy people (Gombart *et al.,* 2020). Throughout this process, free radicals are produced that have their physiological helpful functions. Free radicals, including reactive oxygen species (ROS) and reactive nitrogen species (RNS), are molecules with one or more unpaired electron (figure 2.6). Examples of free radicals include: superoxide [$\bullet O_2$], hydroxyl radical [•OH], and nitric oxide radical [$\bullet NO_2$] (Asmat *et al*., 2016; Żukowski *et al.,* 2018).

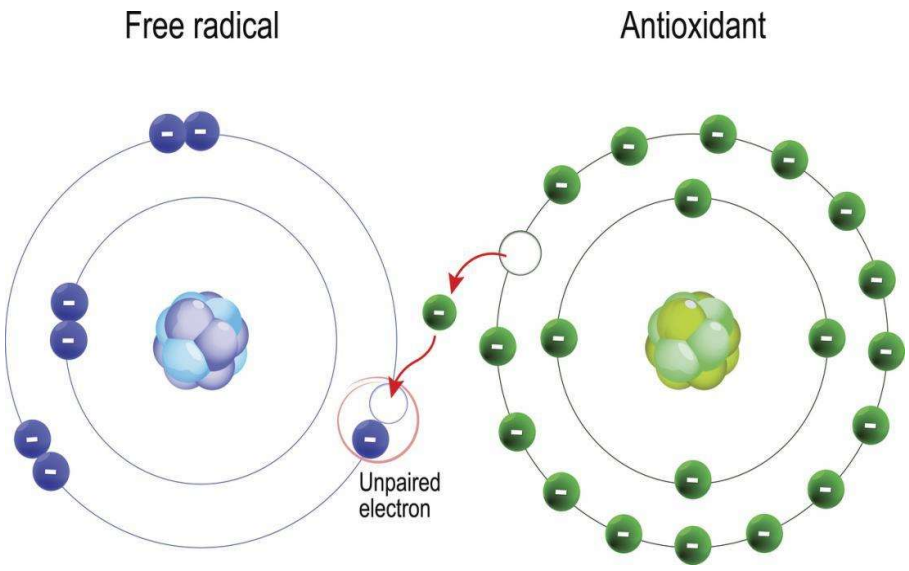


Figure (2.6): Donating Electrons. (Villines, 2017).

Cells contain small organelles called mitochondria, which work to generate energy in the form of adenosine triphosphate (ATP). Mitochondria combine oxygen and glucose to produce carbon dioxide, water, and ATP. Free radicals arise as byproducts of this metabolic process. The problem arises when an equilibrium is broken and when natural defense apparatuses fail, the level of free radicals begins to rise over the capacity of the body to neutralize them, which changes the oxidative status and the body enters the state of oxidative stress, or a state of high risk for the generation of numerous disorders and diseases (Żukowski *et al.,* 2018; Ahmadi-Motamayel *et al*., 2019).

Antioxidants are substances that neutralize free radicals by mean of donating an electron. Examples of antioxidants include vitamins B, C, D and E, lycopene and selenium. Like free radicals, antioxidants come from several different sources. Cells naturally produce antioxidants such as glutathione (Choromańska *et al*., 2017; Balbi *et al*., 2018).

2.10.2 : Salivary Antioxidants System

Organism cells established an antioxidant protective system for preventing the formation of excess free radicals, as well as repairing the damage caused by free radicals. Antioxidant protection consists of cytoprotective enzymes and non-enzymatic antioxidants (Choromańska *et al.,* 2017). In saliva protective mechanisms include the prevention of the occurrence of the damage, and the repairing of the resulting damage, and removing damaged biomolecules before their accumulation causes a new damage.

The effectiveness of the antioxidant system depends on the intake of vitamins and micronutrients through food and supplements as well as the synthesis of antioxidant enzymes, which can be change according to the physical activity, nutrition or aging (Sugiura *et al*., 2015; Ahmadi-

Motamayel *et al.,* 2017, 2019). The salivary levels of antioxidants can be changed in response to an infection, inflammation or disease (Hegde *et al*., 2009).

Antioxidants can be classified as enzymatic elements that include superoxide dismutase (SOD), GSH peroxidase, myeloperoxidase and catalase as well as non-enzymatic elements as minerals, vitamins (B, C, D and E), polyphenols, thiols, uric acid (UA), and albumin (Kamodyová *et al*., 2015; Ighodaro and Akinloye, 2018).

There is another classification of salivary antioxidants into three groups according to their function. The first group consists of preventive antioxidants, those which inhibit the production of free radicals, such as SOD, catalase, carotenoids, GSH peroxidase, transferrin, albumin and haptoglobin. Second group contains what called 'scavenger' antioxidants, such as vitamin C and E, UA, albumin and bilirubin, which eliminate free radicals to inhibit the starting and spreading of cell damage.The last groups are enzymes such as proteases, transferase, lipases, etc, that repair the damage caused in the cells and tissues (Ighodaro and Akinloye, 2018).

Antioxidant salivary protection has been in the last few decades, the interest subject of many medical research. Many studies have proved that oxidative stress plays an important role in the pathogenesis of many inflammatory diseases (Arulselvan *et al*., 2016; Wang *et al.,* 2017). As periodontal disease is the most commonly inflammatory disease of the oral cavity, this role of oxidative stress and antioxidant protection in the pathogenesis of this disease is increasingly mentioned.

Analyzing saliva antioxidant types, as well as its overall total antioxidant capacity (TAC) level estimation, is essential for a better

understanding of pathogenesis of periodontal disease and the introduction of new preventive measures (Pendyala *et al.,* 2013).

2.10.3 : The Role of Oxidative Stress in The Pathogenesis of The Oral Diseases

Oxidative stress that is a result of imbalance between the reactive oxygen species activity and the ability of the human body to detoxify them by antioxidants can lead to the cell damage by micro-damage of the cell membrane, protein deactivation, DNA damage, and prompt of cell signaling molecules that induced the tissue damage (Varadharaj *et al*., 2015; Wang *et al.,* 2017).

Oral tissues are uniquely liable to the free radical damage because the mucous membranes allow rapid absorption of substances throughout their surfaces. In the oral tissues, infection of gingiva can generate oxidative stress. The increase in the formation of free radicals from oxidative stress leads to a further breakdown of cell walls and oral tissues (Akpinar *et al*., 2013; Baltacioglu *et al*., 2014).

Oxidative stress has been determined by the decrease in TAC. In a meta-analysis including 16 studies from different countries reported oxidative stress biomarkers, serum TAC levels were lower in patients with periodontal disease compared to healthy subjects, suggesting that the periodontal inflammation may trigger systemic oxidative stress (Liu *et al*., 2014). Numerous clinical and pre-clinical researches reported that the periodontal inflammation generate ROS which are diffused into bloodstream, and gradually distressed other organs (Tomofuji *et al*., 2007; Baltacioglu *et al*., 2014).

When inflammation occurs, ROS production is extremely increased, this is mainly due to cells of innate immune system, e.g., neutrophils and macrophages (Mittal *et al*., 2014). For explanation, the accepted hypothesis

is that the inflammation can trigger oxidative stress and the oxidative stress state can also induce inflammation. In addition to ROS, the level of serum antioxidants were reported to be lower in the periodontal disease patients (Baltacioglu *et al*., 2006; Konopka *et al*., 2007; Trivedi *et al*., 2015).

Studies proved the presence of periodontopathic bacteria in saliva. Activation of the host response by these periodontal pathogens results in activation and infiltration of neutrophils, which are the primary source of ROS in periodontitis (Ryder, 2010; Scott and Krauss, 2012). ROS production by neutrophils is considered as an important mechanism of bacteria killing (Wang *et al.,* 2017).

However, ROS production by neutrophils might also cause damages of host tissues by lipid peroxidation and DNA damage when ROS are not neutralized by the antioxidant system or in the case of an impaired neutrophils clearance (Hajishengallis, 2014). So that, a proper balance between ROS production and TAC of the host tissue plays an important role in the homeostasis of periodontal tissue and prevents tissue damage (Zhang *et al*., 2016). They have been found a high frequency of *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia* in patients with periodontal disease. The presence of these bacteria was strongly significant in the increase of oxidative stress markers (Wara-Aswapati *et al*., 2009; Suresh *et al*., 2017).

It is reasonable that neutrophils acts upon this inflammation, increasing ROS levels to kill different pathogens (Almerich-Silla *et al*., 2015; Wang *et al.,* 2017).

2.11 : Antioxidant Effect of Folic Acid

Folic acid plays a vital role in DNA synthesis by transferring of single carbon units in the biosynthesis of purine and thymidylate (Khan *et al*, 2019; Batra *et al.,* 2020). Also, folate being a methyl group donor, it fulfills a

fundamental role in the remethylation process of Hcy to methionine amino acid which is catalyzed by a B12- dependent methionine synthetase (Crider *et al*., 2012; Capelli *et al*., 2019).

In case of FAD, remethylation of Hcy can be blocked and will result in the accumulation of Hcy leading to the production of various ROS. So, folic acid is considered to have free-radical-reducing properties (Huang *et al*., 2001; Hsu *et al*., 2013).

Lai *et al*. (2017) found that folate was an antioxidant based on its capability to effectively scavenge various types of free radicals (Lai *et al*., 2017). FAD along with deficiencies in vitamins B6 and B12 has been concerned as the etiological factor in the pathogenesis of many chronic degenerative diseases including cancer, cardiovascular disorders, and Alzheimer's disease (Wang *et al*., 2012; Li *et al.,* 2020).

Gingival inflammation involves excessive release of oxygen-free radicals by inflammatory cells, especially polymorphonuclear leucocytes (PMNLs). PMNLs play an essential role in the host defense and serve as the first line of defense against pathogens invasion and infection in the body. In the oral cavity, after plaque accumulation and the development of clinical inflammation 90% of leukocytes that enter the saliva and 50% of those that infiltrate junctional epithelium are PMNLs. This type of neutrophils helps in controlling the microbial invasion by several intracellular and extracellular oxidative and non-oxidative killing mechanisms (Ahmadi-Motamayel *et al*., 2017).

The oxidative killing mechanism of neutrophils leads to the formation of ROS. In the event of a microbial attack, there is a burst of O_2 consumption at about 10 or 20 times that of resting consumption. This burst of oxygen consumption is the "respiratory burst" phenomenon, and the

excessive uptake of oxygen in neutrophils and macrophages generates ROS that able of damaging either cell membranes or biomolecules. Oral health of the organism depends on the activity of efficient immune defense mechanisms against oxidative damage which induced by FRs/ROS.

Antioxidants, in particular vitamin B, have a protective effect on the periodontium. They neutralize the ROS that can cause oxidative stress, which results in excessive tissue damage. A study in 2019 has been found that antioxidants were significantly elevated after folic acid administration in the mouse in the present study, which may be related to its antioxidant properties. These findings confirmed the previous observations that the folic acid effectively reduces oxidative stress and normalize the concentration of antioxidant enzymes (Zhang *et al.,* 2019).

2.12: Anti-inflammatory Effect of Folic Acid

In epidemiological studies, low folate concentrations were shown to prompt an inflammatory state in the body. Folic acid supplementation improved disease outcomes in subjects with chronic inflammatory diseases by reducing levels of inflammatory markers as TNF-α (Cianciulli *et al*., 2016; Zhang *et al.,* 2019).

A study was conducted in China 2016, aimed to evaluate whether folic acid supplementation would improve cognitive performance by reducing serum inflammatory cytokine concentrations. This study suggested that, daily oral administration of a 400-µg folic acid supplement to mild cognitive impairment (MCI) subjects for 12 months can significantly improve cognitive performance and reduce peripheral inflammatory cytokine levels (Ma *et al*., 2016).

Another study was conducted in 2019, has found that the oral administration of folic acid is well tolerated and that folic acid has notable

anti-inflammatory properties. Reduced inflammation was confirmed histologically, as they observed a reduction in the cytokines appropriate for the maintenance of a cutaneous allergy. Results also demonstrated the significant ability of folic acid to inhibit T-cell proliferation and the secreting of cytokines (Makinoa *et al*., 2019). In addition to this, in healthy overweight subjects, a short-term course of folate supplementation (2.5 mg/day for three months) reduces the circulating level of some inflammatory mediators as IL-6 independently of weight change (Solini *et al*., 2006).

The anti-inflammatory effect of folic acid explained by reduced expression of proinflammatory genes in monocytes by inhibition of NF-κB activation (Feng *et al*., 2011; Lisboa *et al.,* 2020). During the inflammatory disease, monocytes and macrophages produce cytokines in response to different stimuli, such pathogenic microorganisms (Laskin and Pendino, 1995; Samblas *et al*., 2018).

The proinflammatory molecules as TNF-α or ILs released by macrophages in the inflamed regions coordinate the enhancement of monocyte recruitment from blood to tissue. Recruited monocytes differentiate into macrophages to continue the inflammatory response (Shi *et al*., 2011). In contrast, FAD in the same cell line enhanced the expression of proinflammatory genes then inflammation carries on (Kolb and Petrie, 2013).

2.13 : The Role of Folic Acid in Gingival Health and Disease

The vitamin B complex includes eight vitamins involved the folic acid. Oral changes that are common to B-complex deficiencies are gingivitis, and inflammation of the entire oral mucosa. Folic acid-deficient animals demonstrate necrosis of the gingiva, periodontal ligament, and alveolar bone. In humans with sprue and FAD, generalized stomatitis

occurs, which may be accompanied by ulcerative glossitis and cheilitis. Folic acid is utilized by virtually all mammalian cells as a coenzyme for amino acid conversions and synthesis of pyrimidine and purine needed for DNA synthesis (Liew, 2016). It is required for cellular division and new cells production. At the cellular level, the major defect in FAD is an impaired production of DNA expressed as inefficient mitosis, increase in cellular stroma, and asynchronism between protein synthesis and cell division (Erdemir and Bergstrom, 2007).

Interference with DNA synthesis disrupts the production of RNA and prevents the process of cell maturation from reaching completion, a factor that would account for the mucosal changes found in the FAD (Vogel *et al*., 1978).

Histologically, the most prominent FAD-related changes were an interference with the maturation of the epithelial cells, impairment of keratinization, and an increased susceptibility to the ulceration and inflammation (Levy *et al*., 1970). Repair and maintenance of the periodontium generates a high turnover rate of squamous epithelium, thus folic acid is essential for the proper maturation of the rapidly proliferating cells. It is thus conceivable that the gingival epithelium would also be affected in FAD.

Folic acid supplementation can be taken both systemically and topically (Bergstrom 1989). Folic acid is available in various dosage forms like mouthwash, tablets, capsules, oral drops and injections. A supplementation of 2 mg systemic folic acid showed an increase in the resistance of gingiva to the local irritants and thus lead to a reduction in the inflammation over a 30 days period (Vogel, 1976).

In another study, the use of 5 ml folate mouthwash rinse twice daily for 4 weeks for 1 min on established gingivitis in adults showed improved gingival health (Pack, 1984).

Evidence indicates that in a FAD prone situation, the management and maintenance of periodontal health and disease will be positively influenced by folic acid supplementation. It is essential for the maintenance of an intact oral mucosa.

Low levels of serum folate were independently associated with periodontal disease in older adults; hence, it is quite possible to presume that smoking−induced FAD can negatively influence periodontal disease expression and progression. Thus, folic acid supplementation as an adjunct in the management of periodontal disease in smokers will prove to be beneficial. Oral mucosal integrity can be protected by the daily supplements of folic acid (George *et al*., 2013).

A double-blind study evaluated the effects of systemic and topical folate on the gingival inflammation during the fourth and eighth months of pregnancy. They found that, the folate mouthwash produced highly significantly improvement in gingival health in pregnancy (Pack and Thomson, 1980). Another study has reported that a low serum folate level is associated with periodontal disease in non-institutionalized older adults (Yu *et al*., 2013).

Clinically, patients with one of oral and dental specific diseases may need complete blood count and serum folic acid, iron, vitamin B12, Hcy, thyroglobulin antibody, and thyroid microsomal antibody levels to be examined to assess whether these patients have problems like deficiency. (Chiang *et al*[a]., 2020; Chiang *et al*[b]., 2020).

Chapter Three

MATERIALS AND METHODS

CHAPTER THREE

MATERIALS AND METHODS

3.1 : Study design

This is a randomized clinical trial conducted in dental private clinics and scientific laboratories in Mosul city/Iraq, in the period between July/2019 to February/2020. The study was approved by the scientific academic committee of basic science department of college of dentistry/ university of Mosul according to relevant guidelines.

3.1.1 : Patients

The study was carried out on 40 participants (20 females, 20 males) of different ages between a range of (20-40 years) were attended to private dental clinics. Scaling and polishing have been carried out for each volunteer at private dental clinics. In the next day, oral health indices (PI, GI, OHI and CI) for all participants were measured and at 21st day then at 42nd day of study. Diagnosis of chronic gingivitis was established depending on the oral health indices and dental history.

The choice of each volunteer depending on dental diagnosis and achievement of inclusion and exclusion criteria (Table 3.1).

Table (3.1) : Inclusion and Exclusion Criteria

CRITERIA	INCLUDED	EXCLUDED
Age	Between (20-40) years old	out of the range
Systemic Diseases/Oral lesions/Xerostomia	Free	Has one disease/lesion or more
Pregnancy / Lactation	Not	Yes
Folic acid Sensitivity	No	Yes
Other drug / supplements intake	No	Yes
The mouth contains 20 teeth or more	yes	Less than 20 teeth
Smoking	No	Yes
Alcohol Intake	No	Yes
Agree to participate in this study	yes	No

3.1.2 : Record

After selection, patients were divided randomly into 2 groups;

1. Group 1 consisted of 20 chronic gingivitis patients, did not receive any medication (control group).
2. Group 2 consisted of 20 chronic gingivitis patients (study group), received 1 mg/day of oral tablet of folic acid for 42 days.

An informed consent sheet was filled and signed by each volunteer (Appendix 1). Also, case sheet was assigned for each participant (Appendix 2). Patients of both groups were instructed to use the same toothpaste (Biofresh®/Ispain) and same toothbrush, at least twice a day.

3.2: Materials

3.2.1 : Drug Used

Folic acid tablet (1 mg) of SDI Company/ Iraq was used.

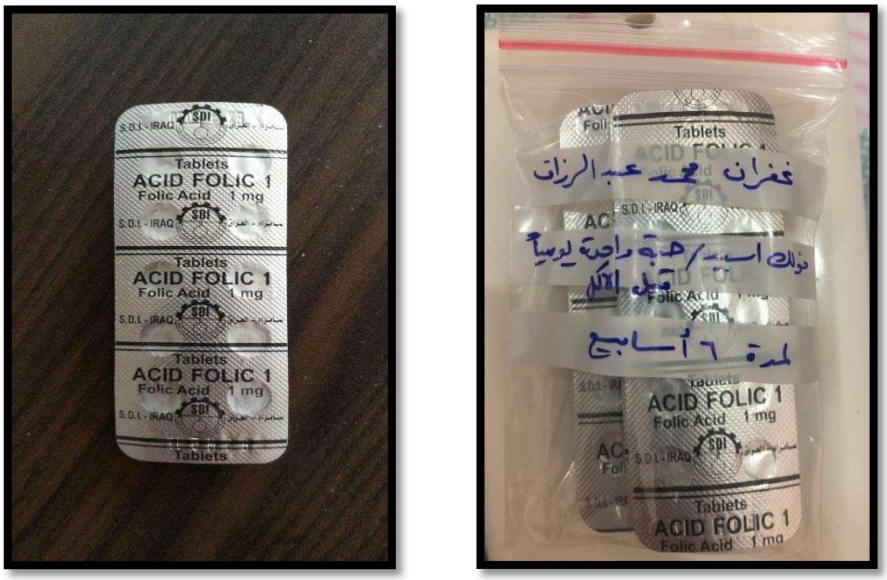


Figure (3.1): Folic acid tablets and the prescription

3.2.2 : Saliva Collection Instruments

1- Plane tube for saliva /China.

2- Eppendorf's tube (2 ml) /China.

3- Disposable syringes 5cc (G 22) /China.

3.2.3 : Diagnostic Set for Gingivitis (Figure 3.2)

1- Dental Probe/China

2- Dental Mirror/China

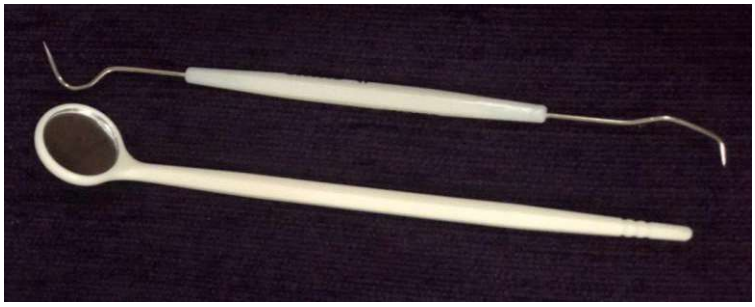


Figure (3.2) : Diagnostic set for gingivitis

3.2.4: Devices

1- ELISA Device /Microplate Reader / Biotek/USA (Figure 3.3 A).
2- Spectrophotometer Device /Zenith Lab Co./China (Figure 3.4).
3- Washer Device /Microplate Washer/Biotek/USA (Figure 3.3 B).
4- Centrifuge / QLS/ ISO-fuge 1424/United Kingdom.
5- Vortex Mixer MX2500/QLS/United Kingdom.
6- Oscillator/KJ 201A/Hinotek/China.
7- Pipettes /Ratiolab/Germany and Watson Nexty/Japan (Figure 3.5 A, B and C).
8- Incubator/JRAD/Syria.
9- Thermostatic Water bath/Taiwan.

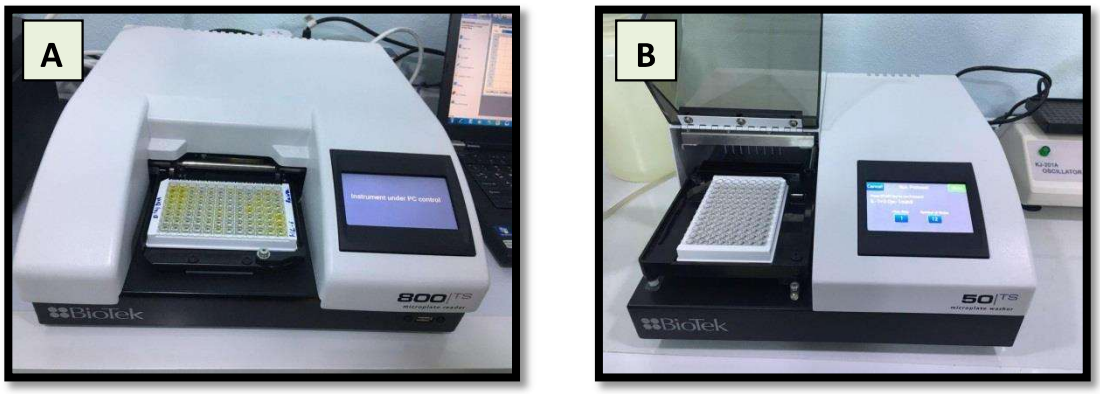


Figure (3.3): A- ELISA Device, B- Washing Machine.

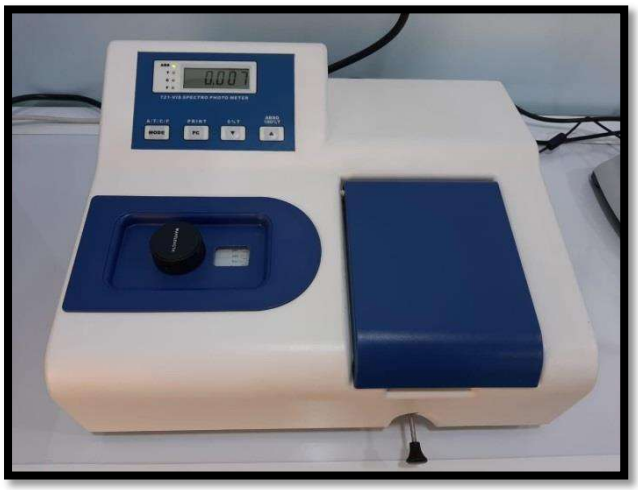


Figure (3.4) Spectrophotometer Device.

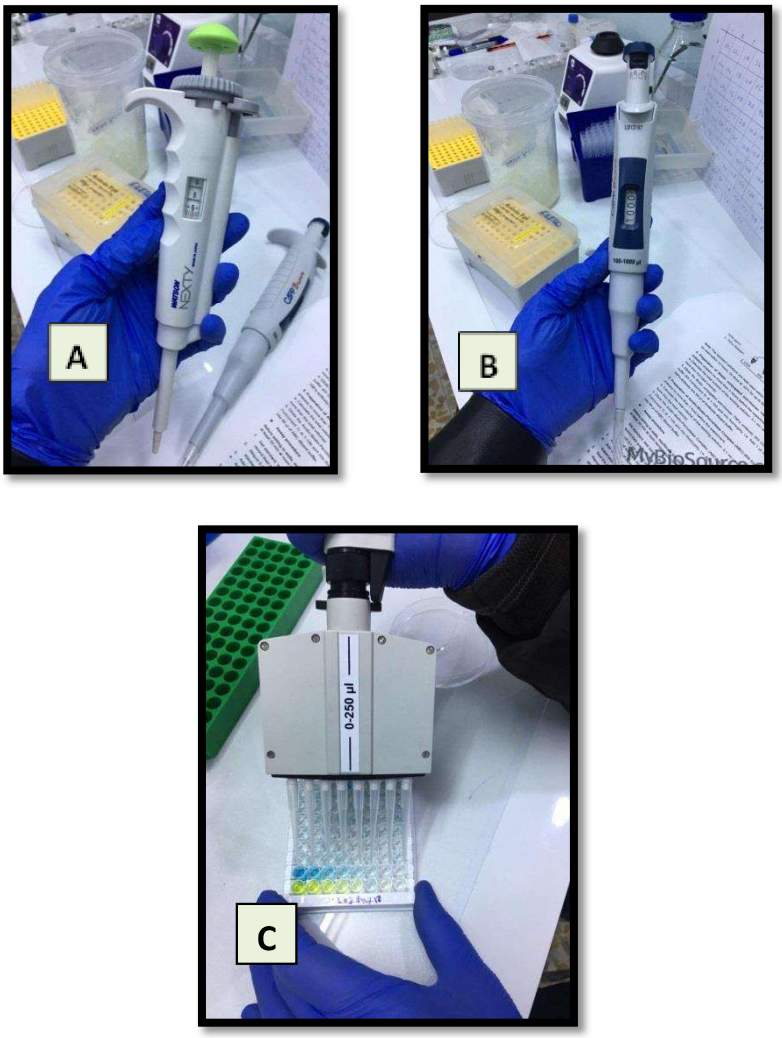


Figure (3.5) : Pipettes used; **A**. Micropipette10-100µl

B. Micropipette 100-1000µl

C. Multichannel pipette 0-250µl.

3.2.5 : Protective Measures

1- Face Mask / Meheco pharmaceuticals / China.

2- Disposable Gloves / China.

3- Laboratory Coat / India.

4- Alcohol Solution / Syria.

5- Cotton Rolls / China.

3.3 : Methods

3.3.1 : Saliva Collection and Storage Procedures

The following instruction have been followed:

1. The participants were refrained from intake of any food or drinks (except for water) 1 hr before collection of saliva, the best time is between 9-11 a.m.
2. The mouth has been rinsed thoroughly with approximately 150 ml of water three times before sample collection.
3. Whole saliva was collected by unstimulated passive drooling. Donors tilted the head forward, in order the saliva to pool on the floor of the mouth, then pass into a plane tube.
4. Storage and stabilization of samples, they are kept in refrigerator within 30 minutes after collecting in order to avoid bacterial growth in the specimen.
5. Unstimulated saliva samples were collected in a plane tubes then drown and kept in Eppendorf's tubes that arranged in a rank at standup position.
6. Then freeze at or below -20ºC until analysis day.
7. At the time of analysis, samples dissolved at room temperature, then centrifugation for all samples is carried out in centrifuge to get a clear saliva sample ready for analysis.

Unstimulated saliva samples which used for measurement of salivary TNF-α, IL-6, TAC and salivary TP were collected from patients of each group as following :

1. Five milliliters of saliva were collected in the day following scaling and polishing, prior to receiving treatment.
2. Five milliliters saliva sample were collected at day 21st of study.
3. Another five milliliters were collected at the end of the study (at day 42nd of the study).

3.3.2: Oral Health Indices (Newman *et al*., 2012)

3.3.2.1 : Measuring of Plaque Index (According to Silness and Löe, 1964)

By using mirror and dental probe, four surfaces (buccal, lingual, mesial and distal) of the six teeth (3, 7, 12, 19, 23, and 28) were examined (figure 3.6) and given a score from 0-3 according to the criteria in table (3.2).

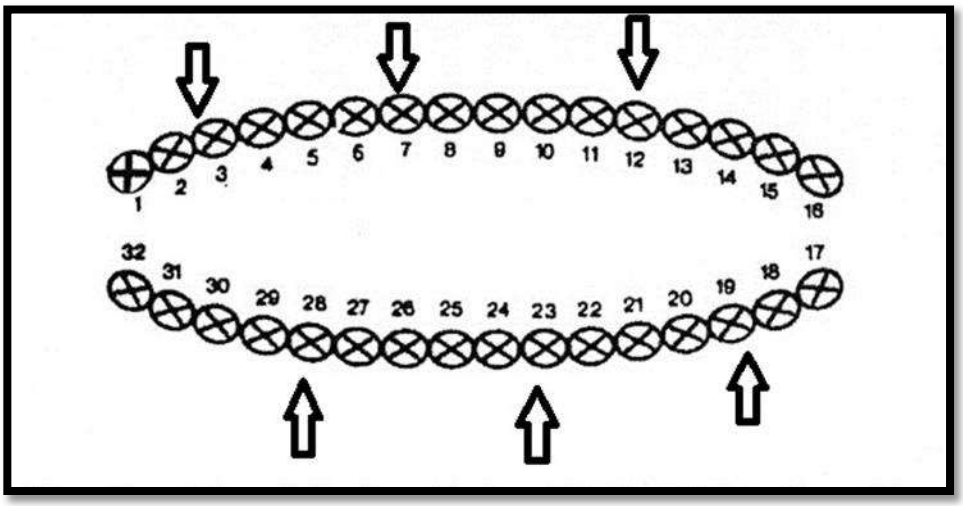


Figure (3.6) : The teeth which examined for oral health indices

Table (3.2) : Plaque index scores and criteria (Newman *et al*., 2012).

Scores	Criteria
0	No plaque
1	A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be seen in situ only after application of disclosing solution or by using the probe on the tooth suface.
2	Moderate accumulation of soft deposits within the gingival pocket, or on the tooth and gingival margin which can be seen with the naked eyes.
3	Abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin.

3.3.2.2 : Measuring of Gingival Index (According to Silness and Löe, 1963)

By using mirror and dental probe, the gingiva of six teeth (3, 7, 12, 19, 23, and 28) were examined and given a score from 0-3 according to the criteria in table (3.3).

Table (3.3) : Gingival index scores and criteria (Newman *et al*., 2012).

Scores	Criteria
0	Normal gingiva
1	Mild inflammation- slight change in color and slight edema but no bleeding on probing
2	Moderate inflammation - redness, edema and glazing, bleeding on probing.
3	Severe inflammation - marked redness and edema, ulceration with tendency to spontaneous bleeding.

3.3.2.3 : Measuring of Oral Hygiene Index (According to Greene and Vermilion, 1960)

It consists of the combined simplified debris Index and simplified calculus index (CI), each component was assessed on a scale of 0 - 3, only mouth mirror and sickle type dental explorer were used for the examination of fourth surfaces of six teeth (3, 7, 12, 19, 23, and 28).

The criteria for scoring the debris and calculus components of the OHI are contained in the tables (3.4) and (3.5). The simplified oral hygiene index score per person is the total of the debris and calculus scores per person.

A. Simplified Debris Index

Table (3.4) : Debris index scores and criteria (Newman *et al*., 2012).

Scores	Criteria
0	No debris or stain present.
1	soft debris covering not more than one third of the surface or the presence of extrinsic stains without other debris, regardless of surface area covered.
2	soft debris covering more than one third but not more than two third of the exposed tooth surface.
3	soft debris covering more than two thirds of the exposed tooth surface.

B. Simplified Calculus Index

Table (3.5) : Calculus index scores and criteria (Newman *et al*., 2012).

Scores	Criteria
0	No calculus present.
1	Supragingival calculus no more than one third of the tooth surface.
2	Supragingival calculus covering more than one third but not more than two thirds of the exposed tooth surface, or the presence of individual flecks of subgingival calculus around the cervical portion of the tooth, or both.
3	Supragingival calculus covering more than two thirds of the exposed tooth surface or a continuous heavy band of subgingival calculus around the cervical portion of the tooth, or both.

3.3.3 : Measurement of Human Salivary TNF-α

Assay Principle

This kit was based on a sandwich enzyme-linked immune-sorbent assay technology (ELISA) of 96 wells (figure 3.7). Anti-TNF-α polyclonal antibody was pre-coated onto 96-well plates and the biotin conjugated anti-TNFα polyclonal antibody was used as detection antibodies. The standards, test samples and biotin conjugated detection antibody were added to the wells subsequently, and washed with wash buffer.

Avidin-Biotin-Peroxidase complex was added and unbound conjugates were washed away and wash buffer. Tetramethylbenzidine (TMB) substrates were used to visualize horseradish peroxidase (HRP) enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the TNF-α amount of sample captured in plate. Optical density absorbance at 450 nm in a microplate reader, was red, and then the concentration of TNF-α can be calculated.

Assay Procedure (according to the kit leaflet)

At the time of an analysis, the freezed samples were dissolved at room temperature, then centrifuged at 1000x g for 10 minutes, and then:

1. Standard, test sample and control wells have been set on the pre-coated plate respectively.
2. 0.1 ml of 1000 pg/ml, 500 pg/ml , 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.2 pg/ml, 15.6 pg/ml standard solution have been aliquotted into standard wells.
3. 0.1 ml of sample / standard diluent buffer was added into the control well.
4. We added 0.1 ml of properly diluted saliva sample into test sample wells.
5. We sealed the plate with a cover and incubated at 37ºC for 90 min.

6. We removed the cover and discarded the plate content. Then we clapped the plate on the absorbent filter paper .
7. We added 0.1 ml of biotin conjugated anti-human TNFα antibody work solution into the above wells (standard, test sample, and control wells).
8. We sealed the plate with a cover and incubated at 37ºC for 60 min.
9. Then we removed the cover and washed plate 3 times with wash buffer using automated washing that aspirated all wells then washed plate 3 times with wash buffer.
10. We added 0.1 ml of ABC working solution into each well (figure 3.8 A), covered the plate and incubated at 37ºC for 30 min.
11. We removed the cover and washed the plate 5 times with wash buffer .
12. We added 0.1 ml of TMB substrate into each well, then covered the plate and incubated at 37ºC in dark place within 30 min .
13. We noticed the shades of blue in the first 3-4 wells (with most concentrated Human TNF-α standard solutions). The other wells showed no obvious color (Figure 3.8 B).
14. We added 0.1 ml of stop solution into each well and mixed thoroughly. The color changed into yellow immediately (Figure 3.8 C).
15. We read the optic density (OD) absorbance at 450 nm in a microplate reader within 30 minutes after adding the stop solution .
16. Calculation of salivary TNF-α concentration as follow:

    Relative $O.D_{450}$ = *the* $O.D_{450}$ *of each well- the* $O.D_{450}$ *of zero well*

    So the concentration is calculated by plotting the relative O.D of each well (Y) vs the respective concentration of blank solution at 450 nm, using a standred curve (figure 3.9).

Figure (3.7): A. Human TNF-α ELISA Kit (MyBioSource®/ USA)

B. Kit components

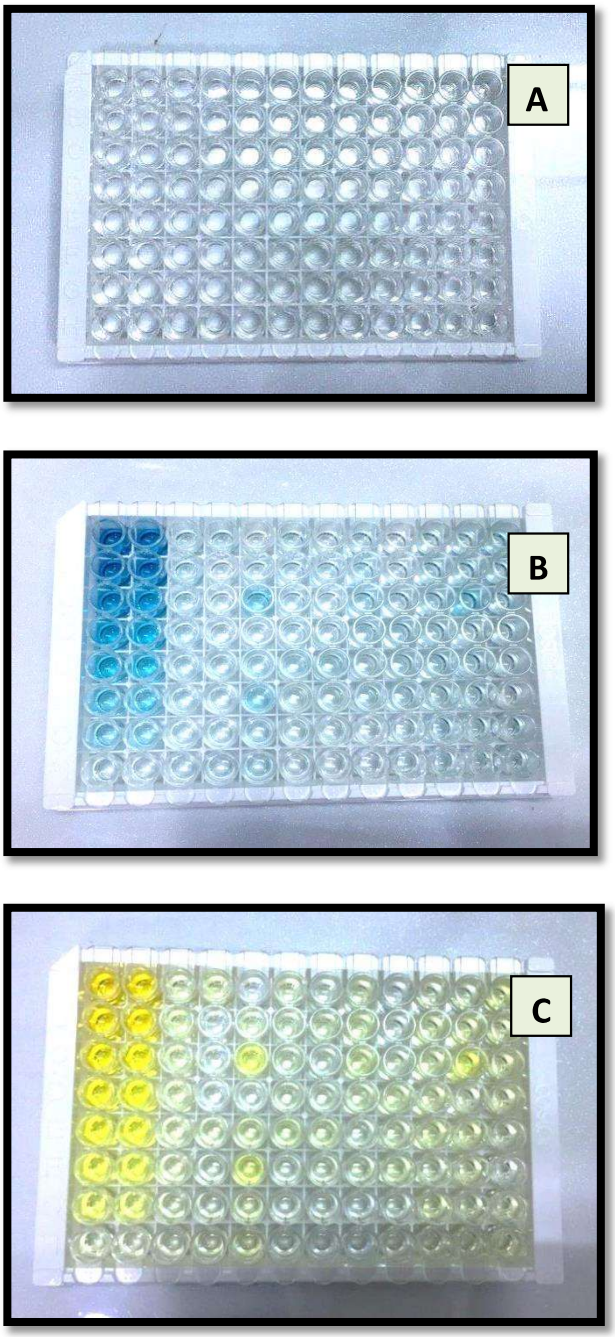


Figure (3.8): Changing the color of salivary TNF-α ELISA kit wells during work. **A.** Color of wells after adding of working solution, **B.** Color of wells after adding of antibody solution, **C.** Color of wells after adding of the stop solution.

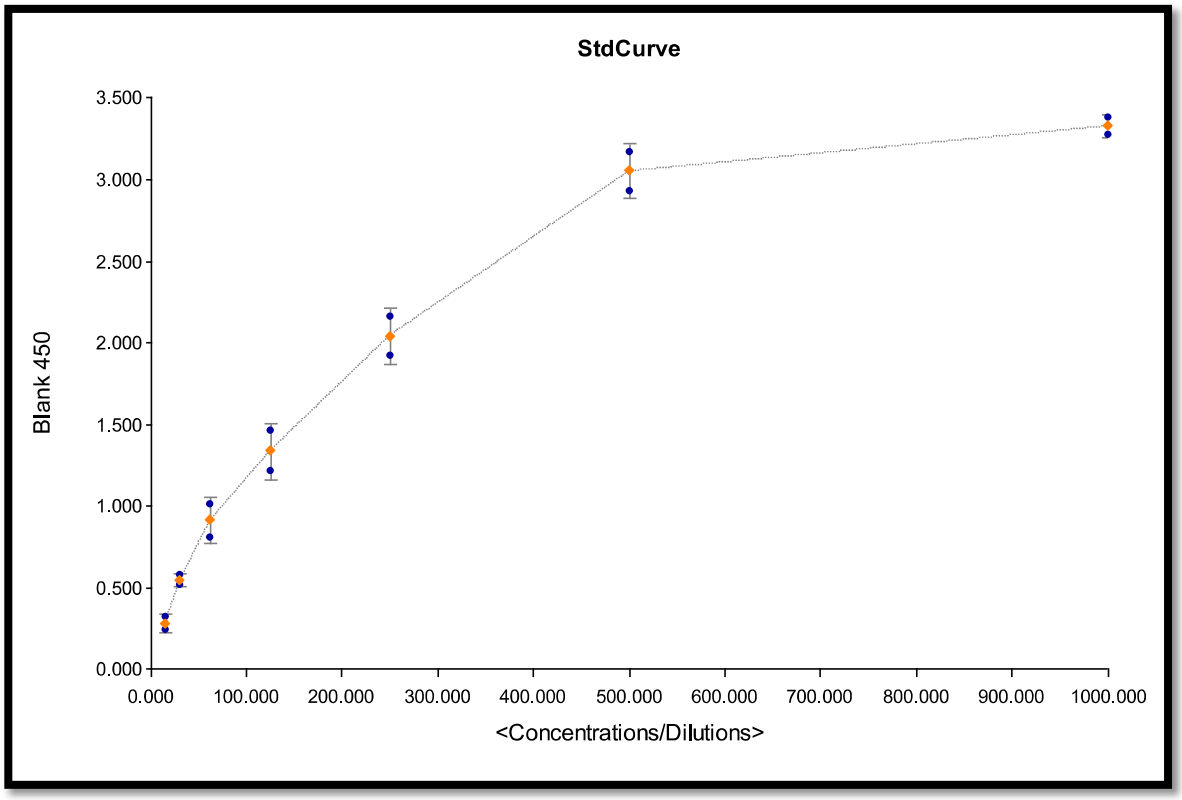


Figure (3.9) : Standard Curve of Human salivary TNF-α ELISA Kit

3.3.4 : Measurement of human salivary IL-6

Test Principle

This is a sandwich ELISA kit, of 96 wells. IL-6 in standards and samples binds to the antibody binding sites on a microtitre plate (Figure 3.10). After incubation, unbound components are washed away. Biotin conjugated to goat antibodies to human IL-6 are added and attach the bound IL-6. After incubation, unbound components are washed away. Streptavidin conjugated to HRP is added and binds to biotin conjugated to the goat antibodies. Bound streptavidin-HRP is measured by the reaction of the horseradish peroxidase enzyme to the substrate TMB. This reaction produces a blue color. A yellow color is formed after stopping the reaction with an acidic solution. The optical density is read on a standard plate reader at 450 nm. The amount of streptavidin- HRP detected is proportional to the amount of IL-6 present in the sample.

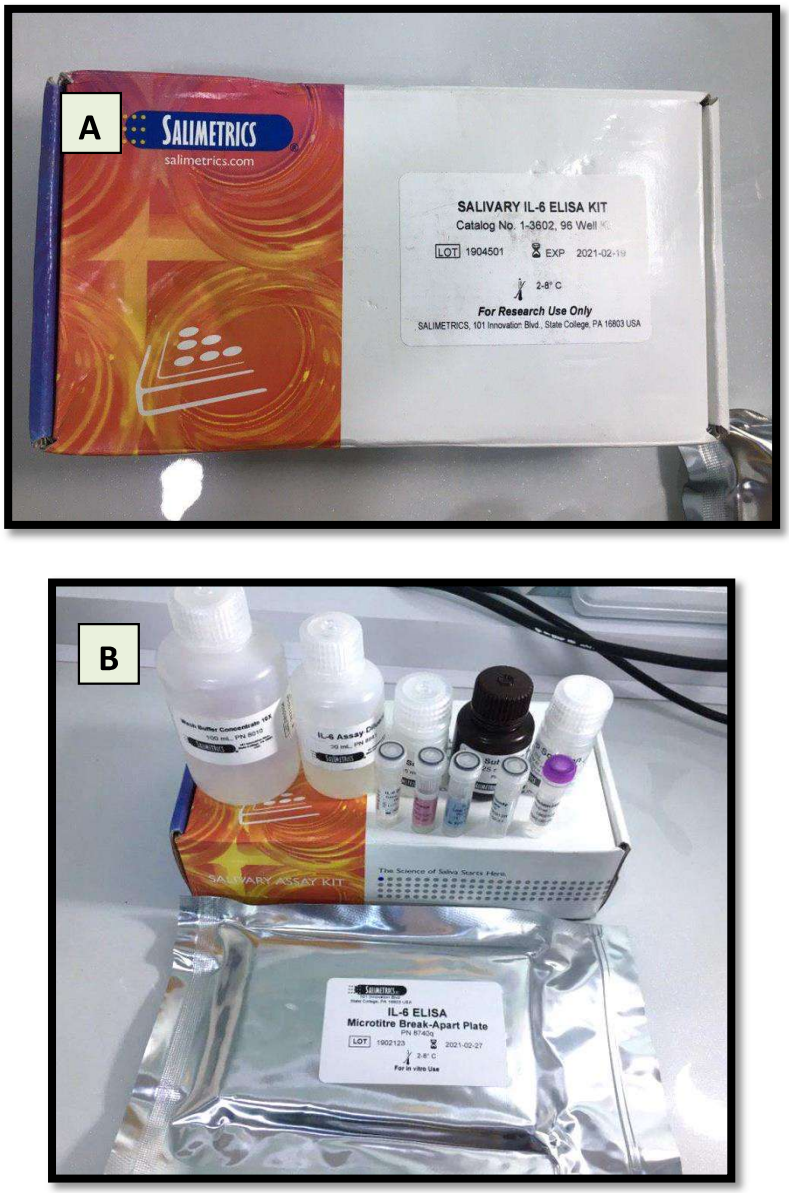


Figure (3.10) : A. Human salivary IL-6 ELISA Kit (Salimetric®/ USA) .
B. Kit components

Test Procedure (according to the kit leaflet)

1. We diluted saliva sample 5X in IL-6 sample diluent using 60 µl saliva to 240 µl IL-6 sample diluent.
2. We pipetted 100 µl of standards, controls, and diluted saliva samples into appropriate wells. Then pipetted 100 µl of IL-6 assay diluent into 2 wells to serve as the zero standard.

3. We placed adhesive cover provided over plate. Mixed plate on a plate rotator continuously at 500 rpm for 1 hour at room temperature.
4. With 1X wash buffer, we washed the plate 4 times by automated washer device, then the plate thoroughly blotted on paper towels before turning upright.
5. The antibody conjugate 1:500 was diluted by adding 24 µl of the antibody conjugate to the 12 ml of IL-6 assay diluent. We immediately mixed the diluted antibody conjugate solution and added 100 µl to each well using a multichannel pipette.
6. Then we placed a new cover over plate. We mixed plate on a plate rotator continuously at 500 rpm for 2 hours at room temperature.
7. With 1X wash buffer, we washed the plate 4 times by automated washer device, then the plate thoroughly blotted on paper towels before turning upright.
8. We diluted the streptavidin-HRP 1:100 by adding 120 µl of the streptavidin-HRP to the 12 ml of IL-6 assay diluent. Immediately we mixed the diluted streptavidin-HRP solution and added 100 µl to each well using a multichannel pipette.
9. Then we mixed plate on a plate rotator continuously at 500 rpm for 20 minutes at room temperature.
10. With 1X wash buffer, we washed the plate 4 times by automated washer device, then the plate thoroughly blotted on paper towels before turning upright.
11. We added 100 µl of TMB substrate solution to each well with a multichannel pipette.
12. Then we mixed on a plate rotator for 5 minutes at 500 rpm and incubated the plate in the dark place at room temperature for 15 minutes.

13. 50 µl of stop solution is added with a multichannel pipette.
14. Then we mixed on a plate rotator for 3 minutes at 500 rpm until all wells turned yellow color. Then we read OD absorbance in a plate reader at 450 nm within 10 min of adding stop solution. Calculation of salivary IL-6 concentration as follow:
15. Relative $O.D_{450}$ = the $O.D_{450}$ of each well- the $O.D_{450}$ of zero well. So the concentration is calculated by plotting the relative O.D of each well (Y) vs the respective concentration of blank solution at 450 nm, using a standred curve (figure 3.11).

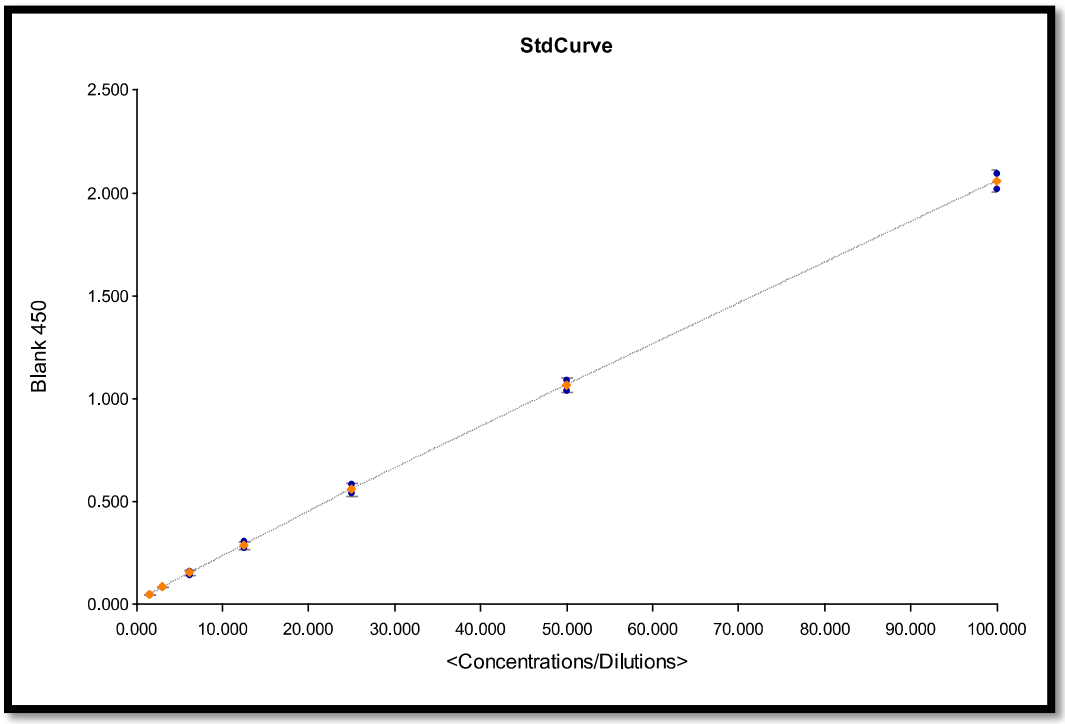
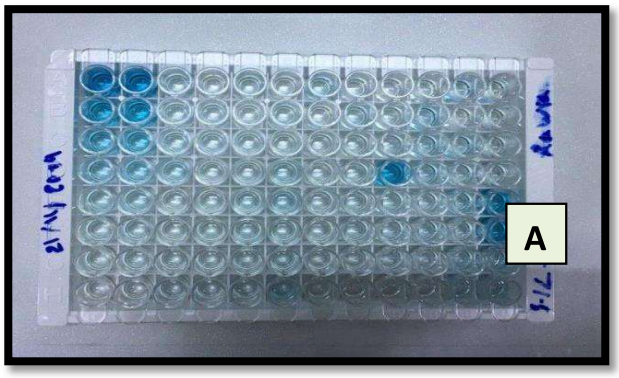


Figure (3.11) : Standard Curve of Human Salivary IL-6 ELISA Kit.



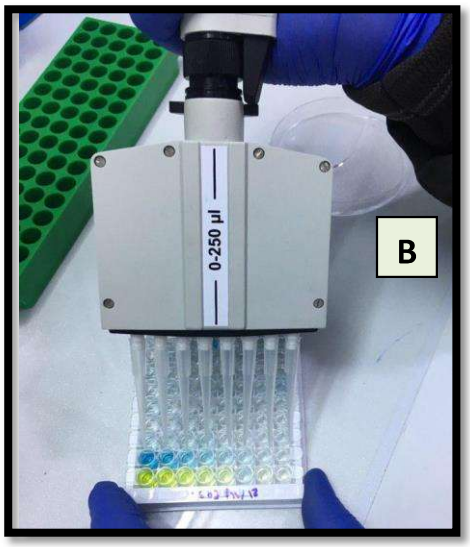


Figure (3.12) : Changing the color of salivary IL-6 ELISA kit wells during work. **A**. Color of wells after adding of TMB solution, **B.** adding of Stop solution by multichannel pipette, **C.** Color of wells after adding of the stop solution.

3.3.5 : Measurement of Total Antioxidant Capacity in Saliva

Detection Principle

A variety of antioxidant macromolecules, antioxidant molecules and enzymes in a system can eliminate all kinds of reactive oxygen species and prevent oxidative stress induced by reactive oxygen species. The total level reflect the total antioxidant capacity in the system. Many antioxidants in the body can reduce Fe^{3+} to Fe^{2+} and Fe^{2+} can form stable complexes with phenanthroline substance. TAC can be calculated by measuring the absorbance at 520 nm.

Measurement procedure (according to the kit leaflet)

1. We centrifuged each saliva sample at 10000x g (Appendix 3) for 10 min at 4℃, Then we took the supernatant for detection.
2. In the Sample tube we added 1.0 mL of reagent 1 to 5 mL EP tube.
3. In Control tube we added 1.0 mL of reagent 1 to 5 mL EP tube.
4. In Sample tube we added 0.1 mL of sample the tube.
5. In control tube we added nothing.
6. We added 2.0 mL of reagent 2 working solution and 0.5 mL of reagent 3 working solution to sample tube and control tube.
7. We mixed fully and incubate the tubes at 37℃for 30 min.
8. We added 0.1mL of reagent 5 to sample tube and control tube.
9. In Sample tube we add nothing.
10. In Control tube we added 0.1 mL of sample.
11. We mixed fully and stand for 10 min at room temperature. Then we set to zero with double-distilled water and measured the OD value of each tube at 520nm with 1cm optical path quartz cuvette.
12. Finally, we calculated TAC value for each sample:

At 37℃, the OD value of the reaction system was increased 0.01 by 1 mL of sample per minute is defined as a unit of TAC.

TAC(U/mL)=(ΔA/0.01)÷30*×V1/V2×f

[Note]:

ΔA: OD Sample – OD Control

***:** The reaction time, 30 min.

V1: The total volume of reaction, mL.

V2: The volume of sample added to the reaction, mL.

f: Dilution factor of sample before tested.

0.01: Constant

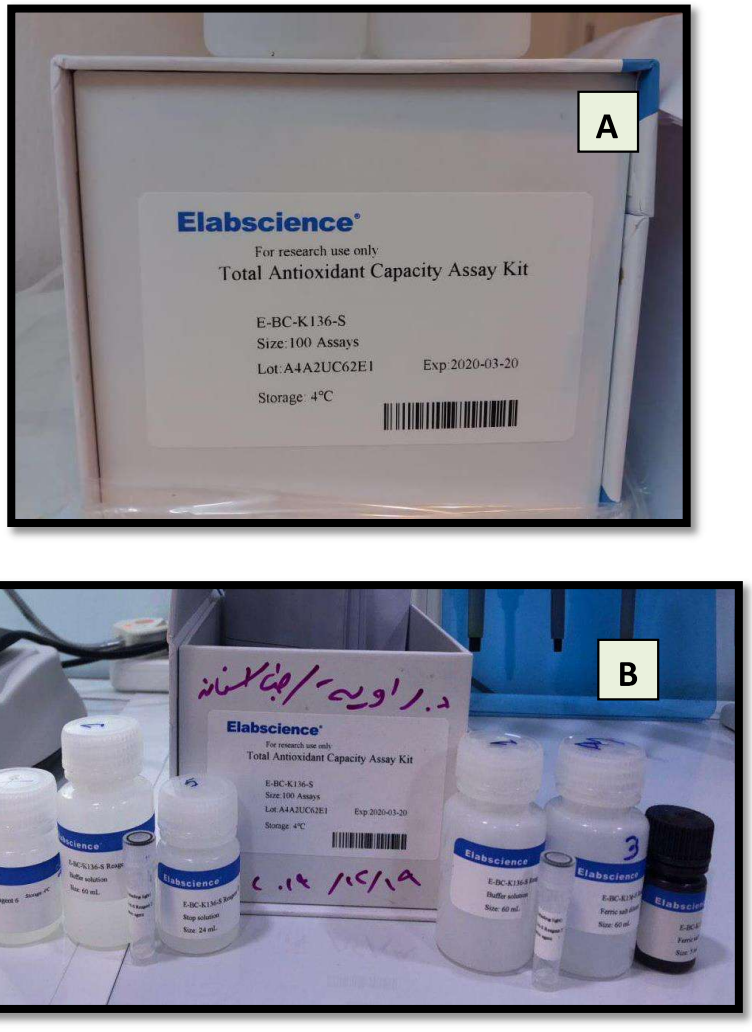


Figure (3.13) : A. Total antioxidant capacity Assay Kit (Elabscience® /USA).

B. Kit components.

3.3.6 : Measurement of Salivary Total Proteins

Assay Principle

Calorimetric method were described by Gornall *et al*. in this kit (figure 3.14). The peptide bonds of proteins react with Cu^{2+} in alkaline solution to form a colored complex which absorbance proportional to the concentration of TP in the specimen is measured at 550 nm. The Biuret reagent contains sodium potassium tartarate to complex cupric ions and maintains their solubility in alkaline solution.

Assay Procedure (According to the kit leaflet)

Total proteins for each sample of saliva was determined using the Biuret method (Itzhaki and Gill, 1964) as in the following steps:

First of all, we dissolved all samples at room temperature then, we made simple mixing for each eppendorf's tube for 5 sec. by using a vortex device, and then we performed:

1. Calibration of ultraviolet visible spectrophotometer device (Figure 3.4) at 550 nm wavelength by using reagent blank which is composed of 20 µL of demineralized water and 1 mL of Biuret reagent which was prepared by mixing of 370 mmol/L of Sodium hydroxide, 10 mmol/L of Na-K Tartarate , 3 mmol/L of Potassium Iodide and 3 mmol/L of Copper II sulphate.

2. Then we prepared standard solution by adding 20 µL of biuret standard (Bovine Albumin) plus 1 mL of Biuret reagent , we recorded its absorbance at 550 nm wavelength.

3. We prepared each saliva sample tube by adding 20 µL specimen plus 1 mL of Biuret reagent then recorded optical density at 550 nm wavelength.

4. We determined salivary total proteins for each saliva sample by using a simple calculation as following:

Total Proteins Concentration = {Abs (sample)/Abs (standard)} X standard concentration

Standard absorbance = 0.355 nm (of this procedure).

Standard concentration = 6 g/dL.

Figure (3.14) : Total Proteins Kit/Biolabo®/France

3.4 : Statistical Analysis

Data analysis and statistical tests

Data tabulation and coding performed via Microsoft Excel-2010. Descriptive and analytic statistics was performed using Minitab (version 18) software statistical program. The descriptive statistics included mean ± Standard Deviation (SD) for measurable variables.

Independent t-test for two means was used for comparing parameters between treatment and control groups. One-way Analysis of Variance test (ANOVA-test) with Tukey's Pair-wise comparisons was used for comparison between the three means parameters recoding throughout the study period. Dependent t-test of two means (paired) was applied for the differences in parameters within each group (beginning – final. (Triola *et al.,* 2018).

Percentage improvement rate was calculated in the two groups as follow (Parker *et al.,* 2009);

$$\%\ \textbf{Improvement Rate} = \frac{\textbf{before intervention} - \textbf{after intervention}}{\textbf{before intervention}} \times \mathbf{100}$$

P-values ≤ 0.05 were considered statistically significant throughout data analysis

Chapter Four

THE RESULTS

CHAPTER FOUR

THE RESULTS

4.1 : Demographic Data

A total of 40 patients was included in the study. They were divided randomly into two groups; treatment group (20 patients) and control group (20 patients). The treatment group consisted of 11 females (55%) and 9 males (45%), the mean of age of patients was 28.2 ± 5.72 years, while the control group consisted of 9 females (45%) and 11 males (55%), the mean of age was 28.9 ± 7.05 years with no significant differences between their gender, p = 0.732 as shown in the (Figure 4.1).

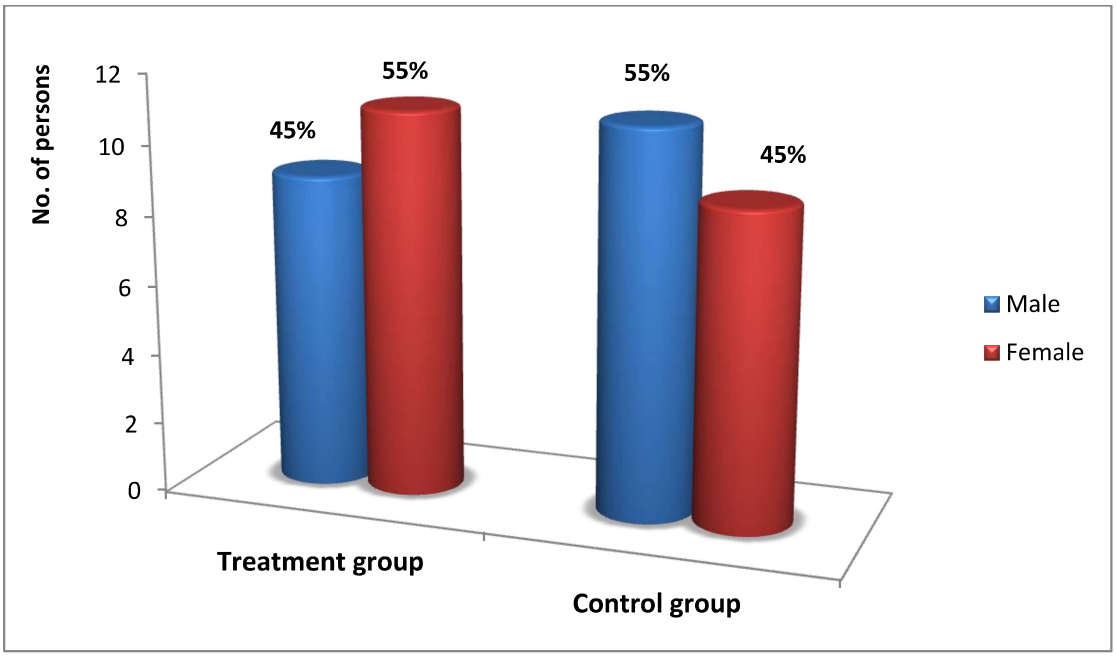


Figure (4.1) : Gender distribution of the study samples population.

4.2: Oral Health Scores

4.2.1: Descriptive Statistics

The mean and standard deviation of oral health scores (PI, GI, OHI and CI) for each participant of both treatment and control groups at the beginning of study were shown in table 4.1.

Table (4.1) : Oral health scores of treatment and control group at the beginning of the study (n = 20).

Oral Health Scores	Treatment Group Mean ± SD	Control Group Mean ± SD
Plaque index	0.167±0.291	0.308±0.293
Gingival index	0.458±0.436	0.550±0.429
Oral hygiene index	0.233±0.348	0.433±0.380
Calculus index	0.117±0.163	0.133±0.206

*SD : Standard deviation

The mean and standard deviation of oral health scores for each participant of both treatment and control group at 21st day of the study period were shown in the table 4.2.

Table (4.2) : Oral health scores of treatment and control group at 21st day of the study period (n = 20).

Oral Health Scores	Treatment Group Mean ± SD	Control Group Mean ± SD
Plaque index	0.191±0.282	0.433±0.348
Gingival index	0.350±0.346	0.541±0.338
Oral hygiene index	0.308±0.394	0.558±0.493
Calculus index	0.191±0.287	0.174±0.282

The mean and standard deviation of oral health of each participant both treatment and control groups at 42nd day of the study period were shown in the table 4.3.

Table (4.3) : Oral health scores of treatment and control group at 42nd day of the study period (n = 20).

Oral Health Scores	Treatment Group Mean ± SD	Control Group Mean ± SD
Plaque index	0.341±0.256	0.433±0.326
Gingival index	0.400±0.317	0.566±0.406
Oral hygiene index	0.441±0.307	0.624±0.515
Calculus index	0.333±0.259	0.250±0.289

4.2.2 : Comparison in oral health scores between both groups

The results are showing that there is no significant difference between means of treatment and control group in PI, GI, OHI and CI at the beginning of the study (Table 4.4).

Table (4.4) : Comparison in oral health scores between the two groups at the beginning of the study.

Oral Health Scores	Treatment Group [N = 20] Mean ± SD	Control Group [N = 20] Mean ± SD	P-Value*
Plaque index	0.167 ± 0.291	0.308 ± 0.293	0.133
Gingival index	0.458 ± 0.436	0.550 ± 0.329	0.457
Oral hygiene index	0.233 ± 0.348	0.433 ± 0.380	0.091
Calculus index	0.117 ± 0.163	0.133 ± 0.206	0.778

*** Independent T-test of two means was used**

This study showed a significant difference between means of treatment and control group in PI, but there is no significant difference in the means between treatment and control group in GI, OHI and CI at day 21st of the study, as illustrated in table 4.5.

Table (4.5) : Comparison in oral health scores between the two groups at 21st day of the study.

Oral Health Scores	Treatment Group [N = 20] Mean ± SD	Control Group [N = 20] Mean ± SD	P-Value*
Plaque index	0.191 ± 0.282	0.433 ± 0.348	**0.022**
Gingival index	0.350 ± 0.346	0.541 ± 0.338	0.086
Oral hygiene index	0.308 ± 0.394	0.558 ± 0.493	0.085
Calculus index	0.191 ± 0.287	0.174 ± 0.282	0.854

*** Independent T-test of two means was used**

No significant difference is found between means of treatment and control group in PI, GI, OHI and CI at 42nd day of the study, as seen in table 4.6.

Table (4.6) : Comparison in oral health scores between treatment and control group at 42nd day of the study.

Oral Health Scores	Treatment Group [N = 20] Mean ± SD	Control Group [N = 20] Mean ± SD	P-Value*
Plaque index	0.341 ± 0.256	0.433 ± 0.326	0.329
Gingival index	0.400 ± 0.317	0.566 ± 0.406	0.156
Oral hygiene index	0.441 ± 0.307	0.624 ± 0.515	0.180
Calculus index	0.333 ± 0.259	0.250 ± 0.289	0.343

*** Independent T-test of two means was used**

4.2.3 : Comparison in oral health scores between study days for both treatment and control group

Comparisons among oral health scores of treatment group during all the study period are showing that there is a significant difference in mean of CI between 1st day and the 21st day of the study, while there is no significant difference in means of the other oral health scores during all the study period as illustrated in the table 4.7 and figure 4.2, 4.3, 4.4 and 4.5.

Table (4.7) : Effect of systemic folic acid treatment on oral health scores during the study period.

Oral Health Scores	Treatment Group			P-Value*
	1st day Mean ± SD	21st day Mean ± SD	42nd day Mean ± SD	
Plaque index	0.167 ± 0.291 A	0.191 ± 0.282 A	0.341 ± 0.256 A	0.106
Gingival index	0.458 ± 0.436 A	0.350 ± 0.346 A	0.400 ± 0.317 A	0.653
Oral hygiene index	0.233 ± 0.348 A	0.308 ± 0.394 A	0.441 ± 0.307 A	0.175
Calculus index	0.117 ± 0.163 B	0.191 ± 0.287 AB	0.333 ± 0.259 A	**0.021**

*** One-way ANOVA-test with Tukey's Pair wise comparisons was used. Means that do not share (A) letter are significantly different.**

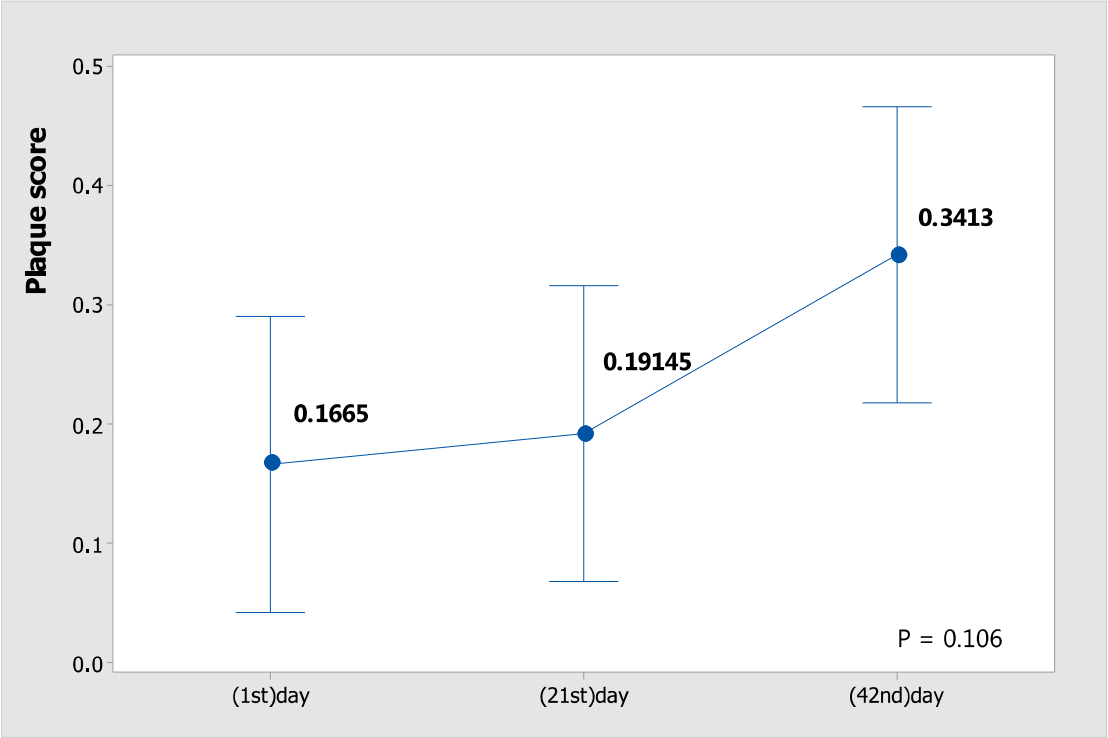


Figure (4.2) : The change in plaque index score after use of folic acid during all the study period**.**

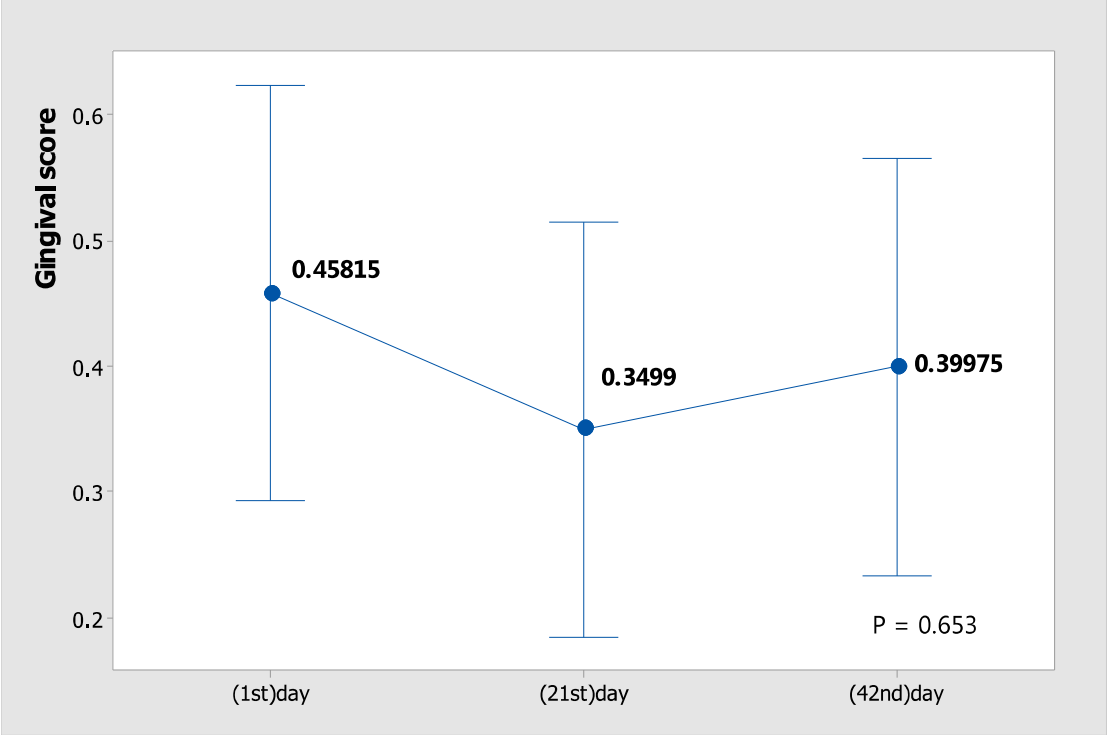


Figure (4.3) : The change in gingival index score after use of folic acid during the study period.

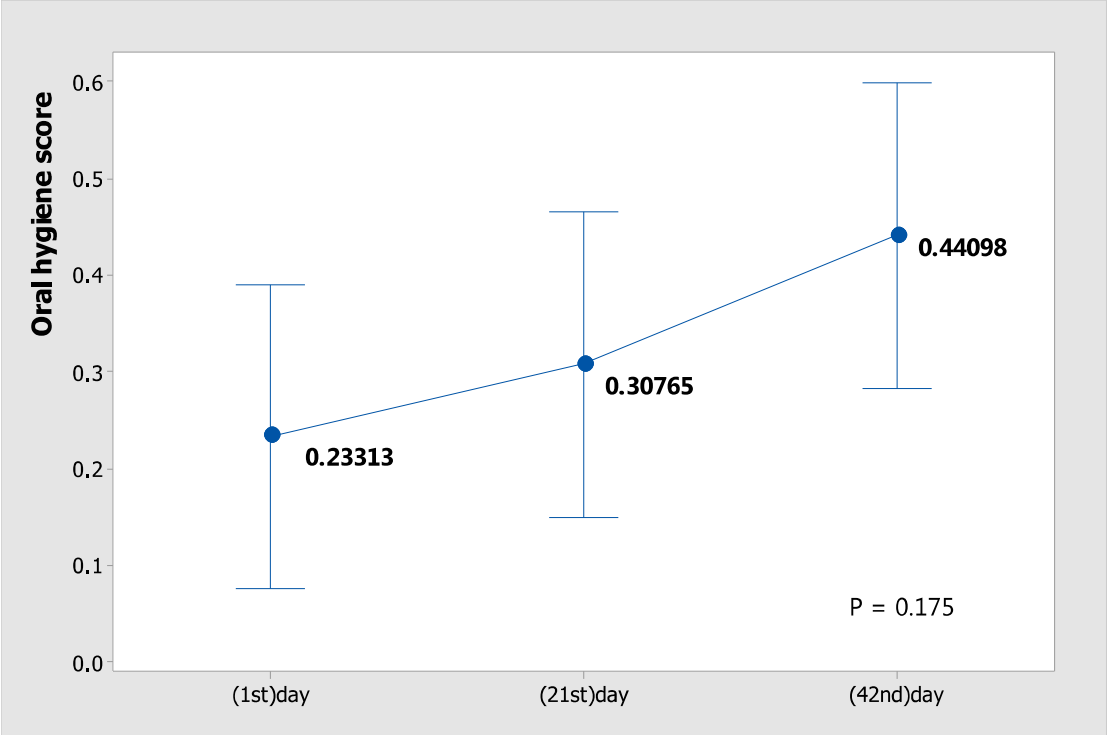


Figure (4.4) : The change in oral hygiene index score after use of folic acid during study period.

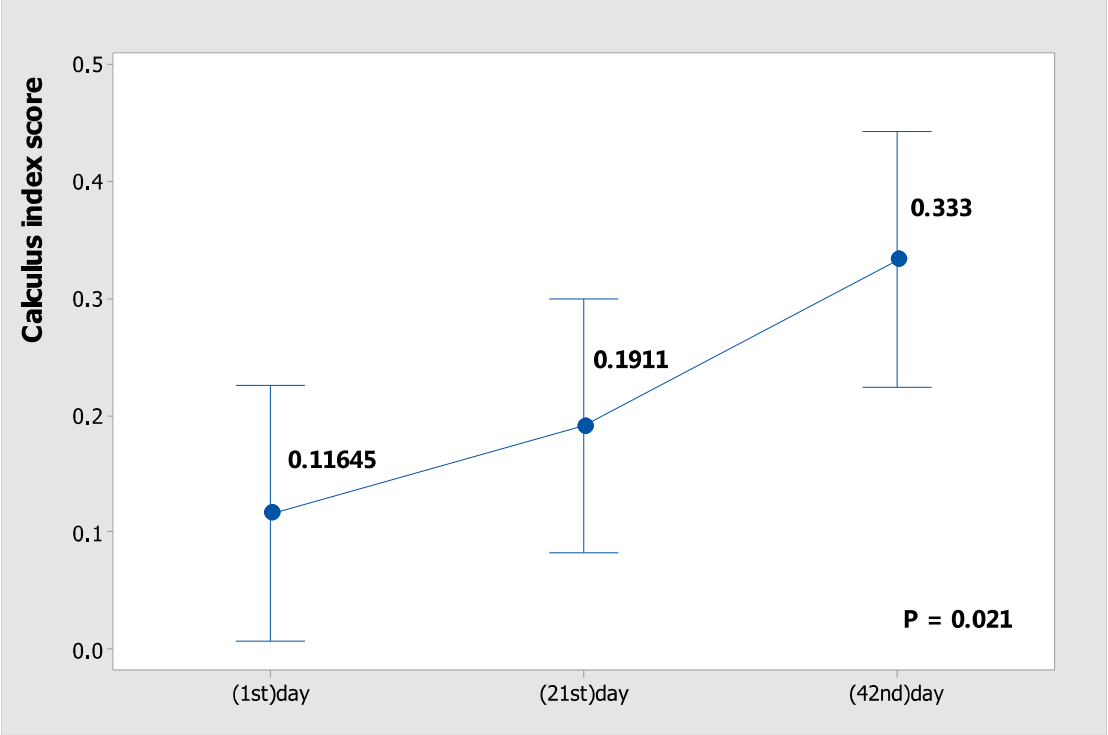


Figure (4.5) : The change in calculus index score after use of folic acid during study period.

Comparisons among oral heath scores of control group during all the study times show no significant difference between means of PI, GI, OHI and CI at the 1st, 21st, 42nd days of study (Table 4.8).

Table (4.8) : Comparison in oral health scores of control group during the study period.

Oral Health Scores	Control Group			P-Value*
	1st day Mean ± SD	21st day Mean ± SD	42nd day Mean ± SD	
Plaque index	0.308 ± 0.293 A	0.433 ± 0.348 A	0.433 ± 0.326 A	0.376
Gingival index	0.550 ± 0.329 A	0.541 ± 0.338 A	0.566 ± 0.406 A	0.974
Oral hygiene index	0.433 ± 0.380 A	0.558 ± 0.493 A	0.624 ± 0.515 A	0.427
Calculus index	0.133 ± 0.206 A	0.174 ± 0.282 A	0.250 ± 0.289 A	0.367

*** One-way ANOVA-test with Tukey's Pair wise comparisons was used. Means that do not share (A) letter are significantly different.**

4.2.4: The Improvement Percentage Rates of Oral Health Scores

Paired T-test and% Improvement Rate that were used to estimate the percentage of changes in oral health scores at day 42 of the study compared to the beginning in treatment group and control group, as illustrated in tables 4.9 and 4.10

Table (4.9) : the Percentage of improvement rates in oral health scores in treatment group.

Oral Health Scores	Beginning Mean ± SD	After 42 Days Mean ± SD	Before – After	% Improvement Rate
Plaque Index	0.167 ± 0.291	0.341 ± 0.256	- 0.174	**104.2**
Gingival Index	0.458 ± 0.436	0.400 ± 0.317	0.058	**12.7**
Oral Hygiene Index	0.233 ± 0.348	0.441 ± 0.307	- 0.208	**89.3**
Calculus Index	0.117 ± 0.163	0.333 ± 0.259	- 0.216	**184.6**

*** Paired T-test of two means was used.**

Table (4.10) : Percentage of improvement rate in oral health scores of control group during the study period.

Oral Health Scores	Beginning Mean ± SD	After 42 Days Mean ± SD	Before – After	% Improvement Rate
Plaque Index	0.308 ± 0.293	0.433 ± 0.326	- 0.125	**40.6**
Gingival Index	0.550 ± 0.329	0.566 ± 0.406	- 0.017	**2.9**
Oral Hygiene Index	0.433 ± 0.380	0.624 ± 0.515	- 0.191	**44.1**
Calculus Index	0.133 ± 0.206	0.250 ± 0.289	- 0.117	**87.9**

*** Paired T-test of two means was used.**

4.3 : Salivary Parameters

4.3.1 : Descriptive Statistics

The mean and standard deviation of salivary parameters level; IL-6, TNF-α, TAC and TP of each participant in both treatment group and control group at the beginning of study were estimated as shown in the table (4.11)

Table (4.11): Mean and standard deviation of salivary parameters levels of treatment and control group at the beginning of the study (n = 20).

Salivary Parameters	Treatment Group Mean ± SD	Control Group Mean ± SD
IL-6 (Pg/mL)	3.35±2.66	2.68±1.64
TNF-α (Pg/mL)	16.65±1.38	17.21±1.55
TAC (U/mL)	5.02±3.77	5.51±4.08
TP (g/dL)	0.76±0.46	0.66±0.39

The mean and standard deviation of salivary parameters level; IL-6, TNF-α, TAC and TP of each participant in both treatment and control groups at the 21st day of the study period were estimated as shown in the table (4.12).

Table (4.12) : Mean and standard deviation of salivary parameters levels of treatment and control group at the 21st day of the study (n = 20).

Salivary Parameters	Treatment Group Mean ± SD	Control Group Mean ± SD
IL-6 (Pg/mL)	2.59±2.11	3.45±2.22
TNF-α (Pg/mL)	16.26±0.56	17.75±1.84
TAC (U/mL)	7.51±4.26	6.22±3.88
TP (g/dL)	0.56±0.31	0.67±0.34

The mean and standard deviation of salivary parameters level; IL-6, TNF-α, TAC and TP of each participant in both treatment and control groups at the 42nd day of the study period were estimated as shown in the table 4.13.

Table (4.13): Mean and standard deviation of salivary parameters levels of treatment and control group at the 42nd day of the study (n = 20).

Salivary Parameters	Treatment Group Mean ± SD	Control Group Mean ± SD
IL-6 (Pg/mL)	2.00±1.79	2.91±1.75
TNF-α (Pg/mL)	16.11±0.05	19.56±5.12
TAC (U/mL)	8.16±4.17	6.49±4.49
TP (g/dL)	0.49±0.31	0.74±0.56

4.3.2 : Comparison in Salivary Parameters between Treatment and Control Group

Independent T-test of two means showing that there is no significant difference between means of treatment group and control group in salivary IL-6, TNF-α, TAC and salivary TP respectively at the beginning of the study, as illustrated in table 4.14.

Table (4.14) : Comparison in mean of salivary parameters between treatment and control group at the beginning of the study.

Salivary Parameters	Treatment Group [N = 20] Mean ± SD	Control Group [N = 20] Mean ± SD	P-Value*
IL-6 (Pg/ml)	3.35 ± 2.65	2.68 ± 1.64	0.345
TNF-α (Pg/ml)	16.65 ± 1.38	17.21 ± 1.55	0.228
Salivary TAC (U/ml)	5.02 ± 3.77	5.51 ± 4.08	0.695
Salivary TP (g/dL)	0.76 ± 0.46	0.66 ± 0.39	0.470

*** Independent T-test of two means was used.**

Independent T-test of two means showing that there is a significant difference between two means of treatment group and control group in salivary TNF-α, while there is no significant difference between means of treatment group and control group in salivary IL-6, TAC and salivary TP respectively at the 21st day of the study, as illustrated in table 4.15.

Table (4.15) : Comparison in mean of salivary parameters between treatment group and control group at the 21st day of the study.

Salivary Parameters	Treatment Group [N = 20] Mean ± SD	Control Group [N = 20] Mean ± SD	P-Value*
IL-6 (Pg/ml)	2.59 ± 2.11	3.45 ± 2.22	0.213
TNF-α (Pg/ml)	16.26 ± 0.56	17.75 ± 1.84	**0.001**
TAC (U/ml)	7.51 ± 4.26	6.22 ± 3.88	0.322
TP (g/dL)	0.56 ± 0.31	0.67 ± 0.34	0.278

*** Independent T-test of two means was used.**

There is a significant difference between treatment group and control group in salivary TNF-α, while there is no significant difference in salivary IL-6, TAC and salivary TP respectively at the 42nd day of the study, as illustrated in table 4.16.

Table (4.16) : Comparison in mean of salivary parameters between treatment and control group at the 42nd day of the study.

Salivary Parameters	Treatment Group [N = 20] Mean ± SD	Control Group [N = 20] Mean ± SD	P-Value*
IL-6 (Pg/ml)	2.00 ± 1.79	2.91 ± 1.75	0.116
TNF-α (Pg/ml)	16.11 ± 0.05	19.56 ± 5.12	**0.005**
TAC (U/ml)	8.16 ± 4.17	6.49 ± 4.49	0.232
TP (g/dL)	0.49 ± 0.31	0.74 ± 0.56	0.107

*** Independent T-test of two means was used.**

4.3.3: Comparison in Salivary Parameters between Study Days for both Treatment and Control Groups

For control group, there is no significant difference in salivary parameters at the 1st, 21st, 42nd days of the study (Table 4.17).

Table (4.17) : Comparison in salivary parameters levels of control group during the study period.

Salivary Parameters	Control Group			P-Value*
	1st day Mean ± SD	21st day Mean ± SD	42nd day Mean ± SD	
IL-6 (Pg/ml)	2.68 ± 1.64 A	3.45 ± 2.22 A	2.91 ± 1.75 A	0.413
TNF-α (Pg/ml)	17.21 ± 1.55 A	17.75 ± 1.84 A	19.56 ± 5.12 A	0.067
TAC (U/ml)	5.51 ± 4.08 A	6.22 ± 3.88 A	6.49 ± 4.49 A	0.745
TP (g/dL)	0.66 ± 0.39 A	0.67 ± 0.34 A	0.74 ± 0.56 A	0.859

*** One-way ANOVA-test with Tukey's Pair wise comparisons was used. Means that do not share (A) letter are significantly different.**

There is a significant difference in mean of salivary TAC level between 1st day and the 21st day of the study, while there is no significant difference in means of the other salivary parameters levels during all the study period as illustrated in the table 4.18 and figures 4.6, 4.7, 4.8 and 4.9.

Table (4.18) : Effect of systemic folic acid treatment on salivary levels of biochemical and antioxidant parameters during the study period.

Salivary Parameters	Treatment Group			P-Value*
	1st day Mean ± SD	21st day Mean ± SD	42nd day Mean ± SD	
IL-6 (Pg/ml)	3.35 ± 2.65 A	2.59 ± 2.11 A	2.00 ± 1.79 A	0.167
TNF-α (Pg/ml)	16.65 ± 1.38 A	16.26 ± 0.56 A	16.11 ± 0.05 A	0.132
TAC (U/ml)	5.02 ± 3.77 B	7.51 ± 4.26 AB	8.16 ± 4.17 A	**0.044**
TP (g/dL)	0.76 ± 0.46 A	0.56 ± 0.31 A	0.49 ± 0.31 A	0.065

*** One-way ANOVA-test with Tukey's Pair wise comparisons was used. Means that do not share (A) letter are significantly different.**

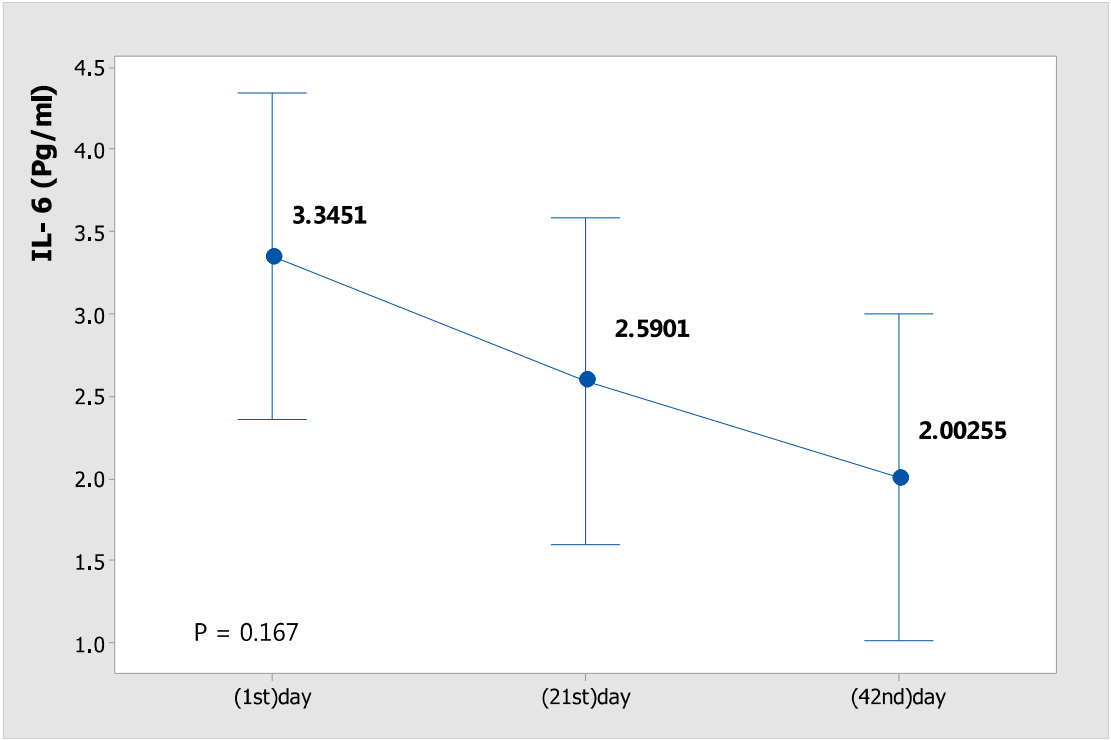


Figure (4.6) : The change in salivary levels of IL-6 after the use of systemic folic acid during the study period.

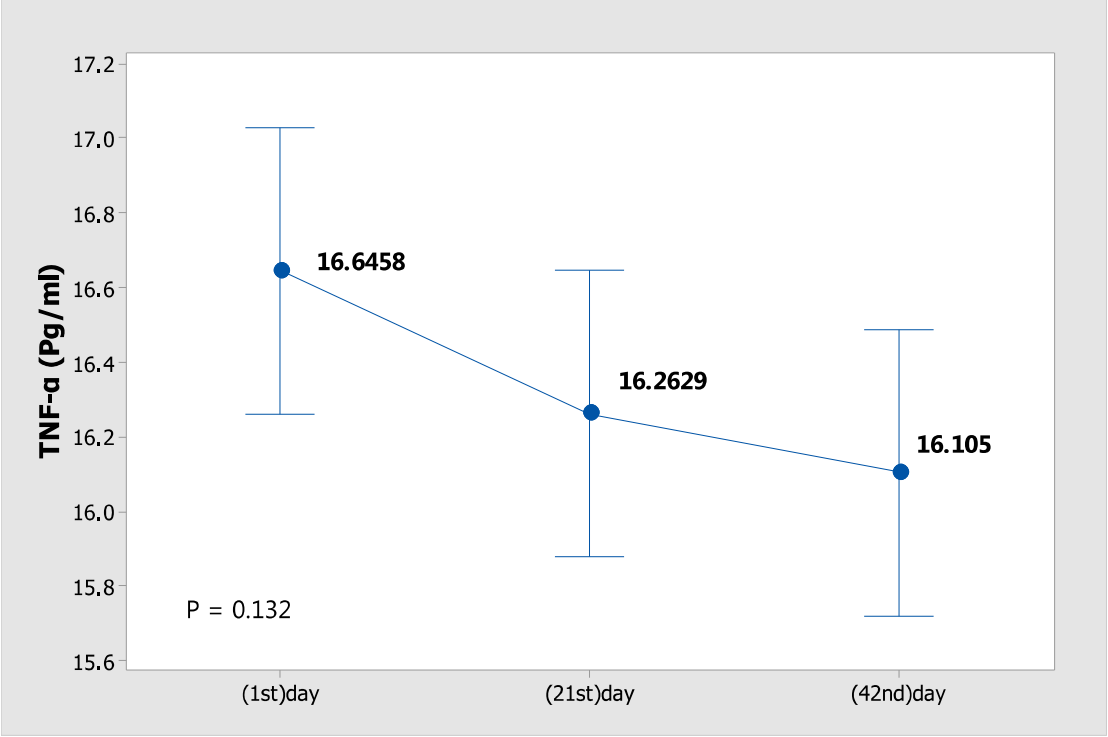


Figure (4.7) : The change in salivary levels of TNF-α after the use of systemic folic acid during the study period.

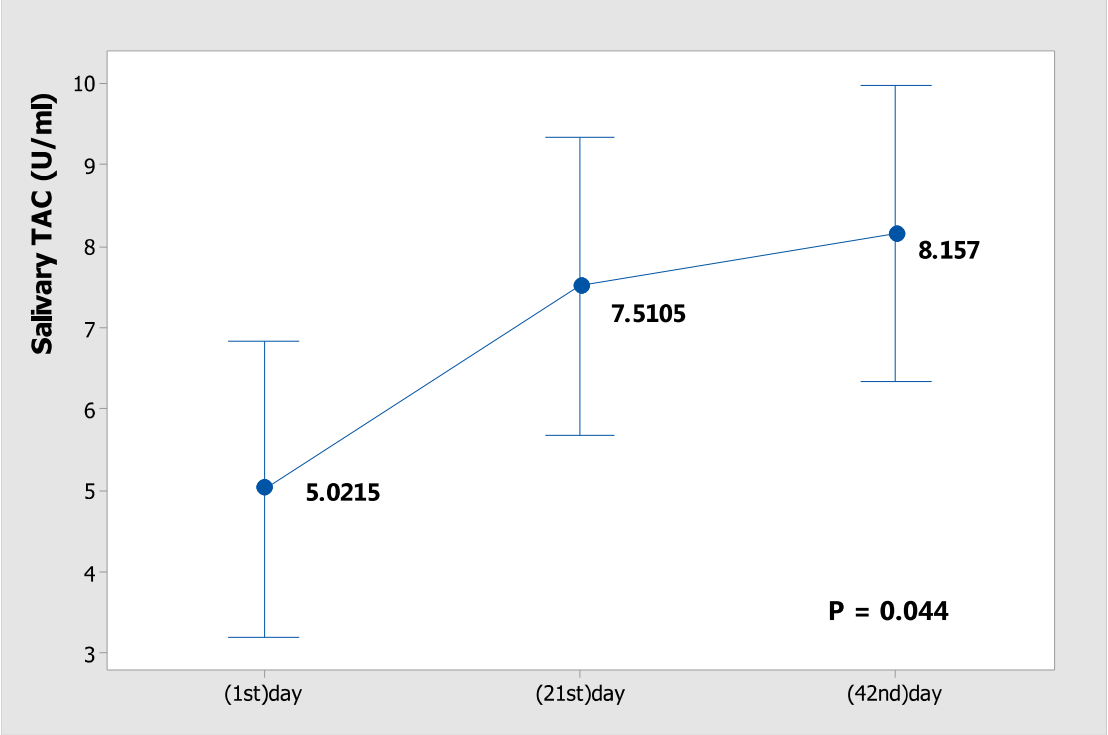


Figure (4.8) : The change in salivary levels of TAC after the use of systemic folic acid during the study period.

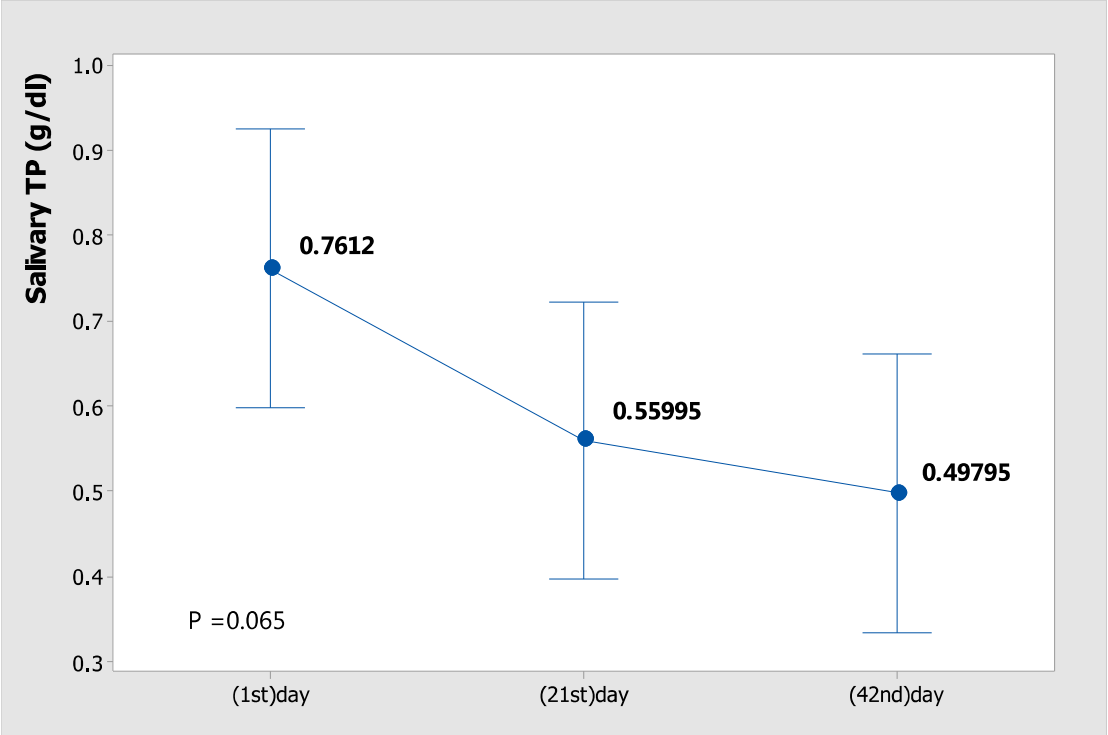


Figure (4.9) : The change in salivary levels of TP after the use of systemic folic acid during the study period.

4.3.4 : The Improvement Percentage Rates of Salivary Parameters

Paired T-test and % Improvement Rate were used to estimate the percentage of changes in salivary parameters at day 42nd of the study compared to the beginning by applying the following calculation :

% Improvement Rate = (mean at the 1st day - mean at 42nd day) /mean at 1st day X 100

Table (4.19) : Percentage improvement rates in salivary parameters levels after the use of systemic folic acid treatment.

Salivary Parameters	Beginning Mean ± SD	After 42 Days Mean ± SD	Before – After	% Improvement Rate
IL-6 (Pg/ml)	3.35 ± 2.65	2.00 ± 1.79	1.35	40.3
TNF-α (Pg/ml)	16.65 ± 1.38	16.11 ± 0.05	0.54	3.2
TAC (U/ml)	5.02 ± 3.77	8.16 ± 4.17	- 3.14	62.6
TP (g/dL)	0.76 ± 0.46	0.49 ± 0.31	0.27	35.5

*** Paired T-test of two means was used.**

Table (4.20) : Percentage improvement rates in salivary parameters levels of control group during the study period.

Salivary Parameters	Beginning Mean ± SD	After 42 Days Mean ± SD	Before – After	% Improvement Rate
IL-6 (Pg/ml)	2.68 ± 1.64 ^{A}	2.91 ± 1.75 ^{A}	- 0.23	8.5
TNF-α (Pg/ml)	17.21 ± 1.55 ^{A}	19.56 ± 5.12 ^{A}	- 2.35	13.7
TAC (U/ml)	5.51 ± 4.08 ^{A}	6.49 ± 4.49 ^{A}	- 0.98	17.8
TP (g/dL)	0.66 ± 0.39 ^{A}	0.74 ± 0.56 ^{A}	- 0.07	12.12

*** Paired T-test of two means was used.**

4.3.5 : Comparison percentage improvement rates of both oral health scores and salivary parameters between the treatment and control groups.

The table (4.21) illustrates the comparison in percentage of improvement rates in oral health scores and salivary parameters between the treatment group and control group at the final day of the study.

Table (4.21) : Comparison in % improvement rates in oral health scores and salivary parameters between the two groups.

Parameters	% Improvement Rate	
	Treatment Group	Control Group
Plaque index	104.2	40.6
Gingival index	12.7	2.9
Oral hygiene index	89.3	44.1
Calculus index	184.6	87.9
IL-6 (Pg/ml)	40.3	8.5
TNF-α (Pg/ml)	3.24	13.7
TAC (U/ml)	62.6	17.8
TP (g/dL)	35.5	12.12

4.4 : Correlation Coefficient

4.4.1 : Correlation Coefficient between Oral Health Scores and Salivary Parameters of Treatment Group at the Beginning of the Study

Table (4.22) demonstrating that there are significant positive correlation between PI and OHI , also between CI and OHI, between GI and salivary IL-6, and between salivary TP and salivary TNF-α with significant negative correlation between salivary TNF-α and salivary TAC.

Table (4.22) : Correlation matrix between different parameters in treatment group at the beginning of the study, (n= 20).

Parameter	Correlation Coefficient*	PI	GI	OHI	CI	IL-6	TNF-α	TAC
GI	r	0.104	---	---	---	---	---	---
	P	0.664	---	---	---	---	---	---
OHI	r	**0.895**	0.193	---	---	---	---	---
	P	**0.000**	0.416	---	---	---	---	---
CI	r	0.184	0.215	**0.526**	---	---	---	---
	P	0.437	0.362	**0.017**	---	---	---	---
IL-6	r	0.141	**0.558**	0.308	0.372	---	---	---
	P	0.555	**0.011**	0.186	0.106	---	---	---
TNF-α	r	-0.208	-0.335	-0.167	-0.077	-0.162	---	---
	P	0.378	0.149	0.483	0.747	0.494	---	---
TAC	r	0.328	0.174	0.175	-0.085	0.268	**-0.430**	---
	P	0.159	0.463	0.460	0.721	0.253	**0.050**	---
S.TP	r	-0.337	-0.225	-0.186	0.244	0.216	**0.506**	-0.412
	P	0.147	0.340	0.433	0.300	0.361	**0.023**	0.071

*** Pearson correlation method (r) was used.**

4.4.2: Correlation Coefficient Between oral health scores and salivary parameters of treatment group at the end of the study

The following correlation matrix (Table 4.23) between salivary parameters and oral health scores of treatment group at the end of the study using Pearson correlation method demonstrating that there are a positive correlation between PI and OHI (Figure 4.10), between CI and OHI (Figure 4.11) and between CI and PI, while there is a negative correlation between salivary TNF-α and salivary TAC.

Table (4.23): Correlation matrix between different parameters in treatment group at the end of the study, (n= 20).

Parameter	Correlation Coefficient *	PI	GI	OHI	CI	IL-6	TNF-α	TAC
GI	r	0.011	---	---	---	---	---	---
	P	0.965	---	---	---	---	---	---
OHI	r	**0.823**	-0.002	---	---	---	---	---
	P	**0.000**	0.994	---	---	---	---	---
CI	r	**0.572**	0.320	**0.660**	---	---	---	---
	P	**0.008**	0.169	**0.002**	---	---	---	---
IL-6	r	-0.138	-0.048	-0.188	-0.130	---	---	---
	P	0.563	0.842	0.427	0.585	---	---	---
TNF-α	r	-0.110	0.333	-0.060	0.172	0.164	---	---
	P	0.646	0.152	0.801	0.468	0.489	---	---
TAC	r	0.001	0.182	-0.147	-0.209	0.069	**-0.617**	---
	P	0.995	0.443	0.538	0.376	0.772	**0.004**	---
TP	r	0.044	-0.280	0.029	0.344	0.222	0.025	-0.422
	P	0.853	0.232	0.903	0.137	0.348	0.917	0.064

*** Pearson correlation method (r) was used.**

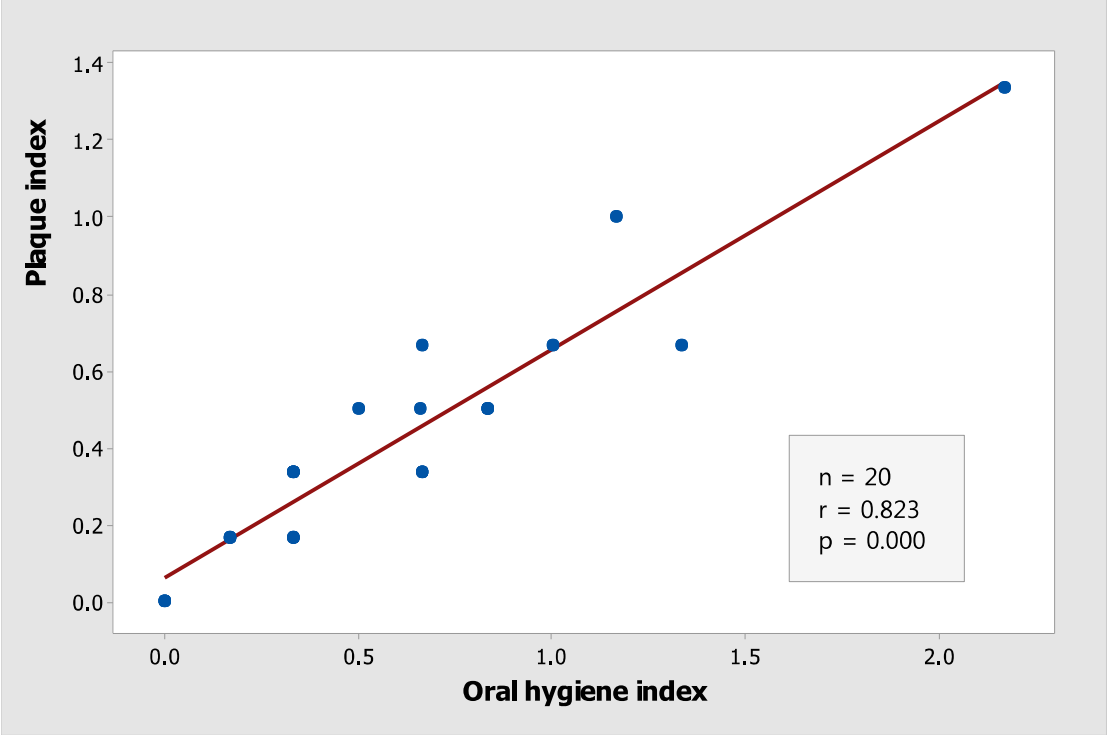


Figure (4.10) : The correlation between OHI and PI in treatment group at the end of the study.

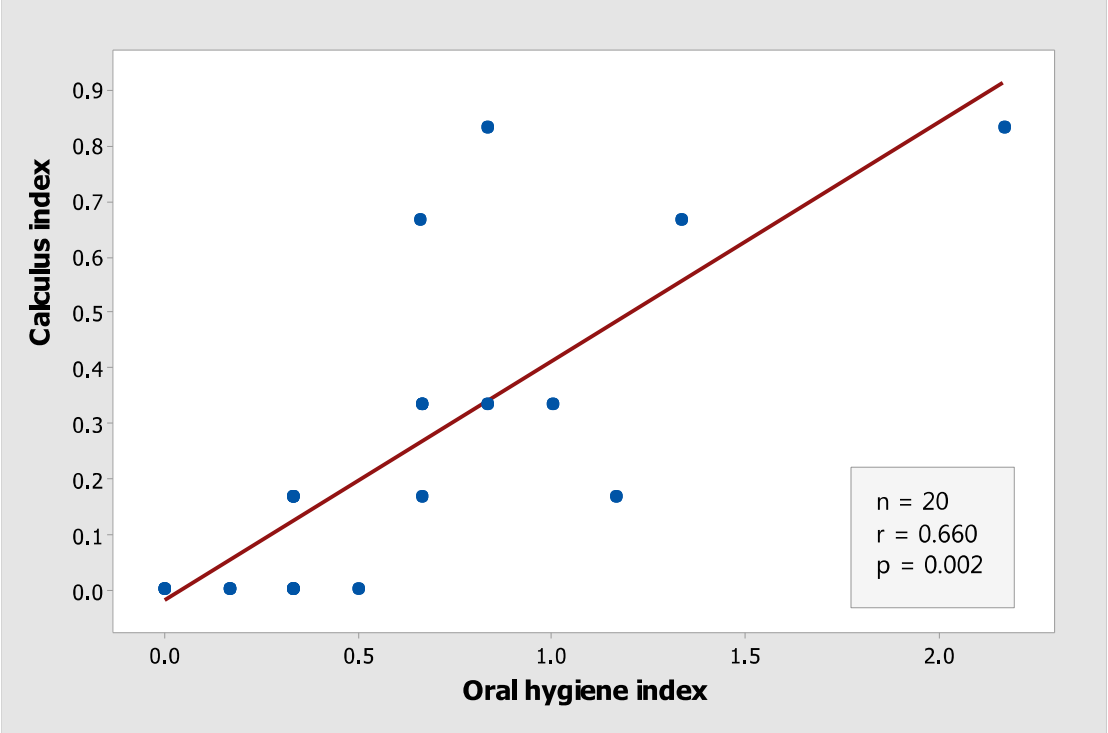


Figure (4.11): The correlation between OHI and CI in treatment group at the end of the study.

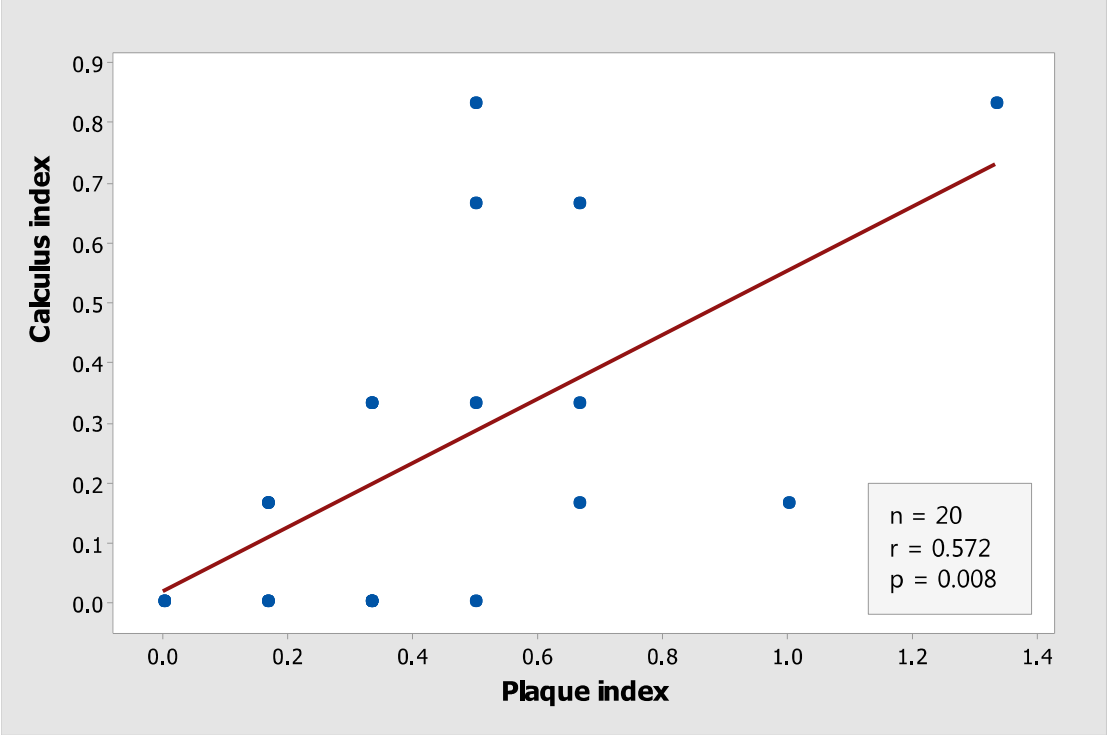


Figure (4.12): The correlation between PI and CI in treatment group at the end of the study.

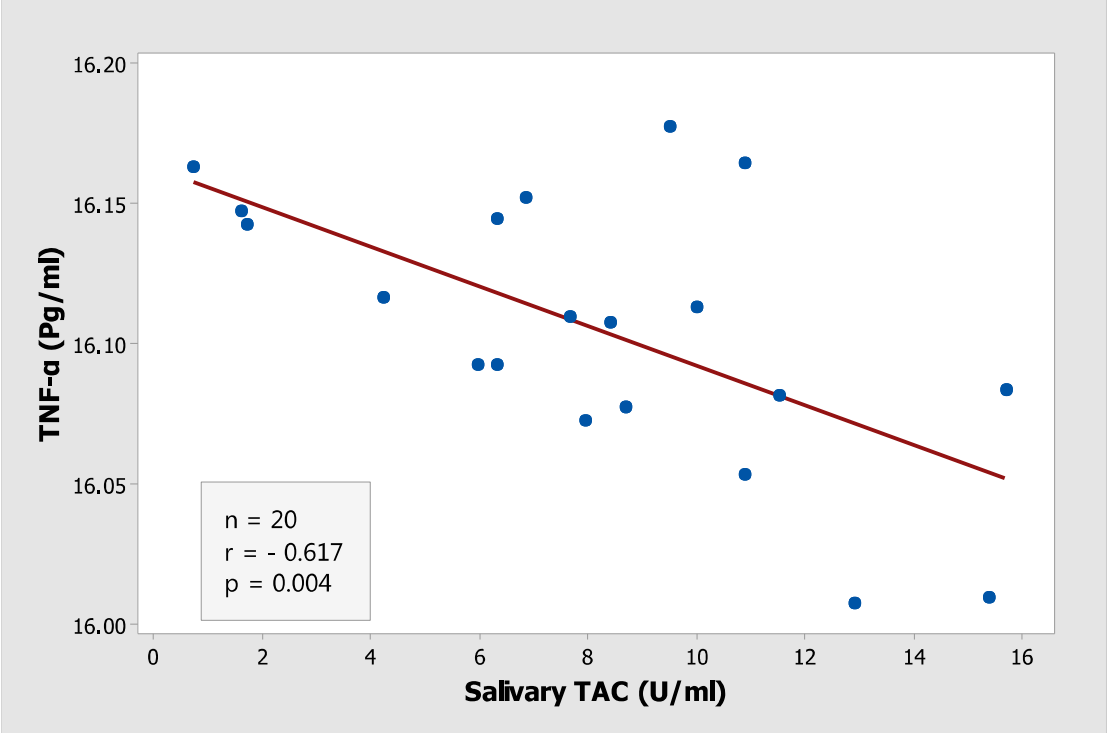


Figure (4.13) : The negative correlation between salivary TAC and TNF-α in treatment group at the end of the study.

4.4.3: Correlation Coefficient Between Oral Health Scores and Salivary Parameters of Control Group at the Beginning of the Study

The table (4.24) is demonstrating that there are significant positive correlation between PI and GI, between PI and OHI , also between CI and OHI, between GI and salivary TAC , between GI and salivary TP and finally between salivary TP and salivary TAC.

Table (4.24) : Correlation matrix between different parameters in control group at the beginning of the study, (n= 20).

Parameter	Correlation Coefficient*	PI	GI	OHI	CI	IL-6	TNF-α	TAC
GI	r	**0.606**	---	---	---	---	---	---
	P	**0.005**	---	---	---	---	---	---
OHI	r	**0.865**	0.413	---	---	---	---	---
	P	**0.000**	0.070	---	---	---	---	---
CI	r	0.154	-0.147	**0.622**	---	---	---	---
	P	0.516	0.538	**0.003**	---	---	---	---
IL-6	r	0.227	0.151	0.163	-0.038	---	---	---
	P	0.335	0.524	0.493	0.874	---	---	---
TNF-α	r	-0.063	-0.138	0.001	0.074	0.349	---	---
	P	0.791	0.561	0.995	0.757	0.132	---	---
TAC	r	0.290	**0.686**	0.231	-0.026	-0.088	-0.049	---
	P	0.215	**0.001**	0.327	0.914	0.712	0.837	---
TP	r	0.276	**0.639**	0.334	0.174	0.144	0.036	**0.616**
	P	0.239	**0.002**	0.149	0.463	0.544	0.881	**0.004**

*** Pearson correlation method (r) was used.**

4.4.4: Correlation Coefficient Between Oral Health Scores and Salivary Parameters of Control Group at the End of the Study

The table (4.25) is demonstrating that there are significant positive correlation between OHI and GI, between PI and OHI , also between CI and OHI, between PI and CI, between PI and salivary TAC , between GI and salivary TAC, between salivary TP and salivary TAC, between salivary TNF-α and salivary TP, between salivary TP and GI and finally between OHI and salivary TAC.

Table (4.25) : Correlation matrix between different parameters in control group at the end of the study, (n= 20).

Parameter	Correlation Coefficient*	PI	GI	OHI	CI	IL-6	TNF-α	TAC
GI	r	0.510	---	---	---	---	---	---
	P	0.022	---	---	---	---	---	---
OHI	r	**0.939**	**0.489**	---	---	---	---	---
	P	**0.000**	**0.029**	---	---	---	---	---
CI	r	**0.605**	0.212	**0.770**	---	---	---	---
	P	**0.005**	0.370	**0.000**	---	---	---	---
IL-6	r	0.433	0.292	0.331	0.036	---	---	---
	P	0.057	0.212	0.154	0.880	---	---	---
TNF-α	r	0.369	0.302	0.398	0.323	-0.149	---	---
	P	0.109	0.196	0.082	0.165	0.530	---	---
TAC	r	**0.585**	**0.776**	**0.619**	0.346	0.339	0.401	---
	P	**0.007**	**0.000**	**0.004**	0.135	0.143	0.080	---
TP	r	0.354	**0.615**	0.437	0.305	0.119	**0.458**	**0.656**
	P	0.126	**0.004**	0.054	0.191	0.616	**0.042**	**0.002**

*** Pearson correlation method (r) was used.**

Chapter Five

DISCUSSION

CHAPTER FIVE

DISCUSSION

5.1 : Study Design

In the present study, patients were selected randomly for clinical trial. They were chosen according to some criteria and grouped randomly into the treatment and control groups.

In accordance with previous studies of the inflammatory markers in gingivitis and periodontitis patients, all participants aged between 20-40 years, taking in consideration that the gingivitis prevalence is higher among patients below 20 years and above 40 years. Studies documented that the gingival lesions which formed in the old individuals are more pronounced to occur and their saliva contained more inflammatory cells than in the young subject saliva sample. Also salivary flow rate is declined by the age progression (Syndergaard *et al*., 2014; Nazir, 2017; Rangbulla *et al*., 2017).

In this study, there is no significant sex-based difference between treatment and control groups to exclude the effect of gender factor on the study outcomes (Jordan *et al*., 2011; Sreenivasan *et al*., 2017).

This study has excluded smokers because their saliva has a higher stimulated flow than of non-smoker due to tobacco irritation. Also, the level of proteins and some cytokines concentration in saliva are increased due to smoking. Even exposure to the environmental cigarette smoke is associated with increased leucocyte counts, chemotaxis and increased release of reactive oxidants from stimulated neutrophils (anderson *et al*., 1991). Studies revealed that there is a decrease in the serum antioxidant levels in a smoker, that will affect on salivary TAC measurement (Rahmadhani *et al*., 2020). Also, organic nitrites, nitrous oxide, cyanates, and isocyanates found in cigarette smoke have been shown to interact with a folic acid, transforming them into biologically inactive compounds and

thereby leading to lower folic acid levels in serum (Mahanonda *et al*., 2009; Kościelniak *et al*., 2012; George *et al*., 2013).

Alcoholic individuals are more likely to have folate deficiency, also alcoholic people may exhibit relevant changes in the volume and composition of the salivary fluid, so they were excluded in this study. (Lewis, 2020; Lyra *et al*., 2020).

Individuals with oral or systemic diseases were also excluded as many chronic diseases affect on salivary oxidative state and salivary protein level (Kościelniak *et al*., 2012; Nazir, 2017; Klimiuk *et al*., 2020).

Participants that receive any medication or supplements were excluded in addition to the drugs that interact with folic acid. Periodontal diseases intensified when there is a diminished salivary flow due to certain medications (Güncü *et al*., 2005). Some medications may cause alteration in the salivary flow rate, due to anticholinergic effects as; antidepressants, anxiolytics, antipsychotics and antihistaminics (Edgar *et al*., 2004; Marcott *et al.,* 2020). Corticosteroids cause hyposalivation and increase the viscosity of saliva which affect on salivary parameters level(Mohiti *et al*., 2020). Also, patients who were takhe store of en supplements which contain antioxidant vitamins (C, D and E) have been excluded from the study to avoid alteration in salivary TAC.

5.2 : Collecting Unstimulated Saliva

Unstimulated whole saliva represents the baseline saliva presents in the oral cavity. Unstimulated whole saliva often correlates to local and systemic clinical conditions more accurately than stimulated saliva (Syndergaard *et al*., 2014; Rangbulla *et al.,* 2017). Materials as drugs or food increase salivary flow rate that may change salivary composition (Edgar *et al*., 2004; Williamson *et al*., 2012). As the salivary flow rate

increases, the concentrations of total proteins, sodium, calcium, chloride, and bicarbonate as well as the pH increases to various levels. In the area of proteomics, analysis of unstimulated saliva samples is usually preferred over stimulated saliva samples because dilution of the proteomic components in stimulated saliva samples has been reported (Yakob *et al*., 2014; Schafer *et al*., 2014). So, unstimulated saliva has been collected in order to avoid changing in proteins and cytokines level in saliva.

Saliva has been widely analyzed as an indicator of oral health (Lee *et al*., 2012; Hartenbach *et al.,* 2020). Investigators have identified cytokines in the saliva which are associated with a periodontal disease that reflect local, rather than systemic, immune responses. Salivary components may originate entirely from the salivary glands or may be derived from the blood by passive diffusion or active transport (Chiappin *et al*., 2007). Also, the changes in the inflammatory mediators present in saliva reflect the changes that occur in gingival tissue (Santos *et al*., 2017).

In cases where components in saliva are derived from the blood, levels of biochemical and immunological components measured in saliva may reflect blood levels. Substitution of saliva samples for blood or CGF in analysis of inflammatory biomarkers is of considerable interest because collection of saliva is a non-invasive, rapid and does not have any of the risks associated with collection of blood or CGF also its collection requires less manpower and materials (Yue *et al*., 2013; Hemadi *et al*., 2017; Rangbulla *et al.,* 2017). This is the major difference between oral fluid and serum, in which the concentrations of the various components can only vary between narrow border values (Aps and Martens, 2005).

5.3 : Dose of Folic Acid

Once absorbed, folic acid needs to be converted to THF to enter in the metabolic pathways, a reaction that is catalyzed by the enzyme DHFR. The human gut has a limited ability to reduce methylated folic acid. So, when folic acid dose exceeds physiologic threshold, the DHFR enzyme can become saturated and the conversion of folic acid to the reduced form is impaired, resulting in the appearance of unmetabolized folic acid form (UMFA) in the circulation (Steluti *et al*., 2020).

In this study, 1 mg oral tablet of folic acid once daily was prescribed for the treatment group, on empty stomach, for continuous 42 days. This dose of folic acid is pharmacologically compatible with tolerable upper intake limit/dose (TUL) that was recommended by Institute of Medicine "IOM" in 1998 which is 1 mg once daily (Institute of Medicine (US) Standing Committee, 1998) intended to reflect a maximum safe intake. The committee considered a number of endpoints associated with UMFA appearance which are; carcinogenicity, decreased efficacy of folate antagonists, and risk of neurological complications in vitamin B12-deficient patients.

Elevated folic acid dose over TUL has been proposed to promote tumorigenesis from preexisting foci of neoplasia and accelerate the growth of established tumors. That hypothesis relates to the role of folic acid in the synthesis of nucleotides for DNA synthesis in rapidly dividing cells (Crider *et al*., 2011; Field and Stover, 2018; Steluti *et al*., 2020).

A recent study suggested that the intervention of folic acid with 5 mg/day, even for just a short time (45 days), produced a several folds increase in serum folate concentrations. They observed a reduced number of NK cells in healthy young participants (Clovis *et al*., 2018). That data corroborate previous findings by Troen *et al*. (2006), that a high folic acid supplementation intake was associated with reduced cytotoxicity of NK

cells in vivo in obese postmenopausal women who consumed the greatest amount of folate from their diet. Also, it is in agree with study performed by Sawaengsri *et al*. (2016) that concluded that; animal models have shown that a high folic acid diet can result in reduced NK cell cytotoxicity in aged mice. Furthermore, the results from a clinical trial showed that a daily dose of 5 mg FA for 90 days is associated with increased serum UMFA, and reduced number and cytotoxicity of NK cells in healthy adults. It is better to know that NK cells play a major role in the host-rejection of tumor cells. They are activated in the response to the macrophage-derived cytokines (Paniz *et al*., 2017).

5.4 : Effects of Folic Acid on Oral Health Scores

Tooth brushing is an effective method in reducing levels of dental plaque; thus, it is the reference technique for control of plaque. Studies showed that after tooth brushing, a significant reduction in the plaque index was observed and considered to be the baseline plaque index. In the next day, the plaque, gingival, calculus and oral hygiene indices score for all participants were measured before treatment to get a base line (but not necessary to be the zero line) (Syndergaard *et al.*, 2014; Rangbulla *et al.,* 2017, Lynch *et al*., 2018, Rubido *et al*., 2018).

Plaque, gingival, oral hygiene and calculus indices have a wide application as the "gold standard for assessment of oral health in general, provide a diagnosis of periodontal disease and to evaluate the effect of folic acid treatment on gingival health in a simple and non-invasive manner. (Zhang *et al*., 2010; Korte and Kinney, 2016).

The regular plaque removal of the causative factors (dental plaque, and calculus) by mechanical cleaning of the teeth has approved efficient in the control of gingivitis by providing a state of homeostasis due to reduced immunopathogenesis in the periodontium (Franco *et al*., 2008; Naiff *et al*., 2020), but the inability of the some people to perform adequate mechanical

tooth cleaning has been induced the researchers for finding effective agents that can improve plaque control and gingivitis (Smiley *et al*., 2015; Rizvi *et al*., 2016).

In a constant quest to enhance oral health, a number of synthetic chemical agents with potential adjunctive benefits were added to the existing measures of gingival treatment (Ramesh *et al.,* 2020)

Vitamins improve the capacity of gingival epithelial cell to act against the attack of pathogens and slow down the inflammatory response in gingiva (Thanoon and Al-Mashhadane, 2020). Also, repair and maintenance of the periodontium including gingival generates a high turnover rate of squamous epithelium, folic acid is essential for the proper maturation of the rapidly proliferating cells, It is thus conceivable that FAD is associated with severe gingival inflammation (Vogel *et al*., 1976; George *et al*., 2013). Among all the analyzed vitamins those with antioxidant capacity and effects on the immune system seems to be useful for the prevention or improvement of periodontal disease (Varela-Lopez *et al*., 2018).

According to the results of this study, there was a non-significant difference in PI after using of folic acid which may indicates that folic acid have no antiplaque effect. In addition to possible non-compliance of the participants in treatment group in following oral hygiene instructions during study period (Ramsay, 2000; Waldron *et al*., 2019).

A number of clinical investigations, maintained that the quantitative relationship between plaque levels and gingivitis severity is tenuous and, possibly, non-existent (Breuer and Cosgrove, 1989). Further support for this view comes from Midda and Cooksey who found that an enzyme-containing toothpaste significantly improved gingival health without significantly changing plaque levels (Midda and Cooksey, 1986).

In the results of this study, there is a positive correlation between

IL-6 and GI at the beginning of the study in treatment group, both parameters levels are high. There is a an improvement in GI at the end of the study in treatment group in comparison with the first day due to intake of folic acid supplement while in control group there was no any improvement in GI scores.

This was in agreement with Vogel *et al* (1976) who used 2 mg folic acid, twice daily for 30 days in their study on gingivitis, the results indicate that folic acid lead to improve in gingival inflammation. FAD is relative to abnormalities in rapidly proliferating epithelial cells, including buccal squamous cells. So, that the junctional epithelium could also be affected (Vogel *et al*., 1976).

Another study was conducted by Pack, in which treatment of patients with mouth wash (MW) of 5 mg folate per 5 ml twice daily for 4 weeks appears to have a positive effect on health of gingiva through gingival color and bleeding index improvement (Pack, 1984).

Also, a study in 2010 concluded that there is a significant relationship between FAD and gingival bleeding in non-smokers in Japan (Esaki *et al*., 2010).

The presence of dental calculus may limit the ability to perform the optimal oral hygiene practices (Pradeep *et al*., 2011). Calculus is considered as an important secondary etiological factor in the development and progression of periodontitis (Akcalı and Lang, 2018).

In this study, comparison in the oral health scores between treatment group and control group demonstrate increament in PI and OHI together with CI at days 21 & 42 of study period. According to these results folic acid might be without any effect against plaque and calculus formation which is in agreement with one research which stated that despite of vitamins are important in the prevention and treatment of oral diseases like gingivitis, there is no sufficient data supporting the need for them for good

oral hygiene including hard dental pathological processes & calculus deposition which is mainly depend on mechanical oral hygiene measures carried out by both dentists and patients (Cagetti *et al*., 2020).

In comparison among study days in treatment group, there was significant increase in CI in association with non-significant increase in PI and OHI for the same factors related to patient cooperation and skill of dental brushing. (Ramsay, 2000; Waldron *et al*., 2019). In control group, comparison among study days showed increase in PI, OHI & CI in association for the same reasons above.

Incidental systemic findings of folate deficiency may be influencing factors for the development of gingival diseases. This factor serves as an example of a stepwise approach toward interdisciplinary patient care when the underlying cause of gingival diseases are not immediately obvious by routine methods of diagnosis like PI, OHI and CI scores (Barbe, 2020).

5.5 : Effects of Folic Acid on Salivary Parameters

Infections and trauma are common factors which can cause the body to become inflamed. Inflammation is the mechanism used to restrict any sort of possible damage spreading, and to repair it (Saladin, 2010; Frank *et al*., 2017).

The presence of distinct microbes in the periodontal environment has been associated with increased levels of host-produced pro-inflammatory cytokines, such as TNF-α, IL-6 in the gingival fluid and tissues. Those will formulate to signal surrounding sensors to begin the necessary mechanisms for tissue repair and restoration, in addition to the effect of salivary cytoprotective proteins (Jiang *et al*., 2016; Evans and Omaya, 2017; Kharaeva *et al*., 2020). So, inflammatory markers and proteins measurement in the saliva are important to determine the presence of gingivitis and evaluation the response to gingivitis treatment either locally or systematically (Syndegaad *et al*., 2014; Gomes *et al*., 2016).

Gingivitis usually requires not only the presence of periodontopathogens, but the persistent host inflammatory immune response against those pathogens results in the increased blood flow and vascularization (angiogenesis), increased vascular permeability, and migration of neutrophils and monocytes/macrophages to the site of infection (Graves *et al*., 2011; Belstrom *et al*., 2017).

Among the host mediators produced after microbial invasion, cytokines such as TNF-α, and IL-6 were the first to have their role in periodontal disease pathogenesis (Musacchio *et al.*, 2009, Garlet, 2010; Khosravi *et al*, 2013).

Results showed that no significant difference in the mean of IL-6 and TNF-α levels in saliva between treatment and control group at the beginning of the study, then their levels have been decreased in response to the folic acid treatment at the 2nd visit (on the 21st day) and there was a significant difference in TNF-α, also there is decrement in IL-6 level, but non significantly compared to control group. At the end of the study, the salivary TNF-α level has been decreased significantly and the salivary level of IL-6 has been decreased in non-significant manner.

Pathogens secrete LPS by their cell wall, those induce inflammatory response by binding with Toll-like receptors (TLRs); class of immune receptors present at cell surface of macrophages. TLRs are considered the activator of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB).

NF-κB is a major transcription factor presents in cytoplasm of all human cells, a key regulator of immune response by controlling the transcription of cytokines which involved in inflammatory process. It is found to be active in many chronic inflammatory diseases as inflammatory

bowel disease, asthma, sepsis, arthritis, atherosclerosis, and periodontal disease.

NF-κB is normally found as inactive form in the cytoplasm associated with inhibitory protein called IκB. Upon stimulation by LPS-TRL complex, there is instant phosphorylation of IκB and releasing of NF-κB into nucleus to start transcription of inflammatory cytokines genes (Doyle and O'Neill, 2006; Hayden *et al*., 2006; Qiu *et al.*, 2020).

Results of of this study in relation to TNF-α & IL-6 can be explained by results of two previous studies that supposed folic acid to subside inflammatory response mediated by IL-6 and TNF-α by inhibition of phosphorylation of IκB (Au-Yeung *et al*., 2006; Feng *et al*., 2011). Our results are also in line with a recent study concluded that 5 weeks, oral intake of 1 mg folic acid has the ability to significantly decrease in T-cell proliferation, so that decrease cytokines production and dermatitis treatment. Folic acid treatment significantly interfered with T-cell proliferation in a dose-dependent manner (Makino *et al*., 2019).

Another study was performed in 2016, examined the effect of 1.25 mg/day of folic acid supplementation for six months on newly diagnosed patients with Alzheimer disease, they found that folic acid improved cognition and decreased markers of inflammation as IL-6 and TNF-α (Chen *et al.*, 2016).

Saliva has buffering, lubricating, antibacterial, and remineralizing tasks, it is also an excellent source of both enzymatic and non-enzymatic antioxidants, which preserves the redox homeostasis, and stop disturbances of them in the oral environment by overproduction of free radicals or reduction in antioxidants synthesis which is called oxidative stress state.

Salivary TAC is mainly associated to some proteins (as albumin),

enzymes (as glutathione peroxidase), UA and ascorbic acid (Żukowski *et al*., 2018). The excessive production of free radicals or antioxidants deficiency have a role in pathogenesis of periodontal disease as gingivitis. It has been proposed, that antioxidant supplementation could decrease or slow gingival tissues injury. Up to date studies affords constructive evidences of antioxidant therapeutic of periodontal disease (Celec, 2017; Ambati *et al*., 2017; Kharaeva *et al*., 2020).

Relationship between oxidative stress and periodontal disease is reasonably strong. The periodontal inflammation enhances the availability of oxidative stress markers, and it tends to enforce aspects of periodontal destruction (da Silva *et al*., 2018).

Accumulation of dental plaque and calculus, both are considered the integral parts for OHI, that play a critical role in periodontal disease as they contain various pathogenic bacteria that induce production of free radicals which leads to inflammation (Albandar *et al*., 1996).

This study results have found that there is a significant effect of folic acid on treatment group by increasing salivary TAC value through the entire period of study. This result is in line with a recent study that confirmed that the folic acid is an antioxidant used with vitamin E in pregnancy to protect the fetus from oxidative damage caused by cigarette smoke, 5 mg/day for 20 days regimen is used (Rahmadhani *et al*., 2020). That can be explained by the dual role of folic acid, the first involves subsiding gingival inflammation by decreasing cytokines production (which produce oxidation), then to neutralize the state of oxidative stress (Vogel *et al*., 1976; Esaki *et al*., 2010; Chen *et al*., 2016; Paniz *et al*., 2017; Makino *et al*., 2019) as discussed previously. The second is explained as in cases of mild case of gingivitis, the normal immune response to bacterial endotoxin (LPS) involves neutrophils

recruiting and production of inflammatory cytokines as IL-6 which activates Nrf2 system. (Matsuoka *et al*., 2016) . Then ROS are produced of in fair level enough to return redox homeostasis of gingival tissue. If there is excessive production of ROS as in moderate to severe cases of gingivitis, the neutrophil infiltrate increased together with a downward regulation of the Nrf2 pathway and following inhibition of antioxidant production, resulting in higher synthesis of ROS leads to tissue damage that may lead to periodontitis. Nrf2 is nuclear factor erythroid 2-related factor 2, it currently recognized as one of main cellular defense mechanism against oxidative stress. It is a transcriptional factor involved in cellular redox homeostasis. In healthy state it is found in cytoplasm often sequestered by special molecule called Klech like- ECH-associated protein 1 (Keap-1).

At inflammatory state when the cell expose to the ROS or pro-inflammatory cytokines, results in conformational changes in cysteine moiety of Keap-1 molecule leads Nrf2 to dissociates from Keap-1 and translocates in nucleus where it performs its role in activating of transcription of various detoxification and antioxidant enzyme genes (Taguchi *et al*., 2011). Expression of endogenous antioxidants is regulated by Nrf2 system (Shaw and Chattopadhyay, 2020).

It was confirmed that folic acid can efficiently scavenge such free radicals (Joshi *et al*., 2001). In the circumstances of extreme oxidative stress, the exhaustion of folates may occur. It was suggested that the main antioxidant activity of the active form of folate (5-MTHF) resides in its pterin core and an electrondonating effect because the higher the electron donating effect, the higher the antioxidant activity (Rezk *et al*., 2003). Folic acid dose-dependently decreased ROS, DNA oxidative damage, and tissue damage (Li *et al*., 2020).

In the beginning of this study, results revealed that there is a negative correlation between salivary TNF-α and TAC of treatment group indicates the presence of inflammation where inflammatory marker is elevated and antioxidant level is depressed. The opposite case is found at the end of the study, where salivary TNF-α level is decreased while TAC level is elevated, indicated the anti-inflammatory and antioxidant effect of folic acid.

Saliva principally consists of 99.5% water, 0.3% proteins and 0.2% trace and inorganic substances. Saliva is one of the innate defense systems of the human body that protects oral cavity tissues by several mechanisms, such as improving tooth enamel by remineralization, neutralizing low plaque pH, rinsing food debris, microorganisms and sugar aggregation, and by its antibacterial properties (Hicks *et al*., 2004; Dawes, 2008). The concentration of proteins and polypeptides present in the saliva is important in the maintenance of oral health and homeostasis, as increased frequency and severity of oral disease are often associated with qualitative and quantitative changes of the saliva proteome (Scarano *et al*., 2010; Dawes *et al*., 2015).

Proteomic molecules, such as histatins, mucin, lactoperoxidase, defensins, proline-rich peptides and lactoferrin regulate the microbial flora of the oral cavity by exerting direct antibacterial effects (Van *et al*., 2004; Schipper[a,b] *et al*., 2007). Therefore, salivary protein composition may play an important role in the etiology of oral disease prevalence as periodontal disease (Van *et al*., 2004; Hartenbach *et al*., 2020).

In gingival inflammation, epithelial cells are released and degraded while the blood capillaries permeability is increased, which results in release of serum components, such as albumin into saliva. While in oral healthy individuals, the chance of detecting serum components, released

from the blood vessels through saliva, is rather little & not significant (Aps and Martens, 2005).

By comparisons of salivary TP levels during all study days within treatment group & in comparison to the control group, there was a decrease in the levels that supposed to be due to anti-inflammatory effect of folic acid (Chen *et al*., 2016; Paniz *et al*., 2017; Makino *et al*., 2019).

There is a positive correlation between salivary TNF-α and salivary TP in the treatment group at the beginning of the study. In response to the chronic infection by pathogenic micro-organisms during the chronic gingivitis condition there is an up-regulation of pro-inflammatory mediators which increase genes expression of oral host defense system proteins in gingival tissues (McAuley *et al*., 2007; Aboodi *et al*., 2016).

The limitations of the current study including the small sample size and the fact that none of the subjects were diagnosed as folic acid deficient. The results showed an important role for folic acid in inducing anti-inflammatory and antioxidant effects and enhancing the improvement in oral health profile in chronic gingivitis patients.

Chapter Six

CONCLUSIONS AND RECOMMENDATIONS

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1: Conclusions

The following conclusions have been drawn from this study:

1. Folic acid supplement of 1 mg/day for 42 days has a noticeable effect on the treatment of chronic gingivitis.
2. Folic acid has no effect on plaque Index and oral hygiene index, but it causes a little decrease in gingival index, so it may provide dentists with additional option during management of gingival inflammation in addition to scaling and polishing.
3. Results showed that there is a difference between means of treatment group with folic acid and control group in TNF-α at 21st and 42nd day, so it may has anti-inflammatory effect.
4. Folic acid supplement within the recommendations can increase the level of total antioxidant capacity in saliva, so it enhance anti-oxidant effect which help to improve oral health.
5. Folic acid causes a little decrease in the level of salivary total proteins at the end of the study.

6.2: Recommendations

The study recommends the following points :

1. Gingivitis is a widely distributed dental disease among the population, so it becomes rationale to consider safest, available, and economic treatment (like vitamins) for it.
2. Further studies of other cytokines levels in whole saliva, GCF, and serum sample in dental diseases.
3. Further studies of specific antioxidants levels in whole saliva, GCF, and serum sample in dental diseases are suggested.
4. Further studies of specific salivary proteins in whole saliva, GCF, and serum sample in dental diseases are suggested.
5. Further studies about the use of folic acid supplement in medically compromised patients are recommended.
6. Further studies about the use of vitamin B12 with folic acid in the treatment of chronic gingivitis are recommended.

REFERENCES

REFERENCES

- Aboodi, G. M., Sima, C., Moffa, E. B., Crosara, K. T., Xiao, Y., Siqueira, W. L., Glogauer, M. (2016). *Salivary cytoprotective proteins in inflammation and resolution during experimental gingivitis—a pilot study*. **Frontiers in cellular and infection microbiology**, 5, 92.

- Ackermann A, Bélanger M, Brogden K, Burnell K, Cavanaugh J, Kohlgraf K, Lu X, Progulske-Fox A, Xie H. (2010). *Defensins attenuate cytokine responses yet enhance antibody responses to Porphyromonas gingivalis adhesins in mice*. **Future Microbiol** 5: 115.

- Ahmadi-Motamayel, F., Goodarzi, M. T., Hendi, S. S., Kasraei, S., Moghimbeigi, A. (2013). *Total antioxidant capacity of saliva and dental caries.* **Medicina oral, patologia oral y cirugia bucal**, 18(4), e553.

- Ahmadi-Motamayel, F., Goodarzi, M. T., Jamshidi, Z., Kebriaei, R. (2017). *Evaluation of salivary and serum antioxidant and oxidative stress statuses in patients with chronic periodontitis: a case-control study.* **Frontiers in physiology**, 8, 189.

- Ahmadi-Motamayel, F., Falsafi, P., Abolsamadi, H., Goodarzi, M. T., Poorolajal, J. (2019). *Evaluation of Salivary Antioxidants and Oxidative Stress Markers in Male Smokers*. **Combinatorial chemistry high throughput screening**, 22(7), 496-501.

- Akalin FA, Isiksal E, Baltacioglu E, Renda N, Karabulut E. (2008). *Superoxide dismutase activity in gingiva in type-2 diabetes mellitus patients with chronic periodontitis.* **Arch Oral Biol**. 53: 44-52.

- Akcalı, A., Lang, N. P. (2018). *Dental calculus: the calcified biofilm and its role in disease development.* **Periodontology 2000**, 76(1), 109-115.
- Akpinar A, Toker H, Ozdemir H, Bostanci V, and Aydin H. (2013). *The effect of non-surgical periodontal therapy on oxidant and anti-oxidant status in smokers with chronic periodontitis*. **Arch Oral Biol**. 58: 717-23.
- Akram, Z., Shafqat, S. S., Aati, S., Kujan, O., & Fawzy, A. (2020). *Clinical efficacy of probiotics in the treatment of gingivitis: A systematic review and meta-analysis*. **Australian Dental Journal**, 65(1), 12-20.
- Albandar, J. M., Brown, L. J., Brunelle, J. A., Löe, H. (1996). *Gingival state and dental calculus in early-onset periodontitis.* **Journal of periodontology**, 67(10), 953-959.
- Alijotas-Reig, J., Esteve-Valverde, E., Ferrer-Oliveras, R., Llurba, E., Gris, J. M. (2017). *Tumor necrosis factor-alpha and pregnancy: focus on biologics. An updated and comprehensive review*. **Clinical reviews in allergy immunology**, 53(1), 40-53.
- Allgrove J, Davison G, Gleeson M. (2009). *Salivary antimicrobial peptides(LL-37 and alpha-defensins HNP1–3), antimicrobial and IgA responses to prolonged exercise*. **Eur J Appl Physiol** 106: 277–284.
- Al-Mashhadane, F. A., Al-Mashhadane, A. A., Taqa, A. A. R. (2018). *Folic Acid Supplementation: A Review of the Known Advantages and Risks.* **Public Health and Preventive Medicine**, 4(3), 51-59.

- Al-Mashhadane, F. A., & Thanoon, A. Y. (2020). *Anti-inflammatory effect of vitamin D3 on chronic gingivitis.* **Al-Rafidain Dental Journal**, 20(1), 73-82.
- Almerich-Silla, J. M., Pastor, S., Serrano, F., Puig-Silla, M., Dasí, F. (2015). *Oxidative stress parameters in saliva and its association with periodontal disease and types of bacteria*. **Disease markers**, *2015*.
- Al-Noori M. J. (2013). *Therapeutic Effect of Indomethacin As A Mouth Wash in Gingivitis*. M.Sc. Thesis. **Mosul University, Dentistry College.** Mosul, Iraq.
- Alpers, D. H. (2016). *Absorption and blood/cellular transport of folate and cobalamin: pharmacokinetic and physiological considerations*. **Biochimie**, 126, 52-56.
- Ambati, M., Rani, K. R., Reddy, P. V., Suryaprasanna, J., Dasari, R., Gireddy, H. (2017). *Evaluation of oxidative stress in chronic periodontitis patients following systemic antioxidant supplementation: A clinical and biochemical study*. **Journal of Natural Science, Biology and Medicine,** 8(1), 99–103.
- Anderson, R., Theron, A. J., Richards, G. A., Myer, M. S. van Rensburg, A. S. (1991). *Passive smoking by humans sensitizes circulating neutrophils.* **American Review of Respiratory Disease**, 144, 570–574.
- Aps, J. K., Martens, L. C. (2005). *The physiology of saliva and transfer of drugs into saliva*. **Forensic science international**, 150(2-3), 119-131.
- Arulselvan, P., Fard, M. T., Tan, W. S., Gothai, S., Fakurazi, S., Norhaizan, M. E., Kumar, S. S. (2016). *Role of antioxidants and*

natural products in inflammation. **Oxidative medicine and cellular longevity**, 2016.

- Asmat, U., Abad, K., Ismail, K. (2016). *Diabetes mellitus and oxidative stress—A concise review*. **Saudi Pharmaceutical Journal**, 24(5), 547-553.

- Augieb, R., Boothe, J. H., Hutcheigs, B., Mowat, J. H., Semb, J., Stokstad, E. L. R., ... Htjltqutst, M. (1945). *Synthesis of a compound identical with the L. casei factor isolated from liver*. **Science (Washington),** 102, 227-228.

- Au-Yeung, K. K., Yip, J. C., Siow, Y. L., O, K. (2006). *Folic acid inhibits homocysteine-induced superoxide anion production and nuclear factor kappa B activation in macrophages*. **Canadian journal of physiology and pharmacology**, 84(1), 141-147.

- Balbi, M. E., Tonin, F. S., Mendes, A. M., Borba, H. H., Wiens, A., Fernandez-Llimos, F., Pontarolo, R. (2018). *Antioxidant effects of vitamins in type 2 diabetes: a meta-analysis of randomized controlled trials*. **Diabetology metabolic syndrome**, 10(1), 18.

- Baltacıoğlu, E., Akalın, F. A., Alver, A., Balaban, F., Ünsal, M., Karabulut, E. (2006). *Total antioxidant capacity and superoxide dismutase activity levels in serum and gingival crevicular fluid in post-menopausal women with chronic periodontitis*. **Journal of clinical periodontology**, *33*(6), 385-392.

- Baltacıoğlu, E., Yuva, P., Aydın, G., Alver, A., Kahraman, C., Karabulut, E., Akalın, F. A. (2014). *Lipid peroxidation levels and total oxidant/antioxidant status in serum and saliva from patients with chronic and aggressive periodontitis. Oxidative stress index: a new*

biomarker for periodontal disease?. **Journal of Periodontology**, *85*(10), 1432-1441.

- Barbe, A. G., Röhrig, G., Hieggelke, L., Noack, M. J., Derman, S. H. M. (2020). *Interdisciplinary assessment and management of a patient with a fibrous gingival enlargement of unknown origin: A case report*. **Clinical case reports**, 8(1), 159.
- Barton BE (2005). *Interleukin-6 and new strategies for the treatment of cancer, hyperproliferative diseases and paraneoplastic syndromes.* **Expert Opin Ther Targets** 9: 737–752.
- Batra, B., Narwal, V., Kalra, V., Sharma, M., Rana, J. S. (2020). *Folic acid biosensors: A review*. **Process Biochemistry**, 92, 343-354.
- Belstrøm, D., Damgaard, C., Könönen, E., Gürsoy, M., Holmstrup, P., Gürsoy, U. K. (2017). *Salivary cytokine levels in early gingival inflammation.* **Journal of oral microbiology**, 9(1), 1364101.
- Blom, H. J., Shaw, G. M., Den Heijer, M., Finnell, R. H. (2006). *Neural tube defects and folate: case far from closed*. **Nature Reviews Neuroscience**, 7(9), 724-731.
- Breuer, M. M., & Cosgrove, R. S. (1989). *The relationship between gingivitis and plaque levels.* **Journal of periodontology**, 60(4), 172-175.
- Brogin Moreli, J., Cirino Ruocco, A. M., Vernini, J. M., Rudge, M. V. C., Calderon, I. M. P. (2012). *Interleukin 10 and tumor necrosis factor-alpha in pregnancy: aspects of interest in clinical obstetrics.* **ISRN obstetrics and gynecology**, 2012.
- Bortolus, R., Blom, F., Filippini, F., van Poppel, M. N., Leoncini, E., de Smit, D. J., ... Mastroiacovo, P. (2014). *Prevention of congenital*

malformations and other adverse pregnancy outcomes with 4.0 mg of folic acid: community-based randomized clinical trial in Italy and the Netherlands. **BMC pregnancy and childbirth**, 14(1), 166.

- Boxmeer, J. C., Smit, M., Utomo, E., Romijn, J. C., Eijkemans, M. J., Lindemans, J., ... Steegers-Theunissen, R. P. (2009). *Low folate in seminal plasma is associated with increased sperm DNA damage.* **Fertility and sterility**, 92(2), 548-556.

- Buczko, P., Zalewska, A., Szarmach, I. (2015). *Saliva and oxidative stress in oral cavity and in some systemic disorders*. **J. Physiol Pharmacol,** 66, 1-7.

- Cabras, T., Iavarone, F., Manconi, B., Olianas, A., Sanna, M. T., Castagnola, M., Messana, I. (2014). *Top-down analytical platforms for the characterization of the human salivary proteome.* **Bioanalysis**, 6(4), 563-581.

- Cagetti, M. G., Wolf, T. G., Tennert, C., Camoni, N., Lingström, P., Campus, G. (2020). *The Role of Vitamins in Oral Health. A Systematic Review and Meta-Analysis*. **International journal of environmental research and public health,** 17(3), 938.

- Capelli, I., Cianciolo, G., Gasperoni, L., Zappulo, F., Tondolo, F., Cappuccilli, M., La Manna, G. (2019). *Folic Acid and Vitamin B12 Administration in CKD, Why Not?*. **Nutrients**, 11(2), 383.

- Celec, P. (2017). *Oxidative stress and antioxidants in the diagnosis and therapy of periodontitis*. **Frontiers in physiology**, 8, 1055.

- Chen, H., Liu, S., Ji, L., Wu, T., Ji, Y., Zhou, Y., ... Huang, G. (2016). *Folic acid supplementation mitigates Alzheimer's disease by reducing*

inflammation: a randomized controlled trial. **Mediators of inflammation**, 2016.

- Chiang[a], C. P., Wu, Y. C., Chang, J. Y. F., Wang, Y. P., Wu, Y. H., Sun, A. (2020). *Anemia, hematinic deficiencies, and gastric parietal cell antibody positivity in atrophic glossitis patients with or without hyperhomocysteinemia*. **Journal of the Formosan Medical Association**, 119(1), 544-552.

- Chiang[b], C. P., Wu, Y. C., Chang, J. Y. F., Wang, Y. P., Wu, Y. H., Sun, A. (2020). *Anemia, hematinic deficiencies, hyperhomocysteinemia, and gastric parietal cell antibody positivity in atrophic glossitis patients with iron deficiency*. **Journal of the Formosan Medical Association,** 119(2), 587-594.

- Chiappin, S., Antonelli, G., Gatti, R., Elio, F. (2007). *Saliva specimen: a new laboratory tool for diagnostic and basic investigation*. **Clinica chimica acta**, 383(1-2), 30-40.

- Choromańska, M., Klimiuk, A., Kostecka-Sochoń, P., Wilczyńska, K., Kwiatkowski, M., Okuniewska, N., ... Maciejczyk, M. (2017). *Antioxidant defence, oxidative stress and oxidative damage in saliva, plasma and erythrocytes of dementia patients. Can salivary AGE be a marker of dementia?*. **International Journal of Molecular Sciences**, 18(10), 2205.

- Chu, D., Li, L., Jiang, Y., Tan, J., Ji, J., Zhang, Y., ... Liu, F. (2019). *Excess folic acid supplementation before and during pregnancy and lactation activates Fos gene expression and alters behaviors in male mouse offspring*. **Frontiers in neuroscience**, 13, 313.

- Cianciulli, A., Salvatore, R., Porro, C., Trotta, T., Panaro, M. A. (2016). *Folic acid is able to polarize the inflammatory response in LPS*

activated microglia by regulating multiple signaling pathways. **Mediators of inflammation**, 2016.

- Clovis p. Juliano F., Maylla R., E. De Carli Patr´ıcia M., Guilherme W.,Cec´ılia Z., Maria S. (2018). *A Daily Dose of 5 mg Folic Acid for 90 Days Is Associated with Increased Serum Unmetabolized Folic Acid and Reduced Natural Killer Cell Cytotoxicity in Healthy Brazilian Adults.* **American Society for Nutrition**

- Corbitt, M., Eaton-Fitch, N., Staines, D., Cabanas, H., Marshall-Gradisnik, S. (2019). *A systematic review of cytokines in chronic fatigue syndrome/myalgic encephalomyelitis/systemic exertion intolerance disease (CFS/ME/SEID).* **BMC neurology**, 19(1), 207.

- Crider, K. S., Bailey, L. B., Berry, R. J. (2011). *Folic acid food fortification—its history, effect, concerns, and future directions.* **Nutrients**, 3(3), 370-384.

- Crider, K. S., Yang, T. P., Berry, R. J., Bailey, L. B. (2012). *Folate and DNA methylation: a review of molecular mechanisms and the evidence for folate's role.* **Advances in nutrition**, 3(1), 21-38.

- Crandall, B. F., Corson, V. L., Evans, M. I., Goldberg, J. D., Knight, G., Salafsky, I. S. (1998). *American College of Medical Genetics statement on folic acid: fortification and supplementation*. **American journal of medical genetics**, 78(4), 381-381.

- Daly, S., Mills, J. L., Molloy, A. M., Conley, M., McPartlin, J., Lee, Y. J., ... Scott, J. M. (2002). *Low-dose folic acid lowers plasma homocysteine levels in women of child-bearing age*. **Qjm**, 95(11), 733-740.

- Dawes C, Pedersen AM, Villa A. (2015). *The functions of human saliva: a review sponsored by the World Workshop on Oral Medicine VI*. **Arch Oral Biol**; 60 (6): 863–874.
- Dawes, C. (2008). *Salivary flow patterns and the health of hard and soft oral tissues*. **The Journal of the American Dental Association**, 139, 18S-24S.
- De Almeida, P. D. V., Gregio, A. M., Machado, M. A., De Lima, A. A., Azevedo, L. R. (2008). *Saliva composition and functions: a comprehensive review*. **J Contemp Dent Pract**, 9(3), 72-80.
- De Almeida, C., Amenábar, J. M. (2016). *Changes in the salivary oxidative status in individuals with temporomandibular disorders and pain*. **Journal of oral biology and craniofacial research**, 6, S1-S4.
- Deshmukh, U., Katre, P., Yajnik, C. S. (2013). *Influence of maternal vitamin B12 and folate on growth and insulin resistance in the offspring. In Maternal and Child Nutrition: The First 1,000 Days* (Vol. 74, pp. 145-156). **Karger Publishers.**
- Denny, P., Hagen, F. K., Hardt, M., Liao, L., Yan, W., Arellanno, M., ... Delahunty, C. M. (2008). *The proteomes of human parotid and submandibular/sublingual gland salivas collected as the ductal secretions*. **Journal of proteome research**, 7(5), 1994-2006.
- Doyle, S. L., O'Neill, L. A. (2006). *Toll-like receptors: from the discovery of NFκB to new insights into transcriptional regulations in innate immunity*. **Biochemical pharmacology**, 72(9), 1102-1113.
- Dye, B. A. (2012). *Global periodontal disease epidemiology.* **Periodontology 2000**, 58(1), 10-25.

- Ebersole, J. L., Nagarajan, R., Akers, D., Miller, C. S. (2015). *Targeted salivary biomarkers for discrimination of periodontal health and disease (s).* **Frontiers in cellular and infection microbiology**, 5, 62.

- Ebersole, J. L., Graves, C. L., Gonzalez, O. A., Dawson III, D., Morford, L. A., Huja, P. E., ... Wallet, S. M. (2016). *Aging, inflammation, immunity and periodontal disease.* **Periodontology 2000**, 72(1), 54-75.

- Ebisch, I. M. W., Peters, W. H. M., Thomas, C. M. G., Wetzels, A. M. M., Peer, P. G. M., Steegers-Theunissen, R. P. M. (2006). *Homocysteine, glutathione and related thiols affect fertility parameters in the (sub) fertile couple.* **Human reproduction**, 21(7), 1725-1733.

- Edgar M, Dawes C, O'Mullane D. (2004). *Saliva and oral health.* 3rd Edn. **Stephen Hancocks company, Wrigley oral healthcare programme, UK**; Pp: 15-16.

- Edgar M., C. daws, and D. O'Mullane. (2012). *saliva and oral health.* 4th Edn. **Stephen Hancocks company, Wrigley oral healthcare programme, UK**; Pp: 3.

- El-Bahr, S. M. (2013). *Biochemistry of free radicals and oxidative stress*. **Sci. Int**. 1,111–117.

- Erdemir, E. O., Bergstrom, J. (2006). *Relationship between smoking and folic acid, vitamin B12 and some haematological variables in patients with chronic periodontal disease*. **Journal of clinical periodontology**, 33(12), 878-884.

- Erdemir, E. O., Bergstrom, J. (2007). *Effect of smoking on folic acid and vitamin B12 after nonsurgical periodontal intervention*. **Journal of clinical periodontology,** 34(12), 1074-1081.
- Esaki, M., Morita, M., Akhter, R., Akino, K., Honda, O. (2010). *Relationship between folic acid intake and gingival health in non-smoking adults in Japan*. **Oral diseases**, 16(1), 96-101.
- Evans, L. W., Omaye, S. T. (2017). *Use of saliva biomarkers to monitor efficacy of vitamin C in exercise-induced oxidative stress*. **Antioxidants**, 6(1), 5.
- Feng, D., Zhou, Y., Xia, M., Ma, J. (2011). *Folic acid inhibits lipopolysaccharide-induced inflammatory response in RAW264. 7 macrophages by suppressing MAPKs and NF-κB activation.* **Inflammation Research**, 60(9), 817-822.
- Field, M. S., Stover, P. J. (2018). *Safety of folic acid*. **Annals of the New York Academy of Sciences**, 1414(1), 59.
- Fratoni, V., Brandi, M. L. (2015). B vitamins, homocysteine and bone health. Nutrients, 7(4), 2176-2192.
- Franco Neto, C. A., Parolo, C. C. F., Rösing, C. K., Maltz, M. (2008). *Comparative analysis of the effect of two chlorhexidine mouthrinses on plaque accumulation and gingival bleeding*. **Brazilian oral research**, *22*(2), 139-144.
- François, M., Bull, C. F., Fenech, M. F., Leifert, W. R. (2019). *Current state of saliva biomarkers for aging and Alzheimer's disease*. **Current Alzheimer Research**, 16(1), 56-66.

- Frank J. Dowd, Barton S. Johnson, Angelo J. (2017). *Pharmacology and Therapeutics for Dentistry*. 7th Edn. **Elsevier Co. Germany**; Pp: 257-258.

- Frodge, B. D., Ebersole, J. L., Kryscio, R. J., Thomas, M. V., Miller, C. S. (2008). *Bone remodeling biomarkers of periodontal disease in saliva*. **Journal of periodontology**, 79(10), 1913-1919.

- Gangula, P., Ravella, K., Chukkapalli, S., Rivera, M., Srinivasan, S., Hale, A., ... Kesavalu, L. (2015). *Polybacterial periodontal pathogens alter vascular and gut BH4/nNOS/NRF2-phase II enzyme expression.* **PloS one**, 10(6).

- Gao, X., Jiang, S., Koh, D., Hsu, C. Y. S. (2016). *Salivary biomarkers for dental caries*. **Periodontology 2000**, 70(1), 128-141.

- Garrett, J. R., Suleiman, A. M., anderson, L. C., Proctor, G. B. (1991). *Secretory responses in granular ducts and acini of submandibular glands in vivo to parasympathetic or sympathetic nerve stimulation in rats*. **Cell and tissue research**, 264(1), 117-126.

- Garlet, G. P. (2010). *Destructive and protective roles of cytokines in periodontitis: a re-appraisal from host defense and tissue destruction viewpoints*. **Journal of dental research**, 89(12), 1349-1363.

- George, A. P. R., & Donald, P. M. (2012). *Estimation of flow rate and study of epithelial cells in stimulated and unstimulated saliva.* **International poster journal of dentistry and oral medicine**, vol. 14 no. 3, poster no. 605.

- George, J. P., Shobha, R., Lazarus, F. J. (2013). *Folic acid: A positive influence on periodontal tissues during health and disease.* **International Journal of Health Allied Sciences**, 2(3), 145.

- Gerald, K. M. (2005). *AHFS drug information.* **Bethesda MD. American Society of Health-System Pharmacists**.
- Gomes, F. I. F., Aragão, M. G. B., Barbosa, F. C. B., Bezerra, M. M., Pinto, V. D. P. T., Chaves, H. V. (2016). *Inflammatory cytokines interleukin-1β and tumour necrosis factor-α-novel biomarkers for the detection of periodontal diseases: a literature review*. **Journal of oral maxillofacial research**, 7(2).
- Gombart, A. F., Pierre, A., Maggini, S. (2020). *A review of micronutrients and the immune System–Working in harmony to reduce the risk of infection.* **Nutrients**, 12(1), 236.
- Gonçalves, L. D. R., Soares, M. R., Nogueira, F. C., Garcia, C., Camisasca, D. R., Domont, G., Alves, G. (2010). *Comparative proteomic analysis of whole saliva from chronic periodontitis patients.* **Journal of proteomics**, 73(7), 1334-1341.
- Gonçalves, L. D. R., Soares, M. R., Nogueira, F. C. S., Garcia, C. H. S., Camisasca, D. R., Domont, G., Alves, G. (2011). *Analysis of the salivary proteome in gingivitis patients.* **Journal of periodontal research**, 46(5), 599-606.
- Gonen, N., Assaraf, Y. G. (2012). *Antifolates in cancer therapy: structure, activity and mechanisms of drug resistance*. **Drug Resistance Updates,** 15(4), 183-210.
- Gonzalez D, Marquina R, Rondon N, Rodriguez-Malaver AJ Reyes R. (2008). *Effects of aerobic exercise on uric acid, total antioxidant activity, oxidative stress, and nitric oxide in human saliva.* **Res Sports Med.** 16: 128-137.

- Graves, D. T., Cochran, D. (2003). *The contribution of interleukin-1 and tumor necrosis factor to periodontal tissue destruction.* **Journal of periodontology**, 74(3), 391-401.
- Graves, D. T., Li, J., Cochran, D. L. (2011). *Inflammation and uncoupling as mechanisms of periodontal bone loss.* **Journal of dental research,** 90(2), 143-153.
- Güncü, G. N., Tözüm, T. F., Çaglayan, F. (2005). *Effects of endogenous sex hormones on the periodontium—review of literature*. **Australian dental journal**, 50(3), 138-145.
- Haigh, B. J., Stewart, K. W., Whelan, J. R., Barnett, M. P., Smolenski, G. A., Wheeler, T. T. (2010). *Alterations in the salivary proteome associated with periodontitis*. **Journal of clinical periodontology**, 37(3), 241-247.
- Hajishengallis, E., Hajishengallis, G. (2014). *Neutrophil homeostasis and periodontal health in children and adults.* **Journal of dental research,** 93(3), 231-237.
- Halliwell, B. (2001). *Free radicals and other reactive species in disease*. **Els J.**, 1-9.
- Hartenbach, F. A. R. R., Velasquez, É., Nogueira, F. C., Domont, G. B., Ferreira, E., & Colombo, A. P. V. (2020). *Proteomic analysis of whole saliva in chronic periodontitis*. **Journal of Proteomics**, 213, 103602.
- Hatem, S. A., Nsaif, Z. N., & AL-Rubaee, E. A. (2020). *Evaluation of Interleukin IL-1β, TNF-α, and VDB Protein Levels in Serum of Patients with Chronic Periodontitis*. **Journal of Biochemical Technology**, 11(1), 14.

- Hayden, M. S., West, A. P., Ghosh, S. (2006). *NF-κ B and the immune response*. **Oncogene**, 25(51), 6758-6780.
- Hegde, A., Rai, K., Padmanabhan, V. (2009). *Total antioxidant capacity of saliva and its relation with early childhood caries and rampant caries.* **Journal of Clinical Pediatric Dentistry**, 33(3), 231-234.
- Heikkilä, K., Ebrahim, S., Lawlor, D. A. (2008). *Systematic review of the association between circulating interleukin-6 (IL-6) and cancer.* **European journal of cancer**, 44(7), 937-945.
- Hemadi, A. S., Huang, R., Zhou, Y., Zou, J. (2017). *Salivary proteins and microbiota as biomarkers for early childhood caries risk assessment.* **International journal of oral science**, 9(11), e1-e1.
- Hendler, S. S., Rorvik, D. (2001). *Physicians' desk reference for nutritional supplements.* **Montvale, NJ: Medical Economics**, 575 . p.161.
- Hendy, J., Warinner, C., Bouwman, A., Collins, M. J., Fiddyment, S., Fischer, R., ... Klaus, L. (2018). *Proteomic evidence of dietary sources in ancient dental calculus.* Proceedings of the Royal Society B: **Biological Sciences**, 285(1883), 20180977.
- Hicks, J., Garcia-Godoy, F., Flaitz, C. (2004). *Biological factors in dental caries: role of saliva and dental plaque in the dynamic process of demineralization and remineralization (part 1)*. **Journal of Clinical Pediatric Dentistry**, 28(1), 47-52.
- Hillman, J. R. (2010). *Adequate Food for All: Culture, Science, and Technology of Food in the 21st Century.*, pp. 436, **Experimental Agriculture**, 46(1), 116-116.

- Ho, E., Galougahi, K. K., Liu, C. C., Bhindi, R., Figtree, G. A. (2013). *Biological markers of oxidative stress: applications to cardiovascular research and practice*. **Redox biology**, 1(1), 483-491.
- Hoffbrand, A. V., Weir, D. G. (2001). *The history of folic acid*. **British journal of haematology**, 113(3), 579-589.
- Hsu, H. C., Chiou, J. F., Wang, Y. H., Chen, C. H., Mau, S. Y., Ho, C. T., ... Chen, C. H. (2013). *Folate Deficiency Triggers an Oxidative-Nitrosative Stress-Mediated Apoptotic Cell Death and Impedes Insulin Biosynthesis in RINm5F Pancreatic Islet β–Cells: Relevant to the Pathogenesis of Diabetes*. **PLoS One**, 8(11).
- Huang D, Ou B, Prior RL. (2005). *The chemistry behind antioxidant capacity assays*. **Agr Food Chem**. 53: 1841-1856.
- Huang, R. F. S., Huang, S. M., Lin, B. S., Wei, J. S., Liu, T. Z. (2001). *Homocysteine thiolactone induces apoptotic DNA damage mediated by increased intracellular hydrogen peroxide and caspase 3 activation in HL-60 cells*. **Life sciences**, 68(25), 2799-2811.
- Humphrey, S. P., Williamson, R. T. (2001). *A review of saliva: normal composition, flow, and function.* **The Journal of prosthetic dentistry**, 85(2), 162-169.
- Hunter, R., Barnes, J., Oakeley, H. F., & Matthews, D. M. (1970). *Toxicity of folic acid given in pharmacological doses to healthy volunteers*. **The Lancet**, 295(7637), 61-63.
- Idriss, H. T., Naismith, J. H. (2000). *TNF alpha and the TNF receptor superfamily: structure-function relationship(s)*. **Microscopy research and technique**, 50(3), 184–195.

- Inan, S. (2019). *The Potential Role of Nutraceuticals in Inflammation and Oxidative Stress. In Nutraceuticals-Past, Present and Future.* **IntechOpen**.
- Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. (1998). *Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline***. National Academies Press (US).**
- İscan, B., Tuzun, F., Eroglu Filibeli, B., Cilekar Micili, S., Ergur, B. U., Duman, N., ... & Kumral, A. (2019). *Effects of maternal folic acid supplementation on airway remodeling and allergic airway disease development*. **The Journal of Maternal-Fetal & Neonatal Medicine**, 32(18), 2970-2978.
- Itzhaki, R. F., & Gill, D. M. (1964). *A micro-biuret method for estimating proteins*. **Analytical biochemistry**, 9(4), 401-410.
- Jiang, H., Zhang, Y., Xiong, X., Harville, E. W., Karmin, O., Qian, X. (2016). *Salivary and serum inflammatory mediators among pre-conception women with periodontal disease*. **BMC oral health**, 16(1), 131.
- Jin, L. J. (2015). *Inter professional education and multidisciplinary teamwork for prevention and effective management of periodontal disease*. **Journal of the International Academy of Periodontology**, 17(1Suppl),74–79.
- Jones KG, Brull DJ, Brown LC et al (2001). *Interleukin-6 (IL-6) and the prognosis of abdominal aortic aneurysms.* **Circulation** 103: 2260–2265.

- Jordan, R. A., Lucaciu, A., Fotouhi, K., Markovic, L., Gaengler, P., Zimmer, S. (2011). *Pilot pathfinder survey of oral hygiene and periodontal conditions in the rural population of The Gambia (West Africa).* **International journal of dental hygiene**, 9(1), 53-59.

- Joshi, R., Adhikari, S., Patro, B. S., Chattopadhyay, S., Mukherjee, T. (2001). *Free radical scavenging behavior of folic acid: evidence for possible antioxidant activity.* **Free Radical Biology and Medicine**, 30(12), 1390-1399.

- Kamodyová, N., Červenka, T., Celec, P. (2015). *Salivary markers of oxidative stress in oral diseases.* **Frontiers in cellular and infection microbiology**, 5, 73.

- Katzung- BG, Master SB, Trevor AJ. (2018). *Therapeutic and clinical pharmacology.* 14th Edn. **Basicocentration Copyright. Mc Grawhill, California, USA**; Pp: 738.

- Keles, S., Anik, A., Cevik, O. et al. (2020). *Gingival crevicular fluid levels of interleukin-18 and tumor necrosis factor-alpha in type 1 diabetic children with gingivitis.* **Clin Oral Invest** .

- Khan, M. Z., Zhang, Z., Liu, L., Wang, D., Mi, S., Liu, X., ... Yu, Y. (2019). *Folic acid supplementation regulates key immunity-associated genes and pathways during the periparturient period in dairy cows.* **Asian-Australasian journal of animal sciences**.

- Khaneghah, A. M., Hashemi, S. M. B., Es, I., Gholamhosseinpour, A., Loizzo, M. R., Giardinieri, A., ... Ferreira, D. S. (2019). *Water-soluble vitamins. In Innovative Thermal and Non-Thermal Processing, Bioaccessibility and Bioavailability of Nutrients and Bioactive Compounds* (pp. 241-266). **Woodhead Publishing**.

- Kharaeva, Z. F., Mustafaev, M. S., Khazhmetov, A. V., Gazaev, I. H., Blieva, L. Z., Steiner, L., ... Korkina, L. G. (2020). *Anti-Bacterial and Anti-Inflammatory Effects of Toothpaste with Swiss Medicinal Herbs towards Patients Suffering from Gingivitis and Initial Stage of Periodontitis: from Clinical Efficacy to Mechanisms*. **Dentistry Journal**, 8(1), 10.

- Khosravi, R., Ka, K., Huang, T., Khalili, S., Nguyen, B. H., Nicolau, B., Tran, S. D. (2013). *Tumor necrosis factor-α and interleukin-6: potential interorgan inflammatory mediators contributing to destructive periodontal disease in obesity or metabolic syndrome.* **Mediators of inflammation.**

- Kishimoto, T. (2010). *IL-6: from its discovery to clinical applications.* **International immunology**, 22(5), 347-352.

- Klimiuk, A., Zalewska, A., Sawicki, R., Knapp, M., & Maciejczyk, M. (2020). *Salivary Oxidative Stress Increases With the Progression of Chronic Heart Failure.* **Journal of Clinical Medicine**, 9(3), 769.

- Kolb, A. F., Petrie, L. (2013). *Folate deficiency enhances the inflammatory response of macrophages*. **Molecular immunology**, 54(2), 164-172.

- Konopka, T., Krol, K., Kopec, W., and Gerber, H. (2007). *Total antioxidant status and 8-hydroxy-2'-deoxyguanosine levels in gingival and peripheral blood of periodontitis patients.* **Arch. Immunol. Ther**. Exp. 55, 417–422.

- Kornman, K. S. (2008). *Mapping the pathogenesis of periodontitis: a new look.* **Journal of periodontology**, 79, 1560-1568.

- Korte, D. L., Kinney, J. (2016). *Personalized medicine: an update of salivary biomarkers for periodontal diseases*. **Periodontology 2000**, *70*(1), 26-37.
- Kościelniak, D., Jurczak, A., Zygmunt, A., Krzyściak, W. (2012). *Salivary proteins in health and disease.* **Acta Biochimica Polonica**, 59(4).
- Kubala, E., Strzelecka, P., Grzegocka, M., Lietz-Kijak, D., Gronwald, H., Skomro, P., Kijak, E. (2018). *A review of selected studies that determine the physical and chemical properties of saliva in the field of dental treatment*. **BioMed research international**, 2018.
- Lai, K. G., Chen, C. F., Ho, C. T., Liu, J. J., Liu, T. Z., Chern, C. L. (2017). *Novel roles of folic acid as redox regulator: Modulation of reactive oxygen species sinker protein expression and maintenance of mitochondrial redox homeostasis on hepatocellular carcinoma*. **Tumor Biology**, 39(6), 1010428317702649.
- Lanska, D. J. (2010). *Historical aspects of the major neurological vitamin deficiency disorders: the water-soluble B vitamins.* **Handbook of clinical neurology**, 95, 445-476.
- Larsen T. Fiehn NE (1996). *Resistance of Streptococcus sanguis biofilms to antimicrobial agents.* **APMIS** 104: 280–284.
- Laskin, D. L., Pendino, K. J. (1995). *Macrophages and inflammatory mediators in tissue injury.* **Annual review of pharmacology and toxicology**, 35(1), 655-677.
- Laurence L. Brunton. (2010). *Goodman Gilman's The Pharmacological Basis of Therapeutics*. 12th Edn. **The McGraw-Hill Companies., California, USA**; Pp : 505.

- Lynch MC, Cortelli SC, McGuire JA, Zhang J, Ricci-Nittel D, Mordas CJ et al. (2018). *The effects of essential oil mouthrinses with or without alcohol on plaque and gingivitis: a randomized controlled clinical study*. **BMC Oral Health**.18(6). DOI 10.1186/s12903-017-0454-6.

- Lee H, Lee YJ, Choi H, Ko EH, Kim JW. (2009). *Reactive oxygen species facilitate adipocyte differentiation by accelerating mitotic clonal expansion*. **J Biol Chem**. 284: 10601-10609.

- Lee, A., Ghaname, C. B., Braun, T. M., Sugai, J. V., Teles, R. P., Loesche, W. J., ... Kinney, J. S. (2012). *Bacterial and salivary biomarkers predict the gingival inflammatory profile.* **Journal of periodontology**, 83(1), 79-89.

- Levy, B. M., Dreizen, S., Bernick, S., Hampton JR, J. K. (1970). *Studies on the biology of the periodontium of marmosets: IX. Effect of parathyroid hormone on the alveolar bone of marmosets pretreated with fluoridated and nonfluoridated drinking water*. **Journal of dental research**, 49(4), 816-821.

- Lewis, M. J. (2020). *Alcoholism and nutrition: a review of vitamin supplementation and treatment*. **Current Opinion in Clinical Nutrition & Metabolic Care**, 23(2), 138-144.

- Li, Y., Huang, T., Zheng, Y., Muka, T., Troup, J., Hu, F. B. (2016). *Folic acid supplementation and the risk of cardiovascular diseases: a meta-analysis of randomized controlled trials*. **Journal of the American Heart Association**, 5(8), e003768.

- Li, W., Ma, Y., Li, Z., Lv, X., Wang, X., Zhou, D., ... Huang, G. (2020). *Folic acid decreases astrocyte apoptosis by preventing oxidative stress-induced telomere attrition.* **p**, 21(1), 62.

- Liew, S. C. (2016). *Folic acid and diseases-supplement it or not?*. **Revista da Associação Médica Brasileira**, 62(1), 90-100.
- Lisboa, J. V. D. C., Ribeiro, M. R., Luna, R. C. P., Lima, R. P. A., Nascimento, R. A. F. D., Monteiro, M. G. C. A., ... & Veras, R. C. (2020). *Food Intervention with Folate Reduces TNF-α and Interleukin Levels in Overweight and Obese Women with the MTHFR C677T Polymorphism: A Randomized Trial.* **Nutrients**, 12(2), 361.
- Liu, Z., Liu, Y., Song, Y., Zhang, X., Wang, S., Wang, Z. (2014). *Systemic oxidative stress biomarkers in chronic periodontitis: a meta-analysis.* **Disease Markers**, 2014.
- Lucock, M. (2004). *Is folic acid the ultimate functional food component for disease prevention?*. **Bmj**, 328(7433), 211-214.
- Lyra, L., Bellani, W., Carolina, E., Brancher, J. A., De Lima, A. A. S., & Amenábar, J. M. (2020). *Changes in salivary composition of chemically dependent subjects*. **Odontología Vital**, (32), 63-70.
- Ma, F., Wu, T., Zhao, J., Song, A., Liu, H., Xu, W., Huang, G. (2016). *Folic acid supplementation improves cognitive function by reducing the levels of peripheral inflammatory cytokines in elderly Chinese subjects with MCI.* **Scientific reports**, 6, 37486.
- Madi, M., Babu, S., Kumari, S., Shetty, S., Achalli, S., Madiyal, A., Bhat, M. (2016). *Status of serum and salivary levels of superoxide dismutase in type 2 diabetes mellitus with oral manifestations: a case control study*. **Ethiopian journal of health sciences**, 26(6), 523-532.
- Mahanonda, R., Sa-Ard-Iam, N., Eksomtramate, M., Rerkyen, P., Phairat, B., Schaecher, K. E., ... Pichyangkul, S. (2009). *Cigarette smoke extract modulates human β-defensin-2 and interleukin-8*

expression in human gingival epithelial cells. **Journal of periodontal research**, 44(4), 557-564.

- Makino, E., Fukuyama, T., Watanabe, Y., Tajiki-Nishino, R., Tajima, H., Ohnuma-Koyama, A., ... Okazaki, Y. (2019). *Subacute oral administration of folic acid elicits anti-inflammatory response in a mouse model of allergic dermatitis.* **The Journal of nutritional biochemistry**, 67, 14-19.

- Malinow, M. R., Duell, P. B., Hess, D. L., anderson, P. H., Kruger, W. D., Phillipson, B. E., ... Upson, B. M. (1998). *Reduction of plasma homocyst (e) ine levels by breakfast cereal fortified with folic acid in patients with coronary heart disease.* **New England Journal of Medicine**, 338(15), 1009-1015.

- Mann, A. E., Sabin, S., Ziesemer, K., Vågene, Å. J., Schroeder, H., Ozga, A. T., ... Frohlich, B. (2018). *Differential preservation of endogenous human and microbial DNA in dental calculus and dentin.* **Scientific reports**, 8(1), 1-15.

- Marcott, S., Dewan, K., Kwan, M., Baik, F., Lee, Y. J., & Sirjani, D. (2020). *Where Dysphagia Begins: Polypharmacy and Xerostomia*. **Federal Practitioner**, 37(5), 234.

- Marsh, P. D. (2012). *Contemporary perspective on plaque control*. **British dental journal**, 212(12), 601.

- Masadeh, M. M., Gharaibeh, S. F., Alzoubi, K. H., Al-Azzam, S. I., Obeidat, W. M. (2013). *Antimicrobial activity of common mouthwash solutions on multidrug-resistance bacterial biofilms*. **Journal of clinical medicine research,** 5(5), 389.

- Mason, J. B. (1990). *Intestinal transport of monoglutamyl folates in mammalian systems. In: Folic Acid Metabolism in Health and Disease* (Picciano, M. F., Stokstad, E.L.R. Gregory, J. F., eds.), pp. 47–64, **Wiley-Liss,** New York.

- McAuley, J. L., Linden, S. K., Png, C. W., King, R. M., Pennington, H. L., Gendler, S. J., ... McGuckin, M. A. (2007). *MUC1 cell surface mucin is a critical element of the mucosal barrier to infection*. **The Journal of clinical investigation**, 117(8), 2313-2324.

- Merrell, B. J., & McMurry, J. P. (2020). *Folic Acid*. **StatPearls Publishing, Treasure Island (FL).** PMID: 32119374

- Mese, H., Matsuo, R. (2007). *Salivary secretion, taste and hyposalivation.* **Journal of oral rehabilitation**, 34(10), 711-723.

- Midda, M., & Cooksey, M. W. (1986). *Clinical uses of an enzyme-containing dentifrice.* **Journal of clinical periodontology**, 13(10), 950-956.

- Miller, C. S., Foley, J. D., Bailey, A. L., Campell, C. L., Humphries, R. L., Christodoulides, N., ... Redding, S. W. (2010). *Current developments in salivary diagnostics.* **Biomarkers in medicine,** 4(1), 171-189.

- Mittal, M., Siddiqui, M. R., Tran, K., Reddy, S. P., Malik, A. B. (2014*). Reactive oxygen species in inflammation and tissue injury.* **Antioxidants redox signaling**, 20(7), 1126-1167.

- Mohiti, A., Ebrahimzadeh Ardakani, M., & Amooei, M. (2020). *The Effect of Systemic Corticosteroid Use on the pH and Viscosity of Saliva.* **Shiraz E-Medical Journal**, (In Press). doi: 10.5812/semj.101710.

- Muhammed, H. O. (2017). *Gingivitis and Toothbrush Contamination*. **Tikrit Journal for Dental Sciences**, 5(1), 126-134.
- Musacchio, E, Valvason, C, Botsios, C, Ostuni, F, Furlan, A, Ramonda, R. (2009). *The tumor necrosis factor-{alpha}-blocking agent infliximab inhibits interleukin 1beta (IL-1beta) and IL-6 gene expression in human osteoblastic cells.* **J Rheumatol** 36:1575-1579.
- Muthukumar, S., Anand, M. V., & Madhankumar, S. (2014). *Relationship between gingival bleeding and anaerobic periodontal infection assessed by BANA (N-Benzoyl-DL-Arginine-β-Napthylamide) assay*. **Journal of pharmacy & bioallied sciences,** 6(Suppl 1), S70.
- Nagler RM, Klein I, Zarzhevsky N, Drigues N, Reznick AZ. (2002). *Characterization of the differentiated antioxidant profile of human saliva.* **Free Radic Biol Med**. 32: 268-77.
- Naiff, P. F., Carneiro, V., Guimarães, M. D. C. M., Bezerra, A. C. B., Oliveira, M. S., Couto, S. C., ... & Muniz-Junqueira, M. I. (2020). *Mechanical Periodontal Therapy Recovered the Phagocytic Function of Monocytes in Periodontitis.* **International journal of dentistry**, 2020.
- Nazir, M. A. (2017). *Prevalence of periodontal disease, its association with systemic diseases and prevention.* **International journal of health sciences,** 11(2), 72.
- Newman MG, Takei HH, Klokkevold PR, Carranza FA (2012). *Carranza's Clinical Priodontology*. 11th ed. **St louis, Saunders, Elsevier, UK**; pp:71-84, 437-452.
- Nibali, L., Fedele, S., D'aiuto, F., Donos, N. (2012). *Interleukin-6 in oral diseases: a review.* **Oral diseases**, 18(3), 236-243.

- Nishimoto N (2010). *Interleukin-6 as a therapeutic target in candidate inflammatory diseases*. **Clin Pharmacol Ther** 87:483–487.
- Nizamuddin, I., Koulen, P., McArthur, C. (2018). *Contribution of HIV Infection, AIDS, and Antiretroviral Therapy to Exocrine Pathogenesis in Salivary and Lacrimal Glands*. **International Journal of Molecular Sciences**, 19(9), 2747.
- Odell. E.W. (2017). *Cawson's Essentials of Oral Pathology and Oral Medicine,*.9th Edn. **Elsevier Ltd., London**. Pp: 114.
- O'Neil, M. J., Smith, A., Heckelman, P. E., Budavari, S. (2001). *The Merck Index-An Encyclopedia of Chemicals, Drugs, and Biologicals.* **Whitehouse Station**, NJ: Merck and Co. Inc, 767, 4342, p.748.
- P.N Bennett and M.J Brown. (2003). *Clinical Pharmacology*. 10th Edn. **Churchill Livingstone, Elsevier, UK**; Pp. 535-537.
- Pack, A. R. (1984). *Folate mouthwash: effects on established gingivitis in periodontal patients.* **Journal of Clinical Periodontology**, 11(9), 619-628.
- Pack, A. R., Thomson, M. E. (1980). *Effects of topical and systemic folic acid supplementation on gingivitis in pregnancy*. **Journal of clinical periodontology**, 7(5), 402-414.
- Packard RRS, Libby P. (2008). *Inflammation in atherosclerosis: from vascular biology to biomarker discovery and risk prediction*. **Clin Chem** 54: 24–38.
- Page, R., Robichaud, A., Arbuckle, T. E., Fraser, W. D., & MacFarlane, A. J. (2017). *Total folate and unmetabolized folic acid in the breast milk of a cross-section of Canadian women.* **The American Journal of Clinical Nutrition**, 105(5), 1101-1109.

- Paniz, C., Bertinato, J. F., Lucena, M. R., De Carli, E., Amorim, P. M. D. S., Gomes, G. W., ... & Green, R. (2017). *A daily dose of 5 mg folic acid for 90 days is associated with increased serum unmetabolized folic acid and reduced natural killer cell cytotoxicity in healthy Brazilian adults*. **The Journal of nutrition**, 147(9), 1677-1685.

- Park, M. C., Lee, S. W., Choi, S. T., Park, Y. B., Lee, S. K. (2007). *Serum leptin levels correlate with interleukin-6 levels and disease activity in patients with ankylosing spondylitis*. **Scandinavian journal of rheumatology**, 36(2), 101-106.

- Parker, R. I., Vannest, K. J., & Brown, L. (2009). *The improvement rate difference for single-case research*. **Exceptional Children**, 75(2), 135-150.

- Pendyala G, Thomas B, Joshi SR. (2013). *Evaluation of total antioxidant capacity of saliva in type 2 diabetic patients with and without periodontal disease: a casecontrol study*. **N Am J Med Sci**. 5: 51-57.

- Pereira, E. M. R., da Silva, J. L. D. C., Silva, F. F., De Luca, M. P., Lorentz, T. C. M., Santos, V. R. (2011). *Clinical evidence of the efficacy of a mouthwash containing propolis for the control of plaque and gingivitis: a phase II study.* **Evidence-Based Complementary and Alternative Medicine**, 2011.

- Petersen, J. M., Parker, S. E., Benedum, C. M., Mitchell, A. A., Tinker, S. C., Werler, M. M. (2019). *Periconceptional folic acid and risk for neural tube defects among higher risk pregnancies*. **Birth defects research**, 111(19), 1501-1512

- Peyyala, R., & Ebersole, J. L. (2013). *Multispecies biofilms and host responses:"discriminating the trees from the forest*". **Cytokine**, 61(1), 15-25.

- Pisoschi, A. M., Pop, A. (2015). *The role of antioxidants in the chemistry of oxidative stress: A review.* **European journal of medicinal chemistry**, 97, 55-74.

- Pradeep, A. R., Agarwal, E., Arjun Raju, P., Rao, M. N., Faizuddin, M. (2011). *Study of orthophosphate, pyrophosphate, and pyrophosphatase in saliva with reference to calculus formation and inhibition.* **Journal of periodontology**, 82(3), 445-451.

- Prasad, V. N., Chawla, H. S., Goyal, A., Gauba, K., Singhi, P. (2004). *Folic acid and phenytoin induced gingival overgrowth—is there a preventive effect.* **J Indian Soc Pedod Prev Dent**, 22(2), 82-91.

- Qiu, W., Hu, J., Magnuson, J. T., Greer, J., Yang, M., Chen, Q., ... & Schlenk, D. (2020). *Evidence linking exposure of fish primary macrophages to antibiotics activates the NF-kB pathway*. **Environment international**, 138, 105624.

- Qu, P., Li, S., Liu, D., Lei, F., Zeng, L., Wang, D., ... & Dang, S. (2019). *A propensity-matched study of the association between optimal folic acid supplementation and birth defects in Shaanxi province, Northwestern China.* **Scientific Reports**, 9(1), 1-9.

- Rahmadhani, S. P., Rita, R. S., & Reza, M. (2020). *Effect of Folic Acid and Vitamin E Administration on Fetal Growth of Pregnant Rats (Rattus norvegicus) Exposed to Cigarette Smoke.* **International Journal of Research & Review,** 6(10):120-124.

- Ramesh, M. V., Puvvadi-Gopala-Krishna-Naveen Kumar, C. R., Allamaprabhu, N. N. K., Yasmeen, S. A., & Praveen, G. (2020). *Evaluation of dentifrices of complementary and alternative medicinal systems on plaque formation and gingivitis: A randomized controlled clinical trial.* **Journal of Clinical and Experimental Dentistry**, 12(3), e227.

- Ramsay, D. S. (2000). *Patient compliance with oral hygiene regimens: A behavioural self-regulation analysis with implications for technology.* **International Dental Journal**, 50(S6_Part1), 304-311.

- Rangbulla, V., Nirola, A., Gupta, M., Batra, P., & Gupta, M. (2017). *SalivaryIgA, Interleukin-1beta and MMP-8 as salivary biomarkers in chronic periodontitis patients.* **Chin. J. Dent. Res**, 20(1), 43-51.

- Rebelo, M. A. B., De Queiroz, A. C. (2011). *Gingival Indices: State of Art, Gingival Diseases-Their Aetiology*. **Prevention and Treatment, Dr. Fotinos Panagakos**.

- Rezk, B. M., Haenen, G. R., van der Vijgh, W. J., Bast, A. (2003). *Tetrahydrofolate and 5-methyltetrahydrofolate are folates with high antioxidant activity. Identification of the antioxidant pharmacophore.* **FEBS letters**, 555(3), 601-605.

- Rizvi, A., Zafar, M. S., Al-Wasifi, Y., Fareed, W., Khurshid, Z. (2016). *Role of enamel deminerlization and remineralization on microtensile bond strength of resin composite*. **European journal of dentistry,** *10*(03), 376-380.

- Rubido S, Caballero LG, Abeleira MT, Limeres J,García M, and Diz P.(2018). *Effect of chewing an apple on dental plaque removal and on salivary bacterial viability*. **PLoS One**. 13(7): e0199812. doi: 10.1371/journal.pone.0199812

- Ruengsitagoon, W., Hattanat, N. (2012). *Simple spectrophotometric method for determination of folic acid*. **In The 4th Annual Northeast Pharmacy Research Conference of "Pharmacy Profession in Harmony** (Vol. 281).

- Russell, R. M., Dhar, G. J., Dutta, S. K. Rosenberg, I. H. (1979). *Influence of intraluminal pH on folate absorption: studies in control subjects and in patients with pancreatic insufficiency.* **J. Lab. Clin. Med**. 93: 428–436.

- Russell, R. M., Kransinski, S. D., Samloff, I. M., Jacob, R. A., Hartz, S. C. Brovender, S. R. (1986). *Folic acid malabsorption in atrophic gastritis: possible compensation by bacterial folate synthesis*. **Gastroenterology** 91: 1476–1482

- Ryder, M.I. (2010). *Comparison of neutrophil functions in aggressive and chronic periodontitis.* **Periodontol.2000** 53,124–137.doi:10.1111/j.1600- 0757.2009.00327.x.

- Saini, R., Saini, S., Saini, S. R. (2011). *Periodontitis: A risk for delivery of premature labor and low birth weight infants*. **Journal of natural science, biology, and medicine,** 2(1), 50.

- Saladin, K.S. (2010). *Anatomy Physiology A Unity of Form and Function*, 5th Edn. **McGraw-Hill, New York, NY, USA**. Pp: 346.

- Samblas, M., Martínez, J. A., Milagro, F. (2018). *Folic acid improves the inflammatory response in LPS-activated THP-1 macrophages*. **Mediators of inflammation**, 2018.

- Santos, M. T. B. R., Diniz, M. B., Guaré, R. O., Ferreira, M. C. D., Gutierrez, G. M., Gorjão, R. (2017). *Inflammatory markers in saliva as indicators of gingival inflammation in cerebral palsy children with and*

without cervical motor control. **International journal of paediatric dentistry**, 27(5), 364-371.

- Sawaengsri, H., Wang, J., Reginaldo, C., Steluti, J., Wu, D., Meydani, S. N., ... Paul, L. (2016). *High folic acid intake reduces natural killer cell cytotoxicity in aged mice.* **The Journal of nutritional biochemistry**, 30, 102-107.

- Scarano E, Fiorita A, Picciotti PM et al. (2010). *Proteomics of saliva: personal experience. Acta Otorhinolaryngol Ital*; 30 (3): 125–130.

- Schafer, C. A., Schafer, J. J., Yakob, M., Lima, P., Camargo, P., Wong, D. T. (2014). *Saliva diagnostics: utilizing oral fluids to determine health status. In Saliva: Secretion and Functions*. **Karger Publishers**, (Vol. 24, Pp. 88-98).

- Schätzle, M., Löe, H., Lang, N. P., Bürgin, W., Ånerud, Å., Boysen, H. (2004). *The clinical course of chronic periodontitis: IV. Gingival inflammation as a risk factor in tooth mortality*. **Journal of Clinical Periodontology**, 31(12), 1122-1127.

- Schipper[a] R, Loof A, de Groot J et al. (2007). *SELDI-TOF-MS of saliva: methodology and pre-treatment effects.* **J Chromatogr**; 847 (1): 45–53.

- Schipper[b], R. G., Silletti, E., Vingerhoeds, M. H. (2007). *Saliva as research material: biochemical, physicochemical and practical aspects*. **Archives of oral biology**, 52(12), 1114-1135.

- Schisterman, E. F., Clemons, T., Peterson, C. M., *Johnstone, E., Hammoud, A. O., Lamb, D., ... Ryan, G. (2020). A Randomized Trial to Evaluate the Effects of Folic Acid and Zinc Supplementation on Male*

Fertility and Livebirth: Design and Baseline Characteristics. **American journal of epidemiology**, 189(1), 8-26.

- Scott, D. A., & Krauss, J. (2012). *Neutrophils in periodontal inflammation. In Periodontal disease.* **Karger Publishers**, (Vol. 15, pp. 56-83).

- Sean C. Sweetman, Paul S Blake, Alison Brayfield, Julie M McGlashan Anne V Parsons. (2009). *Martindale The Complete Drug Reference*. 36th Edn. **Pharmaceutical Press. Royal Pharmaceutical Society , Great Britain**; Pp : 1940.

- Shaila, M., Pai, G. P., Shetty, P. (2013). *Salivary protein concentration, flow rate, buffer capacity and pH estimation: A comparative study among young and elderly subjects, both normal and with gingivitis and periodontitis.* **Journal of Indian society of periodontology**, 17(1), 42.

- Sharma, M., Bairy, I., Pai, K., Satyamoorthy, K., Prasad, S., Berkovitz, B., Radhakrishnan, R. (2011). *Salivary IL-6 levels in oral leukoplakia with dysplasia and its clinical relevance to tobacco habits and periodontitis.* **Clinical oral investigations**, 15(5), 705-714.

- Shaw, P., & Chattopadhyay, A. (2020*). Nrf2–ARE signaling in cellular protection: Mechanism of action and the regulatory mechanisms.* **Journal of cellular physiology**, 235(4), 3119-3130.

- Shi, C., Pamer, E. G. (2011). *Monocyte recruitment during infection and inflammation*. **Nature reviews immunology**, 11(11), 762-774.

- Shimada Y, Komatsu Y, Ikezawa-Suzuki I, Tai H, Sugita N, Yoshie H (2010). *The effect of periodontal treatment on serum leptin, interleukin-6, and C-reactive protein.* **J Periodontol** 81: 1118–1123.

- Si, Y., Ao, S., Wang, W., Chen, F., Zheng, S. (2015). *Magnetic bead-based salivary peptidome profiling analysis for severe early childhood caries*. **Caries research**, 49(1), 63-69.
- Sies, H. (1997). *Oxidative stress: oxidants and antioxidants.* **Experimental Physiology: Translation and Integration**, 82(2), 291-295.
- Silness, J., Löe, H. (1964). *Periodontal disease in pregnancy II. Correlation between oral hygiene and periodontal condition*. **Acta odontologica scandinavica,** 22(1), 121-135.
- Smiley, C. J., Tracy, S. L., Abt, E., Michalowicz, B. S., John, M. T., Gunsolley, J., ... Hujoel, P. P. (2015). *Systematic review and meta-analysis on the nonsurgical treatment of chronic periodontitis by means of scaling and root planing with or without adjuncts*. **The Journal of the American Dental Association**, *146*(7), 508-524.
- Solini, A., Santini, E., Ferrannini, E. (2006). *Effect of short-term folic acid supplementation on insulin sensitivity and inflammatory markers in overweight subjects.* **International Journal of Obesity**, 30(8), 1197-1202.
- Sreenivasan, P. K., Prasad, K. V. V., Javali, S. B. (2016). *Oral health practices and prevalence of dental plaque and gingivitis among Indian adults.* **Clinical and experimental dental research**, 2(1), 6-17.
- Steinweg, D. L., Bentley, M. L. (2005). *Seizures following reduction in phenytoin level after orally administered folic acid.* **Neurology,** 64(11), 1982-1982.
- Steluti, J., Miranda, A. M., De Carli, E., Palchetti, C. Z., Fisberg, R. M., & Marchioni, D. M. L. (2020). *Unmetabolized folic acid is*

associated with TNF-α, IL-1β and IL-12 concentrations in a population exposed to mandatory food fortification with folic acid: a cross-sectional population-based study in Sao Paulo, Brazil. **European Journal of Nutrition**, 1-9.

- Sugiura K, Nagai T, Nakano M, Ichinose H, Nakabayashi T. (2015). *Redox sensor proteins for highly sensitive direct imaging of intracellular redox state*. **Biochem Biophys Res Commun**. 457: 242-248.

- Sun, X., Huang, X., Tan, X., Si, Y., Wang, X., Chen, F., Zheng, S. (2016). *Salivary peptidome profiling for diagnosis of severe early childhood caries*. **Journal of translational medicine**, 14(1), 240.

- Suresh, S., Mahendra, J., Kumar, A. R. P., Singh, G., Jayaraman, S., Paul, R. (2017). *Comparative analysis of subgingival red complex bacteria in obese and normal weight subjects with and without chronic periodontitis*. **Journal of Indian Society of Periodontology**, 21(3), 186.

- Syndergaard, B., Al-Sabbagh, M., Kryscio, R. J., Xi, J., Ding, X., Ebersole, J. L., & Miller, C. S. (2014). *Salivary biomarkers associated with gingivitis and response to therapy*. **Journal of periodontology**, 85(8), e295-e303.

- Taguchi K, Motohashi H, Yamamoto M. (2011) . *Molecular mechanisms of the Keap1-Nrf2 pathway in stress response and cancer evolution.* **Genes Cells** 16 (2): 123–140.

- Thomson, M. E., Pack, A. R. (1982). *Effects of extended systemic and topical folate supplementation on gingivitis of pregnancy*. **Journal of Clinical Periodontology**, 9(3), 275-280.

- Togoo, R. A., Al-Almai, B., Al-Hamdi, F., Huaylah, S. H., Althobati, M., Alqarni, S. (2019). *Knowledge of Pregnant Women about Pregnancy Gingivitis and Children Oral Health*. **European journal of dentistry**, 13(02), 261-270.

- Tomofuji, T., Ekuni, D., Yamanaka, R., Kusano, H., Azuma, T., Sanbe, T., ... Takata, T. (2007). *Chronic administration of lipopolysaccharide and proteases induces periodontal inflammation and hepatic steatosis in rats.* **Journal of periodontology**, 78(10), 1999-2006.

- Tonetti, M. S., Eickholz, P., Loos, B. G., Papapanou, P., Van Der Velden, U., Armitage, G., ... Kocher, T. (2015). *Principles in prevention of periodontal diseases: consensus report of group 1 of the 11th European Workshop on Periodontology on effective prevention of periodontal and peri-implant diseases*. **Journal of clinical periodontology**, 42, S5-S11.

- Tonetti, M. S., Jepsen, S., Jin, L., and Otomo-Corgel, J. (2017). *Impact of the global burden of periodontal diseases on health, nutrition and wellbeing of mankind: a call for global action*. **J. Clin. Periodontol**. 44, 456–462. doi: 10.1111/jcpe.12732

- Tothova L, Celecova V, Celec P. (2013). *Salivary markers of oxidative stress and their relation to periodontal and dental status in children*. **Dis. Markers.** 34: 9-15.

- Triola MM, Triola MF and Roy J. (2018). *Biostatistics for the Biological and Health Sciences*. 2nd ed. **Pearson Education Limited, UK**.

- Trivedi, S., Lal, N., Mahdi, A. A., Singh, B., and Pandey, S. (2015). *Association of salivary lipid peroxidation levels, antioxidant enzymes, and chronic periodontitis.* **Int. J. Period. Restorat. Dent**. 35, e14–e19.

- Troen, A. M., Mitchell, B., Sorensen, B., Wener, M. H., Johnston, A., Wood, B., ... Potter, J. D. (2006). *Unmetabolized folic acid in plasma is associated with reduced natural killer cell cytotoxicity among postmenopausal women.* **The Journal of nutrition**, 136(1), 189-194.

- Tsuji, A., Shibata, K. (2019). *Effects of Biotin Deficiency on the Urinary Excretion of B-Group Vitamins in Mice*. **J Clin Nutr Metab** 3, 1, 2.

- Türer ÇC, Ballı U, Güven B. (2017). *Fetuin-A, serum amyloid A and tumor necrosis factor alpha levels in periodontal health and disease*. **Oral diseases**, 23(3), 379-386.

- Van Nieuw Amerongen A, Bolscher JG, Veerman EC .(2004). *Salivary proteins: protective and diagnostic value in cariology?* **Caries Res**; 38 (3): 247–253.

- Varadharaj, S., Porter, K., Pleister, A., Wannemacher, J., Sow, A., Jarjoura, D. Khayat, R. N. (2015). *Endothelial nitric oxide synthase uncoupling: a novel pathway in OSA induced vascular endothelial dysfunction*. ***Respiratory physiology neurobiology***, *207*, 40-47.

- Varela-López, A., Navarro-Hortal, M. D., Giampieri, F., Bullón, P., Battino, M., & Quiles, J. L. (2018). *Nutraceuticals in periodontal health: a systematic review on the role of vitamins in periodontal health maintenance*. **Molecules**, 23(5), 1226.

- Velsko, I. M., Overmyer, K. A., Speller, C., Klaus, L., Collins, M. J., Loe, L., ... Chaves, E. (2017). *The dental calculus metabolome in modern and historic samples.* Metabolomics, 13(11), 134.

- Velsko, I. M., Yates, J. A. F., Aron, F., Hagan, R. W., Frantz, L. A., Loe, L., ... Warinner, C. (2019). *Microbial differences between dental*

plaque and historic dental calculus are related to oral biofilm maturation stage. **Microbiome**, 7(1), 102.

- Villines, Z. (2017). *How Do Free Radicals Affect the Body?*. **Medical News Today** (accessed July 9, 2018), www. medicalnewstoday. com/articles/318652.php.

- Vogel R., Fink R., Schneider L. and Frank O. (1976). *The effect of folic acid on gingival health.* **J. Periodontol** , November.

- Von Fraunhofer, J. A. (2019). *Vitamin B9 and Health*. **EC Dental Science**, 18, 1331-1335.

- Wald, N. J., Morris, J. K., Blakemore, C. (2020). *Urgent need for folic acid fortification of flour and grains: response to the 2019 UK Government's public consultation*. **Archives of Disease in Childhood**, 105(1), 6-9.

- Wang, T. P., Hsu, S. H., Feng, H. C., Huang, R. F. S. (2012). *Folate deprivation enhances invasiveness of human colon cancer cells mediated by activation of sonic hedgehog signaling through promoter hypomethylation and cross action with transcription nuclear factor-kappa B pathway.* **Carcinogenesis**, 33(6), 1158-1168.

- Wang, Y., andrukhov, O., Rausch-Fan, X. (2017). *Oxidative stress and antioxidant system in periodontitis*. **Frontiers in physiology**, 8, 910.

- Wang, K., Wang, Y., Wang, X., Ren, Q., Han, S., Ding, L., ... Zhang, L. (2018). *Comparative salivary proteomics analysis of children with and without dental caries using the iTRAQ/MRM approach.* **Journal of translational medicine**, 16(1), 11.

- Wang, K., Zhou, X., Li, W., Zhang, L. (2019). *Human salivary proteins and their peptidomimetics: Values of function, early diagnosis, and therapeutic potential in combating dental caries*. **Archives of oral biology,** 99, 31-42.
- Wara-Aswapati, N., Pitiphat, W., Chanchaimongkon, L., Taweechaisupapong, S., Boch, J. A., Ishikawa, I. (2009). *Red bacterial complex is associated with the severity of chronic periodontitis in a Thai population*. **Oral diseases**, *15*(5), 354-359.
- Wien, T. N., Pike, E., Wisløff, T., Staff, A., Smeland, S., Klemp, M. (2012). *Cancer risk with folic acid supplements: a systematic review and meta-analysis.* **BMJ open**, 2(1), e000653.
- Williams RC (2008). *Host Modulation for the Treatment of Periodontal Diseases.* **J of Dentistry India**; 2(5):1-5.
- Williamson, S., Munro, C., Pickler, R., Grap, M. J., Elswick, R. K. (2012). *Comparison of biomarkers in blood and saliva in healthy adults*. **Nursing research and practice**, 2012.
- Willmott, M., Bartosik, D. B., Romanoff, E. B. (1968). *The effect of folic acid on superovulation in the immature rat.* **Journal of Endocrinology**, 41(3), 439-445.
- Wu, Y., Shu, R., Shen, M. H., Ge, L. H. (2007). *Detection and significance of IL-1beta and MMP-8 in patients with periodontitis of whole unstimulated saliva.* **Shanghai journal of stomatology**, 16(2), 127-130.
- Xie, G., Chain, P. S. G., Lo, C. C., Liu, K. L., Gans, J., Merritt, J., Qi, F. (2010). *Community and gene composition of a human dental plaque*

microbiota obtained by metagenomic sequencing. **Molecular oral microbiology**, 25(6), 391-405.

❖ Yakob, M., Fuentes, L., Wang, M. B., Abemayor, E., Wong, D. T. (2014). *Salivary biomarkers for detection of oral squamous cell carcinoma: current state and recent advances*. **Current oral health reports**, 1(2), 133-141.

❖ Yu, Y. H., Kuo, H. K., Lai, Y. L. (2007). *The association between serum folate levels and periodontal disease in older adults: data from the National Health and Nutrition Examination Survey 2001/02.* **Journal of the American Geriatrics Society**, 55(1), 108-113.

❖ Yucel-Lindberg, T., Båge, T. (2013). *Inflammatory mediators in the pathogenesis of periodontitis.* **Expert reviews in molecular medicine**, 15.

❖ Yue, Y., Liu, Q., Xu, C., Loo, W. T., Wang, M., Wen, G., and Ng, E. L. (2013). *Comparative Evaluation of Cytokines in Gingival Crevicular Fluid and Saliva of Patients with Aggressive Periodontitis.* **The International Journal of Biological Markers**, 28(1), 108–112.

❖ Zhang, J., Xuan, D., Fan, W., Zhang, X., Dibart, S., De, W. V., ... Zhang, Y. P. (2010). *Severity and prevalence of plaque-induced gingivitis in the Chinese population.* **Compendium of continuing education in dentistry** (Jamesburg, NJ: 1995), 31(8), 624-629.

❖ Zhang, A., Sun, H., Wang, P., Wang, X. (2013). *Salivary proteomics in biomedical research.* **Clinica chimica acta**, 415, 261-265.

❖ Zhang, T., andrukhov, O., Haririan, H., Müller-Kern, M., Liu, S., Liu, Z., Rausch-Fan, X. (2016). *Total antioxidant capacity and total*

oxidant status in saliva of periodontitis patients in relation to bacterial load. **Frontiers in cellular and infection microbiology**, *5*, 97.

- Zhang, Q., Wu, H., Zou, M., Li, L., Li, Q., Sun, C., ... Wu, L. (2019*). Folic acid improves abnormal behavior via mitigation of oxidative stress, inflammation, and ferroptosis in the BTBR T+ tf/J mouse model of autism*. **The Journal of nutritional biochemistry**, 71, 98-109.

- Zhu, H., Lin, X., Zheng, P., Chen, H. (2015). *Inflammatory cytokine levels in patients with periodontitis and/or coronary heart disease.* **International journal of clinical and experimental pathology**, 8(2), 221.

- Żukowski, P., Maciejczyk, M., Waszkiel, D. (2018). *Sources of free radicals and oxidative stress in the oral cavity.* **Archives of Oral Biology**, 92, 8-17.

APPENDICES

بسم الله الرحمن الرحيم

إني المريضـ ..... أوافق على المشاركة في البحث وذلك بالالتزام بالتعليمات الصحيحة التي يقررها الباحث في اخذ العلاج واتباع روتين يومي بالعناية بالأسنان، حسب تعليمات الباحث، لأجل ذلك وقعت.

التوقيع:
الاســم:
التاريخ:

Appenix 1 : Consent Form

Case Sheet

Date: / / Patient No. :

Patients Name:

Age & Gender: ..... Social condition: ……..

Occupation: ..... . Medical history :……

Drug Intake: …… Folic acid allergy: …….

Address & Phone number: ……

Type of diet: ……..

Plaque Index

Gingival Index

Calculus Index

Oral Health Index

Appendix 2 : Case Sheet

Appendix 3

Relative centrifugal force (RCF) or g force is used to refer to the amount of force applied when using a centrifuge. To convert revolutions per minute (RPM) to relative centrifugal force (RCF), or g force, use the following formula:

$$\mathbf{RCF = (RPM)^2 \times 1.118 \times 10^{-5} \times \mathit{r}.}$$

r: radius of centrifuge rotor in cm.

الخلاصــة

المقدمة : حمض الفوليك هو فيتامين قابل للذوبان في الماء ، وشكله الدوائي قادر على تحسين التهاب اللثة عن طريق مسح الجذور الحرة بكفاءة عالية ويخفض من نسب السيتوكينات. توصف أمراض ماحول السن وخاصة التهاب اللثة بانها واحدة من أكثر الامراض الفموية انتشاراً في العالم مما حث هذا الانتشار الكبير الباحثين على دراسة تأثير العديد من الأدوية أو المكملات الغذائية على التهاب اللثة التي تحسن الحالة بأقل التأثيرات الجانبية و أعلى نسبة توافر في الدم، وفي متناول اليد.

الأهداف : لدراسة تأثير حمض الفوليك على مرض التهاب اللثة المزمن ولتقييم تأثيره على مستويات (IL-6) و(TNF- α) في لعاب المرضى المصابين به و للتحقيق في تأثيره المضاد للأكسدة في لعابهم على صحة الفم كذلك لتقييم تأثير حمض الفوليك على بروتينات اللعاب الكلية.

المواد وطرائق البحث : هذه الدراسة هي تجربة سريرية عشوائية ، أُجريت على ٤٠ شخصًا تم تشخيصهم على أنهم مرضى التهاب اللثة المزمن ، تتراوح أعمارهم بين (٢٠-٤٠) عامًا ، تم تقسيمهم إلى مجموعتين، عشرين شخصاً لكلٍ منهما. تلقت المجموعة الأولى (مجموعة العلاج) قرص فموي من حمض الفوليك بجرعة ١ ملغم مرة واحدة يوميًا لمدة ٤٢ يوماً، ولم تتلقى المجموعة الثانية (مجموعة المراقبة) أي دواء. تمت عمليات تنظيف وتلميع الاسنان لكل مشارك قبل بدء الدراسة. في اليوم التالي (اليوم الأول من الدراسة) ، تم قياس مؤشرات البلاك واللثة والنظافة الفموية والجير للمجموعتين (PI, GI, OHI and CI)، وفي اليوم ٢١ و٤٢ من الدراسة. في تلك الزيارات الثلاث ، تم جمع عينات اللعاب من اجل قياس مستوى (IL-6) و (TNF-α) ،وقياس القدرة الكلية لمضادات الأكسدة وقياس البروتينات الكلية في اللعاب. للحصول على نتائج احصائية تم تطبيق اختبار (ANOVA) واختبار(Tukey) واختبار(t-test) . مع الاخذ بنظر الاعتبار كون قيمة $P < ٠,٠٥$.

النتائج : مجموعة العلاج كانت مكونة من ١١ أناث (٥٥%) و٩ ذكور (٤٥%)، وكان متوسط عمر المرضى ٢٨,٢ ± ٥,٧٢ سنة، بينما كانت مجموعة المراقبة متكونة من ٩ أناث (٤٥%) و١١ ذكور (٥٥%)، وكان متوسط العمر ٢٨,٩ ± ٧,٠٥ سنة، من دون وجود فروق معنوية كبيرة بينها. وقد اظهرت النتائج في بداية الدراسة أنه لا يوجد فرق معنوي بين مجموعة العلاج ومجموعة التحكم في مؤشرات PI وGI وOHI وCI على التوالي ، ولكن كان هناك فرقاً معنوياً بين مجموعة العلاج ومجموعة التحكم في PI في اليوم ٢١ دون وجود فرق معنوي بين مجموعة العلاج ومجموعة التحكم في GI وOHI وCI في اليوم ٢١و٤٢ من الدراسة.

اظهرت نتائج مؤشرات الصحة الفموية في مجموعة العلاج خلال فترة الدراسة كلها وجود فرقاً معنوياً في متوسط CI بين اليوم الأول واليوم ٢١ من الدراسة و أن هناك اختلافا كبيرا بين مجموعة العلاج ومجموعة المراقبة في مستوى TNF-α في يومي ٢١ و ٤٢.

في مجموعة العلاج هناك فرقاً معنوياً ملحوظاً في مستوى TAC في اللعاب ، مع وجود انخفاضاً قليلاً غير معنويًا في مستوى IL-6 و TNF-α و TP في اللعاب خلال جميع فترة الدراسة بينما في مجموعة التحكم لا يوجد فرقاً معنوياً في IL-6 و TNF-α و TAC و TP في اللعاب في الأيام الأولى و٢١ و ٤٢ من الدراسة.

الاستنتاجات : يمكن أن يؤدي استخدام مكملات حمض الفوليك إلى تحسين صحة الفم والحد من التهاب اللثة عن طريق زيادة إجمالي القدرة المضادة للأكسدة اللعابية وتقليل مستوى TNF-α في اللعاب.

اقرار لجنة المناقشة

نشهد إننا أعضاء لجنة تقويم المناقشة قد اطلعنا على الاطروحة الموسومة " التأثيرات المضادة للأكسدة والمضادة للالتهاب لحمض الفوليك على مرض التهاب اللثة المزمن " وناقشنا الطالبة " راوية فهر فاضل اسماعيل " في محتوياتها وفيما له علاقة بها بتاريخ ١ / ١٢ / ٢٠٢٠ وأنها جديرة لنيل شهادة الماجستير في اختصاص علم الأدوية / أدوية الفم والاسنان

التوقيع:
الاسم: د. غادة عبد الرحمن عبد اللطيف
المرتبة العلمية :أستاذ
التاريخ: / /
(رئيس اللجنة)

التوقيع:
الاسم: د. مها طلال فتاح
المرتبة العلمية: أستاذ مساعد
التاريخ: / /
(عضو اللجنة)

التوقيع:
الاسم: د. عباس فاضل الطائي
المرتبة العلمية: أستاذ مساعد
التاريخ : / /
(عضو اللجنة)

التوقيع:
الاسم: د. فيحاء ازهر المشهداني
المرتبة العلمية: أستاذ مساعد
التاريخ : / /
(عضو اللجنة / المشرف)

قرار مجلس الكلية

اجتمع مجلس كلية طب الاسنان بجلسته ( ) المنعقدة بتاريخ / / وقرر منحها شهادة الماجستير في اختصاص علم الادوية / ادوية الفم والاسنان

التوقيع:
الاسم: أ.م.د. نعم رياض سليم
(مقرر مجلس كلية طب الاسنان)
التاريخ : / /

التوقيع:
الاسم: أ.م.د. ريان سالم حامد
(عميد كلية طب الاسنان)
التاريخ : / /

اقرار المشرف

أشهد أن إعداد هذه الرسالة الموسومة بـ (التأثيرات المضادة للأكسدة والمضادة للالتهاب لحمض الفوليك على التهاب اللثة المزمن) جرى تحت إشرافي في جامعة الموصل وهي جزء من متطلبات درجة الماجستير في علم الادوية / ادوية الفم والاسنان

التوقيع

المشرف : أ.م.د. فيحاء ازهر محي الدين المشهداني

التاريخ : / / ٢٠٢٠

اقرار المقوم اللغوي

أشهد بأن هذه الرسالة تمت مراجعتها من الناحية اللغوية وتصحيح ما ورد فيها من أخطاء لغوية وتعبيرية وبذلك أصبحت الرسالة مؤهلة للمناقشة بقدر ما يتعلق الأمر بسلامة الأسلوب وصحة التعبير.

التوقيع

الاسم : أ.م.د. محمد نهاد احمد

التاريخ : / / ٢٠٢٠

اقرار رئيس فرع العلوم الاساسية

بناءً على التوصيات المقدمة من قبل المشرف والمقوم اللغوي ارشح هذه الرسالة للمناقشة.

التوقيع

الاسم : أ.م.د. احمد شهاب الطويل

التاريخ : / / ٢٠٢٠

اقرار رئيس لجنة الدراسات العليا

بناءً على التوصيات المقدمة من قبل المشرف والمقوم اللغوي نرشح هذه الدراسة للمناقشة.

التوقيع

الاسم : أ.م.د. نعم رياض سليم

التاريخ : / / ٢٠٢٠

بسم الله الرحمن الرحيم

{ يَرْفَعِ اللَّهُ الَّذِينَ آَمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ }

صدق الله العظيم

(المجادلة: ١١)

وزارة التعليم العالي والبحث العلمي
جـامـعـة المـوصـل
كلية طب الأسنان

التأثيرات المضادة للأكسدة والمضادة للالتهاب لحمض الفوليك على مرض التهاب اللثة المزمن

رسالة تقدمت بها

راويـة فهـر فاضـل البُصراوي

بكالوريوس علوم صيدلانية

الى

مجلس كلية طب الاسنان- جامعة الموصل

كجزء من متطلبات نيل شهادة الماجستير في اختصاص

علـم الادويـة / ادوية الفم والاسنـان

بأشراف

الاستاذ المساعد

الدكتورة فيحـاء ازهـر مُحـي الدين المشهـداني

٢٠٢٠ م ١٤٤٢ هـ