بسم الله الرحمن الرحيم

Sudan University of Science and Technology

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Assessment of Serum and Salivary Total Protein, Calcium and Salivary pH of Sudanese Pregnant Women with Dental Crises

تقيم مستويات البروتينات الكاملة, الكالسيوم والاس الهايدروجيني في المصل واللعاب لدي النساء السودانيات الحوامل المصابات بتسوس الاسنان

A dissertation submitted in partial fulfillment for the requirements of M.Sc degree in clinical chemistry

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الآية

قال تعالي:

وَلَوْ أَنّهُمْ رَضُوا مَا آتَاهُمُ اللّهُ وَرَسُولُهُ وَقَالُوا حَسْبُنَا اللّهُ سَيُؤْتِينَا اللّهُ مِنْ فَضْلِهِ وَرَسُولُهُ إِنّا إِلَى اللّهِ رَاغِبُونَ

صدق الله العظيم
سورة التوبة الآية(59)

Dedication

To……

M Y Father……..WhoWork hardly for us.

TO……..

M Y M other……Who taught me

How I could be human

M Y beloved brother and sisters

TO……..

The people, whom I love, respect and appreciate.

Acknowledgement

Firstly I thank ALLAH for blessed me with the courage for preparation and completion of this study. With a great deal of respect I want to thank my supervisor Dr. Khalda Mirghani Hamza, all members of clinical chemistry department .It is pleasure to express my respect sincere thanks and gratitude to all test subjects for agreement to participate in this study.

Abbreviations

B	Brushite
Ca^{++}	Calcium
CRP	C reactive protein
GCF	Gingival cervicular fluid
GFR	Glomerular filtration rate
HA	Hydroxyl apatite
HCG	Human chorionic gonadotropin
Ig	Immune globulin
IL	Inter leukin
LNMP	Last normal menstrual period
Mg^{++}	Magnesium
NGF	Nerve growth factor
OCP	Octa calcium phosphate
P	Phosphate
PDL	Periodontal Ligament
pH	Potential of hydrogen
PMNL	Poly morph nuclear leukocyte
PTH	Para thyroid hormone
PTH_ rp	Para thyroid hormone related peptide
PUPPP	Pruritic urticarial papules and plaques of pregnancy
SD	Stander deviation
W	Whitlockite

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Abstract

This is a descriptive analytical case control study, conducted during February to April2014 to evaluate the serum and saliva total protein, calcium and pH in pregnant women with dental diseases.

Sixty pregnant women were enrolled in this study and twenty apparently healthy non pregnant women (control subjects) who aged rang from 14_40 years old.

The blood and salivary samples were collected from pregnant women and control subjects to obtain the serum and clear saliva for estimation of total protein and calcium of each.

The Biuret colorimetric method was used for measurements of total protein levels and the colorimetric Methylthymol blue method was used for estimation of calcium levels.

The result showed that: The serum total protein level is insignificantly different in cases when compared to control subjects (P.value=0.843), and the serum calcium level is significantly decreased in cases compared to contol (P value=0.000). The salivary total protein significantly increased in cases compared to contol (P value=0.000) , calcium and pH levels are significantly decreased in cases when compared to control subjects (P.value=0.000).

The present study showed that the pregnancy cause salivary changes and has effect on protein, calcium and pH levels resulting in dental crises.

ملخص الدراسة

اجريت هذه الدراسة في الفترة ما بين شهري فبراير 2014 و ابريل 2014 بغرض تقييم الصورة الكاملة لمستوي البروتين والكالسيوم في الدم واللعاب عند النساء الحوامل المصابات بتسوس الاسنان .

تضمنت هذه الدراسة60 امراة حامل لتتم مقارنة النتائج بنتائج 20 امراة غير حامل .

ولقد اخذت عينات الدم واللعاب من مجموعتي المرضي والاصحاء للحصول علي المصل واللعاب لاجراء اختبارات موضوع الدراسة.

واظهر التحليل الاحصائي ما يلي:

وجود فروق ذات دلالة احصائية معنوية في المستويات الوسيطية لمعدل البروتينات الكاملة ,الكالسيوم و الاس الهايدروجيني في اللعاب في المرضي مقارنة بالاصحاء

لاتوجد وجود فروق ذات دلالة احصائية معنوية في المستويات الوسيطية لمعدل البروتين الكامل و وجود فرق ذا دلالة احصائية في معدل الكالسيوم في مصل الدم في المرضي الحوامل مقارنة بالاصحاء.

اثبتت هذه الدراسة ان الحمل يحدث تغير في اللعاب وبالتالي يؤدي الي تسوس الاسنان.

CHAPTER ONE

1. Introduction and Literature Review

1.1Introduction

Female steroid sex hormones influence oral health through different mechanisms. Gingival tissue is affected by hormonal changes during puberty and pregnancy, as shown by more pronounced subclinical signs of gingival inflammation during the ovulatory phase of the menstrual cycle and by the worsening of pre-existing gingivitis during human pregnancy. Pregnancy induces an increased response of the gingival tissues to local factors, such as plaque and tartar, through disturbance of tissue metabolism (Salvolini *et al.*, 1998).

Besides the direct effect on tissue metabolism, pregnancy and the menstrual cycle alter the composition of human saliva, as a response to the changed steroid hormone levels. Many human studies have indicated that hormones influence the composition of female saliva, particularly at the time of ovulation. Correlation has been found between the ovulatory status of women and the calcium level. A cyclic variation has been also observed in salivary electrolytes-Saliva plays a critical role in the maintenance of oral health, as it contains many innate and acquired factors with a protective role on oral tissue (Salvolini *et al.*, 1998).

During pregnancy, decreased salivary pH and flow rate have been reported, and changes in salivary electrolyte levels (Salvolini *et al.*, 1998).

According to the previous effects of pregnancy on oral health, the study was conducted to evaluation serum and saliva total protein, calcium and pH of Sudanese pregnant women in different trimesters of pregnancy. The result of the study may add to the knowledge about oral health during pregnancy and what about the precaution needed to minimize the dental disease during pregnancy.

1.2 Literature Review

1.2.1Pregnancy

Pregnancy is the fertilization and development of one or more offspring, known as an embryo or fetus, in a woman's uterus. It is the common name for gestation in humans. A multiple pregnancy involves more than one embryo or fetus in a single pregnancy, such as with twins. Childbirth usually occurs about 38 weeks after conception; in women who have a menstrual cycle length of four weeks, this is approximately 40 weeks from the start of the last normal menstrual period (LNMP).An embryo is the developing offspring during the first 8 weeks following conception, and subsequently the term fetus is used until birth. In many societies' medical or legal definitions, human pregnancy is somewhat arbitrarily divided into three trimester periods, as a means to simplify reference to the different stages of prenatal development. The first trimester carries the highest risk of miscarriage (natural death of embryo or fetus). During the second trimester, the development of the fetus can be more easily monitored and diagnosed. The third trimester is marked by further growth of the fetus and the development of fetal fat stores. The point of fetal viability, or the point in time at which fetal life outside of the uterus is possible, usually coincides with the late second or early third trimesters, and is typically associated with high degrees of morbidity and mortality (Salvolini *et al.,* 1998).

Development of embryo and fetus: The sperm and the egg cell, which has been released from one of the female's two ovaries, unite in one of the two fallopian tubes. The fertilized egg, known as a zygote, then moves towards the uterus, a journey that can take up to a week to complete. Cell division begins approximately 24 to 36 hours after the male and female cells unite. Cell division continues at a rapid rate and the cells then develop into what is known as a blastocyst. The blastocyst arrives at the uterus and attaches to the uterine wall, a process known as implantation. After about 10 weeks of gestational age, the embryo becomes known as a fetus instead. At the beginning of the fetal stage, the risk of miscarriage decreases sharply, when the fetal stage commences, a fetus is typically about 30 mm (1.2 inches) in length, and the heart can be seen beating via ultrasound; the fetus can be seen making various involuntary motions at this stage. During continued fetal development, the early body systems and structures that were established in the embryonic stage continue to develop. Sex organs begin to appear during the third month of gestation. The fetus continues to grow in both weight and length, although the majority of the physical growth occurs in the last weeks of pregnancy (Kalverboer *et al.,* 2001).

Maternal changes: During pregnancy, the woman undergoes many physiological changes, which are entirely normal, including cardiovascular, hematologic, metabolic, renal and respiratory changes that become very important in the event of complications. The body must change its physiological and homeostatic mechanisms in pregnancy to ensure the fetus is provided for. Increases in blood sugar, breathing and cardiac output are all required. Levels of progesterone and estrogens rise continually throughout pregnancy, suppressing the hypothalamic axis and subsequently the menstrual cycle. Pregnancy is typically broken into three periods, or trimesters, each of about three months. Obstetricians define each trimester as lasting for 14 weeks, resulting in a total duration of 42 weeks, although the average duration of pregnancy is actually about 40 weeks. While there are no hard and fast rules, these distinctions are useful in describing the changes that take place over time (Clark *et al*., 1986).

First trimester: Minute ventilation is increased by 40% in the first trimester (up to 13 weeks). The womb will grow to the size of a lemon by eight weeks. Many symptoms and discomforts of pregnancy (further described in later sections) appear in the first trimester (www.nhs.uk\condition of pregnancy and baby).

Second trimester: Weeks 13 to 28 of the pregnancy are called the second trimester. Most women feel more energized in this period, and begin to put on weight as the symptoms of morning sickness subside and eventually fade away. The uterus, the muscular organ that holds the developing fetus, can expand up to 20 times its normal size during pregnancy. Although the fetus begins to move and takes a recognizable human shape during the first trimester, it is not until the second trimester that movement of the fetus, often referred to as "quickening", can be felt. This typically happens in the fourth month, more specifically in the 20th to 21st week, or by the 19th week if the woman has been pregnant before. However, it is not uncommon for some women not to feel the fetus move until much later (www.nhs.uk\condition of pregnancy and baby).

Third trimester: Final weight gain takes place, which is the most weight gain throughout the pregnancy. The woman's abdomen will transform in shape as it drops due to the fetus turning in a downward position ready form birth (Stacey *et al.*, 2011).

1.2.1.1 Symptoms and discomforts of pregnancy:

Common symptoms and discomforts of pregnancy include: Tiredness, Constipation, Pelvic girdle pain, Back pain, Braxton Hicks contractions, Edema (swelling), Increased urinary frequency(A common complaint referred by the gravida, caused by increased intravascular volume, elevated GFR (glomerular filtration rate), and compression of the bladder by the expanding uterus), Urinary tract infection,Varicose veins(Common complaint caused by relaxation of the venous smooth muscle and increased intravascular pressure), Haemorrhoids (piles), Regurgitation, heartburn, and nausea, Striae gravidarum, pregnancy-related stretch marks(Vazquez 2010).

1.2.1.2 Diagnosis of pregnancy

Physical signs: Most pregnant women experience a number of symptoms, which can signify pregnancy. The symptoms can include nausea and vomiting, excessive tiredness and fatigue, cravings for certain foods that are not normally sought out, and frequent urination particularly during the night. A number of early medical signs are associated with pregnancy these signs typically appear, if at all, within the first few weeks after conception. Although not all of these signs are universally present, nor are all of them diagnostic by themselves, taken together they make a presumptive diagnosis of pregnancy. These signs include the presence of human chorionic gonadotropin (hCG) in the blood and urine, missed menstrual period, implantation bleeding that occurs at implantation of the embryo in the uterus during the third or fourth week after last menstrual period, increased basal body temperature sustained for over 2 weeks after ovulation, Breast tenderness is common during the first trimester, and is more common in women who are pregnant at a young age. Shortly after conception, the nipples and areolas begin to darken due to a temporary increase in hormones. This process continues throughout the pregnancy (Qasim *et al.*, 1996).

Pregnancy tests: pregnancy detection can be accomplished using one or more various pregnancy tests. Which detect hormones generated by the newly formed placenta, serving as biomarkers of pregnancy. Blood and urine tests can detect pregnancy 12 days after implantation. Blood pregnancy tests are more sensitive than urine tests (giving fewer false negatives). Home pregnancy tests are urine tests, and normally detect a pregnancy 12 to 15 days after fertilization. A quantitative blood test can determine approximately the date the embryo was conceived.

Testing 48 hours apart can provide useful information regarding how the pregnancy is doing. A single test of progesterone levels can also help determine how likely a fetus will survive in those with a threatened miscarriage (bleeding in early pregnancy) (Verhaegen *et al.*, 2011).

1.2.1.3 Complications of pregnancy

Pregnancy induced hypertension, Anemia, Postpartum depression, Postpartum psychosis, Thromboembolic disorders(The leading cause of death in pregnant women in the US), skin diseases that develop around the 32nd week Pruritic Urticarial Papules and Plaques of Pregnancy (PUPPP), red plaques, papules, itchiness around the belly button that spread all over the body except for the inside of hands and face, Ectopic pregnancy(implantation of the embryo outside the uterus), Hyperemesis gravidarum(excessive nausea that is more severe than morning sickness), pregnancy can enhance susceptibility to periodontal disease and dental caries(Merck 2010).

1.2.2Dental crises that occur during pregnancy

Pregnancy has far reaching systemic effects extending beyond the reproductive system, involving various complex physical and psychological changes that have an impact even on the healthy woman. These effects occur mainly due to hormones on almost every organ. Estrogen and progesterone are the main sex pregnancy hormones. Their level rises until the eighth month of pregnancy and after that it becomes stable until birth. The estrogen level rises slowly until the end of the pregnancy. The high level of hormones in blood and saliva may cause gingival reactions that may increase or cause gingival and periodontal disorders (Barak *et al.,* 2003).

The pregnant women are particularly more prone to periodontal disease due to hormonal changes associated with pregnancy. Pregnancy gingivitis and gingival enlargement have been associated with a variety of local and systemic factors, and therefore, the differential diagnosis becomes an important aspect for complete management of the lesion. Most of the causative factors lead to an unusual hyperplastic tissue response to chronic inflammation associated with local irritants such as plaque, calculus or bacteria and their products. Hormonal changes occurring during pregnancy and puberty significantly potentiate the effects of local irritants on gingival connective tissue. The levels of sex steroid hormones in saliva increases during pregnancy. Some of the most remarkable endocrine related oral alterations occur during pregnancy due to increased plasma hormone levels. Upon fertilization and implantation, the corpus luteum continues to produce estrogen and progesterone while the placenta develops. Progesterone and estrogen reach their peak plasma levels of 100ng/ml and 6ng/ml, respectively, by the end of the third trimester, and the potential biological impact of estrogen and progesterone take place in periodontal tissues during this period (Markou *et al.*, 2009).

1.2.2.1 Mechanisms of Action of Sex Hormones

1.2.2.2.1 Effects of estrogen on the periodental tissues include the following:

Decreases keratinization while increasing epithelial glycogen that results in the diminution in the effectiveness of the epithelial barrier, Increases cellular proliferation in blood vessels, Stimulates Polymorphonuclear Leukocyte (PMNL) phagocytosis, Inhibits PMNL chemotaxis, Suppress leukocyte production from the bone marrow, Inhibits pro-inflammatory cytokins released by human marrow cells, Reduces T-cell mediated inflammation, Stimulates the proliferation of the

gingival fibro-blasts, Stimulates the synthesis and maturation of gingival connective tissues, Increases the amount of gingival inflammation with no increase of plaque (Güncü *et al*.,2005).

1.2.2.2.2 Effects of progesterone on the periodental tissues:

Increases vascular dilatation, thus increases permeability, Increases the production of prostaglandins, Increases PMNL and prostaglandin E2 in the gingival crevicular fluid (GCF), Inhibits collagen and non-collagen synthesis in the periodontal ligament (PDL) fibroblasts, Inhibits proliferation of human gingival fibroblast proliferation, Alters rate and pattern of collagen production in gingiva resulting in reduced repair and maintenance potential, Increases the metabolic breakdown of folate which is necessary for tissue maintenance and repair (Güncü *et al*.,2005).

1.2.2.3 Influences on Gingival Vasculature

The effects of estrogen and progesterone on the gingival vasculature could potentially explain the increased edema, erythema, gingival crevicular exudates (increased protein), and hemorrhagic gingival tissues, noted during pregnancy as well as other stages of the reproductive cycle. An increase in gingival crevicular fluid flow has been correlated to elevated sex steroid levels, which indicates that these hormones may affect vascular permeability in the gingival sulcus (Güncü *et al*.,2005).

1.2.2.4 Microbial Changes during Pregnancy

Microorganisms such as *Aggregatibacter actinomy-cetemcomitans*, *Porphyromonas gingivalis*, and *Prevotella intermedia* are known to synthesize steroid metabolizing enzymes needed for steroid synthesis and catabolism. The steroid metabolites may also contribute to nutritional requirements of the pathogen. this pathogens lead to destroy the underlining tissue and realizing of intracellular enzyme which may lead to gingival changes during pregnancy(Soory *et al* .,1995).

1.2.2.5 Effects of pH changes on dental enamal:

Enamal hardens the outside of the teeth, protecting the dentin and pulp of the teeth from damage. Like any surface, enamal can wear down over time; saliva performs essential function on keeping tooth enamal strong. Calcium and phosphate particles in saliva help strengthen tooth enamal, when enamal weakens bacterial plaque that form on the teeth can reach the inner layer more easily causing cavities so bacteria can easily damage the teeth. Tooth roots begin to

dissolve as in the pH become less than 7.0 and when acidity level dip to pH5.5 or lower teeth will erode, become discolored and be at risk for cavities. Calcium amount variation in demineralization process occur by stomach acid that reach the mouth through vomiting during pregnancy, meaning that the calcium content of enamal increase in salivary fluid due to acidic pH that cause demineralization of calcium from dental enamal(Parus *et a.,l* 2003).

The notion that pregnancy causes tooth loss ("a tooth lost for every child") and that calcium is withdrawn in significant amounts from the maternal circulation to supply fetal requirements has no histologic, chemical or radiographic evidence to support it. Calcium is present in the teeth in a stable crystalline form and, as such, is not available to the systemic circulation to supply a calcium demand. However, calcium is readily mobilized from bone to supply these demands (Butcher *et al.*, 1989).

1.2.3 Salivary glands

Salivary glands are located in the oral cavity. The major salivary glands are the parotid, submandibular, and sublingual glands.They all secrete saliva into oral cavity, the parotid through tubes that drain saliva, called salivary ducts, near upper teeth, submandibular under tongue, and the sublingual through many ducts in the floor of mouth. Besides these glands, there are many tiny glands called minor salivary glands located in the lips, inner cheek area (buccal mucosa), and extensively in other linings of mouth and throat. Salivary glands produce saliva used to moisten the mouth, initiate digestion, and help protect the teeth from decay (Michael *et al*., 2012)**.**

1.2.3.1Salivary fluid function

Saliva contributes to the digestion of food and to the maintenance of oral hygiene. Without normal salivary function the frequency of dental caries, gum disease (gingivitis), and other oral problems increases significantly (Haralampos *et al.,* 2011).

Lubrication: Saliva coats the oral mucosa, mechanically protecting it from trauma during eating, swallowing and speaking. In persons with little saliva (xerostomia), soreness of the mouth is very common, and the food (especially dry food) sticks to the inside of the mouth (Walter and Boron ., 2003).

Digestion: The digestive functions of saliva include moistening food and helping to create a food bolus. This lubricative function of saliva allows the food bolus to be passed easily from the mouth into the esophagus. Saliva contains the enzyme amylase, also called ptyalin, which is capable of breaking down starch into simpler sugars that can be later absorbed or further broken down in the small intestine. Salivary glands also secrete salivary lipase (a more potent form of lipase) to begin fat digestion. Salivary lipase plays a large role in fat digestion in newborn infants as their pancreatic lipase still needs some time to develop (Walter and Boron ., 2003).

Antimicrobial function: Saliva has both a mechanical cleaning action and a specific (immunoglobulins, e.g. IgA) and non-specific immunologic action (e.g.lysozyme, lactoferrin and myeloperoxidase). These factors control the micro-organisms that survive in the mouth. It also has a protective function, helping to prevent dental plaque build-up on the teeth and washing away adhered food particles. Saliva is also key in preventing ascending infections of the salivary glands (e.g. parotitis) (Walter and Boron ., 2003).

Ion reservoir/Buffer function: Saliva is supersaturated with various ions. Certain salivary proteins prevent precipitation, which would form salts. These ions act as a buffer, keeping the acidity of the mouth within a certain range, typically pH 6.2 - 7.4. This prevents minerals in the dental hard tissues from dissolving (Walter and Boron ., 2003).

Hormonal function: Saliva secretes hormone gustin, which is thought to play a role in the development of taste buds (Walter and Boron ., 2003).

Role in taste: Saliva is very important in the sense of taste. It is the liquid medium in which chemicals are carried to taste receptor cells (mostly associated with lingual papillae). Persons with little saliva often complain of dysgeusia (i.e. disordered taste, e.g. reduced ability to taste, or having a bad, metallic taste at all times) (Walter and Boron ., 2003).

Wound licking: A common belief is that saliva contained in the mouth has natural disinfectants, which leads people to believe it is beneficial to "lick their wounds". Researchers at the University of Florida at Gainesville have discovered a protein called nerve growth factor (NGF) in the saliva of mice. Wounds with NGF healed twice as fast as untreated and unlicked wounds; therefore, saliva can help to heal wound in some species. NGF has not been found in human saliva; however, researchers find human saliva contains such antibacterial agents as secretory IgA, lactoferrin, lysozyme and peroxidase. It has not been shown that human licking of wounds disinfects them, but licking is likely to help clean the wound by removing larger contaminants such as dirt and may help to directly remove infective bodies by brushing them away. Therefore, licking would be a way of wiping off pathogens, useful if clean water is not available to the animal or person (Walter and Boron ., 2003).

1.2.3.2 Saliva constituents

Human saliva contain 99.5% water, but it contains many important substances, including electrolytes, mucus, antibacterial compounds and various enzymes(Walter and Boron ., 2003). It is a fluid containing:

- Water
- Electrolytes:
    - 2–21 mmol/L sodium (lower than blood plasma).
    - 10–36 mmol/L potassium (higher than plasma).
    - 1.2–2.8 mmol/L calcium (similar to plasma).
    - 0.08–0.5 mmol/L magnesium.

- 5–40 mmol/L chloride (lower than plasma).
- 25 mmol/L bicarbonate (higher than plasma).
- 1.4–39 mmol/L phosphate.
- Iodine (mmol/L usually higher than plasma, but dependent variable according to dietary iodine intake).

- Proteins (Walter and Boron ., 2003).

1.2.3.3 Salivary proteins

Saliva contains a spectrum of immunologic and non-immunologic proteins with antibacterial properties. In addition, some proteins are necessary for inhibiting the spontaneous precipitation of calcium and phosphate ions inthe salivary glands and in their secretions. This protein includes: Secretary immunoglobulin A (IgA): which is the largest immunologic component of saliva It can neutralize viruses, bacteria, and enzyme toxins. It serves as an antibody for bacterial antigens and is able to aggregate bacteria, inhibiting their adherence to oral tissues. Other Immunologic components, such as IgG and IgM, occur in less quantity and probably originate from gingival fluid (Michael *et al.,* 2012)

Among the non-immunologic salivary protein components, there are enzymes (alpha amylase, lysozyme, lactoferrin, and peroxidase), mucin glycoproteins, agglutinins, histatins, proline-rich proteins, statherins, and cystatins (Michael *et al.,* 2012).

Alpha amylase: is an enzyme catalyzing the hydrolysis of starch into sugars. Amylase present in saliva of human to begin the chemical process of digestion. Alpha amylase is calcium metalloenzyume completely unable to function in the absence of calcium.

Lysozyme: can hydrolyze the cellular wall of some bacteria, and because it is strongly cationic, it can activate the bacterial “autolisines” which are able to destroy bacterial cell wall components.

Gram-negative bacteria are more resistant to this enzyme due to the protective function of their external lipopolysaccharide layer. Other antibacterial mechanisms have been proposed for this enzyme, such as aggregation and inhibition of bacterial adherence (Michael *et al.,* 2012)

Lactoferrin: links to free iron in the saliva causing bactericidal or bacteriostatic effects on various Micro organisms requiring iron for their survival such as the *Streptococcus mutant* group. Lactoferrin also provides fungicidal, antiviral, anti-inflammatory, and immune modulatory functions (Michael *et al.,* 2012)

Peroxidase or sialoperoxidase: offers antimicrobial activity because it serves as a catalyst for the oxidation of the salivary thiocyanate ion by hydrogen peroxide into hypothiocyanate, a potent antibacterial substance. As a result of its consumption, proteins and cells are protected from the toxic and oxidant effects of hydrogen (Michael *et al.*, 2012)

The proline-rich proteins and statherins: inhibit the spontaneous precipitation of calcium phosphate salts and the growth of hydroxyl apatite crystals on the tooth surface, preventing the formation of salivary and dental calculus. They favor oral structure lubrication, and it is probable both are important in the formation of acquired film. Another function proposed for the proline-rich Proteins are the capacity to selectively mediate bacterial adhesion to tooth surfaces (Michael *et al.,* 2012)

The cystatins: are also related to acquired film formation and to hydroxyl apatite crystal equilibrium. Due to its proteinase inhibiting properties, they act in controlling proteolytic activity (Michael *et al.,* 2012)

The histatins: a family of histidine-rich peptides, have antimicrobial activity against some strains of *Streptococcus* mutans and inhibit hemoagglutination of the periopathogen *Porphyromonas gingivallis*. They neutralize the lipopolysaccharides of the external membranes of Gram-negative bacteria and are potent inhibitors of *Candida albicans* growth and development. The bactericidal and fungicidal effects occur through the union of positively loaded histatins with the biological membranes resulting in the destruction of their architecture and altering their permeability. Other functions attributed to these peptides are: participation in acquired film formation and inhibition of histamine release by the mastocytes, suggesting a role in oral inflammation (Michael *et al.,* 2012)

Salivary agglutinin: a highly glycosylated protein frequently associated with other salivary proteins and with secretory IgA, is one of the main salivary components responsible for bacteria agglutination (Michael *et al.,* 2012)

1.2.3.3.1 Salivary protein secretion:

Salivary proteins exhibit vectorial transport from the rough endoplasmic reticulum, where they are synthesized, through a succession of membrane-bounded compartments including the Golgi complex, condenses vacuoles, and secretary granules. The secretary granules migrate to particular locations within the cell close to the apical membrane prior to the release of their contents into the acinar lumen. Exocytosis is the process by which cells release the contents of

their secretory granules. This involves the fusion of the granule membrane with the luminal plasma membrane of the secretory cell followed by the rupture of the fused membranes. This process is continuous in most cells (`constitutive' exocytosis), but it can be greatly accelerated following an appropriate cellular signal such as neural stimulation (`regulatory' exocytosis). In the three major salivary glands, parotid, submandibular and sublingual, exocytotic protein secretion is primarily controlled by the autonomic nervous system; sympathetic stimulation elicits protein release from parotid and submandibular gland acini, and parasympathetic stimulation elicits protein release from sublingual gland acini as well as some release from parotid acini (Quissell et *al.*, 1989).

Salivary protein that present in saliva during this manifestation comes from many sources which include: antimicrobial protein that acts against the pathogens, hydrolyzed enzymes, enzymes result from damaged tissue caused by pathogenic organism,, Cells release these enzymes into the extracellular space during periods of tissue necrosis or trauma which are also detectable in the peripheral circulation. If the periodontal tissue is damaged, due to edema or destruction of a cellular membrane, these intracellular enzymes are increasingly released into the gingival fluid and saliva, exudates fluid and inflammatory mediators that influence in inflammatory process. C-reactive protein (CRP) is an acute-phase reactant synthesized by the liver in response to the inflammatory cytokines interleukin (IL)-6, IL-1, and tumor necrosis factor-alpha. Circulating CRP levels are a marker of systemic inflammation and are associated with periodontal disease. A chronic bacterial infection associated with elevation of pro inflammatory cytokines and prostaglandin. Elevated immunoglobulin G induced by bacterial species associated with destructive periodontal diseases is associated with increase in CRP which has been associated with adverse pregnancy outcomes (Offenbacher *et a.,l* 2006).

1.2.3.4 Salivary calcium

Salivary calcium is related to plasma levels and is about3 mmol/L under resting condition. Like plasma, the major fraction of salivary calcium is diffusible and ionic while the rest is in a bound form, either with protein or as colloidal calcium phosphate. Salivary calcium and phosphorus have been implicated in the deposition of tartar over teeth and formation of salivary calculus. High level of salivary calcium is apparently responsible for the resistance to dental decay. The human body contains more calcium than any of the other essential minerals, as much as 1200 g in a 70 Kg adult. Most skeletal calcium is deposited as a form of hydroxyapatite. In alkaline pH,

calcium plays an important role in remineralization of enamel surface via formation of hydroxyapatite crystals, while in acidic pH salivary calcium plays a role in preventing dissolution of enamel (Kodaka *et al.,* 1988).

Dental calculus represents mineralized bacterial plaque. Recent and old calculus consists of four different crystals of calcium phosphate:

1. $CaH(PO_4)\times 2H2O$= Brushite (B).
2. $Ca4H(PO_4)3\times 2H_2O$= Octa calcium phosphate (OCP).
3. $Ca5(PO_4)3\times OH$= Hydroxyapatite (HA).
4. B–$Ca3(PO_4)2$= Whitlockite (W).

The mineral content is 37%, but ranges from 16 to 51% with some layers yielding a maximal density of minerals of up to 80%.In the presence of relatively low plaque pH and concomitant high Ca/P ratio. In saliva Brushite B is formed which may later on develop into Hydroxyapatite HA and Whitlockite W. When supra gingival plaque mineralizes, Octa calcium phosphate OCP forms and is gradually changed into HA. In the presence of alkaline pH and anaerobic condition and concomitant presence of magnesium or zinc and CO_3, a large amount of Whitlockite W are formed, which are stable form of mineralization (Kodaka *et al.,* 1988).

1.2.4 Serum

In blood, the serum is the component that is neither a blood cell (serum does not contain white or red blood cells) nor a clotting factor; it is the blood plasma after the fibrinogens is removed. Serum includes all proteins not used in blood clotting (coagulation) and all the electrolytes, antibodies, antigens, hormones, and any exogenous substances (e.g., drugs and microorganisms). A study of serum is serology, and may also include proteomics. Serum is used in numerous diagnostic tests, as well as in blood typing. Blood is centrifuged to remove cellular components. Anti-coagulated blood yields plasma containing fibrinogen and clotting factors. Coagulated blood (clotted blood) yields serum without fibrinogen, although some clotting factors remain (Martin and Elizabeth2007).

1.2.4.1 Serum protein

Importance and Function:

All biochemical reactions are catalyzed by enzymes, which contain protein.The structure of cells and the extracellular matrix that surrounds all cells is largely made of the protein group collagens. Collagens are the most abundant protein in the human body.

The transport of materials in body fluids depends on proteins such as transferrin, receptors for hormones are transmembrane proteins, and transcription factors, needed to initiate the transcription of a gene, are proteins.Proteins make up antibodies, which are a major component of the immune system (Michael *et al.,* 2010).

1.2.4.1.1Types of serum proteins

Enzymes: proteins that catalyze chemical reactions. Enzymes are normally present inside cells but are released into the blood in tissue damage, making enzyme measurement a very important diagnostic tool. Examples of groups of enzymes tested in the clinical laboratory are the transaminases, dehydrogenases , and phosphatases (Michael *et al.,* 2010).

Hormones: proteins that are chemical messengers that control the actions of specific cells or organs. Hormones affect growth and development, metabolism, sexual function, reproduction, and behavior. Examples of hormones testing in the clinical laboratory in blood, urine, or saliva are insulin, testosterone, growth hormone, follicle-stimulating hormone, and cortisol (Michael *et al.,* 2010).

Transport proteins: proteins that transport movement of ions, small molecules, or macromolecules, such as hormones, vitamins, minerals, and lipids, across a biologic membrane. Examples of commonly measured transport proteins are hemoglobin, albumin, and transferring (Michael *et al.,* 2010).

Immunoglobulin’s (antibodies): proteins produced by B-cells (lymphocytes) in the bone marrow that mediate the humoral immune response to identify and neutralize foreign objects. Examples of immunoglobulins are IgG, IgM, and IgA (Michael *et al.,* 2010).

Structural proteins: fibrous proteins are the structures of cells and tissues such as muscle, tendons, and bone matrix. Collagen, elastin, and keratin are examplesof structural proteins (Michael *et al.,* 2010).

Storage proteins: proteins that serve as reserves of metal ions and amino acids that can be released and used later without harm occurring to cells during the time of storage. The most widely studied and tested storage protein is ferritin, which stores iron to be later used in the manufacture of hemoglobin (Michael *et al.,* 2010).

Energy source: plasma proteins serve as a reserve source of energy for tissues and muscle (Michael *et al.,* 2010).

1.2.4.1.2 Total proteins abnormalities

The total protein test is a rough measurement of all of the proteins in the plasma. Total protein measurements can reflect nutritional status, kidney diseases, liver diseases, and many other conditions. If total protein is abnormal, further tests must be performed to identify which protein fraction is abnormal, so that a specific diagnosis can be made (Michael *et al.,* 2010).

1.2.4.1.2.1 Hypoproteinemia

One cause of a low level of plasma proteins is excessive loss either due to excretion in the urin in renal disease or leakage into gastrointestinal tract in inflammation of digestive system. Another circumstance producing hypoproteinemia is decreased intake either because of malnutrition or through intestinal malabsorption. A decrease in serum proteins as a result of decreased synthesis is also seen in liver diseases (site of all non immune protein synthesis) or in inherited immunodeficiency disorders, in which antibody production is diminished. Additionally, hypoproteinemia may result from accelerated catabolism of proteins, such as occurs in burns, trauma, or other injuries (Michael *et al.,* 2010).

1.2.4.1.2.2 Hyperproteinemia

Hyperproteinemia, an increase in total plasma proteins, is not an actual disease state but is the result of the underlying cause, dehydration. When excess water is lost from the vascular system, the proteins, because of their size, remain within the blood vessels. Although the absolute quantity of proteins remains unchanged, the concentration is elevated due to a decreased volume of solvent water. Dehydration results from a variety of conditions, including vomiting, diarrhea, excessive sweating and diabetic acidosis. In addition to dehydration, hyperproteinemia may be a result of excessive production, primarily of the globulins. Some disorders are characterized by the appearance of a monoclonal protein or paraprotein in the serum and often in the urine as well. This protein is an intact immunoglobulin molecule, or occasionally, or light chains only. The

most common disorder is multiple myeloma, in which the neoplastic plasma cells proliferate in the bone marrow. The paraprotein in this case is usually IgG, IgA, or light chains. IgD and IgE paraproteins rarely occur. IgM paraprotein is found in patients with Waldenström's macroglobulinemia, a rare type of slow-growing, non- Hodgkin lymphoma. Many disorders, including chronic inflammatory states, collagen vascular disorders, and other neoplasms, may be associated with paraproteins (Michael *et al.,* 2010).

1.2.4.1.3 Total protein abnormalities during pregnancy:

No significant change in serum total proteins concentration, but serum albumin level was significantly lower and serum globulin concentration was significantly higher in all three trimester compared to non pregnant women.The reduction of albumin concentration, combined with a normal slight increase in serum globulin, results in decrease in albumin to globulin ratio similar to that seen in certain hepatic disease. It is necessary to bear in mind this phenomenon of hemodilution in the interpretation of all serum concentration values during pregnancy (Bhatty *et al*., 2001).

1.2.4.2 Serum Calcium

1.2.4.2.1 Distribution of calcium

About 99% of calcium in the body is part of bone. The remaining 1% is mostly in the blood and other extra cellular fluid. Little is in the cytosol of most cells. In fact, the concentration of ionized calcium in blood is 5,000 to 10,000 times higher than in the cytosol of cardiac or smooth muscle cells. Maintenance of this large gradient is vital to maintain the essential rapid inward flux of calcium. Calcium in blood is distributed among several forms. About 45% circulates as free calcium ions (referred to as ionized calcium) 40% is bound to protein, mostly albumin, and 15% is bound to anions, such as HCO_3^-, citrate, PO_4^- and lactate (Michael *et al.,* 2010).

1.2.4.2.2 Calcium level abnormalities

1.2.4.2.2.1Hypocalcemia

When Para thyroid hormone (PTH) is not present, as with primary hypoparathyroidism, serum calcium levels are not properly regulated. Bone tends to "hang on" to its storage pool and the kidney increases excretion of calcium. Since PTH is also required for normal vitamin D metabolism, the lack of vitamin D's effects also leads to a decreased level of calcium .

Parathyroid gland aplasia, destruction, or removal is obvious reasons for primary hypoparathyroidism. Because hypomagnesemia has become more frequent in hospitalized patients, chronic hypomagnesemia has also become recognized as a frequent cause of hypocalcemia. Hypomagnesemia may cause hypocalcemia by three mechanisms: (1) it inhibits the glandular secretion of PTH across the parathyroid gland membrane, (2) it impairs PTH action at its receptor site on bone, and (3) it causes vitamin D resistance. Elevated Mg^{++} levels may inhibit PTH release and target tissue response, perhaps leading to hypocalcemia and hypercalciuria (Michael *et al.,* 2010).

When total calcium is the only result reported, hypocalcemia can appear with hypoalbuminemia. Common causes are associated with chronic liver disease, nephrotic syndrome, and malnutrition. In general, for each 1 g/dL decrease in serum albumin, there is a 0.2 mmol/L (0.8 mg/dL) decrease in total calcium levels. About one half of the patients with acute pancreatitis develop hypocalcemia. The most consistent cause appears to be a result of increased intestinal binding of as calcium increased intestinal lipase activity occurs.

Vitamin D deficiency and malabsorption can cause decreased absorption, which leads to increased PTH production or secondary hyper parathyroidism Patients with renal disease caused by glomerular failure often have altered concentrations of calcium ,PO_4^- , albumin, Mg^{++}, and H^- (PH). In chronic renal disease, secondary hyperparathyroidism frequently develops as the body tries to compensate for hypocalcemia caused either by hyperphosphatemia (PO^-_4 binds and lowers ionized Ca^{++}) or altered vitamin D metabolism. Monitoring and controlling ionized calcium concentrations may avoid problems due to hypocalcemia, such as osteodystrophy, unstable cardiac output or blood pressure, or problems arising from hypercalcemia, such as renal stones and other calcifications (Michael *et al.,* 2010).

Symptoms of hypocalcemia

Neuromuscular irritability and cardiac irregularities are the primary groups of symptoms that occur with hypocalcemia. Neuromusculars symptoms include parasethesia, muscle cramps, tetany, and seizures. Cardiac symptoms may include arrhythmia or heart block. Symptoms usually occur with severe hypocalcemia, in which total calcium levels are below 1.88 mmol/L (7.5 mg/dL) (Michael *et al.,* 2010).

1.2.4.2.2.2 Hypercalcemia

Primary hyperparathyroidism is the main cause of hypercalcemia. Hyperparathyroidism, or excess secretion of para thyroid hormon, may show obvious clinical signs or may be a symptomatic.The patient population seen most frequently with primary hyperparathyroidism is older women. Although beither total or ionized calcium measurements are elevated in serious cases, ionized calcium is more frequently elevated in bsubtle or asymptomatic hyperparathyroidism. In general, ionized calcium measurements are elevated in 90% to 95% of cases of hyperparathyroidism, whereas total calcium is elevated in 80% to 85% of cases. The second leading cause of hypercalcemia is associated with various types of malignancy, with hypercalcemia sometimes being the sole biochemical marker for disease. Many tumors produce PTH-related peptide (PTH-rP), which binds to normal PTH receptors and causes increased calcium levels (Michael *et al.,* 2010).

Symptoms of hypercalcemia: A mild hypercalcemia (2.62–3.00 mmol/L [10.5–12 mg/dL]) is often asymptomatic. Moderate or severe Ca^{++} elevations include neurologic, GI, and renal symptoms. Neurologic symptoms may include mild drowsiness or weakness, depression, lethargy, and coma. GI symptoms may include constipation, nausea, vomiting, anorexia, and peptic ulcer disease. Hypercalcemia may cause renal symptoms of nephrolithiasis and nephrocalcinosis. Hypercalciuria can result in nephrogenic diabetes insipidus, which causes polyuria that result in hypovolemia, which further aggravates the hypercalcemia. Hypercalcemia can also cause symptoms of digitalis toxicity (Michael *et al.,* 2010).

1.2.4.2.3 Calcium abnormalities during pregnancy:

Calcium metabolism is dramatically altered by pregnancy and lactation. The normal fetal skeleton accumulates approximately 30g of calcium by term, proportional to the fetal weight. **The largest proportion (80%) of that accretion occurs in the third trimester, at a rate of about 250-300 mg/day.** Total serum calcium levels fall early in pregnancy, due to hemodilution and the consequent decline in serum albumin. Ionized calcium levels and phosphate levels remain normal throughout pregnancy .Urinary calcium excretion increases early in gestation secondary to an increased calcium load filtered by the kidneys and the increased glomerular filtration rate of pregnancy (Kovacs *et al.*,1997)

1.3 Rationale

Pregnancy is often associated with physiological abnormalities lead to various diseases. Dental disease may result due to hormonal changes and other factors during pregnancy.

In Sudan, few studies about the effects of pregnancy on serum and saliva total protein, calcium and pH was conducted, so the study was carried out to measure these parameters in serum and saliva during pregnancy.

1.4Objectives:

1.4.1 General Objectives

To evaluate the level of salivary and serum total protein, calcium and pH changes in sundaes pregnant women with dental crises.

1.4.2 Specific Objectives:

1. To measure the levels of serum and salivary total protein in pregnant women.
2. To measure the level of serum and salivary calcium and salivary pH in pregnant women.
3. To correlate between the pregnancy and occurrence of dental crises.
4. To correlate between level of total protein and calcium in saliva and serum of Sudanese pregnant women.

CHAPTER TWO

2. Materials and Methods:

2.1 Materials:

2.1.1 Study design:

This is a case control study.

2.1.2. Study area:

The study was carried at Alsudi Hospital for women and labor.

2.1.3. Study population:

The Study included 60 pregnant women from different trimester of pregnancy and 20 apparently healthy non pregnant women.

Inclusion Criteria:

Pregnant women with dental diseases were included who they age range from 17 -40 years old.

Exclusion criteria:

Pregnant women without dental diseases were excluded.

2.1.4 Samples:

The Five ml of venous blood and two ml of salivary samples were collected from each subject at morning. The samples collected under aseptic conditions and placed in sterile plain containers, after blood clotting centrifuged for 3 minutes at 3000 rpm to obtain serum and clear saliva, then they obtained serum and saliva were kept at ⁻20c till the time of analysis.

2.1.5 Ethical consideration:

Subjects who voluntarily accepted to participate in the study were included.

2.1.6 Equipments:

Colorimeter(model JENWAY),Centrifuge, Sterile plain containers, Disposable syringes, 70% alcohol, Tourniquets, Cotton, Micropipettes (automatic pipettes) and Graduated pipettes, litmus paper for pH.

2.1.7. Reagents:

1) Reagents of total protein, BIURET liquid, Colorimetric method was used for estimation of total protein concentrations. They are supplied by Biosystems Company, and reagent,

composed of: copper^{++} acetate 6mmol\l, potassium iodide12mmol/l, sodium hydroxide1.15mol\l ,detergent.

-Protein standard: Bovine albumin concentration 62.4g/l (GornallAG, et al 1949).

2) Reagents of calcium METHYLTHYMOL BLUE, colorimetric method was used for estimation of calcium concentrations. They are supplied by Biosystem company, and working reagent, which composed of: potassium cyanid 7.7mmo/l,etanolamine 1.5mol/l,methylethymol blue0.1mmol\l, hydrochloric acid 10mmol/l, hydroxyquinoline 17mmol/l .

Calcium standard concentration 10mg/dl or 2.5mmol/l (GinderM and KingJD1972).

2.1.8 Quality control:

It is recommended to use the biochemistry control serum level 1 (cod.18005, 18009 and 18042) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality control scheme and procedure for corrective actions if controls do not recover within the acceptable tolerance.

2.1.9Data analysis:

Data were analyzed using the SPSS computer program by using independent T test and one way ANOVA test to obtain mean value± SD and P value at (0.05) is considered as significant

2.2 Methods:

(1). Estimation of total protein concentration using the Biuret method:

Principle of the method:

Total Protein in sample reacts with copper++ ion in alkaline medium forming a coloured complex that can measured by spectrophotometry.

Procedure:

Tube contents		Blank	Standard	Sample
Sample	ml	_	_	0.02
Standard	ml	_	0.02	_
Distilled water	ml	0.02	_	_
Reagent	ml	1.0	1.0	1.0

The mixture was mixed and the tubes were incubated for 10 minutes at 27c. The absorbance of sample (As) and the standard (A std) were measured at 545 nm against the reagent blank. The color is stable for at least 2 hours (GornallAG, et al 1494).

Calculation of total protein concentration:

Total protein concentration in the sample is calculated using the following general formula:

= (As/A std) × Concentration of standard (GornallAG, et al 1494).

(2). Estimation of calcium concentration using the Methylthymol blue colorimetric method:

Principle of method:

Calcium in the sample reacts with methylthymol blue in alkaline medium forming colored complex that can be measured by spectrophotometry. Hydroxyquinoline is included in the reagent to avoid magnesium interference (GinderM and KingJD1972).

Procedure:

Tube contents	Blank	Standard	Sample
Test sample ml	-	-	0.01
Standard ml	-	0.01	-
Distilled water ml		-	-
Reagent ml	1.0	1.0	1.0

The mixure was mixed thoroughly and the tubes let stand for 2 minutes at room 27c. The absorbance of the sample (As) and standard (A std) were readed at 610nm against the reagent blank. The color is stable for at least 1 hour (GinderM and KingJD1972).

Calculation of calcium concentration:

Calcium concentration in the sample is calculated using the following general formula:

(As/A std) × concentration of standard× sample dilution factor(GinderM and King 1972).

(3): Estimation of Salivary pH directly from mouth:

Estimation done directly by using litmus paper place in mouth and the measure the pH value (GinderM and King 1972).

CHAPTER THREE

RESULTS

3. Results:

Sixty pregnant women with dental disorder in which there age rang from (17_40 years old) and 20 apparently healthy non pregnant women were included in this study. I was collected the sample from Alsudi Hospital for women and labor. The samples (serum and saliva) were collected from patients during period from Fepraoury 2014 to April 2014.

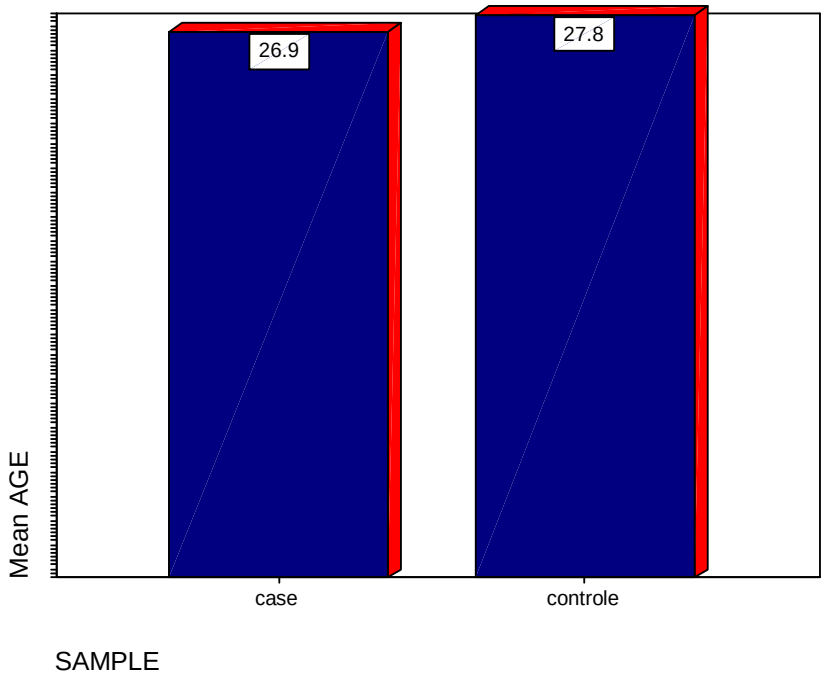

Figure (3.1) The mean of age for study subjects.

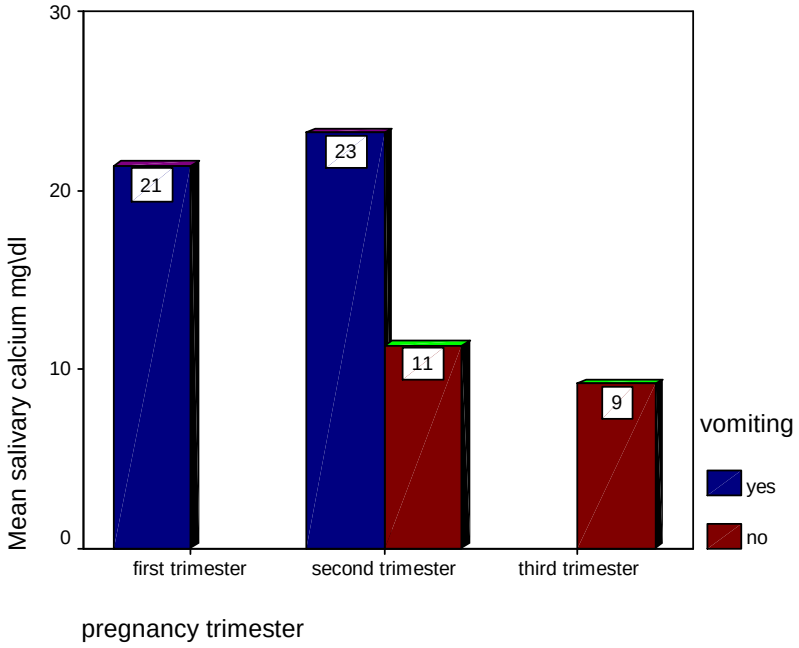

Figure (3.2) show the mean of salivary calcium level in pregnant women with different trimesters in the presence of vomiting

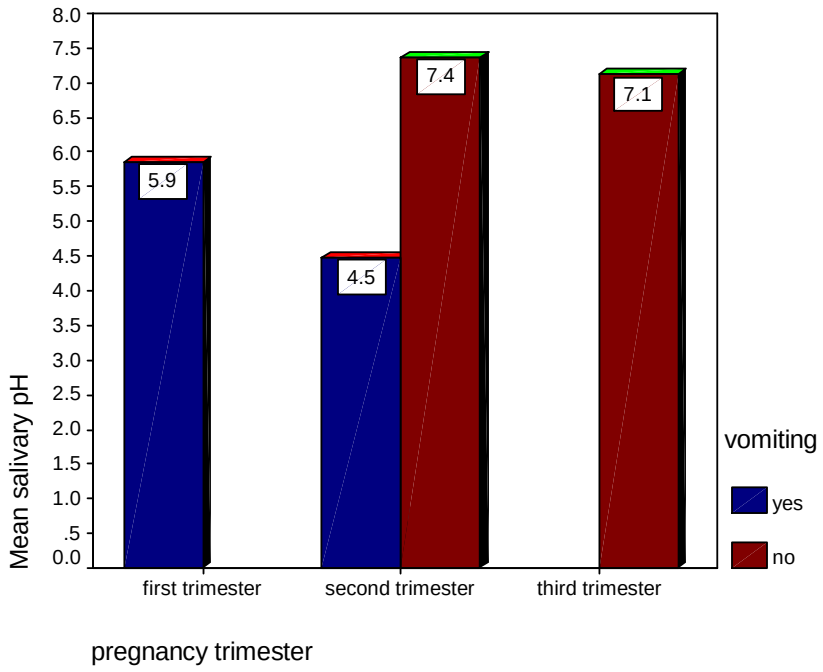

Figure (3.3) show the mean of salivary pH in pregnant women with different trimesters in the presence of vomiting.

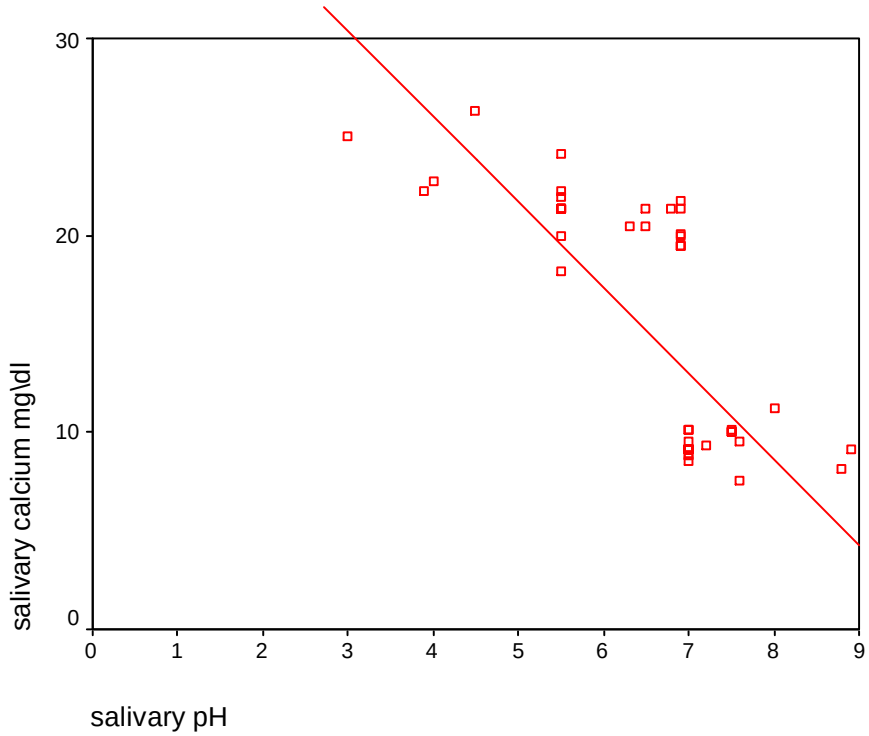

Figure (3.4) a scatter plot show significant strong negative correlation between the salivary pH and salivary calcium(r=0.000, P= -0.755).

Table (3.1): Serum total protein and calcium of test and control group.

Variable	Pregnant women N=60	Control N=20	P. value
Serum total protein (g\l)	66.60±6.2	66.35±4.4	0.843
Serum calcium (mg\dl)	7.79±1.9	8.92±0.6	0.000

The table shows the mean ±SD, and the probability (P). Independent T test was used for comparison. No significant difference of total protein level, and significant difference of calcium level of test compared to control group

Table (3.2): Salivary total protein and calcium of test and control group.

Variable	Pregnant women N=60	Controls N=20	P. value
Salivary total protein (g\l)	32.25±13.1	10.43±0.83	0.000
Salivary calcium (mg\dl)	14.75±6.2	9.32±0.5	0.000

The table shows the mean ±SD, and the probability (P).Independent T-test was used for comparison. There is significant different of salivary total protein and calcium of test compared to control group.

Table (3.3): Comparison between serum calcium and salivary total protein, calcium and pH of test group in different three trimester of pregnancy.

Variables	Pregnancy trimesters	Compare with other trimesters	Mean differences	P value
Serum calcium level mg\dl.	First trimester	Second trimester	0.025	0.943
		Third trimester	3.445*	0.000
	Second trimester	Third trimester	3.445*	0.000
Salivary calcium level mg\dl.	First trimester	Second trimester	7.625*	0.000
		Third trimester	12.150*	.000
	Second trimester	Third trimester	4.525*	.000
Salivary protein level g\l.	First trimester	Second trimester	-1.460	.000
		Third		

		trimester	-27.965*	.000
	Second trimester	Third trimester	-26.505*	.000
Salivary PH	First trimester	Second trimester	0.930*	0.003
		Third trimester	-1.260*	0.000
	Second trimester	Third trimester	-3.30	0.278

(*)The mean difference is significant at the .05 level.

Table (3.5) shows a significant difference between third trimester of pregnancy on serum and salivary total protein and calcium. One way ANOVA test is used for comparisons.

CHAPTER FOUR

4. Discussion, conclusion, and recommendations:

4.1 Discussions:

Pregnancy is thought to be predisposed to the impairment of dental health. As saliva contributes to oral homeostasis, this study aimed to compare the changes of total protein and calcium and concentration in whole saliva and serum between pregnant and non-pregnant Sudanese women. Samples were composed of 60 pregnant and 20 non-pregnant women attending Alsudi hospital for women and labor in Khartoum. Unstimulated whole saliva was collected to determine salivary protein and calcium concentration. Serum protein and calcium level was measured to determine if there is effect on serum contents on saliva contents level and effects on oral health.

In the current study the mean value of age for test group is 26.9years old and for control group is 27.8 years old.

The mean value of salivary calcium in pregnant women with presence of vomiting at first trimester and second trimester found to be increased when compared with pregnant women without presence of vomiting at second trimester and third trimester. The mean value of pH of salivary fluid in pregnant women with presence of vomiting at first trimester is decreased and at second trimester also when compared with pregnant women without presence of vomiting at second and third trimester of pregnancy and this result agree with Larsen M 1999: (gastrointestinal reflux during pregnancy lead to enamel loss, as acid reflux up into mouth which decrease pH in mouth lead to demineralize of calcium from enamel allowing greater bacteria invasion deeper into the mouth and progress tooth destruction) which done in British.

Data collect for research show significant strong negative correlation between salivary pH and salivary calcium (low pH increase calcium in saliva) and this result agree with Larsen M 1999: (gastrointestinal reflux during pregnancy lead to enamel loss, as acid reflux up into mouth which

decrease pH in mouth lead to demineralize of calcium from enamel allowing greater bacteria invasion deeper into the mouth and progress tooth destruction) which done in British.

The mean value of serum total protein level is insignificantly different in cases compared to control subject (P value is 0.843) and this result agree with Bhatty 2001: (no significant change in serum total protein concentration).

The mean value of serum calcium is significantly decreased in cases compared to control (P value is 0.000) and this result disagree with Kovacs 1997: (serum calcium level remain normal throughout pregnancy).

Also the mean value of salivary total protein is significantly increased in cases compared to control (P value is 0.000) and this result agree with Kloetzel 2011: (showed increased total protein in saliva from both paratiod gland and whole saliva which affect microbe adherence result in dental disease reflecting to sex hormone increase during pregnancy), and mean value of salivary calcium is also significantly increased in test compared to control (P value is 0.000) and this result agree with Parus M 2003 : (calcium content of enamel increase in saliva during acid attack).

In comparison between three trimesters of pregnancy found that there is insignificant different in serum calcium level between first and second trimester (P value is 0.943) and there is significant decrease between first and third trimester, and between second and third trimester (P value is 0.000) and this result disagree with Bhatty 2001: (no significant change in serum total protein concentration).

Also there is significant increase in salivary calcium level during first and second trimester due to change in pH caused by vomiting (P value is 0.000) compared to third trimester and these results agree with Larsen M 1999: (gastrointestinal reflux during pregnancy lead to enamel loss, as acid reflux up into mouth which decrease pH in mouth lead to demineralize of calcium from enamel allowing greater bacteria invasion deeper into the mouth and progress tooth destruction) which done in British, also there is significant increase in salivary total protein level in three trimester of pregnancy due to increase progesterone level (P value is 0.000) and this result agree with Kloetzel 2011: (showed increased total protein in saliva from both paratiod gland and whole saliva which affect microbe adherence result in dental disease reflecting to sex hormone increase during pregnancy)

Finally there is significant decrease in salivary pH in first and second trimester (P value 0.000) compared to third trimester, and insignificant difference between second and third trimester (P value is 0.278) and this result agree with Larsen M 1999: (gastrointestinal reflux during pregnancy lead to enamel loss, as acid reflux up into mouth which decrease pH in mouth lead to demineralize of calcium from enamel allowing greater bacteria invasion deeper into the mouth and progress tooth destruction).

Decrease in serum calcium does not affect level of salivary calcium due to the facts that the fetus calcium from mother bone only and the calcium in the teeth is not affected and dental disease occurs due to vomiting and reflection of acid in mouth causing lowering of pH in the oral cavity which lead to dissolve the calcium present in teeth enamel the teeth soft and subjected to bacterial infection. In addition dissolve calcium increases level of salivary calcium. According the result of the present study, no relationship between serum and salivary total protein. The increase of salivary total proteins may be due to inflammatory process caused by action of progesterone hormone.

4.2 Conclusion

- Salivary calcium is significantly increased in cases compared to control group.
- Salivary pH is significantly decreased in first and second trimester compared to third trimester.
- Serum calcium significantly decreased in test compared to control group despite taking of calcium supplements.
- Salivary total protein is significantly increased in cases compared to control group.
- Serum total protein insignificantly changed in test when compared to control group.
- No relationship between salivary and serum calcium and total protein

4.3. Recommendation:

From the result of this study it is recommended that:

- Measure the amount of separated proteins such as amylase, lactoferrin, lysosyme and peroxidase which are reflects body biochemical's status and changes during pregnancy.
- Measurement the level of sex hormone during throughout pregnancy due to it is effect on dental health.
- Regular taking of calcium supplements during pregnancy to maintain serum calcium level in reference value to avoid hypocalcaemia and it is complication.
- Regular check of the oral health for pregnant women to minimize the risk of dental crises.

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بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Medical laboratory sciences

Assessment of Serum and Salivary Total Protein, Calcium and Salivary pH of Sudanese Pregnant Women with Dental Crises

Questioners

Name: ……..

Age: …….

Number of trimester: ……

Presences of vomiting: ……..

Lab investigation:

Test	Result
Serum total protein	……..mg/dl

Serum calcium ……..mg/dl

Salivary total protein ……..mg/dl

Salivary calcium ……..mg/dl