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# **The Influence of Some Biochemical Parameters on Dental Caries in Pregnant Iraqi Women**

A Thesis

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By

**Sana'a Abdulrazzaq Ibrahim Mohammad**

B.D.S / University of Baghdad, 1985

M. Sc. Clinical Biochemistry, Faculty of Medicine /

University of Kufa, 2006

Supervised by

**Professor**

**Dr. Mufeed Jalil Ewadh**

**Professor**

**Dr. Alaa Jafear Mahrath**

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

(وَلْيَعْلَمَ الَّذِينَ أُوتُوا الْعِلْمَ أَنَّهُ الْحَقُّ مِنْ رَبِّكَ فَيُؤْمِنُوا

بِهِ فَتُخَبِتَ لَهُ قُلُوبُهُمْ وَإِنَّ اللَّهَ لَهَادِ الَّذِينَ آمَنُوا إِلَىٰ

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صدق الله العظيم

(سورة الحج اية 54)

## الاهداء

- \* الى سيدتي و مولاتي التي اشتق الله لها اسما من اسمائه.....
- \*الى من قرن الله رضاه من رضاها و سخطه من سخطها.....
- \*الى من سميت بأم ابيها و قال فيها الرسول الاعظم محمد صلى الله عليه وعلى اله فيها ... أنها روعي التي بين جنبي.....
- \*الى من ضحت بولدها ذبيحا في ارض كربلاء.....
- \*الى فاطمة الزهراء البتول عليها السلام عسى ان تشفع لنا يوم الحشر .....
- \* الى من ارضعتني حب اهل بيت النبوة الى والدتي (رحمها الله) .
- \*الى من زرع في قلبي و روعي حب الخير و الفضيلة الى والدي (رحمه الله) .
- \*الى توأم الروح و شريك الحياة د. عادل فاضل علي
- \*الى اولادي الاعزاء و فلذات كبدي
- (د. سارة، د. سرمد، د. سهى، د. سوزان، اهداء خاص الى تقني تحليلات سيف، طالب الصيدلة سجاد).
- \* الى احفادي الاعزاء عسى ان يذكروني بعد رحيلي.
- \*الى اخوتي و اخواتي موضع اعتزازي و تقديري.

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## Summary

Dental caries, also known as tooth decay, is the breakdown of teeth due to acids made by bacteria, primarily *Streptococcus mutans* that metabolize sugars to produce acid, which over time, demineralized tooth structure.

Teeth mineralized organs, surrounded by alveolar bone, and formed by three distinctive hard tissues (enamel, dentin and cementum. The tooth mineralization process occurs parallel to the skeletal mineralization, yet if mineral metabolism is disturbed then the failures will occur similarly to those that occur in bone tissue.

Women are more liable to be influenced to dental caries during pregnancy because the pregnant women faced many physiological changes. These changes may be local and systemic, like those occur in the oral cavity. Oral health is a necessary part of general health, so the problems in the oral cavity in pregnant women must be come across immediately.

Vitamin D3 (1, 25-dihydroxyvitamin D3) is a fat-soluble steroid hormone, to regulate different biological processes, such as bone and teeth formation and metabolism and immunologic response. The most important action of 1, 25-dihydroxy-vitamin D is to increase the active absorption of Ca from the intestinal lumen.

Vitamin D deficiency during pregnancy may be a common health problem worldwide. Vitamin D deficiency is a pandemic issue due to decreased vitamin D3 intake from food cannot meet the daily vitamin D requirement for both adults and children, and sunlight exposure decrease because the use of sunscreens that increase in both summer and winter months.

The aim of this work was to study the risk factors related to dental caries during pregnancy. In this study some variables considered as important risk

indexes for tooth decay such as deficiency of vitamin D3 and others parameters such as calcium, phosphorous, PTH, and dental hygiene habits, and dietary habits.

Blood specimens of (311) females were collected from healthy volunteers. Blood samples have been divided into control group whom they are non – pregnant group, the number of this group were 157 women, and 154 blood samples were collected from pregnant women. Age was one of the socio – demographic characteristic which considered the dependent variable and divided to the three groups as (15 – 25), (26 – 35), (36 – 45) years.

There is a significant decreased in vitamin D3 concentration in pregnant women if compared with non – pregnant women. In study, the vitamin D concentration in pregnant women was 17.2 ng/ml and P value < 0.05 with high significant decreasing.

This study was investigating for serum vitamin D, ionized and total calcium, phosphorous, and selection samples for PTH in two groups, pregnant women and non- pregnant women, to prove and documented that the deficiency of maternal vitamin D3 is a risk factor for dental caries.

Study concluded that the comparison between the pregnant women were with higher risk for progression of dental caries versus non – pregnant women. There are a huge factors affected this result, the most and important one was vitamin D deficiency, even the deficiency of vitamin D3 was more and significant in pregnant women , however it was also insufficient in non – pregnant women and there was dental caries but less than that in pregnant women.

Vitamin D3 level, and calcium must be maintaining at normal level in pregnant women to protect the pregnant women and fetus from many disease and one of them destruction of teeth and bone during pregnancy, and prevented the early childhood caries in newborn babies.

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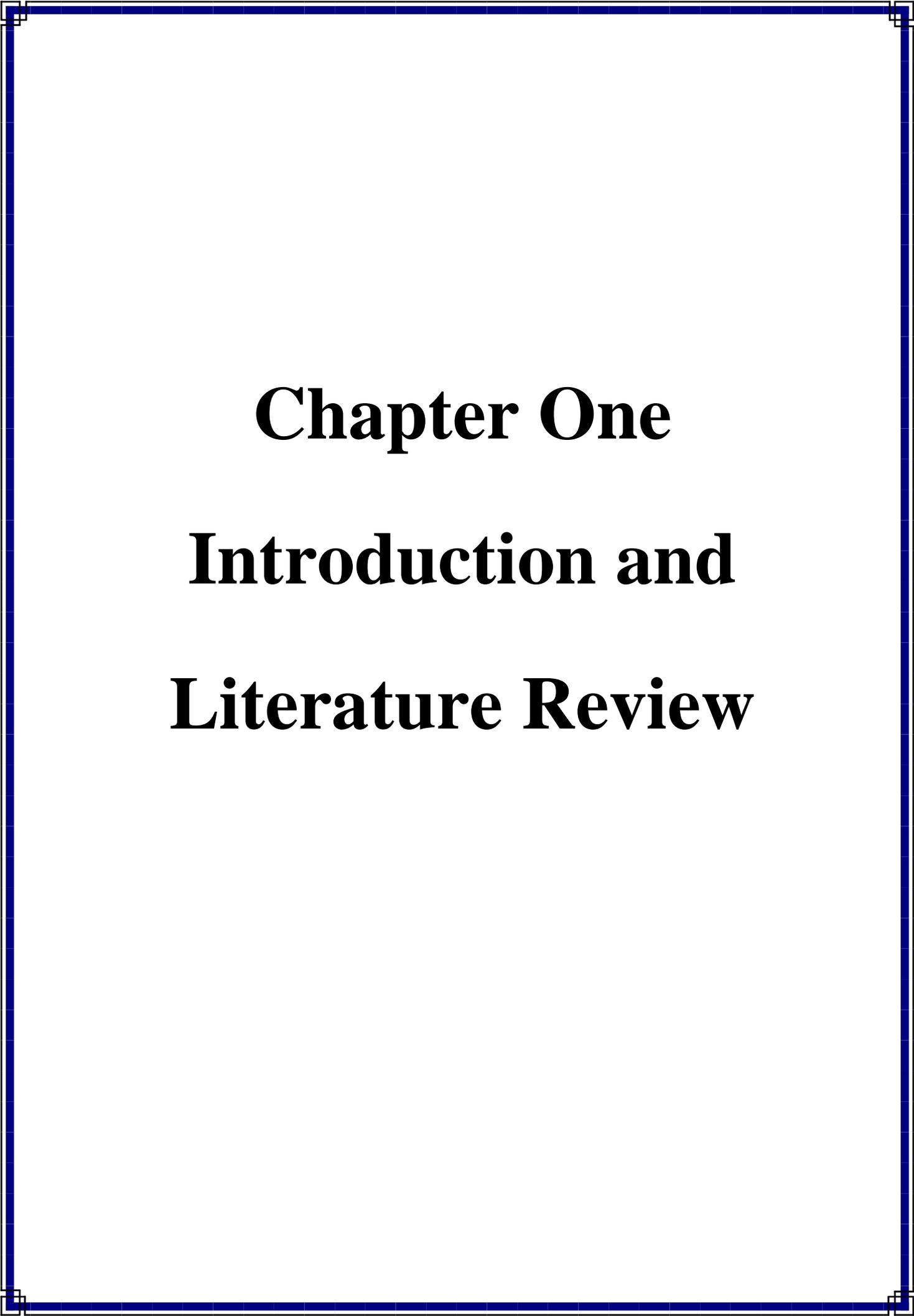
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## Abbreviation

Abbreviation	Meaning
ATPase	Adenosine Tri Phosphatase
Ca <sup>2+</sup>	Ionized Calcium
Ca	Calcium
cAMP	Cyclic Adenosine Mono Phosphate
C-18	columns have octadecyl carbon chain
CYP27B1	Cytochrome enzyme 27B1
CH <sub>3</sub> CN	Acetonitrile
CH <sub>3</sub> OH	Methanol
CI	Confidence Interval
N (n= in tables)	Numbers of sample
DMFT	Decayed Missing Filling Tooth
DMFS	Decayed Missing Filling Surface
ELISA	Enzyme – Linked Immunosorbant Assay
ECC	Early Childhood Caries
FGF	Fibroblast growth factor
FT	Filled Tooth
G	Gravida
HPLC	High Performance Liquid Chromatography
ISE	ion – selective electrode
IU/d	International Unit / day
IQR	interquartile range

<b>Abbreviation</b>	<b>Meaning</b>
(LL-37 or hCAP-18)	the C-terminal part of the only human cathelicidin identified to date called human cationic antimicrobial protein (hCAP18)
Na <sup>+</sup> - Ca <sup>2+</sup>	Sodium – Calcium Channel
1,25(OH) <sub>2</sub> D <sub>3</sub>	1, 25 dihydroxy vitamin D <sub>3</sub>
25(OH)D	25 Hydroxy vitamin D
PTH	Parathyroid Hormone
PTH-rP	Parathyroid Hormone Related Protein
IP <sub>3</sub>	Inositol trisphosphate
Pi	Inorganic Phosphate
SD	Standard Deviation
UVB	Ultra Violet Burn
VDR	Vitamin D Receptor
VDD	Vitamin D Deficiency



**Chapter One**

**Introduction and**

**Literature Review**

## 1.1. Dental Health

Dental health or oral health was defined as a healthy mouth, free of infections, injuries and other problems with teeth and gums (in past). The mouth is the mirror of the body, so any defect in the oral health was important to keeping overall body health. Also the age, lifestyle choices were made in the past played an important role in overall health and quality of life [1].

Disease and other conditions can affect dental health and dental problems can affect other parts of body. Failing to properly care for oral health may lead to other health problems. The goal is to prevent complications such as tooth decay (cavities) and gum disease and to maintain the overall health of the mouth [1].

In simplified terms was the effect in the oral complications at daily function. It was easy explaining. This definition put the oral validity related quality of life equaled the veracity, however in that time it includes proportion were more wide range than validity [1].

Dental diseases caused discomfort, pain, and affect important oral duties like mastication, smiling, talking, also can impact the public roles for every person. As a result from deferent nested researches and clinical attempt explained the dental managements with general social health could be improving the oral validity related fineness of life [2]. Researcher have identified three dental health domains where oral health-related quality of life is significant will be important, called clinical training on dentistry, oral education and dental research[2].

Oral lifestyle related fineness measures, it was exceedingly famed that quality lifestyle measures are not an exchange in evaluating the disease or therapy effects, albeit they are a crucial adjunct to it [2].

Multiple-item surveys are the most common quite used measure, and should be fixed on:

- a) Patient or person should be used
- b) It should consider elements of daily life that are important to them and may be affected by the upset or the situation at hand.

Tools that is relevant to oral health and quality of life

- 1) Socio- dental markers
- 2) Global oral health self-ratings
- 3) Questionnaires with multiple items [3].

## **1.2. Dental Caries.**

Dental caries or cavities, also known as tooth decay, is the breakdown of teeth due to acids made by bacteria, primarily *Streptococcus mutans* that metabolize sugars to produce acid, which over time, demineralized tooth structure as in (figure 1.1). The cavities may be a number of different colors dissolving the hard tissues of the teeth [4]. The three separate hard tissues enamel, dentin, and cementum are what make teeth into mineralized organs that are encased in alveolar bone. Although the mineralization of teeth and skeletons occur simultaneously, if mineral metabolism is disrupted, problems in tooth mineralization will resemble those in bone tissue Enamel is highly mineralized substance covering the tooth crown, hard, and protects the tooth as a barrier. If mineral breakdown outpaces mineral accumulation, as in the enamel begins demineralized, the enamel becomes thin and translucent [5], caries occurs as a result of sources like saliva p H, so is a prevalent chronic infectious, leading to the spread of the effect of bacteria till dentin, hence

causing demineralization of it which eventually leads to deep cavity formation resulting from tooth-adherent specific bacteria [4].



**Fig. 1.1: Cavity of dental caries with different colors and site of dental caries [4].**

The etiology of dental caries can be explained by a simple which consists of recently, a time, which describes the duration of the interaction of the depict diet, dental plaque, or microbial load, and the host. Plaque and dietary factors are interdependent upon each other in the causation of dental caries, as in [6]. These factors are:

- (1) Microbes found in dental plaque as *Streptococcus mutans* [7].
- (2) Time needed for changed the food to acid created by bacteria [7].
- (3) A suitable carbohydrate substrate, fermentable carbohydrates as cariogenic diet in order for dental caries to develop. [8].
- (4) A susceptible tooth surface, enamel or dentin [9].
- (5) Saliva as buffering capacity [9].
- (6) Fluoride as protecting factors, but if there is no fluoride supplement leads to dental caries [10].
- (7) Modifying factors due to the fact that every person sensitivity will differ based on their dental hygiene practices, the shape of their teeth, socioeconomic

and general body health, while the structure of tooth embedded in the bone not affected [11].

*Streptococcus mutans* (*S. mutans*) primarily has links with dental caries initiation [7], and Lactobacilli have links with the progression of dental caries.

The substrates for these bacteria are fermentable carbohydrates and the bacterial generated carbohydrate reserve in the biofilm. As the bacteria metabolize these substrates, they form lactic and other acids..

The formation of lactic acid, along with host factors, lowers down the oxygen coefficient locally, which fosters the rate and progression of dental caries [12]. Repeated cycles of acid generation result in the microscopic dissolution of calcified tissue in the tooth and eventually into cavitation. Studies have shown that enamel demineralization occurs at abrupt plaque pH decline after glucose as pH of 5.5 and below [4]. Remineralization can also take place if the acid is neutralized by saliva or mouthwash, making the process dynamic. Dental varnish or toothpaste with fluoride may help with remineralization [13].

Since saliva lacked the buffering ability to balance the acidic environment brought on by some foods, decreased salivary flow rate is linked to an increase in caries. As a result, illnesses that cause a decrease in the amount of saliva produced by salivary glands, in particular the submandibular gland and parotid gland, are likely to lead to dry mouth by such medical condition as diabetes mellitus and subsequently to extensive dental decay [14]. The chance of developing caries may also rise with tobacco usage. Some smokeless tobacco brands have a high sugar content, which makes people more susceptible to cavities [15].

Poverty line was also a substantial social determinant for good oral health. Dental caries can be viewed as a disease of lower socioeconomic

position and has been connected to [16]. Around 3.9 billion permanent teeth around the world suffer dental caries [17]. About 620 million people, or 9% of the world, experience it with their baby teeth [18]. Adults over the age of 50 experience cavities to a varying extent (29% to 59%) [19], the traditional DMF (decay/missing/filled) index is one of the most used tools for determining the frequency of caries and the need for dental care among population.

This index is based on clinical examinations conducted on people while they were in the field utilizing a probe, mirror, and cotton rolls. The DMF index understates the real incidence of caries and the need for treatment because it is performed without using X-ray imaging. [10]. Nearly all adults, according to the World Health Organization, suffer dental caries at some point [6]. With the aid of a dental mirror, explorer, and a bright light source, all visible tooth surfaces must be examined in order to make a first diagnosis. Smaller lesions can be challenging to spot, while larger areas of dental caries are frequently visible to the naked eye. Dentists routinely use visual and tactile exams, particularly to identify pit and fissure caries. Blown air across the suspicious surface, which eliminates moisture and modifies the unmineralized enamel's optical characteristics, is frequently used to diagnose early, uncavitated caries [20].

### **1.2.1. Dental Caries in Pregnant Women**

Women are more liable to be influenced to dental caries during pregnancy because the pregnant women faced many physiological changes. These changes may be local and systemic, like those occur in the oral cavity as in [figure 1.2].

Oral health is a necessary part of general health, so the problems in the oral cavity in pregnant women must be come across immediately. The dentist should be care about the physiological changes that occur throughout

pregnancy and be apprehensive about his interference by dental treatments, which may have effects on the lives of the mother and the baby in some time. Therefore, the dentist should be taking on all measures requisite to minimize the risk of reverse events [21]. Being pregnant is not a sickness rather it is an indication of good health.



**Fig. 1.2: Vitamin D deficiency in relation with dental caries versus health tooth [5]**

Anyone who was healthy no expected to lose their teeth for no apparent reason. Pregnant women have the same roles. If these women taking steps, they won't have any loss or fault of teeth or have existing dental issues, but if there is a bad oral health in pregnancy and failure to take care will lead to dental problems [22].

The hormone concentrations changing and tooth decay is indirectly caused by pregnancy and directly impacts the gums. In general we know that tooth decay increases during pregnancy. Tooth decay causes severe pain; also tooth losses can be visible.

Dentists can be explained this phenomenon by this ways: morning sickness as 70% of pregnancies are affected by nausea and vomiting. Oral hygiene could be having negative effects by vomiting or may result in the maternal enamel layer being eroded. Calcium concentration decreased while pregnancy. There is no difference in the amount of ionized  $\text{Ca}^{2+}$  during pregnancy in comparison with pre-pregnancy levels, in spite of the fact that pregnancy results in a high level of bone turnover. During pregnancy, improving dental health, cleaning and fluoride application will aid in preventing dental problems [22]. The following variables affect how well oral and dental health are maintained during pregnancy.

1 – Some pregnant women early on in pregnancy, there may be intense curiosity in such type of after consuming certain foods, particularly carbs; they neglect to wash their teeth.

2- Because of how pregnancy hormones (estrogen and progesterone) act, pregnant women gum tissue will be bleeding more every time and this lead to avoid using a toothbrush. Consequently, bacterial plaque accumulated, so, the mouth needs more care.

3- In the first few months, vomiting, during the acidic environment in the mouth gets worse during pregnancy. So after throwing up, pregnant women must be give attention to oral care. In the mouth, an acidic environment will develop, if the teeth are not brushed sufficiently.

4- Because of saliva flow decreases during pregnancy, during this time, dental cavities are more likely to form.

Various studies have found evidence linking together poor maternal oral health, pregnancy outcomes and dental health of the offspring. These may range

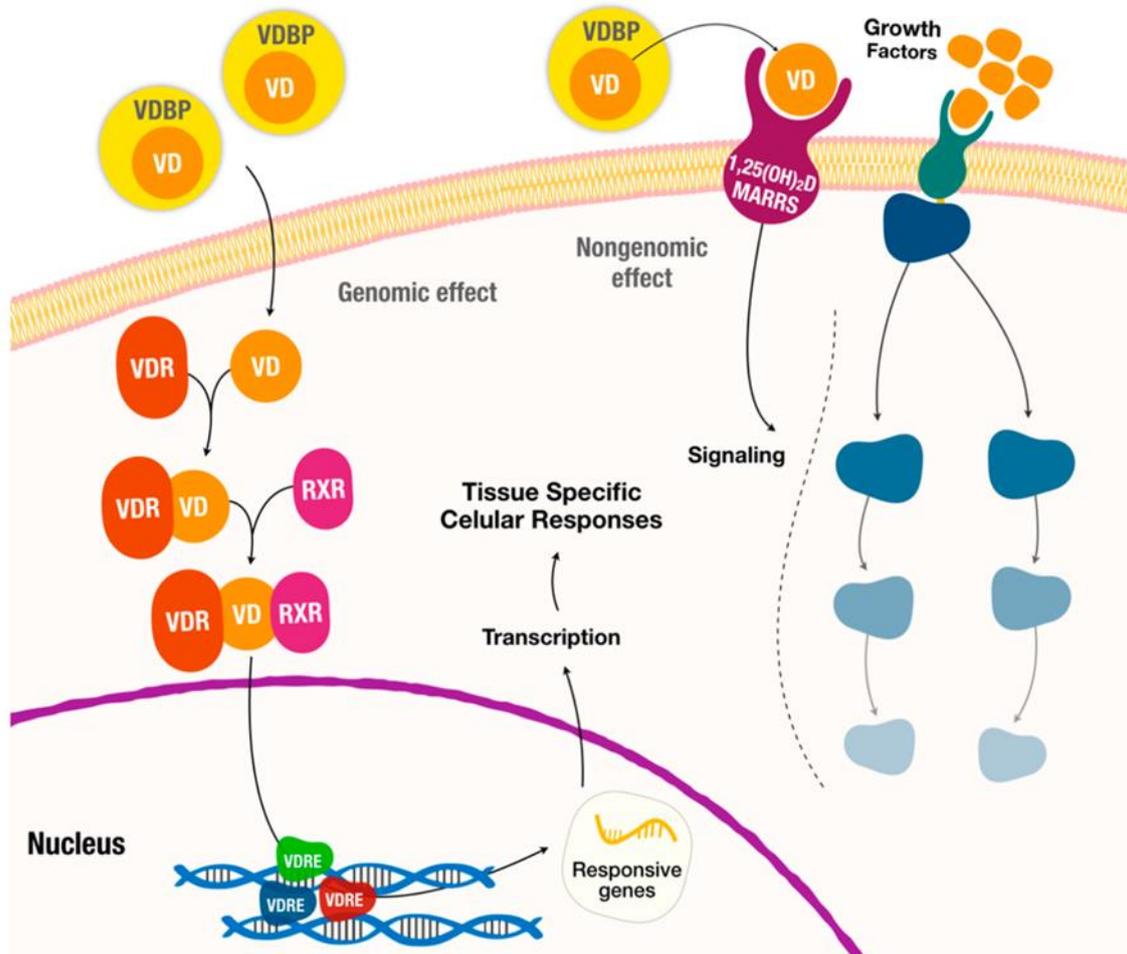
from preterm delivery and low birth weight to higher risk of early caries among infants. Oral health promotion, disease prevention, early detection and timely intervention are crucial aspects for maternal and child oral health.

Pregnant women can neglected their oral and dental health care which ultimately results in dental health declining. The main advantage of finding primary decalcified and benefit of treating lesions with chalky patches with remineralization. Untreated carious lesions increase the incidence of abscess and cellulites [23]. Some psychosocial factors were suggested, such as stress levels, and social support of pregnant women which usually may be get from their families and friends, and these factors were identified to be associated with oral health was social context and environment as health care system, other factors, including demographic and personal characteristics, all of these affected the pregnant women oral health and teeth, another barriers to seeking dental services include lack of knowledge and value, negative oral health experiences, negative attitudes toward oral health professionals and negative attitudes of dental staff toward pregnant women [24].

### **1. 3. Vitamin D3**

Vitamin D3 (1, 25-dihydroxyvitamin D3) is a fat-soluble steroid hormone, to regulate different biological processes, such as bone and teeth formation and metabolism and immunologic response [25]. Vitamin D3 were acted as a regulator of mineral through the mediation of calcium and phosphorus balance and absorption which can influence and promote the quality, growth of healthy bone, enamel, dentin, and involved in immune response, and in recent years has been implicated as a possible risk factor in the etiology of numerous diseases, also functions of vitamin D3 in the body through both an endocrine mechanism as regulation of calcium absorption, and an autocrine mechanism like facilitation of gene expression. Retinoic acid

receptors, in particular, dimerize with vitamin D receptors in order to exert its effects. In this state, a specific DNA region is attached to by the ligand-bound dimerized receptor as in (figure1. 3).



**Fig.1. 3: Hormonal actions of Vitamin D3 with genomic and nongenomic effects. MARRS—membrane-associated, rapid response steroid-binding protein; RDR—retinoid-X receptor; VD—Vitamin D; VDR—Vitamin D Receptor; VDRE—Vitamin D response elements; VDBP—vitamin D binding protein [156].**

Gene expression is either promoted or repressed with the assistance of additional transcription co-activators or co-repressors. There is proof that

vitamin D acts through membrane receptors in a manner similar to how other steroid and thyroid hormones do [26].

The primary function of 1,25 – dihydroxy vitamin D<sub>3</sub> is to promote active Ca absorption from the intestinal lumen. Vitamin D<sub>3</sub> maintains the structure and function of the skeleton and tissues and organs responsible for calcium – phosphate homeostasis, such as bones, kidneys and parotid glands [27]. As a result, calcium concentration in serum is maintained appropriate enabling bone and tooth mineralization [28].

### **1.3.1. Activation of vitamin D<sub>3</sub>**

The active form in humans is 1, 25-dihydroxy vitamin D<sub>3</sub> [1, 25(OH)<sub>2</sub> D<sub>3</sub>], which is derived from provitamin D<sub>3</sub>(7-dehydrocholesterol). On exposure to sunlight (Ultra Violet radiation, 290–315 nm) provitamin D<sub>3</sub> is transformed into cholecalciferol, which undergoes photoisomerization at body temperature and is then released into intracellular space and into the blood as in (figure 1.4). The activation of (25-OH-D), to hormonal form by hydroxylation to 1,25-dihydroxyvitamin D<sub>3</sub>. The first step in hydroxylation occurs in the liver, while the second step takes place in the kidney and extracellular sites. Vitamin D<sub>3</sub> transported from tissues into the circulating blood stream and go to liver and kidney by vitamin D binding protein (DBP) is as essential component of vitamin D<sub>3</sub> [29].

The rate-limiting hormonally regulated step is 1 – $\alpha$ -hydroxylase enzyme, the bioactivation of vitamin D<sub>3</sub> which is cytochrome P450 component (CYP27B1), very important enzyme to activate the 25(OH)D to 1,25 (OH)<sub>2</sub> vitamin D<sub>3</sub>.

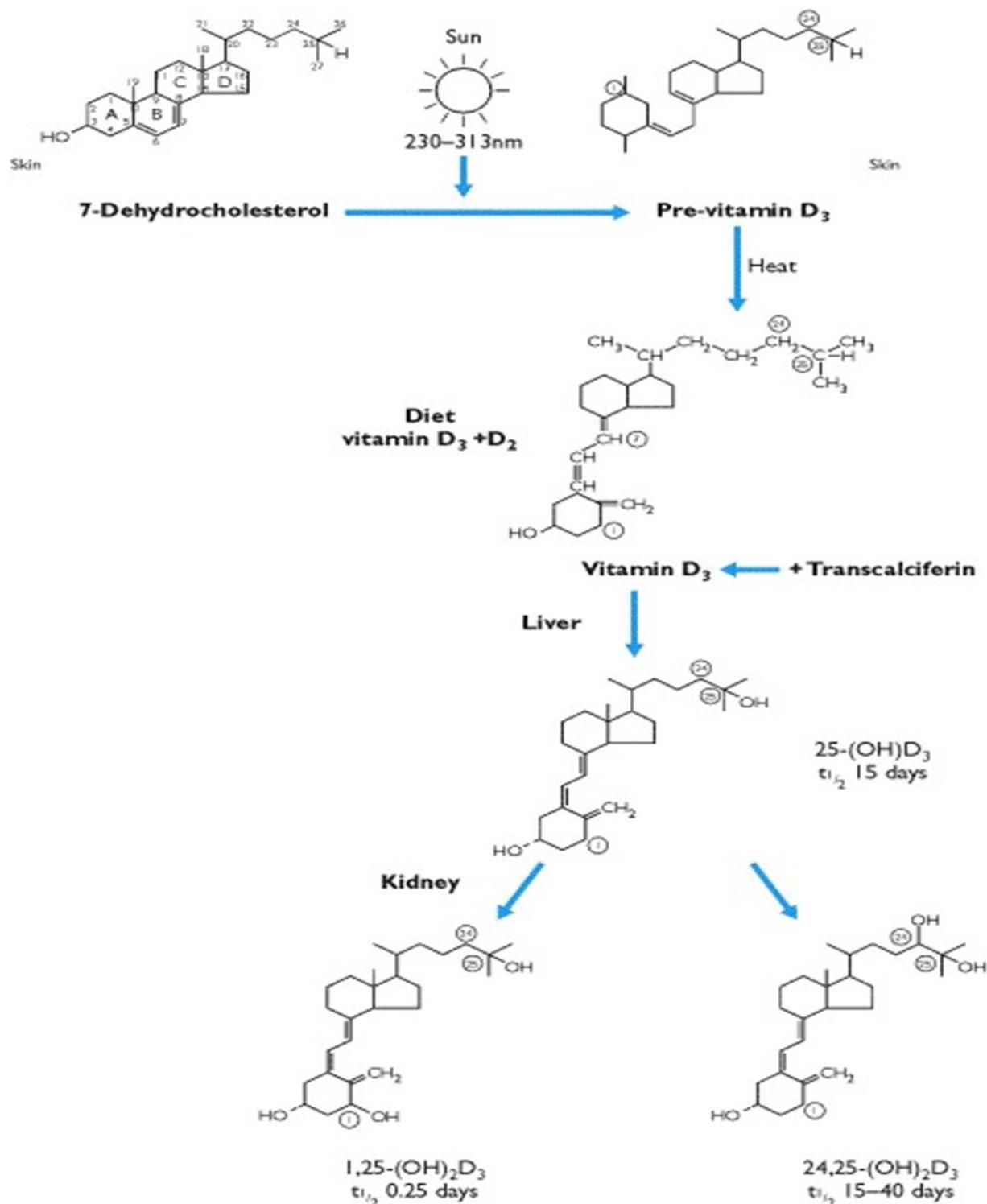


Fig. 1.4: Vitamin D<sub>3</sub> Synthesis [26].

1 $\alpha$ -hydroxylase enzyme thought to be centered regulatory for calcium and phosphate homeostatic system. The enzyme thought to be involved was a mixed function oxidase with a cytochrome P450 component [30]. This enzyme

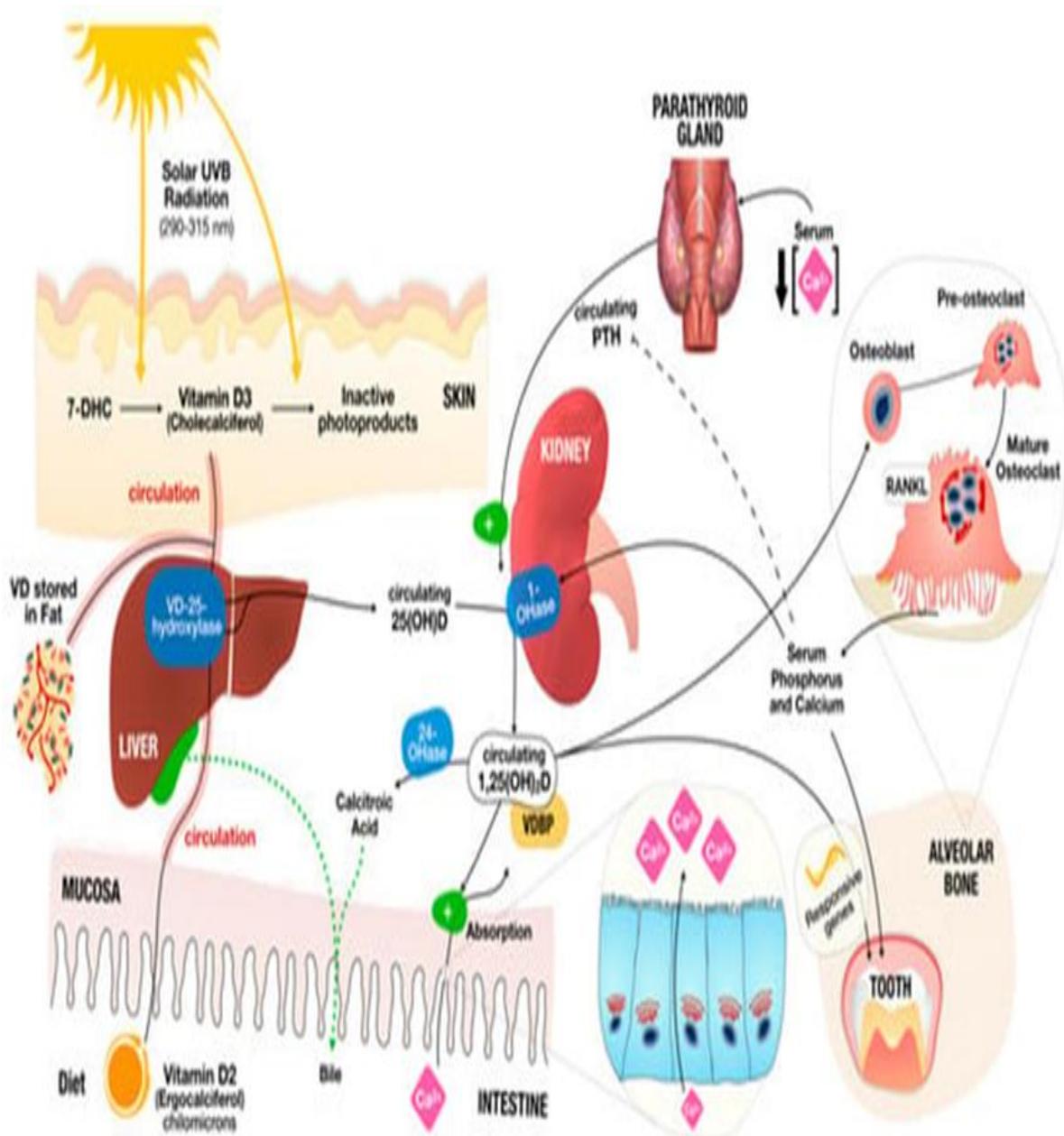
is a mitochondrial enzyme presented in the kidney and extracellular tissue, such as bone, placenta, and macrophage [31] as in (figure 1.5).

### **1.3.2. Vitamin D3 Deficiency**

Due to a decrease in the amount of vitamin D3 consumed through diet, there is a widespread problem of vitamin D deficiency. Vitamin D3 that applies to both adults and children, and sunlight exposure decline in today's because the greater application of sunscreen in the summer and winter. Vitamin D3 levels in serum start to fall as you get older due to reduce in endogenous vitamin D3 synthesis, and limited physical activity and long time spent indoors.

Deficiency of vitamin D can cause bone loss that leads to osteoporosis and fractures in old age, demineralization which eventually leads to rickets in children, and osteomalacia in adults which causing muscle weakness and fractures[5].

Attention to vitamin D3 roles must be notable in clinical conditions including diabetes and heart disease, cancers. Together with minerals, vitamin D3 is particularly beneficial in preserving oral health. Also the calcium-phosphate equilibrium is maintained by vitamin D3 and share in forming of the bone. Vitamin D3 has a connection to dental decay, gum disease, and tooth loss, so acted as oral health-protective agent, and reduced the incidence of caries and periodontitis [5,32, 33].



**Fig1.5: Vitamin D3 and Calcium, Phosphorus and Bone Metabolism.** Vitamin D3 is obtained mostly from sunlight exposure (Vitamin D3) and from diet (Vitamin D2). Vitamin D in the circulation is bound to the vitamin D-binding protein (VDBP), which transports it to the liver. There, Vitamin D is converted to 25-hydroxyvitamin D. Then, 25(OH)D circulates, reaching the kidneys where it is activated by to 1,25-dihydroxyvitamin D3 (1,25(OH)<sub>2</sub>D). Serum phosphorus or calcium can impact renal production of 1,25(OH)<sub>2</sub>D3. 1,25(OH)<sub>2</sub>D3 is recognized in the osteoblasts, causing the induction of mature osteoclast through expression of the receptor activator of nuclear. Mature osteoclasts remove calcium and phosphorus from the bone, maintaining the serum levels of calcium and phosphorus [156].

The primary structural elements of the tooth are minerals including magnesium, calcium, and phosphorus. Vitamin D<sub>3</sub> related with calcium, magnesium, and zinc, these minerals plays a good role with vitamins in strengthening the tooth structure. The maintenance of overall bodily health and the enhancement of oral health require a balanced and healthy diet [34]. Many mechanisms have been explaining a role for vitamin D<sub>3</sub> was reducing possibility for dental caries. The control of serum calcium, phosphate, and parathyroid hormone, which are crucial for the development, calcification, mineralization, and protection of teeth, is one of these systems maintenance of oral bone, hard tissues as well. Hypocalcemia and hypophosphatemia have been linked to enamel and dentin hypoplasia. [32, 35].

### **1.3.3. The action of vitamin D<sub>3</sub> in tooth structure**

Vitamin D has a notable role in ontogenesis [32, 36]. Vitamin D<sub>3</sub> excites the mineralization of tooth enamel involves binding to vitamin D receptor expressed by direct the transcription of a number of target genes in ameloblasts and odontoblasts, both of which are cells that make up bone and tooth tissue [32, 35, 37] Together with calcium-binding proteins and several extracellular matrix proteins, vitamin D receptor induces the production of structural gene products in dentin.

Gene of vitamin D receptor regulates the function biologically of vitamin D<sub>3</sub> metabolite, and as such plays a crucial function in the development of teeth, particularly in the mineralization of enamel, and dentin. As a result, it is possible for enamel hypoplasia and other developmental abnormalities to occur.

Vitamin D deficiency means the vitamin D<sub>3</sub> and at the vitamin D receptor molecular level impact the formation of tooth germ; viands of organization for dentin and enamel development and structure of tooth ; also

the process of growing a dental crown is organized [32, 35]. Vitamin D3 regulates and adjusts the innate and immunological system with effect. Vitamin D3 plays an immunological role by promoting the organization of certain antimicrobial peptides, such as defensins and cathelicidin (LL-37), which fight a variety of infections, including oral bacteria, helps to reduce the risk of cavities, these proteins have antibacterial effects to fight bacteria that caused cavities. [32, 38]. Cathelicidin (LL-37 or hCAP-18) was barred with vitamin D3, in which both antiendotoxin as well as antibiotic estate [33]. Tooth and the tooth's hard tissues are severely impacted by vitamin D deficiency during the development. An advantage exists linked throughout undernutrition, enamel hypoplasia, and caries in children's primary teeth. For cases of mineral deficiency due to absorption disorders, cause delayed tooth eruption, increased tendency to bleeding gums, bone resorption by destruction patterns in alveolar bone , and early tooth loss occur by periodontal disease, and enamel or dentin hypoplasia [34,39].

#### **1.3.4. Vitamin D3 during Pregnancy**

Vitamin D3 levels in children must be improved in the life at early stage, it considered as vital undertaking. Pregnancy must be recognized for this factor. The danger of vitamin D3 insufficiency should be assessed and pregnant women should constantly have their vitamin D3 levels checked during the three trimesters of pregnancy. Early childhood caries and the development of primary dentition may be influenced by prenatal vitamin D3 levels. [32, 39, 40].

Worldwide, vitamin D3 insufficiency during pregnancy could be a prevalent health issue. Because the baby totally depends on the maternal stocks of vitamin D3 for fetal skeletal growth and development, the vitamin D3 status during pregnancy has a crucial impact on the fetus [41]. 1,25(OH)<sub>2</sub>D3 appears

to be dependent on available 25(OH)D levels but independent on calcium metabolism, which is a unique feature of pregnancy that allows such high levels of 1,25(OH)<sub>2</sub>D<sub>3</sub>. Therefore, maintaining high enough levels of 25(OH)D are important to sustain the increased levels of 1, 25 (OH)<sub>2</sub> D<sub>3</sub> during pregnancy.

Such levels are still yet to be determined but studies have shown that maternal vitamin D<sub>3</sub> status is associated with various health outcomes during pregnancy. Deficient in vitamin D<sub>3</sub> gave birth to child suffering from hypocalcaemia, tetanic convulsion and enamel defects of teeth [42].

However, several researchers believe that ideal levels should be higher (beyond 75 nmol/L or 30 ng/mL). To obtain and maintain what many believe to be ideal levels in the body, it has been recommended that a daily supplementation dose of 1000–1600 IU (25–40 mg/d) of vitamin D<sub>3</sub> may be required [43].

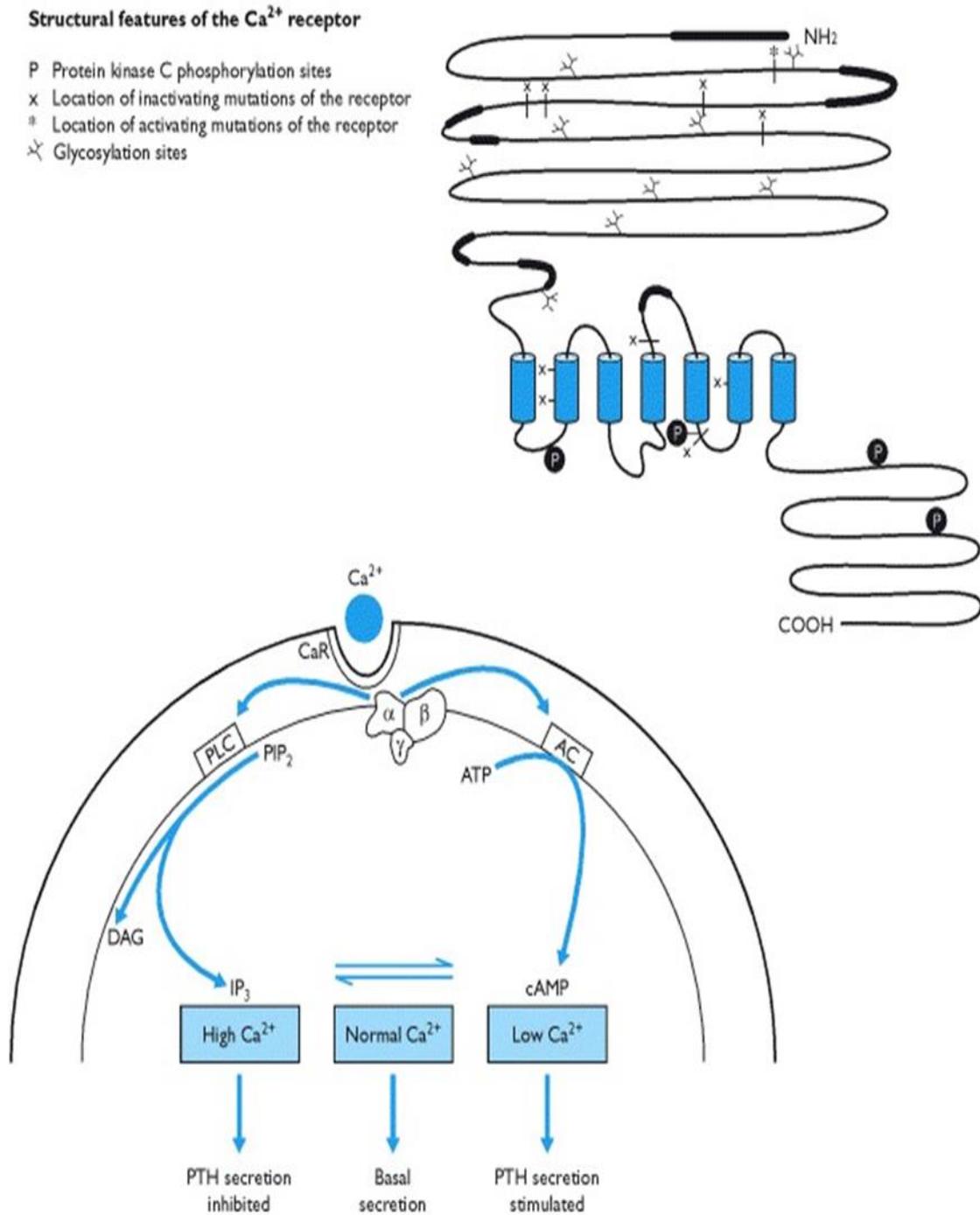
#### **1.4. Parathyroid Hormone (PTH)**

There are four parathyroid glands, one behind each of the upper and lower poles. The parathyroid glands grow embryologically from the third and fourth branchial arches. Parathyroid hormone is initially synthesized as a larger prohormone and subsequently cleaved to a biologically active 84 amino acid peptide. The more abundant main cells of the parathyroid glands carry out this synthesis. PTH does not have a serum-binding protein and the  $t_{1/2}$  of circulating PTH is about 5 min; it is rapidly cleared by the liver and kidney. Parathyroid glands control the amount of calcium in the blood and within the bones. The major function of the parathyroid glands is to maintain the body's calcium level within a very narrow range, so that the nervous and muscular systems can function properly.

### **1.4.1. Control of Parathyroid hormone secretion**

Calcium circulating levels changes always so can be reveal and change PTH production by a negative feedback mechanism. Chief cells regulate blood Concentration of Ca via a special calcium receptor with a G-protein attachment. If phospholipase C is induced by an increase in Ca binding and is inhibited by adenylate cyclase so the results in cAMP levels dropped and phosphatidylinositol triphosphate levels increased, which lead to decline PTH secretion. If there is decreasing in the activation of this receptor this lead to decrease the production of inositol triphosphate IP<sub>3</sub>, so elevated the production of cAMP leading to an increase in PTH releasing. A result, PTH secretion is inversely proportional to serum calcium concentration and the limit point for PTH secretion is around 1.3 mmol/l.

Maximal rates of secretion are achieved at a serum calcium concentration of about 1.15 mmol/l, as in (figure 1.6)[44].

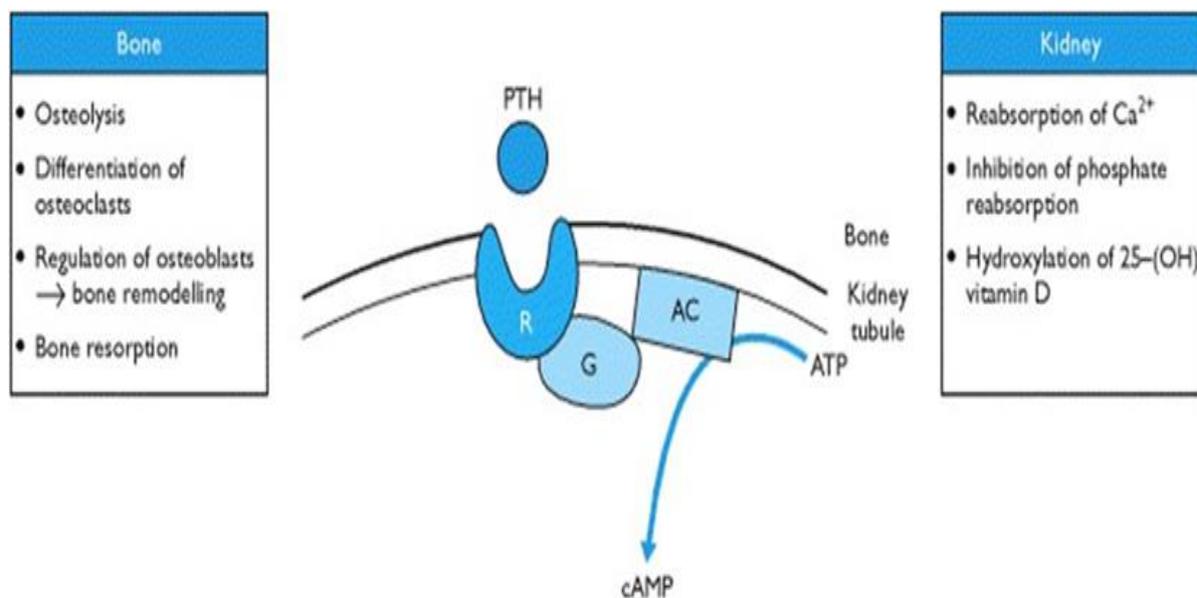


**Fig.1.6: The effect of calcium on the secretion of PTH [44].**

### 1.4.2. Actions of PTH

Parathyroid hormone regulates calcium levels in the blood, largely by increasing the levels when they are too low. It does this through its actions on the kidneys, bones and intestine:

**Bones** – Parathyroid hormone acts on osteoblasts in bone, at 1 - 2 hours, parathyroid hormone induces a process known as osteolysis, in which calcium in the tiny fluid-filled channels (canaliculi/lacunae) is picked up by syncytial processes of osteocytes and transported to the external surface of the bone and eventually into the extracellular fluid. The release of calcium from large calcium stores in the bones into the bloodstream. A few hours later, it promotes the resorption of mineralized bone, releasing calcium and Phosphorous into the extracellular fluid during the process. The most dramatic impact of PTH on the kidney is to prevent reabsorption of Phosphorous in the proximal tubule and significantly increase its excretion, which causes the phosphorous to be eliminated from circulation quickly. This increases bone destruction and decreases the formation of new bone. As in (figure1.7).

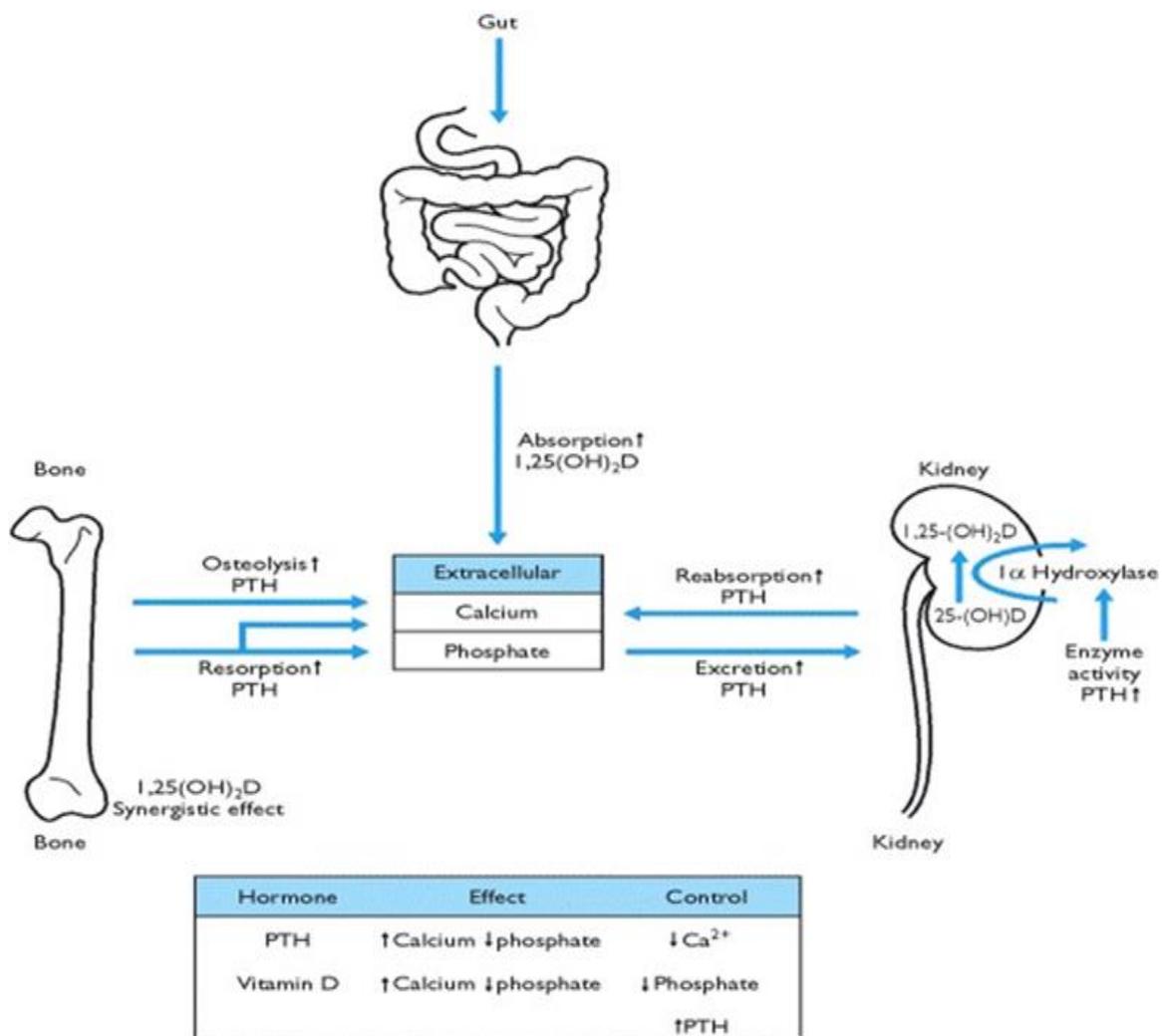


**Fig. 1.7: The relation of calcium with PTH in bone and kidney [44].**

**Kidneys** – Parathyroid hormone acts on G-protein-linked receptors in the kidney stimulate the adenylate cyclase enzyme, which then produces cyclic AMP in the kidney's tubular cells. This second messenger stimulates the signaling pathways that activate diverse bone cell activities. PTH also improves Ca reabsorption by enhancing the active uptake of calcium by  $\text{Ca}^{2+}$ -ATPase

and a  $\text{Na}^+ - \text{Ca}^{2+}$  antiporter in the ascending loop of Henley and the distal convoluted tubule, reduced calcium excretion rate. As previously mentioned, PTH also enhances the kidney's C-1 hydroxylation of 25-hydroxyvitamin D; indirectly boosting the gut's reabsorption of Ca. Parathyroid hormone reduces loss of calcium in urine.

**Intestine** – parathyroid hormone indirectly increases calcium absorption from food in the intestine, via its effects on vitamin D3 metabolism in the kidney (Figure 1.8)[44, 45].



**Fig. 1.8: The role of PTH in Calcium and Phosphate Regulation [44]**

### 1.4.3. The Role of PTH in Calcium Regulation

Calcium metabolism in pregnancy is a complex process. PTH modulates calcium and phosphate homeostasis, as well as bone physiology. Normal serum concentrations of phosphate and calcium are maintained through interactions of three central hormones: PTH, 1, 25-dihydroxyvitamin D<sub>3</sub>, and fibroblast growth factor (FGF) (figure 1.9). These hormones act on four primary target organs: bone, kidney, intestine, and parathyroid glands. Loss of any one of these hormones can have important consequences for the adult [46].

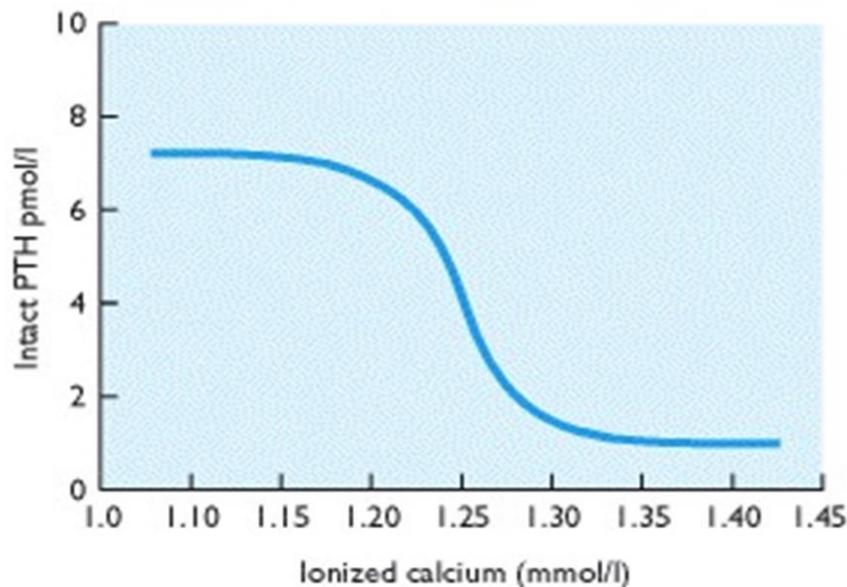


Fig. 1.9: The relation of calcium to PTH

### 1.4.4. Disease States of Parathyroid Gland

It is known that both increased and decreased parathyroid hormone secretion can lead to significant disease in both humans and animals.

A - Parathyroid hormone is secreted too much (hyperparathyroidism)

- A parathyroid tumor (adenoma) that secretes the hormone uncontrollably is the most prevalent cause of primary hyperparathyroidism, which affects the parathyroid gland. Chronically elevated blood calcium levels

(hypercalcemia) kidney stones, and bone decalcification are common symptoms of this condition.

- When a condition outside of the parathyroid gland causes an excessive release of parathyroid hormone, this condition is known as secondary hyperparathyroidism. Kidney disease is a prominent cause of this problem; as blood calcium levels decline as a result of impaired renal absorption of calcium, parathyroid hormone is continuously secreted to maintain normal calcium levels in the blood. Inadequate nutrition, such as diets lacking in calcium or vitamin D3 or too high in phosphorus (such as all meat), can also lead to secondary hyperparathyroidism. Decalcification of bone, which can result in pathologic fractures or rubber bones, is a significant side effect of secondary hyperparathyroidism [47].

B – Hypoparathyroidism is the condition where there is insufficient production of the parathyroid hormone.

Usually causes blood to have higher phosphorus concentrations and lower calcium concentrations, hyperphosphatemia (leading to ectopic calcifications), hypercalciuria (leading to nephrolithiasis and nephrocalcinosis). The surgical removal of the parathyroid glands and diseases that cause the loss of the parathyroid glands are common causes of this illness. The ensuing hypocalcemia can be immediately life-threatening and frequently causes tetany and convulsions. Low bone turnover and possible neurological sequelae from basal ganglia calcifications. With vitamin D deficiency, the serum calcium may be normal or reduced (but not as low as in hypoparathyroidism), serum phosphorus is low, and undermineralization of the skeleton leads to rickets or osteomalacia.

Loss of FGF23 causes hyperphosphatemia, extraskeletal calcifications, and early mortality. The goal of treatment is to get the patient's blood calcium

levels back to normal using vitamin D3 therapy, calcium infusions, and dietary supplements [48].

### **1.4.5. The Role of PTH during Pregnancy**

By the end of full-term gestation, the average fetus accretes about 30 g of calcium, 20 g of phosphorus, and 0.8 g of magnesium to mineralize its skeleton and maintain normal physiological processes. Due to the potential health effects on both mother and fetus, substantial research has been done on physiological changes during gestation that attempt to provide enough calcium for the baby' growth. PTH and other calcium homeostatic hormones are necessary to boost the mother's calcium absorption during pregnancy. [49].

The blastocyst is implanted and fertilized with the help of calcium from the maternal decidual; after that, the rate of transfer from mother to fetus significantly increases. The third trimester is when most of the calcium and phosphate that was in the fetal skeleton at the end of gestation passed the placenta and came primarily from the mother's nutrition.

Due to 1,25-dihydroxyvitamin D3 (calcitriol) and other variables, intestinal calcium and phosphate absorption doubles during pregnancy, and this appears to be the primary adaptation via which women meet the mineral demands of pregnancy. [50]. Due to hemodilution, the serum albumin and hemoglobin decrease during pregnancy; the albumin level is low until delivery.

The total serum calcium then drops to levels that may be far below the normal range as a result of the albumin's reduction. The ionized or free fractions of calcium as well as albumin-bound, bicarbonate, and citrate-complexes make up the total calcium. It is confirmed that the decrease in total calcium is only an aberration that is typically disregarded by the fact that the physiologically significant component of calcium, ionized calcium, remains constant during pregnancy. The serum level of calcium, however, cannot be

relied upon to identify hypercalcemia or hypocalcemia due to the artifactual fall in total calcium. During pregnancy, serum phosphate and magnesium concentrations are consistent with normal levels [50].

Parathyroid hormone was initially detected using assays that showed elevated circulating levels during pregnancy. The notion of physiological secondary hyperparathyroidism in pregnancy was developed in response to the discovery of low total serum calcium and an ostensibly high PTH. PTH participates in a number of functions, including the maintenance of ionized calcium in the blood, elevating the level of calcium phosphate released from bone tissue, preserving calcium, lowering tubular phosphate reabsorption, and increasing intestinal calcium absorption through vitamin D. Extracellular calcium concentrations have a major role in controlling PTH secretion; when calcium levels are low in the blood, PTH production rises. PTH levels rise over the course of pregnancy, reaching a mid-normal range by the third trimester [49]. The observed variations in PTH concentrations between the first and third trimesters may be explained by these changes.

Additionally, it has been noted that nephrogenous excretion of cyclic adenosine monophosphate (cAMP), an indicator of parathyroid activity, declines during the first and second trimesters of pregnancy but returns to normal during the third [51]. In cases of maternal hyperparathyroidism and hypercalcemia, the transfer of calcium to the fetus increases, which leads to the suppression of fetal parathyroid glands [52].

Numerous fetal problems, including spontaneous miscarriages, intrauterine growth restriction, stillbirths, temporary neonatal tetany, and persistent hypoparathyroidism in the newborn, are known to be caused by maternal hyperparathyroidism and hypercalcemia [53]. The placental transfer of calcium, on the other hand, declines in hypoparathyroidism prenatal hyperparathyroidism develops as a result of both hypocalcemia and the

activation of the fetal parathyroid glands. In severe cases of prenatal hyperparathyroidism, the long bones bow, the ribs and limbs are broken inside the womb, the osteitis fibrosa cystica develops, the fetus spontaneously aborts, and the fetus may even die [53]. Understanding the factors that influence having enough PTH concentrations throughout pregnancy is crucial. Studies in women of reproductive age and less commonly in pregnancy have mostly focused on the effects of dietary and plasma calcium and 25(OH) D levels on PTH, as well as the association between PTH and 25(OH) D levels[54].

The study wanted to additional variables, such as socioeconomic determinants and lifestyle factors, that might affect PTH concentrations in addition to the impact of circulating 25(OH) D concentrations on PTH levels [55].

#### **1.4. 6. Parathyroid Hormone – Related Protein (PTH-rP)**

Several organs, including keratinocytes and the placenta, generate PTH-rp, amnion, decidua, umbilical cord and the breast and fetal parathyroid glands have PTH-like effects. It is produced from a gene thought to have originated from a common ancestor with the PTH gene. Several N-terminal, mid-molecule, and C-terminal peptides that are produced by the prohormone PTHrp have different biological functions and specificities. The levels of none of these peptides have, however, been routinely assessed during pregnancy. The big molecule, made up of 1-86 amino acids, has been the subject of the greatest research. Beginning in the third to thirteenth week of pregnancy, PTHrP levels start to climb, and they typically continue to rise until the middle of the second or third trimester of pregnancy by three-fold in comparison to the baseline pre-pregnancy level by term as noted in longitudinal studies [56]. The significant rise in PTHrP may up regulate calcitriol and suppress PTH, although there is evidence that PTHrP may not be as potent as PTH in stimulating the renal 1-

alpha- hydroxylase in vivo [57]. An important source for PTHrP appears to be the placenta. In a case report of a pregnant woman with severe hypercalcemia and very high levels of PTHrP, serum calcium normalized following cesarean section and delivery of the placenta [58]. The reductions in PTH which is secondary to rises in calcitriol and PTHrP also contribute to the hypercalciuria [46]. The hypercalciuria observed during pregnancy has been associated with an increased risk of renal stones during pregnancy. The rises in calcitriol and PTHrP seen in pregnancy in women with hypoparathyroidism may result in lower requirements for calcium and calcitriol supplements during pregnancy as noted in many case reports.

Maternal calcium may be affected directly or indirectly by changes in PTH-related protein (PTHrP) and calcitriol during the first trimester as well as by the flux of other hormones during pregnancy, including estradiol, progesterin, placental lactogen, and insulin-like growth factor I [50].

## 1.5. Calcitonin Hormone

Calcitonin is a hormone, also called thyrocalcitonin, a protein hormone synthesized and secreted in humans by the parafollicular cells known as C-cells of the thyroid gland, the calcitonin protein is made up of 32 amino acids. The thyroid gland is located inside the front of your lower neck above the collar bones. Calcitonin is involved in helping to regulate levels of calcium and phosphate in the blood, opposing the action of parathyroid hormone. This means that it acts to reduce calcium levels in the blood. Both increased calcitonin secretion and increased calcitonin activity are relatively short-lived, lasting only a few days[59].

Calcitonin reduces calcium levels in the blood by two main mechanisms:

1. **Bone:** It inhibits the activity of osteoclasts, which are the cells responsible for breaking down bone. When bone is broken down, the calcium contained

in the bone is released into the bloodstream. Therefore, the inhibition of the osteoclasts by calcitonin directly reduces the amount of calcium released into the blood. However, this inhibition has been shown to be short-lived.

2. **Kidney:** It can also decrease the resorption of calcium in the kidneys, again leading to lower blood calcium levels [60].

Serum calcitonin levels are increased during pregnancy and may derive from maternal thyroid, breast, decidua, and placenta. Whether calcitonin plays an important role in the physiological responses to the calcium demands of pregnancy is unknown. It has been proposed to protect the maternal skeleton against excessive resorption during times of increased calcium demand [61].

The concentration of ionized calcium outside of cells is the main factor influencing calcitonin secretion. Secretion of calcitonin is substantially stimulated by elevated blood calcium levels and is inhibited when calcium concentrations fall below normal. In some circumstances, it has been demonstrated that a number of different hormones can promote the release of calcitonin. Bone remodeling and bone-preserving hormone are thought to be influenced by calcitonin hormones [62].

## **1.6. Calcium and Phosphorus**

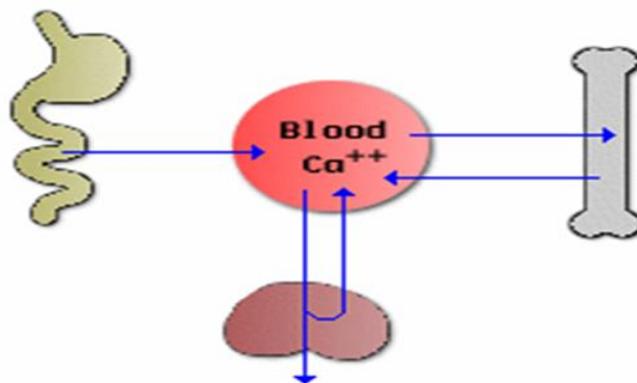
Calcium and phosphorus homeostasis relies on a complex, tightly regulated system involving many ions and hormones. The regulation of calcium and phosphorus is controlled by the actions of these ions and hormones on the intestine, kidneys and bone. The skeleton is the reservoir of calcium for many physiological functions, and it serves a similar but not so unique role for phosphorus and magnesium [63].

### 1.6.1. Calcium and Phosphate Fluxes

Frequent adjustments, which can be thought of as calcium fluxes between blood and other physiological compartments, are necessary to maintain stable calcium concentrations in the blood.

Calcium is supplied to blood by three organs, which can also remove it as necessary.

- 1 - **Small intestine:** Dietary calcium is absorbed in the small intestine. Importantly, the production of a calcium-binding protein in epithelial cells is necessary for effective calcium absorption in the small intestine.
- 2 – **Bone:** A huge calcium reserve exists in bones. Calcium and phosphate are released into the blood when net bone mineral resorption is stimulated, and calcium can be deposited in bone when this effect is suppressed.
- 3 – **Kidney:** The kidney plays a crucial role in maintaining calcium homeostasis. Nearly all of the calcium that enters glomerular filtrate is reabsorbed from the tubular system back into the blood under conditions of normal blood calcium concentrations, maintaining blood calcium levels. When tubular calcium reabsorption declines, calcium is lost through excretion into urine. [63] (Figure 1.10).



**Fig. 1.10: Fluxes of calcium.**

### 1.6.2. Distribution of Calcium and Phosphorus in the Body

The body contains three main calcium storage areas:

- **Intracellular calcium:** The mitochondria and endoplasmic reticulum are where the vast majority of intracellular calcium is stored. Because of release from cellular reserves or influx from extracellular fluid, intracellular free calcium concentrations vary widely, ranging from around 100 nM to more than 1  $\mu$ M. Calcium plays an essential part in intracellular signaling, enzyme activation, and muscle contractions.
- **Calcium in extracellular fluid:** The proportion of calcium in blood and extracellular fluid: that is bound to proteins is approximately 50%. About 1 mM, or 10,000 times the baseline concentration of free calcium in cells, is the average and nearly constant quantity of ionized calcium in this compartment. Additionally, the levels of phosphorus and calcium in blood are nearly comparable.
- **Bone calcium:** Bone contains the vast bulk of the body's calcium. The remaining 1% of calcium in bone is in a pool that can quickly interchange with extracellular calcium, while 99% of the calcium in bone is bound up in the mineral phase.

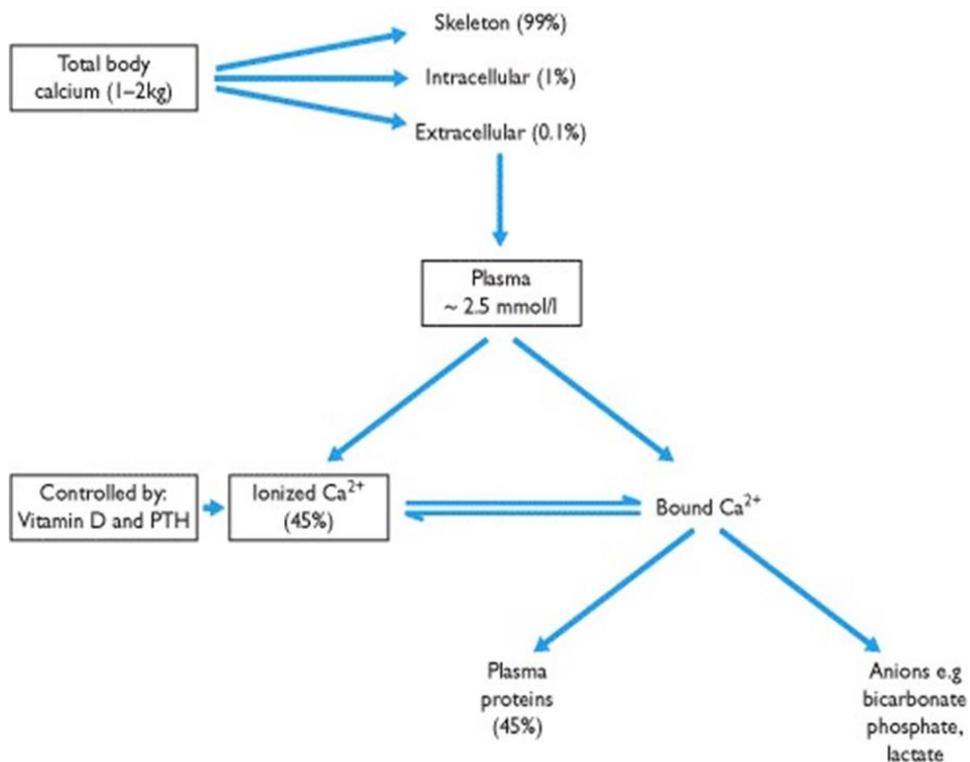
Similar to calcium, the mineral phase of bone contains the majority of the body's phosphate (about 85%). The remaining phosphate in the body is found in a wide range of inorganic and organic substances that are dispersed throughout intracellular and extracellular compartments. Phosphate levels in normal blood are quite comparable to calcium levels. [64].

### 1.6.3. Calcium Metabolism

Calcium weighs about 1.1 and 1.3 kg in an adult human body. Bones and teeth hold the majority of this (99%) information. Soft tissue cells and

extracellular fluid, which includes blood plasma and interstitial fluid, make up the remaining 1%. Blood plasma only contains 8.7 mmol (350 mg) of calcium, which is present at a concentration of about 2.5 mmol/L (10 mg/dL).

Around 40% of these 350 mg are protein-bound, primarily as albumin but also as globulins, and 10% are complexes with various anions (bicarbonate, lactate, phosphate, etc.). Around 1.25 mmol/L of "free" ionized calcium ( $\text{Ca}^{2+}$ ), or the remaining 50%, circulates in the blood. (Figure 1.11).



**Fig.1.11: Distribution of normal body calcium [44].**

Only the ionized calcium fraction is physiologically active, despite the fact that all three calcium fractions seen in blood plasma are in balance [50]. Only 1% of the calcium in bone can be easily exchanged with plasma. Calcium homeostasis in the non-pregnant state is primarily maintained by the complex interactions between parathormone (PTH) and vitamin D3. Through its impact on osteoclasts, PTH, which is released by the parathyroid glands in response to a drop in serum calcium level, facilitates calcium resorption from the skeleton.

By increasing the kidney's 1-alpha hydroxylase, which encourages the conversion of 25-hydroxyvitamin D into 1,25-dihydroxyvitamin D<sub>3</sub> [1,25(OH)<sub>2</sub> D<sub>3</sub>], it also improves renal tubular reabsorption of calcium. Consequently, intestinal calcium absorption is aided [50].

#### **1.6.4. Homeostasis of Calcium during Pregnancy**

To accommodate the increased calcium requirements of the mother and fetus, calcium homeostasis in pregnancy differs slightly from that in non-pregnant states.

In a term neonate, there are roughly 30 g of calcium. The majority of this active calcium transfer occurs in the third trimester, when the collagen matrix is rapidly ossifying. Pregnancy lowers total calcium levels due to hemodilution-related low albumin. The calcium demand may be met by increased resorption of maternal skeleton, increased absorption or decreased urinary excretion. Much of the calcium conservation observed during pregnancy is due to increased intestinal absorption of calcium. This occurs mainly due to the increased

generation of 1,25 (OH)<sub>2</sub>D<sub>3</sub>. [2,3] Compared to non-pregnant state, the 24-hour urinary excretion of calcium during pregnancy is higher but fasting urinary calcium levels are similar. Hence, it is likely to be a reflection of increased absorption of calcium (absorptive hypercalciuria) and to a lesser extent due to higher calcitonin levels seen in pregnancy [49]. Serum levels of magnesium and phosphorus are usually within normal limits during pregnancy [65]. Greater intestinal calcium absorption, decreased urine calcium losses, and enhanced bone resorption are the adaptive mechanisms used in maintaining homeostasis during these times of elevated calcium demands on the mother [65].

### **1.6.5 .Pregnancy's Intestinal Calcium Absorption**

Increased dietary calcium consumption can help the body meet its increased calcium needs during pregnancy, which double as early as 12 weeks. It is commonly accepted that improving intestinal calcium absorption efficiency or a reduction in calcium losses in the urine are explained by doubling or tripling calcitriol levels. Pregnancy has been linked to an increase in appetite, but in developing nations, access to calcium-rich foods is constrained by a lack of resources. Of course, improved intestinal calcium absorption efficiency continues to be the key factor improving the mother's access to calcium. In humans, it has been seen that intestinal calcium absorption is significantly more effective in the later stages of pregnancy. Additionally, there is a higher of intestinal phosphate absorption during pregnancy [50].

### **1.6.6. Calcium Preservation in the Kidneys**

Another technique the body may use to increase the calcium availability to the fetus is renal conservation of calcium. However, due to an increase in GFR in the last trimesters of pregnancy, more urine components such amino acids, glucose, and calcium are excreted. If a woman has a vitamin D3 deficit, she may experience calcium conservation during pregnancy because she is more likely to experience hypocalciuria. Hypocalciuria and pregnancy-related hypertension have also been linked, according to reports [66].

### **1.6.7. Transport of Placental Calcium**

Calcium is actively pumped across the placenta to suit fetal demand. This opinion is reinforced by the finding that the fetus' serum calcium level is 1-2 mg/dL greater than the mother's. In the later stages of pregnancy, the placenta begins to produce calcium-binding protein along with an increase in

the rate of calcium transport. This protein has characteristics with intestinal calcium-binding protein, which is linked to calcium absorption from vitamin D3 in the intestine. As a result, vitamin D3 might help with placental calcium transfer. It's possible that vitamin D3 by itself is not necessary for the active transport of calcium across the placenta. It appears that the peptide linked to parathyroid hormone (PTHrP) is also involved in the transfer of placental calcium [67].

Both calcium and phosphorous, as well as magnesium, are transported to blood from bone, renal, and GI cells, and vice versa [68]. These transport mechanisms can be through cells called transcellular and around cells called paracellular. The cellular transport is mediated by the membrane structures and by binding transport proteins .

The paracellular transport is generally passive and mediated by mineral gradients. These mechanisms also involve corresponding co-transportation and exchange-transportation with other ions, notably sodium, potassium, chloride, hydrogen, and bicarbonate, some of which are powered by ATP hydrolysis. Similar mechanisms allow for the intracellular distribution of calcium, where it partitions primarily between the mitochondria and cytosol [69].

The idea that tooth loss occurs during every pregnancy and that the mother's teeth provide the fetal body with the calcium needed for intrauterine growth is unsupported by science [70].

In this study hypothesized proving that [There is evidence to support the notion that tooth loss occurs during every pregnancy and that the mother's teeth provide the unborn baby with the calcium it needs for intrauterine growth].

Although wanted to determining that by the effect of vitamin D deficiency and its effect on calcium demand during pregnancy and relation of

PTH levels have been effect in this research for the pregnant women in our population, with the other effects such as the ethnicity, type of food, soft drink, health status, socioeconomic status, psychological status, season, age, all these conditions affected the levels of PTH secretion [55].

General health includes oral health, which is even more crucial during this time because it affects both the mother and the fetus. Additionally, it should be remembered that poor oral and dental hygiene during pregnancy can result in preeclampsia, early birth, low birth weight babies, and other issues in addition to concerns like tooth decay and tooth loss.

In order to preserve both the mother's and the unborn child's health during pregnancy, the mother must abide by a set of regulations. Mothers can safeguard their oral health during this time by taking the required steps, which will help them avoid any potential dental issues.

Early detection of dental caries in pregnant women is crucial for both preventive and curative treatment. Pregnant women should pay extra attention to early dental caries detection and treatment. Modern dental medicine aims to provide practitioners with cutting-edge diagnostic tools so they can objectively measure dental cavities. Clinical examinations are only helpful and required if they are carried out carefully, completely, and with delicate, non-traumatic instrumentation. Two fundamental approaches are necessary for the medical evaluation for the early detection of carious lesions in order to properly approach dental caries therapy :

- 1 - Review of risk variables that have a serious chance of causing tooth decay but haven't really caused any harm .
- 2 - Clinical and paraclinical investigation may allow for the early identification of demineralized areas before they are objectified.

**1.7. Aims:**

- 1 To assess the level of serum vitamin D3 concentration, in pregnant women in comparison with control group.
- 2 To examine the association between vitamin D3 level and dental caries in pregnant women in comparison with non – pregnant women.
- 3 To estimate the relationship of Vitamin D3 level, PTH, Calcium, Phosphorous levels in pregnant women in comparison with non – pregnant women in relation with dental caries.

# **Chapter Two**

## **Materials and Methods**

## 2.1. Subjects and Study Design

During 4 months (May, June, July and, August at 2022), 311 blood specimens, all of them from healthy participants, females were taken. There are 2 groups one of them as controls, whom they are non – pregnant group, the number of this group were 157 women, and 154 blood samples were collected from pregnant women attending to Hospital and private dental clinic and some primary health centers in City of AL-Najaf and Babylon city. They were all Iraqis who lived in this society with varying levels of wealth, including some who led affluent lives. The average family income was around the same.

A questionnaire contains name, past medical history, psychological history, their status, dietary habits, oral hygiene habits, brushing teeth as time, and frequency of dental appointments, ethnicity, and socio-economic status, educational level, and clinical diagnosis as decayed teeth, gingivitis, plaque will be complicates for all individuals . The study subjects will be matched for age.

The study was described in details for all participants and their consent was obtained. The study will be approved by Babylon Medical College Ethical Committee. According to them document number 21421and the date18/5/2022 to get this approval.

The chart of the questionnaire included questions about independent variables of non – pregnant and data on the socio-demographics of expectant mothers, employment status (Age, Number of children, Family monthly income (Student, wife, part-time job, full-time job, and occupation), Health status, Psychological status, Level of education as ( Master, Four Year college, Two year College, High School, Less than high school, Secondary or middle or primary School, None),

Type of nutrition, Use of soft drinks, Use brush and toothpaste and the oral hygiene status ( Never brush, Sometimes, Once a day or more),

Periodically review the dentist (Private, Public, None), Last visits for dentist with the past ( 6 Months, 1 Year, 2 Years, 3Years, 4 Years, Never been going to dental clinic, Number of dental caries, Number of filling, Number of extraction teeth, and gingival condition) [71] .

For pregnancy women chart in addition to the questions above also there are other questions as, (Number of Previous Pregnancies, Stage of pregnancy) figure 2.1.

**University of Babylon  
College of Medicine**

الأسئلة الخاصة بالدراسة البحثية العلمية الخاصة ببحث الدكتوراه للكيمياء الحيوية للنساء الحوامل و غير الحوامل مع فحص الأسنان و تحديد تسوس الأسنان مع الموافقة الخطية للبحث.

---

المحلة برقم الموبايل:  رقم الاستمارة:  التاريخ:

العمر:  الاسم:

الجنس: أنثى: حامل 1, غير حامل 2  رقم الحمل أو الطفل 1, 2, 3

الحمل في أي شهر  الحالة الاجتماعية المعيشية: ضعيف 1, متوسط 2, جيد 3

الحالة الصحية والبدنية العامة: جيدة 1, غير جيدة 2

الحالة النفسية:  التحصيل العلمي:

نوع التغذية: جيد و متنوع 1, قليل 2, اعليه حلويات 3  تناول المشروبات الغازية: نعم 1, كلا 2

استعمال الفرشاة والمعجون في المنزل (كم مرة يوميا)

هل يتم مراجعة طبيب الأسنان دوريا: نعم 1, كلا 2  متى آخر مراجعة لطبيب الأسنان:

عدد الأسنان الدائمة:

---

الأسدان المصابة بالتسوس:

عدد الأسنان المعالجة بالحشوات:

عدد الأسنان المقلوعة:

---

حالة اللثة: لا يوجد التهاب 1, التهاب بسيط 2, التهاب قوي 3

وجود حالات غير طبيعية للأسنان (تتكرر):

أوافق على الاشتراك بالبحث بفحص الأسنان و المعطومات و سحب الدم

اسم المشاركة و التوقيع:

اسم طبيب الأسنان الفاحص وتوقيعه

Fig. 2.1: Chart of questionnaire

### 2.1.1. Sample Size Estimation

Sample size depends on an online estimation program cited at <http://Raosoft>, which depend on the margin of error accept, 5% is a common choice. The confidence level accepts, 95%, and the population size. The estimating sample size of the test study is highly variable, so 154 pregnant women and 157 non- pregnant women, they will be randomly selected from the population (figure no. 2.2)

**Sample size calculator**

What margin of error can you accept?  %  
5% is a common choice  
 The margin of error is the amount of error that you can tolerate. If 90% of respondents answer yes, while 10% answer no, you may be able to tolerate a larger amount of error than if the respondents are split 50-50 or 45-55.  
 Lower margin of error requires a larger sample size.

What confidence level do you need?  %  
Typical choices are 90%, 95%, or 99%  
 The confidence level is the amount of uncertainty you can tolerate. Suppose that you have 20 yes-no questions in your survey. With a confidence level of 95%, you would expect that for one of the questions (1 in 20), the percentage of people who answer yes would be more than the margin of error away from the true answer. The true answer is the percentage you would get if you exhaustively interviewed everyone.  
 Higher confidence level requires a larger sample size.

What is the population size?   
If you don't know, use 20000  
 How many people are there to choose your random sample from? The sample size doesn't change much for populations larger than 20,000.

What is the response distribution?  %  
Leave this as 50%  
 For each question, what do you expect the results will be? If the sample is skewed highly one way or the other, the population probably is, too. If you don't know, use 50%, which gives the largest sample size. See below under **More information** if this is confusing.

Your recommended sample size is **384**  
 This is the minimum recommended size of your survey. If you create a sample of this many people and get responses from everyone, you're more likely to get a correct answer than you would from a large sample where only a small percentage of the sample responds to your survey.

Online surveys with Vovici have completion rates of 66%!

**Alternate scenarios**

With a sample size of	<input type="text" value="100"/>	<input type="text" value="200"/>	<input type="text" value="384"/>	With a confidence level of	<input type="text" value="90"/>	<input type="text" value="95"/>	<input type="text" value="99"/>
Your margin of error would be	9.80%	6.93%	5.00%	Your sample size would need to be	271	384	663

Save effort, save time. Conduct your survey online with Vovici.

**More information**

If 50% of all the people in a population of 20000 people drink coffee in the morning, and if you were repeat the survey of 377 people ("Did you drink coffee this morning?") many times, then 95% of the time, your survey would find that between 45% and 55% of the people in your sample answered "Yes".

The remaining 5% of the time, or for 1 in 20 survey questions, you would expect the survey response to more than the margin of error away from the true answer.

When you survey a sample of the population, you don't know that you've found the correct answer, but you do know that there's a 95% chance that you're within the margin of error of the correct answer.

Try changing your sample size and watch what happens to the alternate scenarios. That tells you what happens if you don't use the recommended sample size, and how M.O.E and confidence level (that 95%) are related.

To learn more if you're a beginner, read *Basic Statistics: A Modern Approach* and *The Cartoon Guide to Statistics*. Otherwise, look at the more advanced books.

Fig. 2.2: Sample size calculator

The selected cases were:

Random cases: Three hundred and eleven samples at nine o'clock in the morning, patients were seen, and venous blood was taken ( all of them females), criteria for selection of the study subjects include apparently healthy participants with ages range between (15 - 45) years with healthy, dental Caries, filling, extracted teeth, and gingivitis( simple and difficult types).

## 2.2. Methods and Procedures

### 2.2.1. Equipment and Instruments.

**Table 2.1: The types of equipment and instruments**

No.	Types	Auxiliary Devices
<b>1 -</b>	<b>High Performance Liquid Chromatography (HPLC) consist of [72]</b>	1 – Flow controller valve
A -	Pump for liquid chromatography	2 – Solenoid valve unit
B -	UV-Visible detector	3 – Helium degasser
C -	System controller	4 – Reservoir tray
D -	Column	
E -	Auto injector	
F -	Oven	
G -	Controller as computer system	
<b>2 -</b>	<b>Electrolyte Analyzer (ISE) [73]</b>	
<b>3 -</b>	<b>Enzyme–Linked Immunosorbant Assay (ELISA) [74]</b>	
<b>4 -</b>	<b>UV-visible Spectrophotometer [75]</b>	
<b>5 -</b>	<b>Incubator Memmert</b>	
<b>6 -</b>	<b>Vortex</b>	

### 2.2.2. Materials

**Table 2.2: The types of chemical materials used in analysis**

Numbers	Types of analysis
2.2.2.1.	HPLC analysis
A.	Acetonitrile for HPLC (CH <sub>3</sub> CN) 90%
B.	Methanol for HPLC (CH <sub>3</sub> OH) 10%
A+B.	Mobile phase
C	Vitamin D3 the commercial DEVIT – 3, 1ampoule x1ml, with 300,000 I.U. of vitamin D3
2.2.2.2.	Electrolyte Analysis
A.	Calcium kit as in table 2.3
2.2.2.3.	ELISA analysis
A.	Human parathyroid hormone ELISA kit as in tables (2.4, 2.5 and 2.6), and figures (2.3, and 2.4)
2.2.2.4.	In organic phosphorus determination as a kit in table 2.7

**Table 2.3: Component of the kit of electrolyte analyzer**

Components	Standard A	Standard B
K <sup>+</sup> (mmol/L)	4.00	8.00
N <sup>+</sup> (mmol/L)	140.00	110.00
Cl <sup>-</sup> (mmol/L)	100.00	70.00
Ca <sup>+2</sup> (mmol/L)	1.25	2.50
Li <sup>+</sup> (mmol/L)	1.00	0.50
P H	7.40	7.00

**Table 2.4: Reagent of ELISA kit**

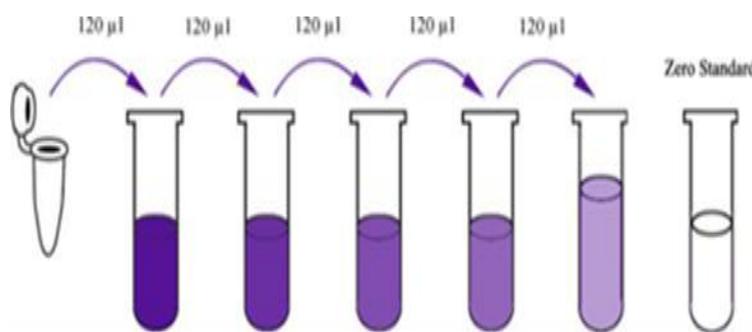
Components	Quantity (96T)	Quantity (48T)
Standard Solution (1200pg/ml)	0.5ml x1	0.5ml x1
Pre-coated ELISA Plate	12 * 8 well strips x1	12 * 4 well strips x1
Standard Diluent	3ml x1	3ml x1
Streptavidin-HRP	6ml x1	3ml x1
Stop Solution	6ml x1	3ml x1
Substrate Solution A	6ml x1	3ml x1
Substrate Solution B	6ml x1	3ml x1
Wash Buffer Concentrate (25x)	20ml x1	20ml x1
Biotinylated Human PTH Antibody	1ml x1	1ml x1
User Instruction	1	1
Plate Sealer	2 pics	2 pics
Zipper bag	1 pic	1 pic

**Table 2.5: Dilution of standard solutions**

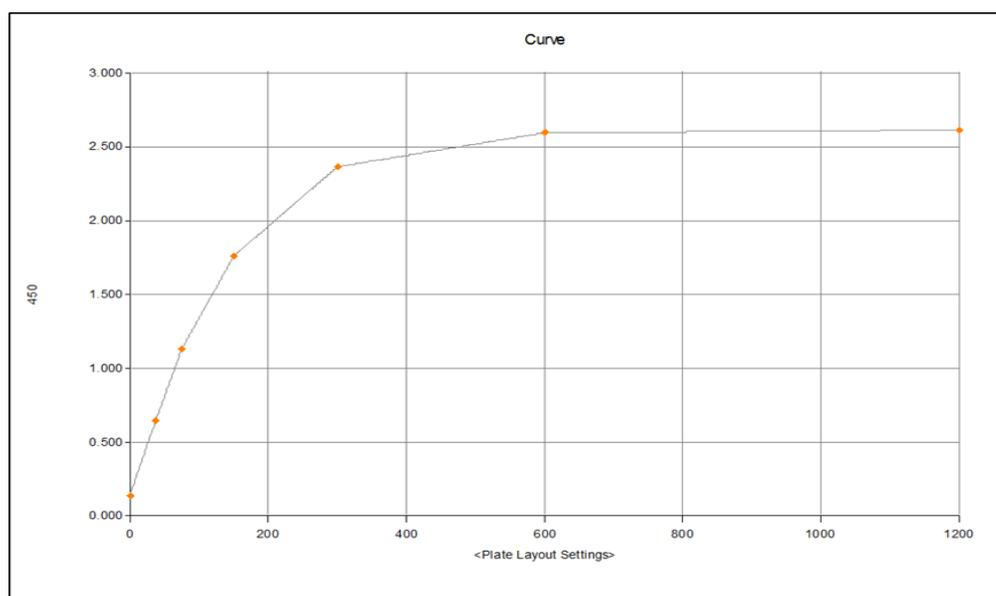
600pg/ml	Standard No.5	120µl Original Standard + 120µl Standard Diluent
300pg/ml	Standard No.4	120µl Standard No.5 + 120µl Standard Diluent
150pg/ml	Standard No.3	120µl Standard No.4 + 120µl Standard Diluent
75pg/ml	Standard No.2	120µl Standard No.3 + 120µl Standard Diluent
37.5pg/ml	Standard No.1	120µl Standard No.2 + 120µl Standard Diluent

**Table 2.6: Standard tubes of ELISA**

Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
1200pg/ml	600pg/ml	300pg/ml	150pg/ml	75pg/ml	37.5pg/ml



**Fig.2.3: Dilutions of standard reagents of ELISA**



**Fig.2.4: Curve for the Optical Density of PTH in (Y) axis and Concentration of PTH in (X) axis**

**Table 2.7: Manual Procedure for the phosphate measurement**

	Blank	Specimen blank	Standard	Assay
Reagent	1 ml		1 ml	1 ml
Saline Solution		1 ml		
Demineralized water	20µL			
Standard			20µL	
Specimen		20µL		20µL

Calculation:

The results calculated as

Serum

Result = Abs (assay) – Abs (specimen blank)

\_\_\_\_\_ x standard concentration

Abs(standard)

### **2.2.3. Pretreatment of Blood**

Usually from the interior of the elbow, blood was taken from the vein, without tourniquet. Five ml of venous blood were drawn using a disposable, sanitized syringe. (BIOZEK medical), the transfer of blood to gel and clot activator tube, they can improve serum surface and prevent blood substance exchange between blood cells and serum; these tubes are used for separation and examination of serum.

Then left for several minutes to clot and centrifuged for 15 minutes with 1600 xg. Then 3 ml of separated serum was isolated in serum tube.

One milliliter was used from this serum to determine 1.25-dihydroxy vitamin D3 concentration.

From this separated serum was used 200 µL for calcium determination. Twenty µL used for phosphate determination, and 40 µL used for determinate PTH by ELISA.

The serum stored at deep freeze -20°C until the time of analysis, to prevent any unfavorable decomposition. When the time for analysis had arrived the serum was kept at room temperature with suitable time.

#### **2.2.4. HPLC- UV spectrophotometer.**

The chosen wave length in this investigation, which was essentially the most suitable one, was used to measure vitamin D3 was 256nm and this related to the type of HPLC that used in lab technique [76]. The detector of the HPLC was modified to reach these criteria for optimum sensitivity.

#### **2.2.5. The extraction of vitamin D3**

Polar solvent (acetonitrile 90% + methanol 10 %) was used to extract the vitamin D binding protein (DBP), which has been successfully utilized to deproteinize or denaturize, is extracted from serum to release vitamin D3 metabolites. [77]. Selective lipid extraction and cleaner extracts are obtained with more polar solvents which often have been used for extraction of more polar dihydroxylated metabolite[78].The previous step separates vitamin D3 metabolites, lipid, and some other substances from another serum constituent. One milliliter of serum and one milliliter of acetonitrile were combined, vortexed for two minutes, and centrifuged at (1600xg) for fifteen minutes.

The supernatant was drawn off to a glass tube for further analysis and quantitation. All plastic tubes were avoided to prevent the loss of vitamin D which may precipitate on plastic walls.

#### **2.2.6. HPLC preparation for direct measurements**

The HPLC separation utilizes an isocratic approach, which allows for the use of a single pump for chromatography at a temperature of 37°C with a reversible phase column. The UV-detector picks up chromatograms at 256 nm.

Retention period of vitamin D3 was (3.4) minutes for every run. Results that were given quantified by the delivered serum calibrator and calculated by the external standard method by integration of the peak area. The

acetonitrile solution was used as eluent solution. The solvents have been filtered through fine membranes (0.22 micron), to prevent any fine particles from getting inside the column. The greatest enemy of HPLC is fine particles.

Columns require high pressure to maintain a convenient flow of the eluting solvent, usually in the range 1.0 ml / min. Upon correctly priming the pump, the system delivered a mobile phase exits the outlet with no interruptions; inlet should be free of air bubbles.

The pump controller was used to set the flow rate for mobile phase. The pump should be restarted, watched the system pressure gauge to see that it was not exceeding the maximum level allowed for various components, then the sample was run.

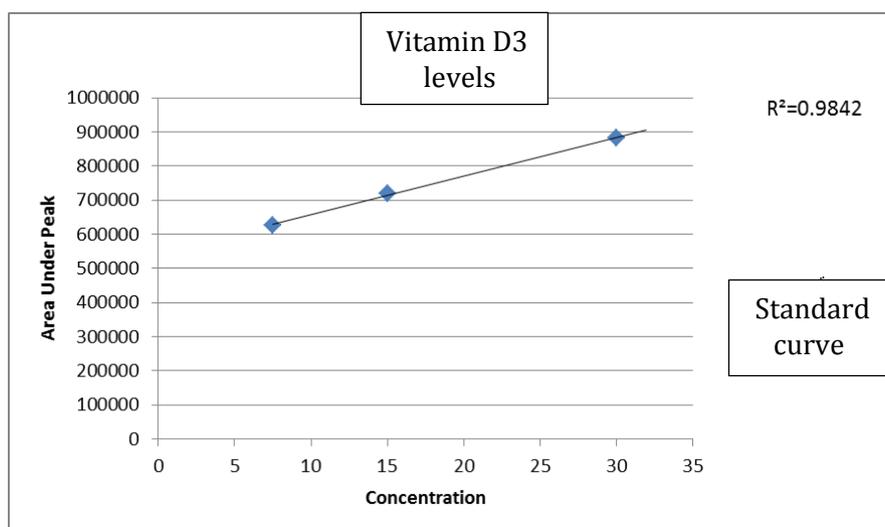
Sample injection, as auto injection for 50  $\mu$ l of sample was injected smoothly.

Then the data system recording was started.

2- Chromatogram or data analysis evaluation.

3- Vitamin D3 measurement by working standard curve.

The standard curve consisted of the concentrations, 15, 30, 75 ng /ml in (figure 2.10).



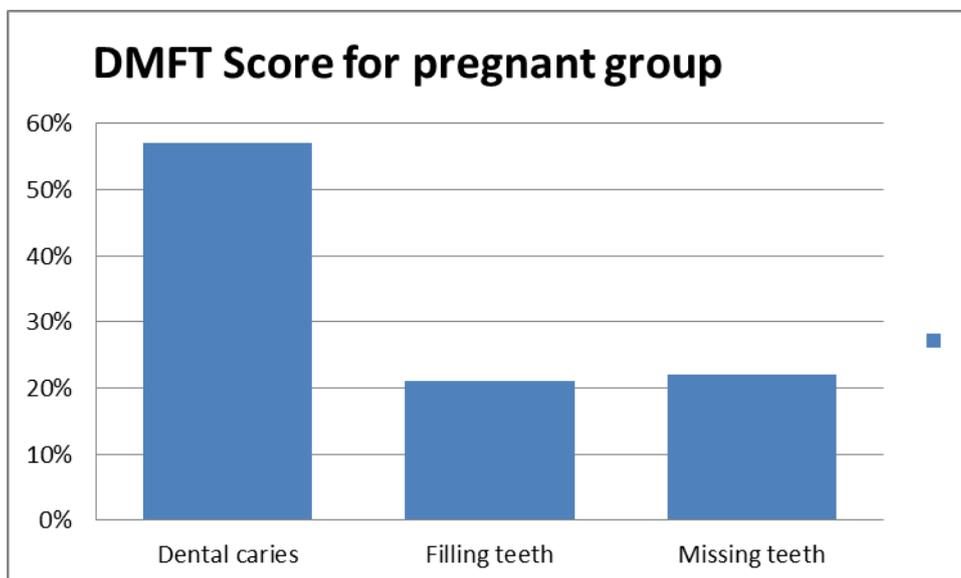
**Fig. 2.5: The standard curve of the vitamin D3 concentrations**

Mothers were invited to examine at dental room in public clinics and seated on dental chair and used a special dental instrument as, probe and mirror to check the oral cavity. The DMFT index was used to determine the prevalence of dental caries, and the DMFS index to determine the severity of those caries experiences. [79, 80].

DMFT scores were classified as: very low (0–1.1), low (1.2–2.6), moderate (2.7–4.4), severe (4.5–6.5) and very severe (6.6 or higher) [81].

In figure 2.11, showed the DMFT score of pregnant Iraqi women in this study as 57% with dental caries, 21% filled teeth, and 22% missing teeth.

All participants were voluntary; they signed paper consent collected from pregnant and non – pregnant ladies who concur to share in this study. The consents for the two groups started from age 15 – 45 years. All aspects of this study, even the consent form that received approval from the Scientific Research Ethics Committee of College of Medicine / University of Babylon.



**Fig.2.6: Percentage of DMFT score in Iraqi pregnant women.**

### 2.3. Statistical Analysis

The socio-economic and demographic characteristics and the laboratory statistics of 311 of pregnant and non-pregnant women are shown as means and standard deviations or as percentages.

Data were analyzed using SPSS software version 26. Categorical variables were presented as frequencies and percentages while continuous variables were presented as mean and SD. Shapiro - Wilk test was significant for PTH which indicated that it was not normally distributed, thus Mann - Whitney U test was used for comparisons between the cases and controls[82]. Kruskal-Wallis test used for comparison of different groups among cases and controls in regard to trimester of pregnancy, gravida and parity. Independent t test was used for comparison of mean Phosphate (mg/dl), Total calcium (mg/dl), Ionized calcium (mg/dl) and D3 (ng/ml) between cases and controls, and among cases and controls in regard to presence of filling, caries and extraction. Chi square test was used to assess the relationships between categorical variables. Clustered bar charts were used as needed. ANOVA test for comparison between the trimester status of pregnant women.

Risk indicators associated with tooth decay were identified using the 95% confidence intervals (95% CI) were calculated. The P value < 0.05 will be selected to define statistical significance.

# **Chapter Three**

## **Results & Discussion**

### **3. Results and discussion**

#### **3.1. General Characteristics, Socio- economic, Demographic, and Laboratory tests of the Studied Population**

Dental health and management of pregnant women considered to be a very principle part. It is suitable to appreciate patient's present dental health status, and then to educate these women about the expectant changes during pregnancy, and then can be helpful to prevent the pain and stress. A total of (311) Iraqi woman was included in the study, of which 154 were pregnant women. There are socio – demographic and behavioral characteristics, which obtained from interview of the women for two groups. Health status, psychological status, and medical history were including, in pregnant and non – pregnant women. Lifestyle factors, as educational levels, type of the diet, soft drink for pregnant; all these were summarized in (table 1). In contrast there are 157 non – pregnant women with the same characteristics and lifestyle factors summarized in (table 2).

The number of each group was showing in (figure 1), and how distributed.

In our study the socio- economic status was using scale for two groups as good, median, and poor as in (table 1, and 2).

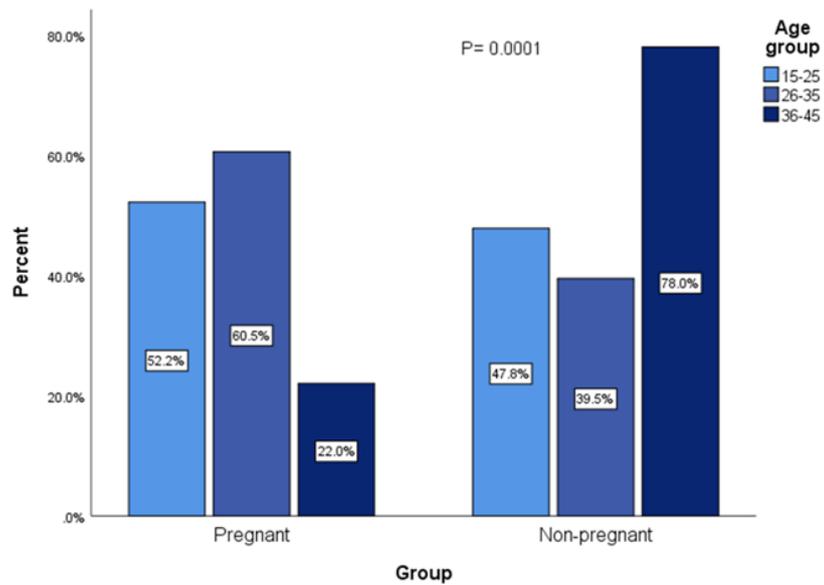
The pregnant women should understand the important relation between the oral health and dental caries experience DMFT. Index for pregnant and non - pregnant was carried, of statistical significance differences observed in the total percent for pregnant women for medium and poor 123(79.9%), while the good 31(20.1%), when compared with the non – pregnant women as medium and poor 90(57.3%), while the good 67(42.7%), this explained in (figure 2) with P value < 0.05

**Table 3.1: Distribution of pregnant women samples according to age, gravida, socioeconomic status, health status, psychological status, educational status, nutrition, soft drink**

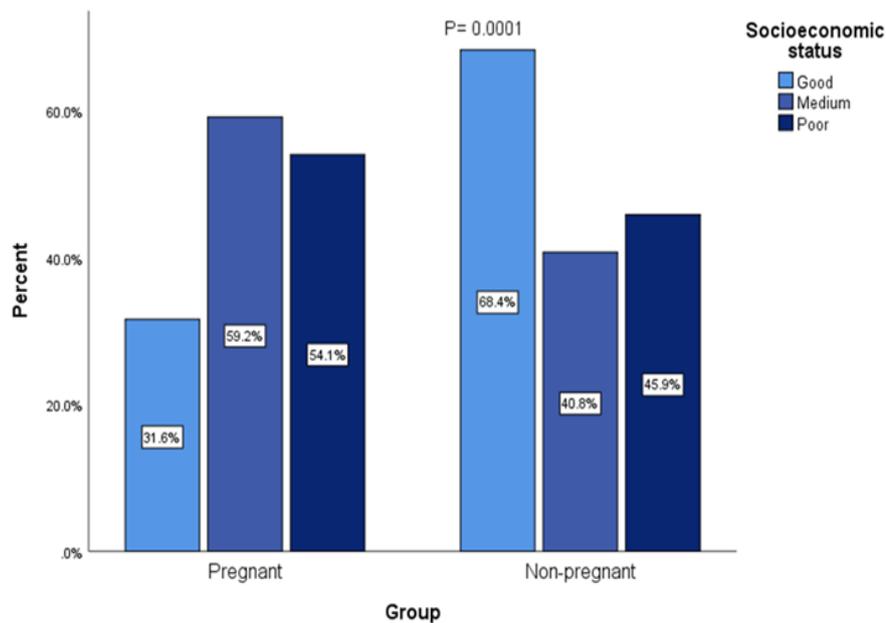
Variables		Age group			Total	P
		15-25 (n=72)	26-35 (n=69)	36-45 (n=13)		
Gravida	1.00	36 (50%)	11 (15.9%)	0 (0%)	47 (30.5%)	0.0001
	2.00	22 (30.6%)	9 (13%)	1 (3.1%)	32 (28.8%)	
	3.00	14 (19.4%)	45 (65.2%)	5 (38.5%)	64 (41.6%)	
	4.00	0 (0%)	4 (5.8%)	7 (53.8%)	11 (7.1%)	
Socioeconomic status	Good	12 (16.7%)	19 (27.5%)	0 (0.0%)	31 (20.1%)	0.08
	Medium	47 (65.3%)	33 (47.8%)	10 (76.9%)	90 (58.4%)	
	Poor	13 (18.1%)	17 (24.6%)	3 (23.1%)	33 (21.4%)	
Health status	Good	67 (93.1%)	60 (87%)	10 (76.9%)	137 (89%)	0.6
	Not	5 (6.9%)	9 (13%)	3 (23.1%)	17 (11%)	
Psychological status	Good	67 (93.1%)	62 (89.9%)	11 (84.6%)	140 (90.9%)	0.6
	Depressed	5 (6.9%)	7 (10.1%)	2 (15.4%)	14 (9.1%)	
Education	Higher education	14 (19.4%)	19 (27.5%)	6 (46.2%)	39 (25.3%)	0.2
	Secondary	6 (8.3%)	9 (13%)	3 (23.1%)	18 (11.7%)	
	Intermediate	8 (11.1%)	6 (8.7%)	0 (0%)	14 (9.1%)	
	Primary	35 (48.6%)	24 (34.8%)	3 (23.1%)	62 (40.3%)	
	Not educated	9 (12.5%)	11 (15.9%)	1 (7.7%)	21 (13.6%)	
Nutrition	Good	45 (62.5%)	46 (66.7%)	7 (53.8%)	98 (63.6%)	0.3
	Poor	25 (34.7%)	20 (29%)	4 (30.8%)	49 (31.8%)	
	Mostly sweets	2 (2.8%)	3 (4.3%)	2 (15.4%)	7 (4.5%)	
Soft drink	Yes	44 (61.1%)	27 (39.1%)	8 (61.5%)	79 (51.3%)	0.03
	No	28 (38.9%)	42 (60.9%)	5 (38.5%)	75 (48.7%)	

**Table 3. 2: Distribution of non- pregnant women sample according to age, number of children, socioeconomic status, health status, psychological status, educational status, nutrition, soft drink.**

		Age group			Total	P
		15-25 (n=66)	26-35 (n=45)	36-45 (n=46)		
Number of child	Nulliparous	50 (75.8%)	8 (17.8%)	7 (15.2%)	65 (41.4%)	0.0001
	1-3	15 (22.7%)	26(57.8%)	24(52.2%)	65 (41.4%)	
	4+	1 (1.5%)	11 (24.4%)	15 (32.6%)	27 (17.2%)	
Socioeconomic status	Good	31 (47%)	15 (33.3%)	21 (45.7%)	67 (42.7%)	0.3
	Medium	25 (37.9%)	21 (46.7%)	16 (34.8%)	62 (39.5%)	
	Poor	10 (15.2%)	9 (20%)	9 (19.6%)	28 (17.8%)	
Health status	Good	62 (93.9%)	39 (86.7%)	39 (84.8%)	140 (89.2%)	0.6
	Not	4 (6.1%)	6 (13.3%)	7 (15.2%)	17 (10.8%)	
Psychological status	Good	66 (100%)	38 (84.4%)	41 (89.1%)	145 (92.4%)	0.006
	Depressed	0 (0.0%)	7 (15.6%)	5 (10.9%)	12 (7.6%)	
Education	Higher education	37 (56.1%)	22 (48.9%)	19 (41.3%)	78 (49.7%)	0.1
	Secondary	7 (10.6%)	0 (0.0%)	4 (8.7%)	11 (7%)	
	Intermediate	6 (9.1%)	5 (11.1%)	6 (13%)	17 (10.8%)	
	Primary	7 (10.6%)	9 (20%)	13 (28.3%)	29 (18.5%)	
	Not educated	9 (13.6%)	9 (20%)	4 (8.7%)	22 (14%)	
Nutrition	Good and diverse	44 (66.7%)	33 (73.3%)	36 (78.3%)	113 (72%)	0.7
	Poor	18 (27.3%)	10 (22.2%)	9 (19.6%)	37 (23.6%)	
	Mostly sweets	4 (6.1%)	2 (4.4%)	1 (2.2%)	7 (4.5%)	
Soft drink	Yes	33 (50%)	19 (42.2%)	12 (26.1%)	64 (40.8%)	0.04
	No	33 (50%)	26 (57.8%)	34 (73.9%)	93 (59.2%)	

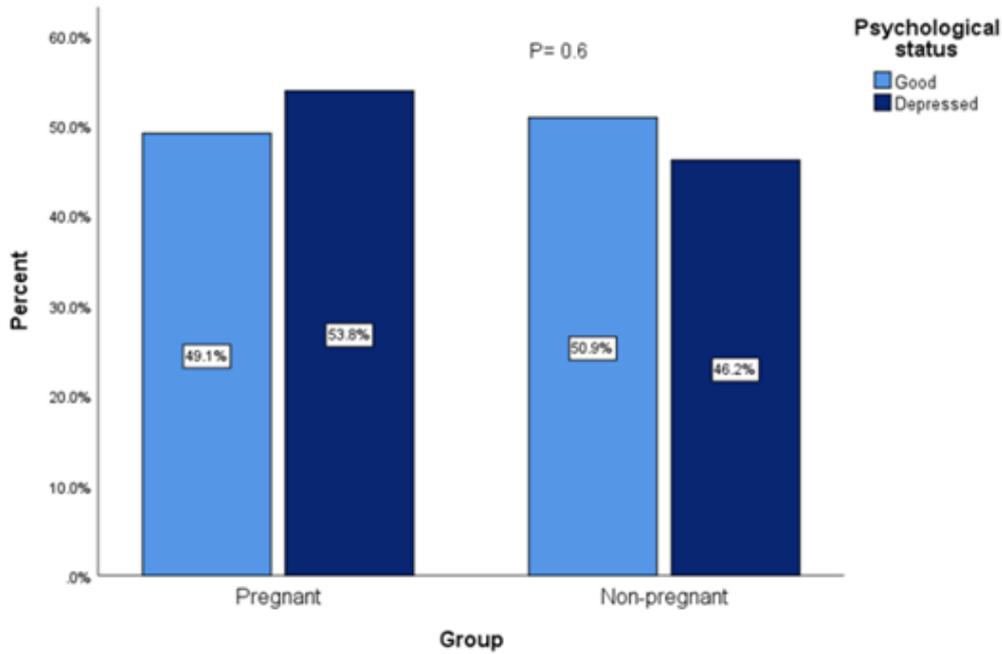


**Fig. 3. 1: Distribution of two groups according to the age and number of each group**



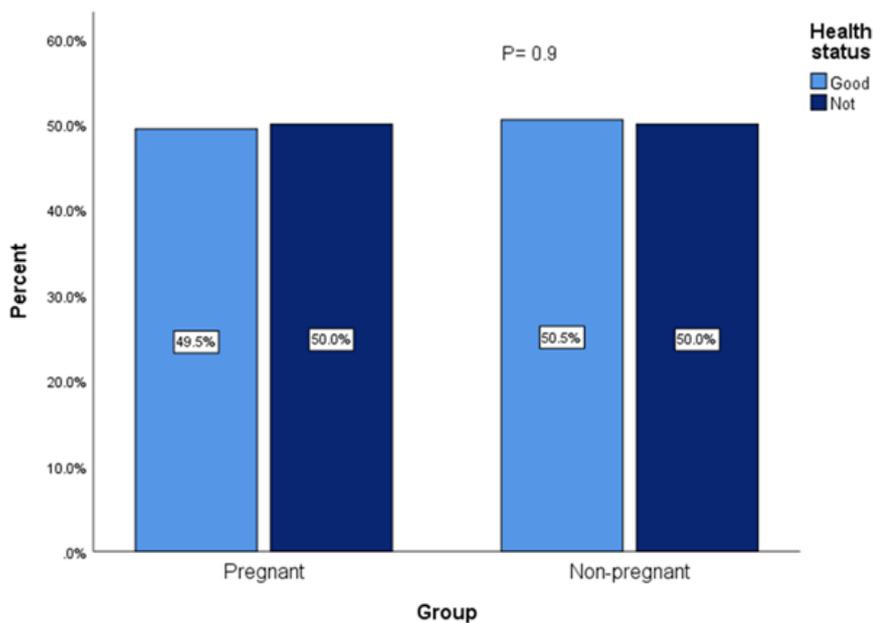
**Fig. 3. 2: Distribution of two groups according to the socioeconomic status**

Psychological status significant in the non-pregnant women ( $P < 0.05$ ) when compared with the pregnant women ( $P > 0.05$ ), as in (figure 3)

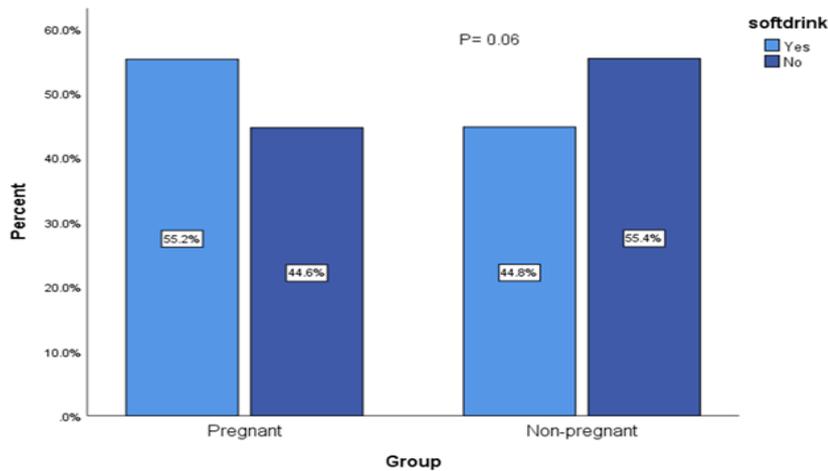


**Fig. 3.3: Distribution of two groups according to the psychological status**

Another demographic characteristics and life style factors were the same as health status, soft drink which more in pregnant women, gingivitis, and using of teeth brush was better in the non – pregnant women, this explained for two groups, as in (figures 4, 5, 6, and 7).



**Fig. 3.4: Distribution of two groups according to the health status.**



**Fig. 3.5: Prevalence of two groups with diet and cola drink**

### 3.2. Dental examination and behavioral

After splitting the socio – demographic and behavioral characteristics, variances were found in the dental caries experience in our study.

Dental history and dental examination for dental caries were including filling and teeth extraction. Appointment of dental clinic visit, brushing the teeth was examined.

Most of pregnant women 154 (97.4%), they felt it was risky for them to receive dental treatment during pregnancy, so they did not visit the dental clinic for checkup or cleaning or any dental work, and they believed to lose a tooth because the pregnancy. If compared with non – pregnant women 127(81.4%), they not visited the dental clinic as in (table 3, and 4).

Level of education was important factor to demonstrate correct way to using teeth brush and this explained in (figure 7), that the group of pregnant women (62.8%) with high level of them no brushing them teeth, if compared with non – pregnant women with (37.2%) no brushing them teeth. In our study the younger pregnant women was the more number who not used teeth brush, at the same more number of low education, and more number of dental caries.

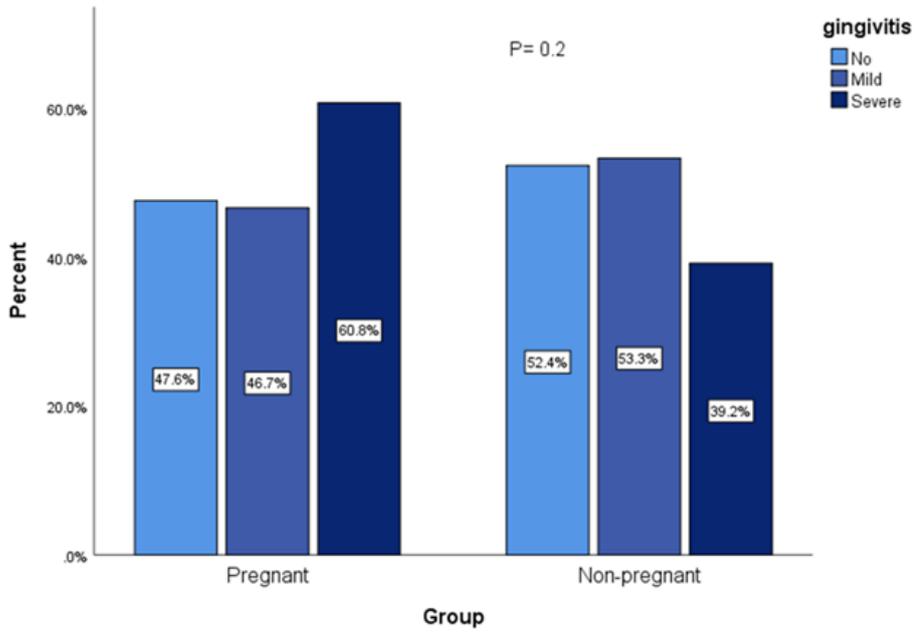
Also the two groups were the same in the filling, and teeth extraction (FT) component, which reflect dental treatments for the pregnant women 62 (40.3%), ( $P < 0.05$ ) compared with non – pregnant the filling 95 (60.5%) ( $P > 0.05$ ), as in (figures 8), and (tables 3, and 4).

**Table 3.3: Distribution for dental caries, filling, and teeth extraction in pregnant women group**

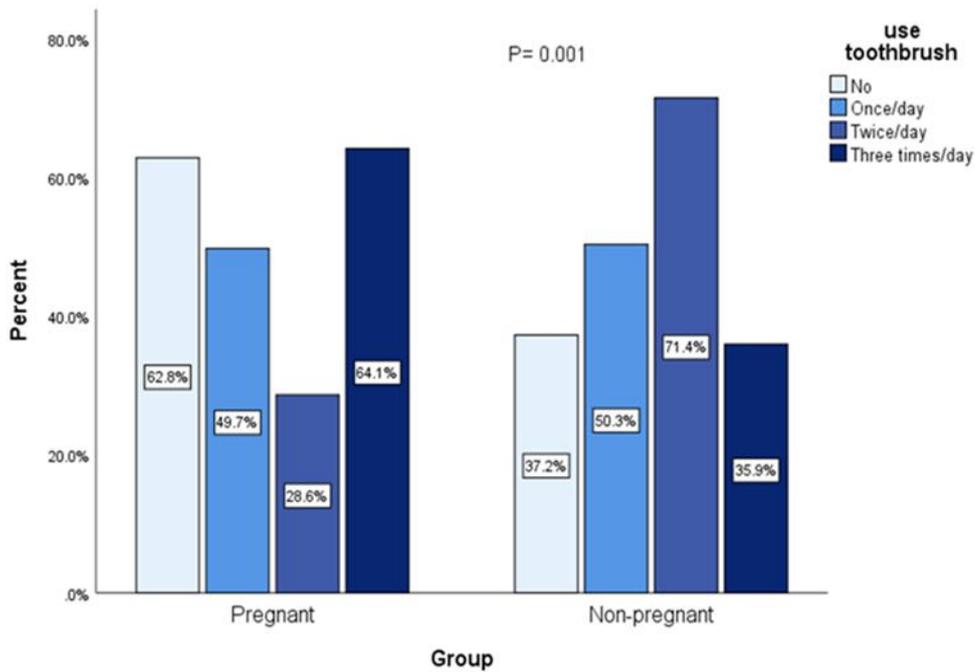
Variables		Age Groups			Total	P
		(15 – 25) yrs (n=72)	(26 – 35 ) yrs (n=69)	(36 – 45) yrs (n=13)		
Caries	Free	7 (7.9%)	11 (15.9%)	1 (7.7%)	19 (12.3%)	0.05
	With	65 (90.3%)	58 (84.1%)	12 (92.3%)	135 (87.7%)	
Filling	Free	55 (76.4%)	34 (49.3%)	3 (23.1%)	92 (59.7%)	0.0001
	With	17 (23.6%)	35 (50.7%)	10 (76.9%)	62 (40.3%)	
Extraction	Yes	18 (25%)	40 (58%)	9 (69.2%)	67 (43.5%)	0.0001
	No	54 (75%)	29 (42%)	4 (30.8%)	87 (56.5%)	
Gingivitis	No	34 (47.2%)	37 (53.6%)	10 (76.9%)	81 (52.6%)	0.2
	Mild	23 (31.9%)	19 (27.5%)	0 (0%)	42 (27.3%)	
	Severe	15 (20.8%)	13 (18.8%)	3 (23.1%)	31 (20.1%)	
Dental visit	Yes	1 (1.4%)	2 (2.9%)	1 (7.7%)	4 (25.3%)	0.4
	No	71 (98.6%)	67 (97.1%)	12 (92.3%)	150 (97.4%)	
Use teeth brush	No	14 (19.4%)	9 (13%)	4 (30.8%)	27 (17.5%)	0.3
	Once/day	40 (55.6%)	40 (5%)	6 (46.2%)	86 (55.8%)	
	Twice/day	4 (5.6%)	11 (15.9%)	1 (7.7%)	16 (10.4%)	
	Once every 2 days	14 (19.4%)	9 (13%)	2 (15.4%)	25 (16.2%)	

**Table 3.4: Distribution for dental caries, filling, and teeth extraction in the non – pregnant women**

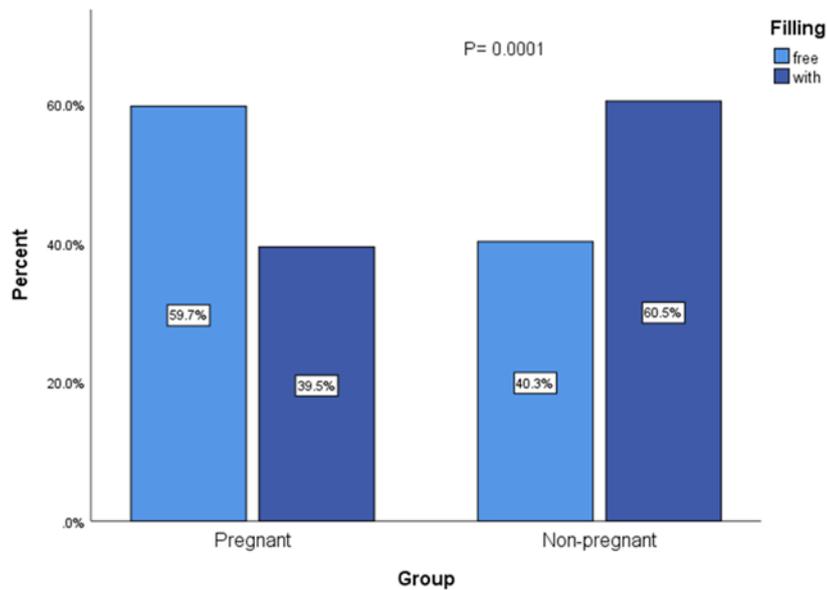
Variables		Age Group			Total	P
		(15 – 25) yrs (n=66)	(26 – 35) yrs (n=45)	(36 – 45) yrs (n=46)		
Caries	Free	21 (31.8%)	6 (13.3%)	12 (26.1%)	39 (24.8%)	0.08
	With	45 (68.2%)	39 (86.7%)	34 (73.9%)	118 (75.2%)	
Filling	Free	34 (51.5%)	18 (40.0%)	10 (21.7%)	62 (39.5%)	0.007
	With	32 (48.5%)	27 (60.0%)	36 (78.3%)	95 (60.5%)	
Extraction	Yes	20 (30.3%)	21 (46.7%)	38 (82.6%)	79 (50.3%)	0.0001
	No	46 (69.7%)	24 (53.3%)	8 (17.4%)	78 (49.7%)	
Gingivitis	No	38 (57.6%)	22 (48.9%)	29 (63%)	89 (56.7%)	0.4
	Mild	22 (33.3%)	14 (31.1%)	12 (26.1%)	48 (30.6%)	
	Severe	6 (9.1%)	9 (20%)	5 (10.9%)	20 (12.7%)	
Dental visit	Yes	14 (21.2%)	1 (2.2%)	14 (31.1%)	29 (18.6%)	0.002
	No	52 (78.8%)	44 (97.8%)	31 (68.9%)	127 (81.4%)	
Use teeth brush	No	6 (9.1%)	4 (8.9%)	6 (13%)	16 (10.2%)	0.4
	Once/day	35 (53.0%)	23 (51.1%)	29 (63.1%)	87 (55.4%)	
	Twice/day	21 (31.8%)	12 (26.7%)	7 (15.2%)	40 (25.5%)	
	Once every 2 days	4 (6.1%)	6 (13.3%)	4 (8.7%)	14 (8.9%)	



**Fig.3.6: Prevalence of two groups with gingivitis**

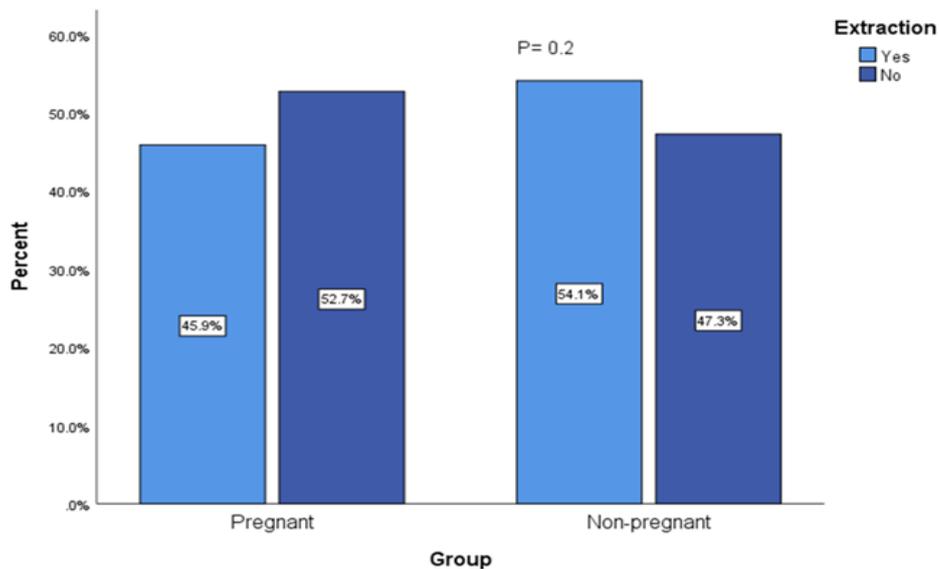


**Fig. 3.7: Prevalence of two groups with teeth brushing**



**Fig. 3. 8: Prevalence of two groups with filling**

The teeth extraction for pregnant 67 (43.5%), ( $P > 0.05$ ), versus the non- pregnant group 79 (50.3%) ( $P < 0.05$ ) as in (figure 9), and (table 3, and 4).



**Fig. 3. 9: Prevalence of two groups with extraction**

For dental caries as explained in (table 3), we showed that 135 (87.7%) of pregnant women had dental caries. In contrast with non – pregnant women were 118 (75.2%) in (table 4).

Tooth decay was more in lower age group and lower educational group. Education was an important determinant of dental caries experiment, so there was statistically a variation between groups. Most of pregnant women at age (15 – 25) yrs, 35 (48.6%) with primary school education and 9 (12.5%) no educated, so that mean 44 (61.1%) with low education for this age only. while if compared with non – pregnant women we had 37 (56.1%) high educated at this age (15 – 25) yrs, who was high education level had low DMFT scores.

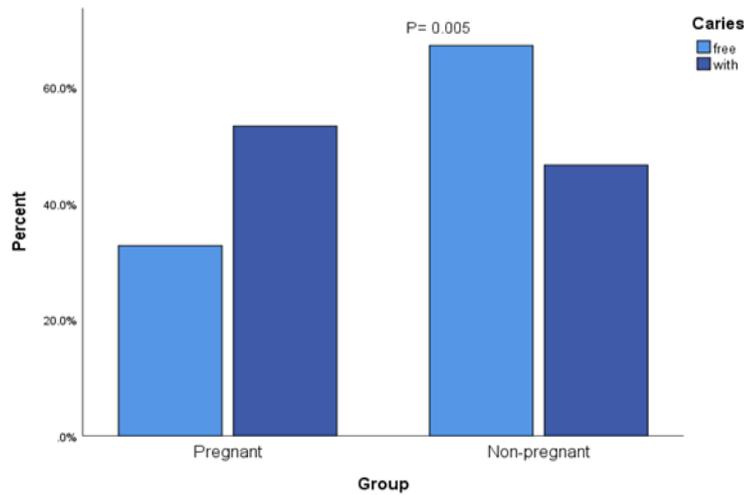
**Table 3.5: Distribution for dental caries for the pregnant and non – pregnant women**

Age group	Pregnant Dental caries		Non – pregnant Dental caries		Total	P
	Free	With	Free	With		
(15 – 25)	7 (7.9%)	65 (90.3%)	21 (31.8%)	45 (68.2%)	138 (44.3%)	0.001
(26 – 35)	11(15.9%)	58 (84.1%)	6 (13.3%)	39 (86.7%)	114 (36.7%)	0.07
(36 – 45)	1 (7.7%)	12 (92.3%)	12 (26.1%)	34 (73.9%)	59 (19%)	0.02
Total	19 (6.1%)	135 (43.4%)	39 (12.5%)	118 (37.9%)	311 (100%)	0.005
P	0.05		0.08			

In this table 5, the differences were very clear, which mean the dental caries in pregnant women was ( $P = 0.05$ ) more than the non – pregnant women was ( $P > 0.05$ ), especially in non – pregnant with the age (15 – 25) years the ( $P < 0.05$ ), and age (26 – 35) years, the ( $P > 0.05$ ) and age (36 – 45) years, the ( $P < 0.05$ ), and the total ( $P < 0.05$ ).

The frequency of dental caries was higher among lower age group of pregnant women [83], so 7.9% free from tooth decay, while 90.3% with tooth decay as in (table 5).

Figure 10 represented the prevalence of dental caries in both groups, showed the inclined toward caries – free in non – pregnant women.



**Fig. 3.10: Prevalence of two groups with dental caries**

The aim of this work was to study the risk index related to dental caries during pregnancy. A risk index may be supposed a risk factor [84]. We did in this study some variables considered as important risk indexes for tooth decay such as dental hygiene habits, and dietary habits.

### 3.3. Vitamin D3, Calcium, Phosphorous, and PTH Concentration

In table 6, the study explained statistically significant differences with ( $P < 0.05$ ) of the variables. In age (15 – 25) years for pregnant group, serum phosphate concentration was increased ( $5.1 \pm 2.8$ ), while non – pregnant group with lower limiting as ( $4.3 \pm 1.4$ ). Serum ionized calcium was with lower normal limiting ( $4.4 \pm 1.1$ ), if compared with non – pregnant group was ( $4.1 \pm 0.9$ ), with ( $P = 0.05$ ). Serum vitamin D3 in pregnant group was very low ( $14.6 \pm 7.3$ ) with ( $P < 0.05$ ), if compared with non – pregnant group was ( $19.9 \pm 11.7$ ).

In age (26 – 35) years for pregnant group, serum phosphate concentration was increased ( $5.3 \pm 2.7$ ) with ( $P < 0.05$ ), while non – pregnant

group with normal limiting as  $(4.2 \pm 1.4)$ . Serum ionized calcium was normal  $(4.9 \pm 0.9)$ , if compared with non – pregnant group was  $(4.3 \pm 1.1)$ . Serum vitamin D in pregnant group was low  $(19.6 \pm 11.2)$ , if compared with non – pregnant group was  $(21.8 \pm 9.8)$ .

In age (36 – 45) years for pregnant group, serum phosphate concentration was increased and very high  $(7.5 \pm 3.2)$ , while non – pregnant group with normal limiting as  $(4.3 \pm 1.6)$ . Serum ionized calcium was normal limiting  $(4.8 \pm 1.1)$ , if compared with non – pregnant group was  $(4.3 \pm 0.8)$ . Serum vitamin D3 in pregnant group was low  $(18.2 \pm 10.5)$ , if compared with non – pregnant group was  $(25.9 \pm 12.3)$ , with  $(P < 0.05)$ .

**Table 3. 6: Comparison in concentrations of vitamin D3, ionized and total calcium and phosphorus based on age of two groups**

	Group		95% Confidence Interval of the Difference		P
	Pregnant (n=154) mean±SD	Non-pregnant (n= 157) mean±SD	Lower	Upper	
15-25 years					
Phosphate (mg/dl)	$5.1 \pm 2.8$	$4.3 \pm 1.4$	0.02624	1.54235	0.04
Ionized calcium (mg/dl)	$4.4 \pm 1.1$	$4.1 \pm 0.9$	-0.00529	0.67827	0.05
Total calcium (mg/dl)	$6.6 \pm 1.2$	$6.8 \pm 1.4$	-0.55183	0.32569	0.6
D3 (ng/ml)	$14.6 \pm 7.3$	$19.9 \pm 11.7$	-8.56322	-2.04188	0.002
26-35 years					
Phosphate (mg/dl)	$5.3 \pm 2.7$	$4.2 \pm 1.4$	0.27346	1.99605	0.01
Ionized calcium (mg/dl)	$4.9 \pm 0.9$	$4.3 \pm 1.1$	0.33732	1.09041	0.0001
Total calcium (mg/dl)	$7.2 \pm 1.1$	$6.9 \pm 1.5$	-0.20359	0.77099	0.3
D3 (ng/ml)	$19.6 \pm 11.2$	$21.8 \pm 9.8$	-6.26875	1.83432	0.03
36-45 years					
Phosphate (mg/dl)	$7.5 \pm 3.2$	$4.3 \pm 1.6$	1.86954	4.44849	0.0001
Ionized calcium (mg/dl)	$4.8 \pm 1.1$	$4.3 \pm 0.8$	-0.07592	1.04612	0.1
Total calcium (mg/dl)	$6.8 \pm 1.04$	$7.2 \pm 1.6$	-1.37708	0.50303	0.4
D3 (ng/ml)	$18.2 \pm 10.5$	$25.9 \pm 12.3$	-15.24945	-0.17931	0.05

**Table 3. 7: Comparison in concentrations of parathyroid hormone based on age of two groups.(Using Mann- Whitney test)**

Age	Group		P
	Pregnant (n=43) Median (IQR)*	Non-pregnant (n=45 ) Median (IQR)	
15-25 years (n=30)	74.2 (98.2)	102.9 (53.7)	0.5
26- 35 years (n=28)	95.5 (72.6)	114.9 (39.1)	0.4
36-45 years (n=29)	101.4 (39.5)	112.9 (192.3)	0.6

\*IQR = interquartile range

The normal value for serum PTH is 14 – 65 pg/ml or ng/ml.

In table 7, we explained the serum concentrations of PTH were increased in pregnant and non – pregnant groups. Note that the measurement of PTH was done to enhance the study, and separate samples were taken from the two groups and because of the presences of high measurement readings, we were taken the median.

In table 8, we were taken the differences in the (1st, 2nd, 3rd ) stages of pregnancy, mean of the serum phosphate increased in the 1st stage and more in 2nd stage and more in 3rd stage , serum ionized calcium was with normal in all stages but the limit in the 1st stage more and reduced in 2nd stage and more reduced in the 3rd stage , serum vitamin D3 considered insufficient in the 1st stage and the deficiency in the 2nd stage and more deficient in the 3rd stage , as explained in table with P values.

**Table 3. 8: Comparison in maternal serum vitamin D3 concentrations, ionized and total calcium, and phosphate at different stages of pregnancy status (1st , 2nd , 3rd )**

variables	Trimester			P
	First (n=32)	Second (n=60)	Third (n=60)	
Phosphate (mg/dl)	5.1 ± 3.03	5.2 ± 2.7	5.7 ± 2.9	0.6
Ionized calcium (mg/dl)	4.9 ± 0.9	4.8 ± 0.97	4.4 ± 1.1	0.02
Total calcium (mg/dl)	7.3 ± 1.2	7.1 ± 0.9	6.6 ± 1.2	0.005
D3 (ng/ml)	20.8 ± 14.4	17.3 ± 8.03	15.2 ± 7.6	0.03

In table 9, we take serum concentration of PTH was decreased also in the 3rd trimester than that of 2nd and 1st trimesters, according to the Kruskal- Wallis test [85].

**Table 3. 9: Comparison in maternal serum PTH according to the pregnancy trimester (Using Kruskal- Wallis test)**

	Trimester			P
	First (n=13) Median (IQR)*	Second (n=13) Median (IQR)*	Third (n=16) Median (IQR)*	
PTH(pg/ml)	111.8 ( 79.6)	100.2 ( 62.9)	90 (66.2)	0.5

**Table 3. 10: Comparison in maternal serum vitamin D3 concentrations, ionized and total calcium, and phosphate at different gravida of pregnancy status**

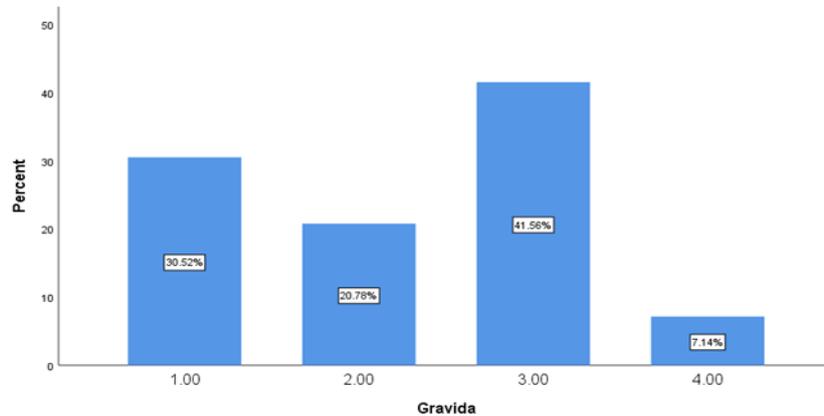
	Gravida				P
	G1 (n=47)	G2 (n=32)	G3 (n=64)	G4 (n=11)	
Phosphate (mg/dl)	5.5 ± 2.9	4.7 ± 2.5	5.6 ± 2.9	5.4 ± 2.8	0.5
Ionized calcium (mg/dl)	4.7 ± 0.9	4.5 ± 1.2	4.7 ± 1.1	4.9 ± 1.4	0.6
Total calcium (mg/dl)	7.02 ± 1.1	6.7 ± 1.2	6.9 ± 1.2	7.2 ± 1.4	0.5
D3 (ng/ml)	17.2 ± 10.4	14.6 ± 6.5	16.9 ± 7.7	26.04 ± 18.5	0.009

In table 10, the gravida 4 was very low number because the old age of pregnant women was low especially from (36 – 45) years. The serum phosphate was increased in all gravida but slightly increased in G2. The serum ionized calcium with normal limiting for all gravida and the less one in the G2. The serum vitamin D3 was reduced in all gravida but more reducing in G2, and deficient in G1, G2, G3, and insufficient in G4. Also the table 9, showed the serum PTH according to the gravida, was increased in all gravida but more increased in G2.

**Table 3. 11: Comparison in maternal serum PTH at different gravida in pregnancy status (Using Kruskal- Wallis test)**

	Gravida				P
	G1 (n=9) Median (IQR)*	G2 (n=12) Median (IQR)*	G3 (n=14) Median (IQR)*	G4 (n=7) Median (IQR)*	
PTH (pg/ml)	96.1 (128.7)	126.5 (78.7)	92.6 (52.3)	99.8 (15.2)	0.6

In the gravida for the pregnant women with vitamin D3, the result was significant ( $P < 0.05$ ) in table 8, and number of children for the non-pregnant women with vitamin D, the result was in table 9 ( $P > 0.05$ ), and in (figures 11, 12).



**Fig. 3.11: Prevalence of pregnant women according to the gravida**

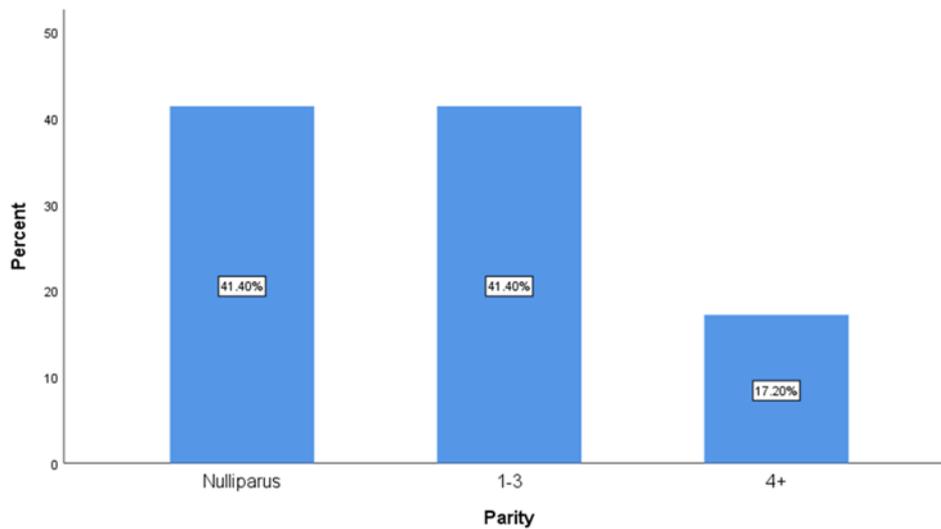
In table 12, showed the serum phosphate was with normal limited but the more less one in childe number 4, and serum ionized calcium was less than the normal limiting in all number of children. Serum vitamin D3 was insufficient in all number but nulliparous women were less than other groups in non-pregnant women. Serum PTH was increased in nulliparous, the number of children from 1-3, but number 4 was the less increased as in (table 11), and (figure 12).

**Table 3. 12: Comparison in serum vitamin D3 concentrations, ionized calcium, and phosphate according to number of children in non-pregnant women**

	Number of children			P
	Nulliparous (n=65)	1-3 (n=65)	4+ (n=27)	
Phosphate (mg/dl)	4.3 ± 1.4	4.4 ± 1.5	3.9 ± 1.5	0.4
Ionized calcium (mg/dl)	4.1 ± 0.9	4.3 ± 0.8	4.2 ± 1.03	0.4
D3 (ng/ml)	20.2 ± 10.4	23.6 ± 11.5	23.9 ± 12.4	0.2

**Table 3. 13: Comparison in PTH according to the number of children in non- pregnant women**

	Number of children			P
	Nulliparous (n=14) Median (IQR)*	1-3 (n=25) Median (IQR)*	4+ (n=6) Median (IQR)*	
PTH (pg/ml)	110.4(88.4)	113.9(78.7)	88.2 (44.6)	0.2

**Fig. 3.12: Distribution of non- pregnant according to the number of children****Table 3. 14: comparison between Vitamin D3, ionized and total calcium, phosphorus, based on dental caries in teeth among pregnant.**

variables	Dental caries		
	Free (n=19)	With (n=135)	P
Phosphate (mg/dl)	4.6 ± 2.5	5.5 ± 2.9	0.05
Ionized calcium (mg/dl)	4.9 ± 1.1	4.7 ± 1.04	0.01
D3 (ng/ml)	19.9 ± 14.8	16.8 ± 8.8	0.05

**Table 3. 15: Comparison between Vitamin D3, ionized and total calcium, phosphorus, based on dental caries in teeth among non- pregnant.**

variables	Dental caries		
	Free (n= 39)	With (n= 118)	P
Phosphate (mg/dl)	4.8 ± 1.2	4.1 ± 0.2	0.01
Ionized calcium (mg/dl)	4.4 ± 0.9	4.1 ± 0.9	0.2
D3 (ng/ml)	26.1 ± 13.9	20.9 ± 10.5	0.02

**Table 3. 16: Evaluate the serum vitamin D3 concentration, in pregnant women and non – pregnant women.**

	Group		P
	Pregnant (n=154)	Non pregnant (n=157)	
D3 (ng/ml)	17.2 ± 9.7	22.3 ± 11.6	0.0001

**Table 3. 17: Evaluate the serum parathyroid hormone, in pregnant women and non – pregnant women (Using Mann-Whitney test)**

	Group		P
	Pregnant (n=43)	Non pregnant (n=45)	
PTH (pg/ml)	100 (67.7)	109.4 (50.4)	0.2

In table 14, showed the comparison between dental caries in teeth among pregnant women in relation to concentration of serum phosphate which

slightly increased in teeth which free from caries, but highly increased in cases of teeth with dental caries. In serum ionized calcium, even with normal limited but less in the pregnant women with dental caries. Serum vitamin D3 was very low or deficient in the pregnant women with dental caries with significant differences and ( $P$  value  $\leq 0.05$ )

If compared with non – pregnant women in table 15, we showed the comparison as, serum phosphate was increased in free group while normal limited with dental caries non – pregnant women. Serum ionized calcium was normally in free group, and decreased with dental caries non – pregnant serum vitamin D was very clear that high in free group than that with dental caries of non – pregnant women.

In table 16, we showed the differences between the concentration of vitamin D into two groups, as in pregnant women were 17.2 ng/ml, with ( $P < 0.05$ ), while in non – pregnant women were 22.3 ng/ml.

In table 17, we showed the differences between the concentrations of PTH into two groups, by using Mann-Whitney test to enhance the study by this test, the ( $P > 0.05$ ).

**Note:** vitamin D3 levels were considered as deficient ( $< 20\text{ng/mL}$ ), and considered insufficient ( $20\text{-}29\text{ng/mL}$ ). The severe deficiency was considered as ( $< 10\text{ng/mL}$ ) [86].

The normal ranges considered were ionized serum calcium ( $4.4\text{--}5.21$  mg/dl), and total serum calcium ( $8.41\text{--}11.62$  mg/dl) [87]. The normal range of PTH ( $10\text{--}65$  pg/mL)[88]. Normal range for inorganic phosphorus ( $2.7\text{--}4.5$  mg/dl) [89].

This data answered approximately all questions, which we were put it previously. There are many studies based on that a good oral health during

pregnancy not only make better to the life quality of pregnant women, but also reduced the difficulty throughout pregnancy and reduced the risk for her child development, as Early Childhood Caries (ECC) in future.

Most pregnancies have a fault idea about the oral cavity health during pregnancy, which lead to neglected them to take care of the oral cavity during this period [79].

This study investigated pregnant and non- pregnant Iraqi women, from many centers and hospitals, and even from private dental clinic. The investigation included the oral cavity health confidences and behaviors and estimated their dental caries experiments.

The samples for two groups randomly selected, and approximately shared the same socio – demographic characteristic.

The results for pregnant group with low educational level, low monthly incomes, and irregular dental visits, and used soft drink, all the pregnant women suffered from dental caries.

These pregnant women also had other oral treatments as fillings, and extraction teeth, and these treatments also related to dental caries in past.

According to investigation by used WHO, Basic Oral Health Survey Methods [80], 87.7% of our pregnant sample was having dental caries. About 57% of DMFT score in our pregnant sample untreated dental caries, with about 21% of the same score with dental filling, and 22% of DMFT score was extracted teeth in this sample. These results explained the high needed of treatment for this group. We take some data published in different areas of the world about pregnant women included dental caries experience, which showed significant differences in these areas.

In Finland, DMFT score of pregnant women (DMFT=18), in Brazil (DMFT=14), and in Hungary (DMFT = 12.57), while in Iran (DMFT = 5.4), in India (DMFT=3.6 and 4.8), which was indicated the lower loaded for the disease in the developing countries of Africa and Asia [90 – 95]. Our result in Iraqi pregnant group (DMFT = 6.2) [81].

These great differences in dental caries experience which happened in by world's various regions are described, the distinction of the culture structures, and socio – economic status, and other characteristics, as changing living conditions, and health their life style, of the samples in each study.

In other hand, the socio –economic status of pregnant group play a role in presented of dental caries, about 79.9% of our sample with low middle and poor socio – economic status, while 20.1% belonged good socio – economic status. There is a verse relationship between the socio – economic status and dental caries, and this increased in adults [96], and especially in pregnant women [97].

Pregnant woman at first time of pregnancy considered a sensible group of peril women; she may be affected by low health education, so she has poor health knowledgement [98]. Socio – economic status is related with oral health in general because people with low socio – economic status had a bad oral and general health if compared to people with the high socio – economic status group, and this due to neglect with poor oral practice, lack of knowledge, improper food intake [99], for pregnant women were founded she needed many important pregnancy requirement and treatment, therefore neglected dental care and leave the dentist's visiting except when feeling pain due to the high cost [100,101] .

Level of education was important factor for dental caries experience, because these women were able to use a correct way of brushing teeth, so

reducing the plaque accumulation on their teeth, in addition showed that level of education considered a factor that affected the personal dental care access, community and organization level [102].

There is a wrong belief about oral health during pregnancy played an important role in defect the teeth, especially dental caries experience, and this belief was strongest foreteller of high level of disease. The belief said that pregnant women must be losing a tooth or teeth in pregnancy, and they also thought that visiting dentists during pregnancy for dental care was unsafe.

These wrong beliefs made us to do this study to document the main causes of tooth decay in pregnant women and to know and prove them as the risk factor for tooth decay and tooth loss, and this relation included serum vitamin D deficiency in pregnant women [103].

In this study was investigating for serum vitamin D<sub>3</sub>, ionized and total calcium, phosphorus, and selection samples for PTH in two groups, pregnant and non- pregnant women, to calculated and documented the deficiency of maternal vitamin D<sub>3</sub> and others parameters as risk factor for dental caries.

The action and metabolism of mineral in normal non – pregnant adult women were maintaining by the concentration of calcium, and phosphorus in the extracellular fluid must be at the narrow normal limiting in plasma, but this processing happened by the action of vitamin D<sub>3</sub>, PTH, and calcitonin on bone, intestine, and kidney. Ionized calcium is the active fraction in metabolic processing, involved many physiological functions, as control of cell membrane excitation, enzyme activation, and exocytosis [104]. The recommendation of calcium daily is 800 – 1200 mg, with normal serum concentration is 4.4 – 5.21mg /dl, and as 47%, while the total body of phosphorus is 650 – 850 g, which mean is presented in skeleton as 85%, and normal serum concentration in adult is 2.7 – 4.5mg/dl Vitamin D<sub>3</sub> has

important role to maintaining the mineral and hemostasis of the body by enhancing the intestinal tract's ability to absorb both phosphorus and calcium. PTH secreted by action of low ionized calcium to activation of vitamin D3 by increased absorption from the intestine, and acting on bone to release calcium and phosphorus, and on kidney by increased activation and formation of vitamin D3, and increased releasing of phosphorus by renal tubule with the urine. Calcitonin action is by reducing bone resorption [104]. The maternal vitamin D3 and mineral in normal pregnancy acted on formation of normal fetus chondrogenesis of limbs and chondral ossification and tooth germ formation start from 32 days and continues to 45 – 56 days of gestation [105].

Skeleton of full normal fetus contained 25 – 30 g of calcium, and depended on mother completely to supply by vitamin D3; calcium, phosphorus, and magnesium, calcium needed more and increased in the 3rd trimester or during latter half of pregnancy [106]. Calcium needed for fetal skeleton mineralization about 140 – 400 gm/d or 100mg /kg fetal mass/d [104].

Calcium homeostasis was a little different in pregnant than the non – pregnant condition, in order to provide the calcium requested for mother and fetus. The total maternal calcium decreased during pregnancy due to hemodilution, but after correction of albumin, the ionized calcium stay almost with normal value, and this explained in this study in (table 6) for all ages of pregnant women [107]. The calcium requested for the fetus came from the skeleton resorption of mother, increased absorption and decreased excretion of calcium with the urine.

In the intestine, much of calcium conservation detected during pregnancy by increased the action of vitamin D3 by double; this is probable to be reversal to increased calcium absorption, and to minimal range released due to high calcitonin level during pregnancy [49, 65].

Theoretically the serum phosphorus level may be usually within the normal limits during pregnancy [107]. In this study the phosphorus level was increased, starting from the first group of pregnant women with age (15 – 25) years, and then more to the older group as hyperphosphataemia, this would be pertinent to Asian pregnant women and infants.

One serious inclusion advanced hyperparathyroidism in Asian pregnant women, which was the possible effect of hyperparathyroidism on bones, especially in the third trimester during pregnancy [85], as the same in the study.

This may had been participated and associated the hyperparathyroidism, because when we investigated some cases of pregnant women showed increasing level of PTH gradually from younger age to older age in pregnant women and this explained in (table 7).

There are different studies were done in different population around the world in order to determine the rates of vitamin D deficiency among pregnant women in different countries in the world, and this studies were conducted in the year 2015 from about 86 studies, and found different concentrations of vitamin D, the differences in maternal average of vitamin D3 concentration founded to be highly variable among these regions and may be variable in same region [108], according to that will be mentioned the mean of vitamin D3 concentration , in America population about (18.8 – 26 ng/ml), in Europe was between (6 – 28.8 ng/ml), in South – East Asia about (8 – 20.8 ng/ml), in Eastern Mediterranean between ( 5.2 – 24 ng/ml), and in Western Pacific about (16.8 – 28.8 ng/ml). There is only one study done African pregnant women, they founded the vitamin D3 concentration mean was (38 ng/ml), this included that the prevalence of vitamin D deficiency or insufficiency of vitamin D3 concentration between (10 – 20 ng/ml) in pregnant women [109]. A study was done in North India showed the vitamin D3 concentration in pregnant women was (< 22.5 ng/ml) [107]. There was a study done for Iraqi non – pregnant

women to determine the concentration of vitamin D3 for the Iraqi pregnant population, the mean was (14.06 ng/ml)[110].

In this study, there was a significant decrease in vitamin D concentration in pregnant women if compared with non – pregnant women, and this explained in (table 16).

In all nationalities, ethnicity, and religions, there was a severe deficiency of vitamin D3 in pregnant women.

In this study showed, there was significant decrease of vitamin D3 in the pregnant women according to the age, pregnancy status, different gravida of pregnancy, and in dental caries as between “free” and “with”.

Almost all pregnant women are around the world have a vitamin D deficiency, because during pregnancy, the fetus was completely dependent on mother in supply of vitamin D3 and other minerals.

Some of these studies done in the world as a comparison done between Caucasian and Asian pregnant women, in this study both groups have a significant decrease in vitamin D3 concentration, but Asian pregnant women were have a vitamin D3 concentration significantly lower than that in Caucasian pregnant women [85], and this gave a strong defense for the study because the Iraqi pregnant women are part of Asian pregnant women.

The other fact in the study that there was increased in PTH levels when measured for some pregnant women as a reinforcement of the study, as in (table 17) and this increase is considered normal in Asian pregnant women to compensate for the deficiency in vitamin D3, as explained that the lower concentration of vitamin D in Asian pregnant women reflected in the significant elevated concentration of PTH in serum of Asian pregnant women and Caucasian pregnant women, this increased in PTH concentration whether

stimulated the kidney to produce 1, 25 (OH)<sub>2</sub> D<sub>3</sub>, or made greater metabolism of bone and mother skeleton to producing more calcium for fetal growth, or to conserve the renal calcium lose, or to compensate the PTH sensitivity during pregnancy [85].

Another study, was done previously in the Colombo District in Sri Lanka, explained the prevalence of vitamin D deficiency in pregnant women was 62%. The prevalence of vitamin D deficiency observed in this study was high with other factors were determinant serum PTH as serum vitamin D<sub>3</sub>, calcium, weeks of pregnancy, and educational levels of pregnant women.

There was significant inverse relationship observed in this study between vitamin D<sub>3</sub> and PTH, the pregnant deficient women with (< 20ng/ml) had a higher level of PTH [111]. There are another factors effected the level of PTH done in the study considered potential factors, such the general population as non – pregnant, if compared to pregnant women [112], ethnicity [113], age [114], and age of gestation [115], also the season and the increasing PTH concentration in African American women if Caucasian women are contrasted with African American women [85, 116], When compared to pregnant Swiss and German women in late stages of gestation, findings of this study also revealed that women of African as well as Middle Eastern background have a low level of PTH in spite of low concentration of vitamin D<sub>3</sub> [117 – 119], the color of the pregnant women [120, 121]. Other factors showed important relation between the concentration of PTH and vitamin D<sub>3</sub> involved physiological change in PTH during pregnancy, socio – demographic characteristic like educational status which cause a good indicated for lowering PTH level [122, 123], economic status with good lifestyle and healthy [124], the demanded of calcium and extracellular phosphate balance during pregnancy could impact indirectly or directly increasing concentration of PTH [125, 126].

The circadian rhythm of PTH might affect the level of PTH at different time at a day especially it influenced by maternal dietary calcium and PTHrP [50].

Researchers showed that calcium intake causes increased in level of PTH even there was low concentration of vitamin D3 (< 10 ng/ml) if the calcium less than 800 mg/day [127]. The work and spend time outdoor as exposed to sun which caused increased in vitamin D3 level if compared with insufficient low vitamin D3 indoor [119]. The concentration of PTH increased more in third trimester [128].

In our study, because the vitamin D3 concentration in pregnant women was 17.2 ng/ml and P value < 0.05 and this mean high significant, and concentration of PTH was 100 pg/ml by using Mann-Whitney test, therefore there was inverse relationship between the low serum concentration of vitamin D3 with high serum level of PTH in group of pregnant women.

The pregnant women demanded high calcium, so bone turnover high, so bone mineral density decline, and this decline suggested occurring influenced by PTH, and other many hormones, as growth hormone, prolactin, estrogen, nutritional habit, and life style [107].

In all past studies were done, calcitonin concentration was increased during pregnancy, so may be induced relative resistant to PTH by continual calcium. Also, because needed of fetus to more transferred maternal calcium [129]. Calcitonin elevated and then decreased after delivery and returned to increasing during lactation. They suggested that increased in calcitonin to protect the skeleton in pregnant women from demineralization, and increased in second and third trimester always, and had a role in calcium metabolism and bone ossification of fetus mostly in third trimester, so it considered a positive calcium balance during pregnancy for mother and fetus.

PTH secretion was increased during pregnancy that means increased absorption of calcium double from the intestine, PTH increased osteolytic activity, so calcitonin reduced this processing and allowed calcium to keep the action of PTH to spending on the kidney and gut while the fetus taken the calcium, and protected the maternal skeleton at the same time [130].

Through perusal and research regarding dental caries in pregnant women and the oral care and hygiene of the mouth and teeth during pregnancy, I found that most researchers believe that tooth decay has multiple causes, and this is a realistic fact and depends on the type of food, including carbohydrates, the process of cleaning the mouth, civilized, the type of food and drinks that contain sugars, sweets, using of fluoride, and other causes.

They added other reasons for tooth decay in pregnant women, including the acidity of vomiting, lack of regular visit to dental clinic, failure to treat infected teeth, lack of correct diagnosis by the dentist, and changes in some hormones in pregnant women which causes infections in the mouth and tooth decay. Decay of teeth as a result of these reasons, but there are more important reasons, and because I am a dentist and biochemistry specialist, I would like to prove here in this study that we did, the main and important cause of tooth decay is vitamin D deficiency and the accompanying biochemical changes in the rest of the vital variables such as calcium, phosphate, parathyroid hormone.

The fetus's need for these requirements to form and build the skeleton and the formation of tooth buds during pregnancy from 25 – 55 days of the fetus's life in the first trimester of pregnancy, and includes the tooth buds of deciduous and permanent teeth, and then strengthening, building and growth of the skeleton in the last stage of pregnancy (third trimester), and these are all factors that negatively affect the pregnant woman and lead to an increase in the rate of caries, this by that vitamin D deficiency has a significant effect to

changes in salivary level to the selected physicochemical characteristics (pH, flow rate, calcium ion, and phosphorus ion) [5].

In addition to that recent studies have proven the relationship of vitamin D deficiency genetically.

Through the tables in results, presented and discussed, showed this relationship, which is vitamin D deficiency in the serum of pregnant women. It is a risk factor for the occurrence and presence of tooth decay. Therefore, this risk factor must be observed and treated by the consultation between the gynecologist and dentist to avoid this problem. This threatens the teeth of pregnant women a lot and thus leads to damage to the teeth and ends with extraction.

In addition, all studies have shown that vitamin D deficiency is the main cause of several diseases in pregnant women, including high blood pressure (preeclampsia), gestational diabetes, premature births, and low fetal weight during pregnancy and after childbirth, termination of pregnancy by caesarean section, and exposure of pregnant women to fracture, and defect in teeth formation of fetus which lead to early childhood caries (ECC). Bone, and joint pain in pregnant women, hair loss, muscle pain and other health conditions, but we explained that the maternal vitamin D deficiency was a risk factor for dental caries.

As a hormone, vitamin D has endocrine functions to regulate the intestinal tract's maintenance of phosphate and calcium equilibrium [131, 132]. Autocrine and paracrine mechanisms of vitamin D<sub>3</sub> action agents, by controlling cell differentiation, cell mature, also acts on immunity of body [133 – 135].

Vitamin D receptors (VDR) were responsible for the cellular effects of vitamin D [133, 135 –137].

Vitamin D<sub>3</sub> depended in its actions on genomic effects of VDR on the membrane associated protein to nongenomic effects [138]. So vitamin D modulated about 5 – 10% of gene expression [133].

There was a public realization about vitamin D deficiency around the worldwide and increased prevalence of this deficiency [131, 139– 144].

The prevalence distributed and focuses on special groups, as children, pregnant women, and some forms of cancer [145 – 148].

Both dental decay and gum disease considered popular diseases connected to VDD and had pathophysiological procedure [149 – 152].

The new research detected that VDD effected odontogenesis and caused hypominerized dentition which lead to dental caries and fracture of tooth [150]. Also during pregnancy VDD caused a periodontitis in addition to caries [148, 153, 154]. Teeth are mineralized organs, contained three hard tissues; enamel, dentin, and cementum, surrounded by alveolar bone. Tooth mineralization happened with the skeletal mineralization, any defected in mineral metabolic distributing or failure occurs in both bone and tooth. Vitamin D<sub>3</sub> has important role in mineralization of tooth and bone [155, 156]. The central biological rule were depending on the reality that sever VDD (< 10ng/ml) caused secondary hyperparathyroidism together with hypophosphatemia and hypocalcemia [157, 158]. The increased calcium absorption from intestine caused by this hyperparathyroidism, also activated renal production of 1,25 – dihydroxy vitamin D, and decreased serum inorganic phosphate level [155, 158]. The first hypophosphatemia severely declined, so vitamin D<sub>3</sub> loosed singling mechanisms in tooth cells with low levels of calcium and phosphate ions concentration caused inhibition for proper mineralization of teeth defects occur [155].

During pregnancy rapid caries development, the most causes of this caries is due to vitamin D deficiency in this period, the needs of fetus for more calcium and phosphate to form and build up the skeleton lead to demineralization of both bone and teeth.

Already the main function of vitamin D3 is to formation and maintenance a good and healthy bone and teeth, when vitamin D will be reduced and deficient already this processing will be defected [159].

Now a day there are many studies to explaining that dental caries is a genetic disease [159]. These studies focused on the important factors that influenced the dental caries, and this factor is vitamin D through the action of vitamin D3 for tooth and bone formation and development and biomineralization [160, 5]. The cause of dental caries was genetic cause as well as the environmental factors [161, 162].

# **Conclusion and Recommendation**

**Conclusion:**

1 – From this study, we concluded that the comparison between the pregnant women versus women who are non – pregnant focused on many parameters included vitamin D3, ionized calcium, phosphorus and parathyroid hormone. These parameters influenced the dental caries in Iraqi pregnant women. There are a huge factors affected this result, the most and important one was vitamin D deficiency, even the deficiency of vitamin D was more and significant in pregnant women , however it was also insufficient in non – pregnant women and there was dental caries but less than that in pregnant women.

2 – In addition to other factors as socio – economic status, educational status, nutrition and soft drink, gravida, periodic visit of dental clinic, good oral hygiene and using of brush.

3 – The maternal demanded of vitamin D during pregnancy with mineral needed to the normal fetus skeleton formation and development will be the cause for demineralization of bone and teeth during pregnancy, and maternal vitamin D deficiency lead to more and sever skeletal defect in this period and one of these defect in teeth.

4 – Vitamin D deficiency was strongly involved the oral cavity disease and had been associated with dental decay danger, also other oral cavity defect like oral soft tissues. Dental health was a component of general health and should be take care for both mother and fetus.

5 – The result from this study was to provide that confirm the value and import of vitamin D concentration were lower in Iraqi pregnant women and considered as risk of dental caries.

**Recommendation:**

1 – doing and documented future studies for Iraqi pregnant women, in order to confirm this matter and take the necessary measures to reduce tooth decay in pregnant women and reduce their tooth loss, as well as ensuring the health of teeth in newborns and controlling oral health.

2 – Vitamin D level, and calcium must be maintaining at normal level in pregnant women to protect the pregnant women and fetus from many disease and one of them destruction of teeth and bone during pregnancy, and prevented the early childhood caries in newborn babies.

3 – Consultation between the gynecologist doctor of pregnant women and the dentist to treat the carious tooth during pregnancy. There is good time for treatment a pregnant woman, including the second trimester, in this time can be doing any type of oral treatment. Educating pregnant women about the importance of teeth and taking care of them through the use of brushing and visiting the dentist periodically.

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## الخلاصة:

تسوس الأسنان، المعروف أيضًا باسم نخر الأسنان، هو انهيار الأسنان بسبب الأحماض التي تصنعها البكتيريا، في المقام الأول العقديّة الطافرة التي تستقلب السكريات لإنتاج الحمض، والذي يؤدي بمرور الوقت إلى إزالة المعادن من بنية الأسنان

تتكون الاسنان من ثلاث طبقات تحتوي على مواد صلبة منها الكالسيوم و الفوسفور ، وتتكون من ثلاثة أنسجة صلبة مميزة (المينا ، العاج ، السمّنت). تحدث عملية اضافة المعادن للأسنان بالتوازي مع اضافة المعادن للهيكل العظمي ، ولكن إذا حدث اضطراب في التمثيل الغذائي للمعادن ، فسيحدث الفشل بشكل مشابه لتلك التي تحدث في أنسجة العظام.

تعتبر النساء أكثر عرضة للتأثر بتسوس الأسنان أثناء الحمل لأن النساء الحوامل يواجهن العديد من التغيرات الفسيولوجية. قد تكون هذه التغييرات موضعية أو جهازية أي تشمل اجزاء و اجهزة الجسم ، مثل تلك التي تحدث في تجويف الفم. تعد صحة الفم جزءًا ضروريًا من الصحة العامة، لذلك يجب أن تظهر مشاكل تجويف الفم عند النساء الحوامل على الفور.

يعتبر فيتامين د هو هرمون ستيرويدي قابل للذوبان في الدهون ، لتنظيم العمليات البيولوجية المختلفة ، مثل تكوين العظام والأسنان والتمثيل الغذائي والاستجابة المناعية.

أهم عمل لفيتامين د هو زيادة الامتصاص النشط للكالسيوم من تجويف الأمعاء.

يعد نقص فيتامين د مشكلة وبائية بسبب انخفاض تناول فيتامين د 3 من الطعام لا يمكن أن يلبي الاحتياجات اليومية لفيتامين د لكل من البالغين والأطفال ، كما أن التعرض لأشعة الشمس ينخفض في الوقت الحاضر بسبب استخدام واقيات الشمس التي تزداد في كل من أشهر الصيف والشتاء.

ينظم جين مستقبل فيتامين (د) وظيفة مستقبل فيتامين (د) بيولوجيًا ، وعلى هذا النحو يلعب وظيفة حاسمة في نمو الأسنان ، لذلك يلعب دورًا رئيسيًا في مجموعة الأسنان ، وخاصة في تمعدن المينا وعاج الأسنان. نتيجة لذلك ، نقص نمو المينا مثل نقص تنسج المينا. قد يكون نقص فيتامين د أثناء الحمل مشكلة صحية شائعة في جميع أنحاء العالم. حالة فيتامين د 3 أثناء الحمل لها تأثير مهم على الجنين لأنها تعتمد بشكل كامل على مخازن الأمهات من فيتامين د 3 لنمو الهيكل العظمي للجنين وتطوره

كان الهدف من هذا العمل هو دراسة عوامل الخطر المتعلقة بتسوس الأسنان أثناء الحمل. في هذه الدراسة تم اعتبار بعض المتغيرات بمثابة مؤشرات خطر مهمة لتسوس الأسنان مثل نقص فيتامين د

وغيرها من العوامل مثل الكالسيوم والفوسفور وهرمون الغدة جار الدرقية وعادات نظافة الأسنان والعادات الغذائية.

تم جمع عينات دم من (311) أنثى من متطوعين أصحاء. تم تقسيم عينات الدم إلى مجموعة ضابطة وهي مجموعة غير حامل، وبلغ عدد هذه المجموعة 157 امرأة، وتم جمع 154 عينة دم من النساء الحوامل. وكان العمر أحد الخصائص الاجتماعية الديموغرافية التي اعتبرت المتغير التابع وقسمت إلى المجموعات الثلاث (15 – 25)، (26 – 35)، (36 – 45) سنة.

هناك انخفاض كبير في تركيز فيتامين د3 لدى النساء الحوامل بالمقارنة مع النساء غير الحوامل. فكان تركيز فيتامين د عند النساء الحوامل 17.2 نانوجرام/مل وقيمة الانخفاض واضحة جدا.

أجريت هذه الدراسة لفحص فيتامين د في الدم، والكالسيوم المتأين والكلى، والفوسفور، وعينات مختارة من هرمون الغدة جار الدرقية في مجموعتين، النساء الحوامل وغير الحوامل، لإثبات وتوثيق أن نقص فيتامين د وكذلك العوامل الأخرى لدى الأمهات هو عامل خطر لمرض تسوس الأسنان.

يجب الحفاظ على مستوى فيتامين د3، والكالسيوم عند المستوى الطبيعي لدى النساء الحوامل لحماية المرأة الحامل والجنين من العديد من الأمراض ومنها تدمير الأسنان والعظام أثناء الحمل، ويمنع الإصابة بالتسوس في مرحلة الطفولة المبكرة عند الأطفال حديثي الولادة.



جمهورية العراق  
وزارة التعليم العالي والبحث العلمي  
جامعة بابل  
كلية الطب

تأثير بعض المتغيرات الكيموحيوية على تسوس الاسنان للنساء  
الحوامل في العراق

اطروحة مقدمة الى

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كجزء من متطلبات نيل درجة دكتوراه فلسفة في الكيمياء الحياتية  
السريية

من قبل

سناء عبد الرزاق ابراهيم محمد شريف الربيعي

بكالوريوس طب وجراحة الفم و الاسنان – جامعة بغداد 1985

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بإشراف

الاستاذ الدكتور

علاء جعفر محراث

الاستاذ الدكتور

مفيد جليل عوض