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Study of risk factors and some biochemical, hematological, hormonal and oxidative stress markers to predict and follow up of gestational diabetes women in El-Oued region

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Abstract:

Gestational Diabetes Mellitus (GDM) is one of the most common pregnancy complications, has increased by more than 30 (%) within one or two decades in a number of countries forming an emerging worldwide, the aim of our work is to evaluate some risk factors and biological markers and stress oxidative status for determining some predictive factors of GDM in patients of El Oued region. Our socioeconomic risk factors study was conducted on 140voluntary women divided into70 healthy women, mean age 28.145±0.767years and 70 GDM patients mean age 34.938±0.736 years. For biological study, we have selected 15 healthy control mean age 27.67±1.77years and 15 women GDM patients mean age 34.64.± 1.03 years. Some biochemical, hematological, trace elements, hormonal and oxidative stress markers were analyzed in our study.

The results of risk factors study illustrate the high relationship between some socioeconomic comportments such as passive smoking, social problems and clinical factors such as obesity, contraceptive pills and gestational diabetes, but family history and chronic disease are the most dangerous risk factors, (OR =58.10, OR =33.22) respectively, for GDM. While sports and daily activity are important protective factors against this disease. For biological study, our results reveal a significant change ($P<0.05$) of hematological, biochemical, hormonal, oxidative stress and some minerals markers in GDM patients as compared to control, with a significant ($P< 0.05$) relationship between the changes of both thyroid hormones (T3 and TSH) and the oxidative stress markers (MDA , CAT and ORAC) on the one hand and the changes in levels of serum zinc and serum copper and the level of oxidative stress in both GDM with and without pregnancy on the other.From this study we found that some of oxidative stress markers have a high sensitivity, specificity and AUC values which qualify them to be important markers for diagnosing and predicting Gestational diabetes disease in women.

Finally, we conclude that there are a most socioeconomic and clinical risk factors and predictive factors for GDM disease. Also a change in the serum level of thyroid hormones and the level of mineral elements in relation with oxidative stress of women with gestational diabetes contributes to the development or complication of the disease after pregnancy.

Key Words: GDM; Risk factors; Oxidative stress; Thyroid hormone, Zn, Cu.

الملخص:

يعتبر داء سكري الحمل أكثر مضاعفات الحمل شيوعا ،فقد زاد بنسبة أكبر من 30% في غضون عقد أو عقدين لعدد من البلدان الناشئة في العالم. وعليه فإنالهدف من عملنا هذا هو تقييم بعض عوامل الخطر ،تغير بعض المعايير البيولوجية ومعايير الإجهاد التأكسدي لتحديد بعض عوامل تنبؤ هذا الداء لمرضى من منطقة الوادي.

أجرينا دراسة تحديد عوامل الخطر على 140 امرأة متطوعة قسمن إلى 70 سليمة (كشواهد) متوسط عمرهن28.145±0.767 سنةو70 امرأة مصابة بداء سكري الحمل متوسط عمرهن 34.938±0.736سنة ولدراسة تغيرات المؤشرات البيولوجية اخترنا 15 امرأة شاهدة بمتوسط عمر 27.67±1.77سنة و15 امرأة مصابة بهذا المرض بمتوسط عمر34.64±1.03سنة.تم إجراء تحاليل لمكونات الدم ،هرمونات الغدة الدرقية ، معادن وعوامل الإجهاد التأكسدي كمعايير بيولوجية لتقييم المرض في هذا العمل.

توضح نتائج دراسة عوامل الخطر ارتباطا قويا بين العوامل الاجتماعية والاقتصادية مثل التدخين السلبي والمشاكل الاجتماعية والعوامل السريرية مثل السمنة ،مداومة تناول حبوب منع الحمل وبين الإصابة بداء سكري الحمل ،لكن التعرض للأمراض المزمنة والعامل الوراثي هما الأخطر بقيمة (OR=58.10;OR=33.22)على التوالي .في حين أن ممارسة الرياضة و حركية الإنسان اليومية عاملان مهمان للحماية من هذا المرض دائما حسب النتائج.

بالنسبة لدراسة المعايير البيولوجية عند مرضى سكري الحمل ،تشير نتائجنا إلى حدوث تغير كبير في مكونات الدم ،المعايير البيوكيميائية ،الهرمونية ومؤشرات الإجهاد التأكسدي وفي مستوى بعض المعادن الأساسية لمرضى داء سكري الحمل مقارنة بالشواهد مع الإشارة إلى وجود ارتباط معنوي ($P<0.05$)في العلاقة بين اضطراب هرمونات الغدة الدرقية (T3, TSH) وظهور الإجهاد التأكسدي (MDA, CAT, ORAC) من ناحية، وأيضا بين تغير في مستويات عنصر الزنك والنحاس في المصل ومستوى الإجهاد التأكسدي عند المصابات بالمرض الحوامل منهن وغير الحوامل من ناحية أخرى. في هذه الدراسة لاحظنا أيضا أن بعض عوامل الإجهاد التأكسدي لها حساسية عالية وخصوصية كبيرة والتي تؤهلها لتكون معاييرا مهمة لتشخيص مرض سكري الحمل والتنبؤ به.

في النهاية، يوجد العديد من عوامل الخطر المتعلقة أساسا بالسلوكيات الاجتماعية وطريقة حياة النساء الحوامل كما أكدنا على إبراز دور بعض المعايير التنبؤية لداء سكري الحمل ،أيضا التغير في الهرمونات و العناصر المعدنية له علاقة بالإجهاد التأكسدي للنساء المرضى والتي تؤدي لتطور هذا الداء أو ظهور مضاعفاته أثناء أو بعد الحمل .

الكلمات المفتاحية: داء سكري الحمل ،عوامل خطر،إجهاد تأكسدي، هرمونات الغدة الدرقية ،زنك ,نحاس.

Summary

Summary

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Figures list

Tables list

ALP: Alkaline phosphatase.

AUC: Area under curve.

BHT: Butylated hydroxytoluene.

BMI: Body mass index .

CAT: Catalase.

$CuSO_4$: Copper sulphate.

CuZn SOD: Copper-zinc super oxide dismutase.

DNA: Deoxyribonucleic acid.

DTNB: 5,5′-Dithiobis(2-nitrobenzoic acid) .

EDTA: Ethylene diamine tetraacetic acid.

ERS: Endoplasmic reticulum stress

FFA: Free fatty acid.

FHD: Family history of diabetes mellitus

FNS: Hematological analysis.

GDM: Gestational diabetes mellitus.

GLUT4: Glucose transporter type 4

GSH: Reduced Glutathion.

GSH-Px: Glutathione peroxidase

H_3PO_4: Phosphoric acid.

H_2O_2: Hydrogen peroxide

H_2SO_4: Sulfuric acid

Hcg: Human chorionic gonadotropin.

HCl: Hydrochloric acid.

Hct: Hematocrit.

HDL: High density lipoprotein.

Hgb: Hemoglobin.

***HNF4A*:** Hnuclear factor 4 alpha

HNO_3: Nitric acid.

ID: Iron deficiency.

IDA: Iron deficiency anemia.

IGF-1: Insulin-like growth factor 1.

JNK: c-Jun N-terminal kinase.

KH_2PO_4: Phosphate-buffered.

LDL: Low density lipoprotein.

LYM: Lymphocytes.

MDA: Malondialdehyde.

NaCl: Sodium Chloride.

NO: Nitric oxide.

OGTT: Oral glucose tolerance test.

OR: Odd Ratio.

ORAC: Oxygen radical absorbance capacity.

OS: Oxidative stress.

P-IRE-1α: Phosphorylation of inositol-requiring enzyme 1α.

PLT: Platets.

P-Ser-IRS-1: Insulin receptor substrate 1 at serine307.

RBC: Red blood cell.

ROS: Reactive oxygen species.

SHS: Second-hand smoke.

T_3: Triiodothyronine.

T_4: Thyroxine.

TBA: Thiobarbituric acid.

TBG: Thyroid binding globulin.

TCA: Trichloroacetic acid.

TG: Triglyceride.

TH: Thyroid hormone.

TNF: Tumour necrosis factor.

TSH: Thyroid stimulating hormone.

VLDL: Very low density lipoprotein.

WHO: World Health Organization.

$Zn(O_2CCH_3)$: Zinc nitrat .

Introduction

Introduction

Pregnancy is a period of rapid growth and cell differentiation for both the mother and fetus(Al-Jameil et *al*., 2014), the pregnant woman presents a diagnostic challenge as physiological ; anatomical and biochemical changes of pregnancy may mask symptoms and signs, as well as the pregnancy itself being the source of the problem (Graham et *al*., 2015). Acute complications of pregnancy can appear in all trimesters, their diagnosis and management are great challenges factors affecting pregnancy outcome are socioeconomic status, smoking status and other health related conditions and behaviors (Khaskheli et *al*., 2010). Pregnancy induces changes in maternal glucose metabolism and insulin sensitivity, pregnant women are able to meet the increased insulin demand, but in some cases these needs are not met resulting in poor glycemic control and consequently GDM (Shelley et *al*., 2014).

Gestational Diabetes Mellitus (GDM) is a non-communicable disease affecting pregnant women (Ferrara, 2007). Globally the median estimates of GDM range from 6 to 13% (Zhu & Zhang, 2016).In Algeria, statistics 2015 show that 6 % of pregnant women have gestational diabetes, with national data showing that of 100 pregnant women, 6 are diagnosed with gestational diabetes, which requires early diagnosis and accurate medical follow-up to avoid complications. Until now , there is not any directly cause of GDM but several risk factors are introduced as causative factors of developing gestational diabetes mellitus such as advanced age (≥35 yrs.), overweight or obesity, family history of diabetes (Larrabure et *al*., 2018),excessive gestational weight gain, excessive central body fat deposition, short stature (<1.50 m), excessive fetal growth, hypertension or preeclampsia in the current pregnancy, and polycystic ovary syndrome (Renata et *al*., 2015). GDM is a substantial and growing health concern in many parts of the world has serious, long-term consequences for both baby and mother (Reece et *al*., 2009),including increased risk of miscarriage, stillbirth, preterm delivery, congenital malformations, fetal macrosomia, neonatal hypoglycemia, obesity and insulin resistance in women and childhood, followed by impaired glucose tolerance and type 2 diabetes later in life (Byrn & Penckofer, 2013; Rasmussen & Harlow , 2010).

Many studies have suggested that the cause of progression GDM is the oxidative stress (Sudharshana et *al*., 2018) that reported increased free radical production and antioxidant depletion in gestational diabetes to be a causative factor in increasing the risk of

congenital anomalies and some investigators have reported increased lipid peroxidation and significant depletion in antioxidant capacity during the development of gestational diabetes (Vida& Zamzam, 2017).

In light of these data, the aim of our work is based on the realization of two following complementary aspects:

- **The first part:** is to study the risk factors associated with gestational diabetes that several types of factors are studied in this context: socioeconomic, environmental and clinical factors.

- **The second part:** is an biological study concerns the determination of the variation and specificity of some biochemical, hematological and oxidative stress markers in the prediction and diagnosis following up on gestational diabetes and finally to study the relationship of mineral and hormonal metabolism dysfunction with gestational diabetes as an agent of initiation or progression of this disease in women of El-Oued population.

First part

Theory part

I. Diabetes mellitus:

I.1. Definition:

Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia , resulting from a deficiency in secretion or the action of insulin or both related (Drouin et *al*., 1999;Anuradha et *al*.,2015). Insulin which is produced in β-cells is a critical regulator of metabolism (Zhuo et *al*., 2014). The chronic hyperglycemia of diabetes is associated with longterm damage dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels (ADA ,2009).

I.2.Classification:

I.2.1. Diabetes mellitus type 1:

I.2.1.1.Definition:

Type 1 diabetes (T1DM, formerly insulin-dependent diabetes) an autoimmune disease that destroys beta cells in the islets of Langerhans in the pancreas (Lepercq 2007).Resulting an insulin deficiency, which causes the symptoms of hyperglycemia as thirst, weight loss involuntary and fatigue abdominal pain (Rachel,2019). T1D is one of the endocrine and metabolic conditions occurring in childhood (Anastasia et *al*., 2017). About 10% to 15% of people with diabetes have type 1 diabetes (AMA, 2015).

I.2.1.2. Pathophysiology:

There are several risk factors of type 1 diabetes, but the main of its include:

- **Family history**: Parent or sibling with type 1 diabetes increases the risk of a person having the same type, and the risk is even higher when both parents have type 1 diabetes.
- **Genetics**: Specific genes may increase the risk of type 1 diabetes.
- **Viral infections:** Certain viruses may trigger the development of type 1 diabetes by causing the immune system to turn against the body such as decoxavirus.
- **Race/ethnicity:** Certain ethnicities have a higher rate of type 1 diabetes.
- **Geography:** The people who live in northern climates are at a higher risk for developing type 1 diabetes (Daphne & Pharm , 2015 ;Stavroula et *al*., 2018).

I .2.2.Diabetes mellitus type 2:

I .2.2.1.Definition:

Type 2 diabetes (T2DM, non-insulin-dependent diabetes) is the consequence of insulin resistance poorly compensated by abnormal insulin secretion (Lepercq, 2007).

Insulin secretion due to a deficiency in β cells of the pancreas and a decrease in the effect of intestinal hormones stimulating postprandial secretion of insulin whose pathophysiological elements include increased resistance of peripheral tissues (liver, muscles, adipose tissue) to the action of insulin (Tripathy &Chavez, 2010). It represents 85% to 90% of all cases of diabetes (AMA, 2015), concerned mostly individuals over the age of 45 years (Jennifer,2019).

I.2.2.2. Pathophysiology:

Type 2 diabetes is a common chronic disease resulting from a complex inheritance-environment interaction along with other risk factors (Yanling et *al*.,2014) as age, high blood pressure, a low level of HDL (good) cholesterol, or a high level of triglycerides (Hannele, 2011), overweight or obese , a family history has been linked with increased risk for type 2 diabetes mellitus, and physical inactivity identify those individuals at highest risk (Fletcher et *al*., 2014). T2DM is a silent epidemic of increasing proportions (Moshe et *al*., 2015).

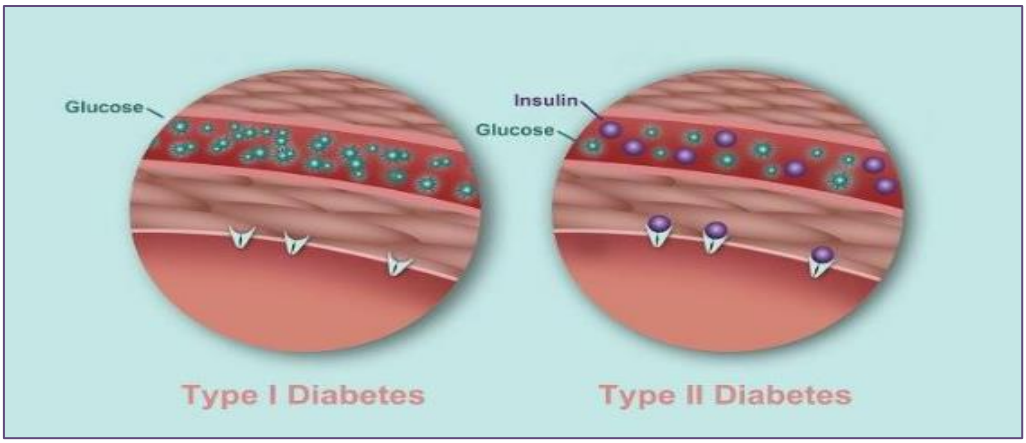

Figure 01 : Difference between diabetes type I and II (Chris , 2017).

I.3. Other types of diabetes:

There are other specific diabetes types as :genetic defects of insulin action, diseases of the exocrine pancreas (e.g. pancreatitis, cystic fibrosis), endocrinopathies (e.g. acromegaly , pheochromocytoma), drug induced (e.g.glucocorticoids, neuroleptics),genetic defects of the β-cell function (e.g. MODY forms) and gestational diabetes that glucose tolerance impairments when first appear or are first diagnosed during pregnancy (Kerner & Brückel, 2014).

I.4. Diabetes treatment:

The major goal in treating diabetes is to control blood sugar (glucose) levels within the normal range, with minimal excursions to low or high levels. Diabetes is treated with: Diabetes medications (oral) or injected insulin, weight reduction by diet and sport exercises (Robert, 2018).

I.5. Prevention:

Eat healthy foods: more vegetables and fruits, less carbohydrates, fats, sugars and salts; exercise regularly: At least 30 minutes of brisk walking every day will do you good ;take the stairs, not the lift; don't board a vehicle (Hanaire & Picard,2003);encourage children to have daily physical exercises; avoid smoking and alcohol, It's good to know you are healthy; visit your nearest health facility for checkups for diabetes. Early diagnosis and proper treatment prevent complications of diabetes (William et *al*., 2012).

II. Pregnancy and gestational diabetes:

II.1.Pregnancy:

II.1.1.Definition:

Pregnancy also known as gestation or gravidity is an event inducing many physiological changes in the mother, in which an embryo(s) implants into maternal uterus and subsequently develops into a fetus (David et *al*., 2018). Pregnancy starts at conception, when an ovum is fertilized by a spermatozoon to form a zygote, and ends in childbirth, abortion or miscarriage (Azab et *al*., 2017 ; Eledo et *al*., 2015). Normal pregnancy last 40 weeks from last menstruation or 38 weeks from conception date (Melissa,2019). These modifications in the mother as well as the intrauterine development of the fetus, require a lot of energy and these needs increase with the progress of the pregnancy (Marey , 2017).

II.1.2.Placenta:

The placenta is a complex organ situated at the interface between mother and foetus that separates maternal from foetal blood. The placental surfaces exposed to the two bloodstreams are different trophoblasts and endothelial cells are in contact with the maternal and foetal circulation respectively (Hiden et *al*., 2006).

II.1.3. Physiological changes during pregnancy:

Two studies, Hytten and Leitch (1971) established that 12.5 kg as the physiological norm for average weight gain for a full-term pregnancy of 40 weeks, increases in maternal tissues and become progressively insensitive to insulin including the uterus and mammary glands, adipose tissue (fat), maternal blood volume and extracellular fluid. (Williamson, 2006). Skeletal muscle and adipose tissue are the main whole-body glucose disposable

sites. In normal pregnancy, insulin-mediated whole-body glucose disposal decreases 50% and in order to maintain a glycemic state, the woman must increase her insulin secretion by 200%-250% (Kampmann et *al*., 2015). The size of the thyroid gland remains unchanged and therefore the presence of goiter should always be investigated. The thyroid gland is 25% larger in patients who are iodine deficient (Kate et *al*., 2016).

II.1. 4. Pregnancy and hematological markers:

During pregnancy, about erythrocyte RBC, the total blood volume increases by about 1.5L, mainly to supply the needs of the new vascular bed. Almost 1 liter of blood is contained within the uterus and maternal blood spaces of the placenta. Expansion of plasma volume by 25%–80% is one of the most marked changes (Ramsay, 2010).In pregnancy, the normal Hgb reference range is 11-12 g/dL. The critical values for Hgb include: Hgb<5 g/dL and Hgb>20 g/dl. Hgb level begins to decline from the 16th week of gestation as a result of increased plasma volume. Similar trends are seen in RBC count and hematocrit (Hct) (David et *al*., 2018).Large cross sectional studies done in pregnancy of healthy women showed that the platelet count does decrease during pregnancy, particularly in the third trimester (Surabhi et *al*., 2012). The neutrophil count begins to increase in the second month of pregnancy and decrease in the second or third trimester, at which time the total white blood cell counts ranges from 9000 to 15,000 cells/µL(Michael et *al*., 2010).

II.1.5. Hormonal changes during pregnancy:

In pregnancy , the effects of increased levels of oestrogen and progesterone, peripheral vasodilatation and resultant decrease in systemic vascular resistance (SVR) begin to occur by 8th week of gestation (Pradeep & Swati ,2018). Estrogen increases the hepatic production of certain proteins, there is greater protein binding of corticosteroids, sex steroids and thyroid hormones (Kate et *al*.,2016). Progesterone is largely produced by the corpus luteum until about 10 weeks of pregnancy (Pratap & Navneet , 2012).

Pregnancy is associated with a relative iodine deficiency ,the causes for this are active transport of iodine from the mother to the foeto-placental unit and increased iodine excretion in the urine (Caron et *al*., 1997). In pregnancy the alterations in total TH levels are the direct consequence of the marked increase in serum binding globulin TBG total T_4 and T_3 levels increase significantly during the first half of gestation. Levels of serum T_4 rise sharply between 6 and 12 weeks, progress more slowly thereafter, and stabilize around mid gestation , for serum T_3 the rise is more progressive (Glinoer ,1997) .Serum

concentrations of TSH are decreased slightly in the first trimester in response to the thyrotropic effects of increased levels of human chorionic gonadotropin , levels of TSH increase again at the end of the first trimester (Priya et *al.*, 2016).The table (01) show the thyroid function in pregnancy :

Table 01 :Thyroid function in pregnancy (Priya et *al*., 2016).

Thyroid function	**Non-pregnant**	**1st trimester**	**2nd trimester**	**3rd trimester**
fT_4 (pmol/l)	9–26	10–16	9–15.5	8–14.5
fT_3 (pmol/l)	2.6–5.7	3–7	3–5.5	2.5–5.5
TSH (mU/l)	0.3–4.2	0–5.5	0.5–3.5	0.5–3.5

II.1.6.Trace elements change during pregnancy:

Iron deficiency (ID) is the most common pathologic cause of anemia in pregnancy, (Graham et *al*., 2015), ID and related anaemia (IDA) during pregnancy are highly prevalent worldwide in both developed and developing nations although the causes are often different, this is due to increased demands of the fetus, growing uterus, placenta, and poor nutritional habits (Abdul-Aziz et *al*., 2014), IDA will arise during pregnancy depends on two factors: the woman's iron stores at the time of conception and the amount of iron absorbed during gestation. The fact that anemia frequently does arise indicates both that pre-existing stores are often inadequate and that physiological adaptations are insufficient to meet the increased requirements (Lawrence, 2010). During pregnancy, fetal growth causes an increase in the total number of rapidly dividing cells, which leads to increased requirements for folate. Inadequate folate intake leads to a decrease in serum folate concentration, resulting in a decrease in erythrocyte folate concentration, a rise in homocysteine concentration Lynn et *al*.,2015). Folate deficiency during this period has

been associated with a number of complications such as preeclampsia, miscarriage, stillbirth, low birth weight, and neural tube malformations (Erika et *al*., 2017).

In addition, the prevalence of calcium deficiency during pregnancy is common in pregnant women (Benali & Demmouche , 2014)**.** During pregnancy increased intestinal absorption of calcium from the gut mainly due to higher generation of calcitriol (1,25 dihydroxy vitamin D) helps in maintaining maternal calcium levels (Shriraam et *al*., 2012).

Zinc (Zn) is an essential micronutrient which is prone to maternal depletion during pregnancy (Ejezie & Nwagha, 2011) ,a structural constituent that is essential for cell growth, development and differentiation. Several earlier reports demonstrate that maternal zinc deficiency during pregnancy is linked with adverse pregnant outcomes including abortion, preterm delivery, stillbirth and fetal neural tube defects (Hua et *al.*, 2015) and risk of spontaneous abortion, gravidic toxemia, treatment-resistant anemia, abnormally prolonged gestation and difficult delivery for the mother (Favier & Favier ,1990).

Copper (Cu) is an essential micronutrient which has an important role in the human body. The serum copper increases during pregnancy and is doubled at full term. Lower levels of serum copper in pregnancy are connected with some pathological conditions (Vukelić et *al*., 2012).In pregnancy, excess copper levels can be associated with intrauterine growth restriction, preeclampsia and neurological disease (Walker et *al*., 2011).

II.1.7.Nutrition recommendations in pregnancy:

The body has increased nutritional needs during pregnancy require more micronutrients and macronutrients. Micronutrients are dietary components, such as vitamins and minerals, that are only required in small amounts. Macronutrients are nutrients that provide calories, or energy. These include carbohydrates, proteins, and fats (Moghissi, 1978). Folate and vitamin B12 for the prevention of neural tube defects, folic acid supplementation has no clear effect on cleft palate/lip or congenital cardiovascular defects, therefore all women are advised to take 400 mcg/day folic acid prior to conception until the 13th week of pregnancy with higher doses in certain circumstance to ensure fetal and placental growth due to its critical role in DNA synthesis and cell replication (Alison et*al*., 2016). The body needs more vitamins during pregnancy, especially vitamin A, vitamin B6 , vitamin C, vitamin D and vitamin K (Kiran,2017). Therefore, it is important to evaluate and make changes to improve maternal nutrition both before and during pregnancy (Oladapo,2000).

Table 02 :Calories and trace elements recommendations for pregnant women (Moghissi, 1978 ;Darnton-Hill ,2013;Rama , 1990)

Nutrient	Daily requirements for pregnant women
Calories	Additional 300, in second and third trimesters
Calcium	1200 milligrams
Folate	600–800 micrograms
Iron	27 milligrams
Zinc	9.6 milligrams
Copper	2 milligrams

II.1.8. Complications during pregnancy:

During pregnancy there are many complications as having pre-eclampsia in one pregnancy is a poor predictor of subsequent pregnancy , but a strong predictor for recurrence of pre-eclampsia in future gestations (Hernández et *al*., 2009), hypertension, occurs when arteries carrying blood from the heart to the body organs are narrowed, this causes pressure to increase in the arteries, in pregnancy this can make it hard for blood to reach the placenta (Andrea et *al* ., 2013),which provides nutrients and oxygen to the fetus (Gude et *al*.,2004), diabetes in the mother and risk of fetal chromosomal abnormalities increase with the mother's age, women aged 35 and older are at increased risk of problems such as high blood pressure, gestational diabetes and complications during labor (Reeta et *al*., 2009).

II.2. Gestational diabetes mellitus (GDM):

II.2.1.Definition:

GDM is defined by the World Health Organization (WHO) as an anomaly of carbohydrate homeostasis leading to hyperglycemia of variable severity (Vanderijst et *al*., 2012; Debjyoti & Jai ,2010), beginner or diagnosed for the first time during pregnancy of the third trimester and responsible for feto-maternal complications (Sophie et *al.*,2017) glucose intolerance was not present or recognized prior to pregnancy.GDM affect 7% of pregnancies each year (Bonaventura et *al*., 2015). After birth many pregnant women with previously undiagnosed type 2 diabetes are often mistakenly diagnosed as having gestational diabetes (Reece et *al*., 2009).

II.2.2.Symptoms:

Feeling thirsty, that want to drink a lot more than you usually do, feel thirsty even when eaten something salty, run around on a hot day, or done something else that would make you want an extra glass of water ,being tired, feel fatigued even early in the day ,having a dry mouth, frequent urination and blurred vision (Lisa,2017).

II.2.3. Diagnostic of gestational diabetes:

Fifty years ago, screening for GDM was done by taking patients' history alone. In 1973, Mahan and O'Sullivan proposed using the 1- hour 50- g oral glucose tolerance test (OGTT) for screening. As stated previously, women with a positive 50-g OGTT need further diagnostic testing with either the 75 or the 100-g OGTT. There is a debate in the literature over which test is a better diagnostic tool. Both tests are administered after an overnight fast of at least 8 hours, but not more than 14 hours and after at least 3 days of unrestricted diet including > 150 g of carbohydrate per day.

Patients need to remain seated and should not smoke throughout the test if using the 100-g OGTT, the cutoff values should be fasting < 95 mg/dl, 1-hour _> 180 mg/dl, 2-hour _> 155 mg/dl and 3-hour > 140 mg/dl . Two or more abnormal values must be measured for the test to be considered a positive diagnostic test. When using the 2-hour 75-g OGTT, the cut-offs are the same at 1 and 2 hours. Again, two or more abnormal values are needed for a positive diagnosis. However, studies have shown that mothers with only one abnormal value are at increased risk for macrosomic infants and other morbidities.(Vambergue,2010; Sophie et *al*., 2017), the table (03)shows the results of diagnostic diabetes and GDM :

Table 03 : The results of diagnostic diabetes and GDM (Perkins et *al*., 2007)

Time of measurement	Glucose concentration (mg/dl)	
	Type 1 or type 2 diabetes	*GDM*
Random	≥200	—
After overnight fast	≥126	95
1 hour postchallenge		180
2 hour postchallenge		155
3 hour postchallenge		140

II.2.4.Pathophysiology of GDM:

Maternal age is an established risk factor for gestational diabetes mellitus (GDM) (Kalok et *al.*, 2018), in clinical practice maternal age of ≥25 years should be adopted instead of ≥35 years or 40 years as a risk factor for the development of GDM (Terence et *al*., 2006). Maternal obesity in early pregnancy is an important risk factor for GDM .The relationship between maternal body mass index (BMI) and hyperglycemia is poorly characterized because mass index (BMI) has not been calculated accurately in early pregnancy (Farren et *al*., 2015),glucose intolerance and obesity may play a role in the development GDM, a strong family history of diabetes mellitus (FHD) has emerged as a risk factor for GDM (Nuriye, 2015), exactly as the results of Moosazadeh's (2016) study found that the odd odds ratio (OR) of gestational diabetes appears to be primarily associated with family history of diabetes, which is to be a powerful diagnostic and predictive factor of this disease which contribute also to mainly improve the health care measures of the pregnant woman (Moosazadeh et *al*., 2016).Polycystic ovary syndrome, a history of delivering big baby, history of recurrent abortions, and hypertension, or pregnancy-related hypertension are other risk factors for GDM and taking some types of anti-psychotic or steroid medications (NDSS,2016), unhealthy diet and race (Brenda , 2019)**.**

II.2.5.Complications:

II.2.5.1. For mother:

Women with gestational diabetes mellitus have a higher risk of pre-eclampsia caractized by elevation of blood pressure and presence of protein in the urine (Sanjay& Girija, 2014), shoulder dystocia, caesarean section increase the risk of developing type 2 diabetes in the future for the mother. Preterm delivery neonatal , hypoglycemia and admission to the neonatal intensive care unit was shown to be associated with maternal hyperglycemia (Howard et *al*., 2016).

II.2.5.2. For baby:

Risks for the baby above average weight, which can make birth more difficult. Hypoglycemia (abnormal glucose drop) at birth because their own insulin production is high, severe episodes of hypoglycemia may provoke seizures in the baby. An increased risk of type 2 diabetes in adulthood (Stewart& Malhotra, 2015)**,**excessive birth weight**,** extra glucose in bloodstream crosses the placenta, which triggers the baby's pancreas to make extra insulin (Radmin,2018). This can be the cause for baby to grow too large (macrosomia) and preterm delivery. The aforementioned increase in placental transport of

glucose, amino acids, and fatty acids stimulate the fetus's endogenous production of insulin and insulin-like growth factor 1 (IGF-1) (Jasmine et *al*., 2018).

II .2.6 GDM and hormonal system:

II.2.6.1GDM and thyroid hormones:

Thyroid hormones are chemical substances made by the thyroid gland. They help regulate growth and the rate of chemical reactions (metabolism) (Rashmi et *al* .,2014), and are involved in the circadian rhythms, among other essential functions. The two most important thyroid hormones are thyroxine (T_4) and triiodothyronine (T_3) (Bridget,2019). Thyroid stimulating hormone (TSH), which is produced by the pituitary gland acts to stimulate hormone production by the thyroid gland. The pituitary gland is stimulated to make TSH by the hypothalamus gland in the brain (William, 2018). The relationship between thyroid disorders and diabetes mellitus is characterized by a complex interdependent interaction (Hage et *al*., 2011).Women in early pregnancy who have high levels of a certain thyroid hormone may be at greater risk for gestational diabetes, compared to women who have normal levels of the hormone (Rawal, 2018).There is a correlation between thyroid disease and GDM , blood glucose levels during pregnancy is influenced by various physiological hormones such as estrogen, thyroid-binding globulin (TBG), human chorionic gonadotropin (hCG), and placental insulin enzyme, all of which are affected by maternal thyroid function (Shuai et *al*., 2016).

II.2.6.2 Insulin resistance in normal pregnancy and with GDM:

Pregnancy is associated with alterations in the regulation of glucose metabolism caused by the actions of human placental growth hormone, prolactin, cortisol, and progesterone; these hormones antagonize the action of insulin, particularly during the 2^{nd} and 3^{rd} trimesters. In early pregnancy, insulin secretion increases (Amit et *al*., 2014) while insulin sensitivity is unchanged , decreased , or may even increase and in late gestation, maternal adipose tissue depots decline, while postprandial free fatty acid (FFA) levels increase and insulin-mediated glucose disposal worsens (Barbour et *al*., 2007). Insulin resistance develops in the second trimester and continues until birth, but is believed to be related to the production of hormones, cytokines, or adipokines by the placenta. Insulin secretion also increases, resulting in normal glucose concentrations (Karen et *al*., 2016). Insulin resistance involves impaired activities of the glucose transport system in insulin

target tissues by GLUT4 glucose transporter protein in adipose tissues from the pregnant women (Okuno et *al*., 1995). Hormones secreted by the placenta starting with the second trimester reduce the effectiveness of insulin, which leads to increased transplacentar nutrient transport as the fetus develops and promotes its growth (Elvira et *al*., 2016).

About gestational diabetes is a metabolic abnormalities occurring during pregnancy associated with carbohydrate metabolism (Butte, 2000). Decreased maternal pregravid insulin sensitivity (insulin resistance) coupled with an inadequate insulin response are the pathophysiological mechanisms underlying the development of gestational diabetes (Catalano et *al* 2003). Caractised by altering of levels and function of insulin receptors , control of insulin-dependent processes in the human placenta will change from mother to foetus throughout gestation particular interest in conditions associated with altered maternal or foetal insulin levels (Hiden et *al.*, 2006).Gestational diabetes mellitus is a disease that associates with endoplasmic reticulum stress (ERS) (Hong et *al*., 2016),it plays a role in the pathogenesis of diabetes, contributing to pancreatic beta-cell loss and insulin resistance (Eizirik et *al*., 2008). Increased phosphorylation of inositol-requiring enzyme 1α (P-IRE-1α) resulting in higher c-Jun N-terminal kinase (JNK) activity causes phosphorylation of insulin receptor substrate 1 at serine307(P-Ser-IRS-1) ending in lower insulin receptor (IR)-associated cell signaling in response to insulin , thus inhibiting insulin signaling pathway, a condition that turns into a stage of insulin resistance due to defective downstream signaling, including reduced protein kinase P/Akt activation and NO synthesis (Sobrevia et *al*., 2016).

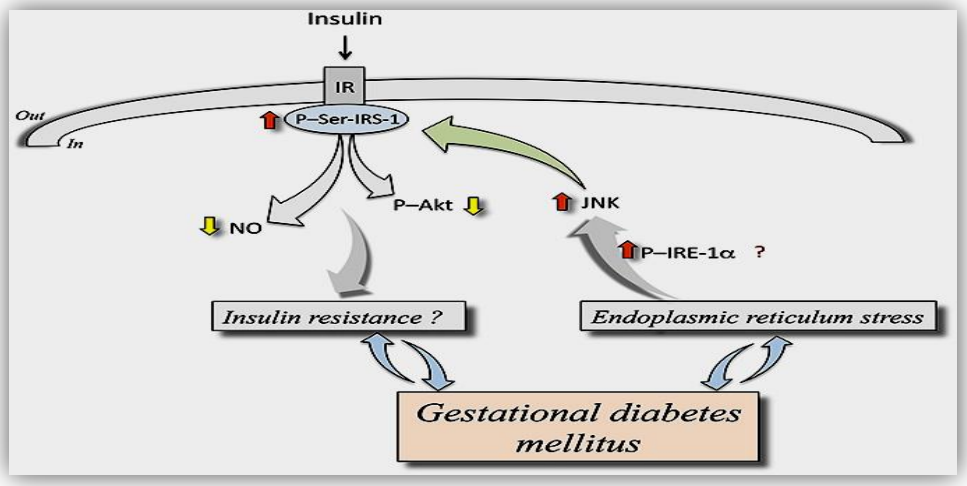

Figure 02: Mecanism of insulin resistance in GDM (Sobrevia et *al*., 2016)

II.2.6.3 Relationship between β-cell dysfunction, insulin resistance, and GDM:

During normal pregnancy, β-cells undergo hyperplasia and hypertrophy in order to meet the metabolic demands of pregnancy. Blood glucose rises as insulin sensitivity falls. During gestational diabetes, β-cells fail to compensate for the demands of pregnancy when combined with reduced insulin sensitivity, this results in hyperglycemia (Hongjie,2010). Following pregnancy, β-cells, blood glucose, and insulin sensitivity may return to normal or may remain impaired on a pathway toward GDM in future pregnancy or T2DM. Pancreas image obtained from The Noun Project under the terms and conditions of the Creative Commons Attribution (CC BY) licens represented in figure (03) (Jasmine et *al*., 2018).

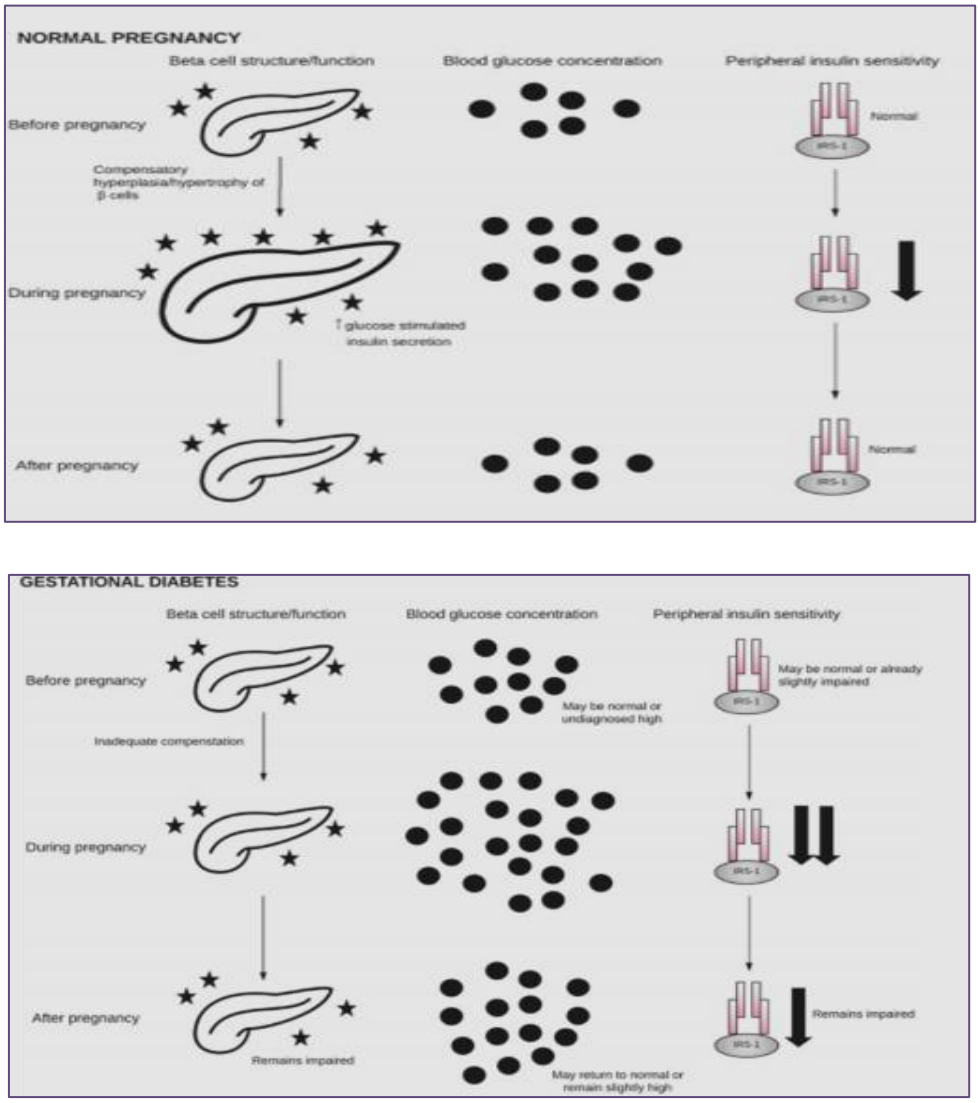

Figure 03: Relationship between β-cell dysfunction, insulin resistance, and GDM (Jasmine et *al.*, 2018).

II.2.7. Treatment:

Treatment of gestational diabetes dietetics, glycemic self-monitoring, insulin therapy if necessary, that reduces severe perinatal complications, fetal macrosomia and preeclampsia (Jacqueminet & Jannot, 2010).Treatment strategy with glibenclamide (known in the as glyburide) requiring progression to insulin in around 4% of casesaction of glyburide is to increase insulin secretion, decreasing hepatic glucose production with resultant reversal of hyperglycemia and indirect improvement of insulin sensitivity (Silva et *al* ., 2006; Langer et *al*., 1989), and metformin are effective treatments for gestational diabetes. Metformin resulted in similar outcomes to initial insulin treatment in gestational diabetes (Lindsay et *al*., 2011).

II.2.8. Prevention:

Which might include controlling excessive gestational weight gain or glycemic control. Physical activity during pregnancy, exercise is believed to play a role in reducing the risk of complications such as preterm birth and pre-eclampsia, and may help prevent excess pregnancy weight gain and postpartum weight retention (Emily et *al*., 2017). Should have a reasonable diet, replenish trace elements, therefore reducing the occurrence of adverse pregnancy outcomes (Shen et *al*., 2015).

II.2.9. Development of gestational diabetes:

Most likely, gestational diabetes should go away about six weeks after delivery. However, because women had gestational diabetes, she is at a higher risk (a 33-50% increased risk) for having it again in another pregnancy. She is also at a higher risk for developing type 2 diabetes (Grazia,2019) is related to the continuum between the level of maternal hyperglycemia and the occurrence of perinatal complications (Lepercq, 2007).

III.Oxidative stress:

III.1. Definition:

Oxidative stress can be defined as a state of disrupted balance between reactive oxygen species and the mechanisms of detoxification and repair. Reactive oxygen species (ROS) are formed in every living cell during the physiological process of breathing, a molecule of ROS contains an atom of oxygen with an unpaired electron (Anna& Krzysztof, 2013). Its enhanced state has been associated with many of the chronic diseases such as cancer, diabetes, neurodegenerative and cardiovascular diseases (Pollyanna et *al*.,2014).

III.2. Free radicals:

A free radical is defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital (Lobo et *al*., 2010).

Reactive oxygen species is a collective term that includes all reactive forms of oxygen, including both radical and non radical species that participate in the initiation and/or propagation of chain reaction (Alugoju,2015).Table (04) shows the main (ROS):

Table 04: Main oxygen reactive species and its performance (Monique & João, 2010)

Species	Chemical Structure	Description	Occurence	Action
Superoxide radical	$O_2^{\cdot}$	Most potent radical in the induction of cellular damage	Almost all aerobic cells	Majority of reactions as a reducing agent
Hydroxyl radical	OH•	$O_2^{\cdot}$ acid conjugate, highly reactive	Formed through water radiolysis	DNA, proteins, carbohydrates and lipids
Hydroperoxyl radical	HO_2•	Protonated form of the $O_2^{\cdot}$	From hydrogen peroxide	Biological membranes
Hydrogen peroxide	H_2O_2	It's not a free radical, because did not submit electrons paired in the last layer	Reactions for the production of OH •	Proteins and lipids
Singlet oxygen	$^1O_2$	Excited form of molecular oxygen. It's not a free radical, because did not submit electrons paired in the last layer	Generated by phagocytes, luminous induction and catalyzed reactions by peroxidases	DNA changes

III.3.Sources of free radicals:

The human body is continuously exposed to potentially harmful oxidative stresses during the course of life time, these may arise from exogenous as well as endogenous sources (Ray *et al*., 2001), figure (04) shows the sources of free radicals.

III.3.1.Endogenous sources of ROS:

The main source of ROS in vivo is aerobic respiration. ROS are also produced by peroxisomal β-oxidation of fatty acids, microsomal cytochrome P450 metabolism of xenobiotic compounds, stimulation of phagocytosis by pathogens or lipopolysaccharides, arginine metabolism and tissue specific enzymes (Nicholls , 2000).

III.3.2.Exogenous sources of ROS:

Excessive levels of free radicals are produced from pollution exposure; toxin exposure, including exotoxins such as heavy metals like mercury, lead and cadmium. Other exotoxins include anticancer drugs, anesthetics and analgesics (Egbuna & Ifemeje, 2017).

Toxin exposure also includes endotoxins such as those produced from bacteria, yeast, viruses and parasites; trauma; radiation; electromagnetic fields; alcohol; cigarette smoke; medications; stress; allergens; cold; excessive exercise; dietary factors such as excess sugar, saturated fat and fried oils; malnutrition and various disease states (Cadenas & Davies, 2000).

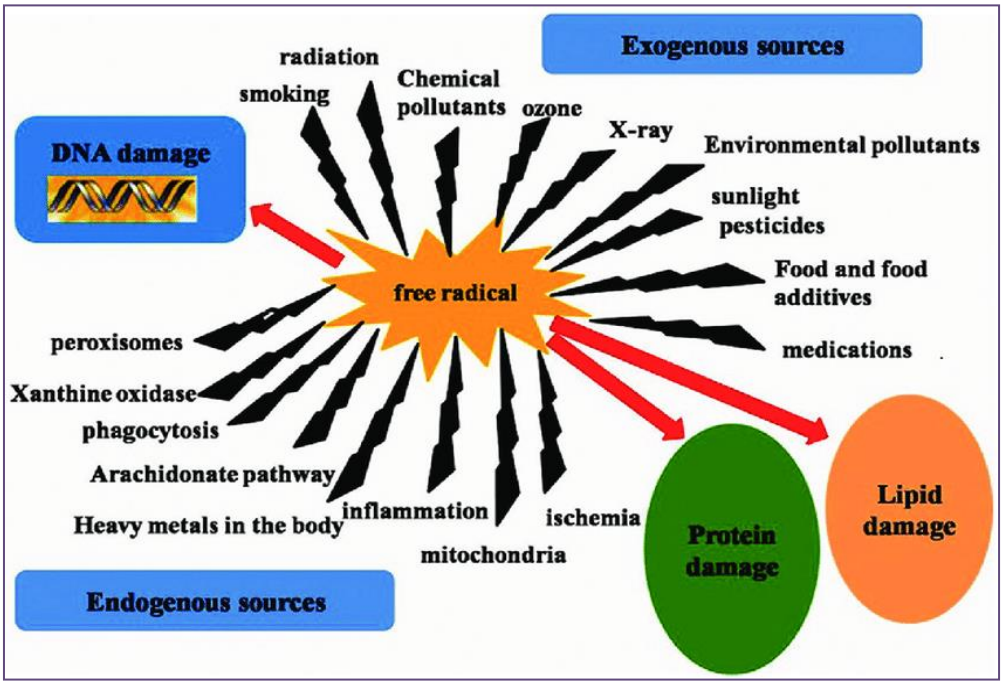

Figure 04: Endogenous and exogenous sources of free radicals (Saheem et *al*., 2017)

III.4. Antioxidants:

Antioxidants are a class of chemical substances naturally, which can prevent or reduce the oxidative stress of the physiological system being fantastic free radical scavengers help in preventing and repairing the cell damage caused by these radicals (Mamta et *al*., 2014). Antioxidants are found in many foods, including fruits and vegetables. Although oxidation reactions are crucial for life, they can also be damaging; plants and animals maintain complex systems of multiple types of antioxidants (Anuj et *al*., 2016).

The classification of antioxidant enzymatic and no enzymatic , the enzymatic antioxidant defense systems is copper-zinc super oxide dismutase (CuZn SOD) that converts super oxide radicals to hydrogen peroxide (H_2O_2), glutathione peroxidase (GSH-Px) and catalase (CAT) will then convert H_2O_2 to a water molecule. Cu-Zn SOD, GSH-Px, and CAT together provide the primary antioxidant defense mechanism (Moharram&

Mohammed ,2014). The non-enzymatic antioxidant defense system includes ascorbic acid (vitamin C), α- tocopherol (vitamin E), glutathione (GSH), β-Carotene, and vitamin A. There is a balance between both the activities and intracellular levels of these antioxidants that are essential for the survival of organisms and their health. Such as glutathione, vitamin C, vitamin A, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxides (Vaisi et *al*., 2007). Figure(06) shows the classification of antioxidants:

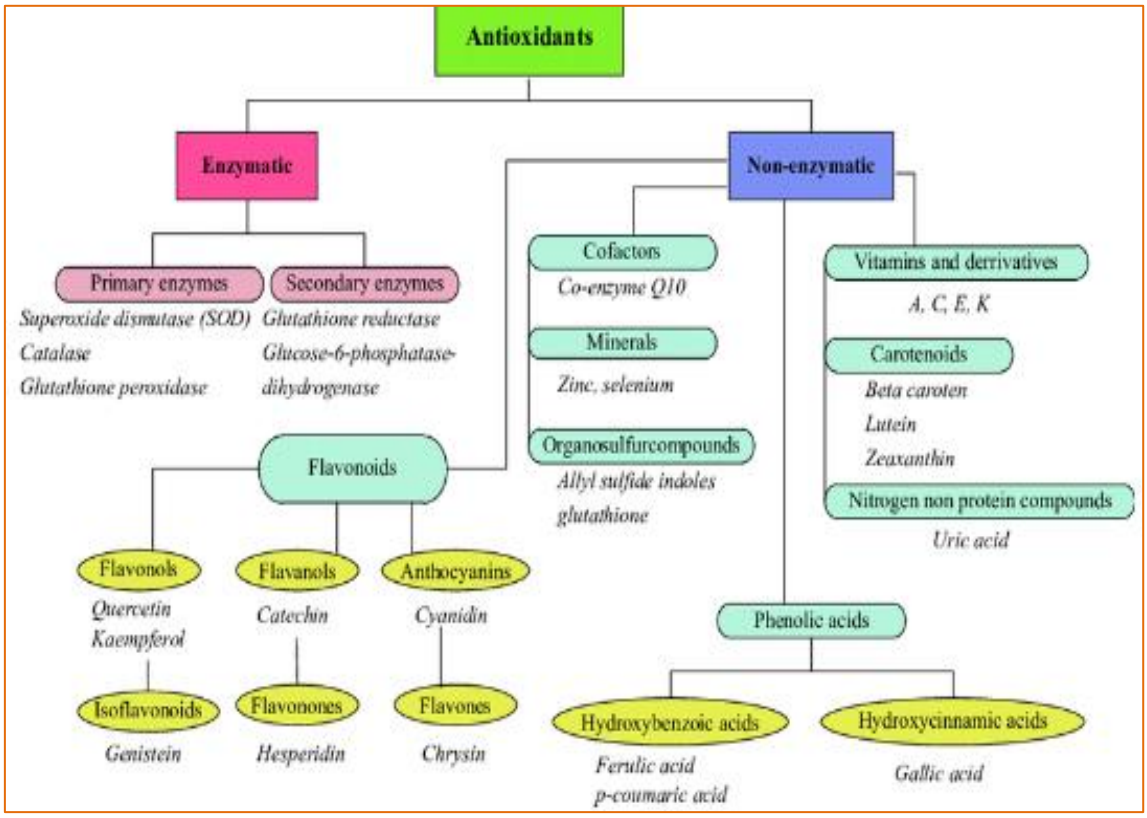

Figure 05: Classification of antioxidants (Iryna , 2018)

III.5.Oxidative stress and pregnancy:

Pregnancy is physiological conditions in which women are prone to oxidative stress due to imbalance between pro-oxidant and antioxidant factors that increased mitochondria in placenta and increased partial pressure of oxygen in pregnant women (Saikumar et *al.*, 2013). Oxidative stress is implicated in the pathophysiology of many reproductive complications including infertility, miscarriage, pre-eclampsia, fetal growth restriction and preterm labour (Kate et *al*., 2016). It plays a pivotal role in the development of placental related diseases ,the mechanisms involved in the alterations of oxygen (O_2) homeostasis and evaluated the consequences for human pregnancy (Eric et *al*., 2006).

During a normal pregnancy, oxidative stress enhances antioxidant mechanisms that are capable of reacting by way of enzyme activity and non-enzyme free radical deactivators, which this adaptation and balance (Anna & Krzystof, 2013), oxidative stress plays important roles in embryo development, implantation, placental development and function, fetal development, and labor antioxidant enzymes include glutathione peroxidase and superoxide dismutase (Kate et *al*., 2016).

The placenta, a hormone-rich tissue, is an important source of pro-oxidizing agents and of antioxidant enzymes that maintaining lipid peroxidation under control which increases during normal pregnancy (Sainz et *al*., 2000). The serum MDA levels in the second trimester are significantly higher than the first trimester (Saikumar et *al*., 2013) because during pregnancy is based on the products of lipid peroxidation produced in the placenta could pass into maternal blood and act as agents triggering damage in other tissues, which are secreted mainly on the maternal side of the placenta and remain in the maternal circulation for some time (Npso et *al*., 2018). Increased lipid peroxidation markers are observed during normal pregnancy, disturbances in the maternal compartment can affect the placental gene methylation state and increase placental oxidative stress, resulting in changes in placental function (Jayasri et *al*., 2015). The increase in lipid peroxidation and the significant decrease in GSH and total antioxidant power ORAC in the serum and erythrocytes of pregnant women during the third trimester of pregnancy clearly show the evolution of the state of stress oxidative therapy associated with pregnancy in pregnant women (Derouiche et *al*., 2018). An oxidative burst occurs during pregnancy due to the large consumption of oxygen in the tissues and an increase in metabolic demands in response to maternal physiological changes and fetal growth (Lucca et *al*., 2016).

III.6. Oxidative stress and GDM:

Pathologic pregnancies including gestational diabetes mellitus (GDM), are associated with a heightened level of oxidative stress, owing to both over production of free radicals and/or a defect in the antioxidant defenses. The relative immaturity of the antioxidant system facilitates the exposure of embryos and fetuses to the damaging effects of oxidative stress (Martha et *al.*, 2011),the increase secretion of tumor necrosis factor- (TNF-) from the placenta and adipose tissue from women with GDM under conditions of high glucose and propose that an altered oxidative homeostasis in women with GDM may be involved (Coughlan et *al*., 2003). Limited data in gestational diabetes mellitus (GDM) suggest that

products of lipid peroxidation maybe increased and antioxidant enzyme activities decreased. As in type 2 diabetes mellitus, glycemic levels in patients with GDM correlate with concentrations of lipid peroxides (Xinhua & Theresa , 2005). Radical phenomena plays an important role in the reproduction, the nesting of the fertilized egg and the development of the embryo, but an imbalance between their production, intense during gestation, and their elimination can generate oxidative stress and iron supplementation during pregnancy contributes to the increase of oxidative stress in pregnant women who take it, in particular an increase in maternal and placental plasma MDA (Devrim et *al*.,2006). Increased oxidative stress is a widely accepted participant in the development and progression of diabetic tissue damage and induced changes in the activities of antioxidant enzymes in various tissues (Derouiche et *al* .,2018).

Second part

Experimental part

Materials & Methods

I.1. Patients and reagents:

I .1.1. Study period:

Our study was organized over a period of 7 months (from the beginning of September 2018 to the end March 2019) to Faculty of Natural Sciences and Life at the University of Echahid Hamma Lakhdar El-Oued.

I.1.2.Risk factors study:

In our study, we used the questionnaires (Annex 01) and asked for 140 volunteers divided into 70 healthy women as a control and 70 women gestational diabetes patients they are the origin of El Oued region.

I.1.3. Biological study:

For biological study, our study was applied to 30 volunteers women of age between 16-45 years were divided into two groups; a group of 15 healthy control women with mean age 27.67± 1.77year, and the other group of 15 women has diabetes with mean age 34.64±1.03 year.

- ✓ **Inclusion criteria:**
- ❖ Voluntary women live in the El Oued region.
- ❖ Control women in good health, does not have any pathology.
- ❖ Women suffering from gestational diabetes during or after pregnancy period.

- ✓ **Exclusion criteria:**
- ❖ Women are suffering from other acute or chronic pathology.

I.1.4.Reagents:

Ethylene diamine tetraacetic acid (EDTA), Hydrogen Peroxyde (H_2O_2), Hydrochloricacid (HCl) , Thiobarbituric acid (TBA) , Salicylic acid , Methanol, Tris Trichloroacetic acid (TCA) , Copper sulfate ($CuSO_4$) , Nitric acid (HNO_3) , Ascorbic acid, DTNB (5,5′-Dithiobis(2-nitrobenzoic acid)), Phosphoric acid (H_3PO_4) , Sodium chloride (NaCl) , Zinc nitrat ($Zn(O_2CCH_3)$), Butylated hydroxytoluene (BHT), Phosphate-buffered (KH_2PO_4,K_2HPO_4), Coomassie Blue.

I.2. Methods:

I.2.1. Collection of data:

Each volunteer submitted the questionnaire including social and clinical data that can give us different factors associated with the pathology.

I.2.1.1. Sample collection:

Performed blood sampling for both groups is done morning fasting. It is performed in the vein of the end of the elbow. After the blood sampling, the blood is collected in two tubes. Dry tubes are centrifuged at 3000 rpm for 10 minutes, then recover the serum to achieve the dosage of biochemistry parameter: Glucose, urea, creatinine ,cholesterol, triglyceride, amylase activity, alkaline phosphatase activity, calcium,copper, zinc, iron,T3, T4, TSH and total antioxidant ORAC.

The anticoagulant tube (EDTA) is mixed well and then assays the hematological and oxidative stress (MDA, GSH and CAT activity) parameters.

I.2.2. Biochemical parameters assay:

Serum glucose, urea, creatinine, calcium, iron, triglyceride and cholesterol Were determined by Auto analysis (BIOLIS24j) use commercial kits from Spinreact, (Spainref: glucose-20121, urea-20141, creatinine-20151,calcium: 20051, iron-20061, cholesterol-20111, triglyceride-20131,) and enzyme marker were also measured using commercial kits (Spinreact, ref: phosphates-20015,amylase -20031).

I.2.3. Hormonal parameters assay:

T3, T4 and TSH level are measured by Enzyme Linked Fluorescent Assay (ELFA) andPLC used:Vidas PC, biomerieux.

I.2.4. Method of Hematological analysis:

Hematological analysis (FNS) is performed by the hematology Auto analyzer.

I.2.5. Method of estimating oxidative stress parameters:

I.2.5.1. Preparation of erythrocyte homogenate:

Blood EDTA tubes contents are centrifuged at 2000 rpm for 10 min and removed the plasma. The cap of EDTA tube was lysis with 50 ml of TBS buffer (EDTA 2.92M;tris 1.21M; pH=7) and incubated 30 min in freezer. After incubation centrifuged at 2500 rpm for 10 min and the obtained supernatant (erythrocyte homogenate) was used for the determination of antioxidant activity (Miller et *al*., 1988).(Annex02).

I.2.5.2. Leukocyte separation:

After removing the plasma and separation of erythrocyte, the rest of EDTA tube contents centrifuge at 2000 rpm for 10 min. Wash pellet with lysis buffer and shake incubate in freezer for 30 min. After incubation centrifuged at 2500 rpm for 10 min. followed this step by washing with lysis buffer until the Leukocyte pairing and then recovered to make the dosage of stress tests (Miller et *al*., 1988). (Annex 03)

I.2.5.3. Collect saliva:

Saliva collected in a dry tube was fasting in the morning. Centrifuge at 3000 rpm for 10 minutes. And then used for oxidative stress assay (GSH ,CAT and MDA).

I.2.5.4. Determination of Leukocyte Protein concentration:

❖ **Principle:**

Protein concentration was measured according to the method of Bradford M.M., 1976 that uses Comassie blue as reagent. The latter reacts with the amino groups (-NH) of the proteins to form a blue complex (The appearance of the blue color reflects the degree of ionization of the medium and the intensity corresponds to the concentration of the proteins).

❖ **Operating mode:**

- Take 40µl of erythrocyte.
- Add 1 ml of Coomassie Blue.
- Shake and let stand for 5 min for color stabilization.
- Read the optical density at 595 nm, against the control.

The obtained optical density is reported on a calibration curve previously drawn.

The concentration of the proteins is determined by comparison with a standard range of bovine serum albumin (1 mg / ml) previously carried out under the same conditions.(Annex03).

I.2.5.5. Determination of catalase activity:

The catalase activity consists in measuring the catalase-induced H_2O_2 disappearance contained in the sample by measuring the absorbance of H_2O_2 at 560 nm using a UV / visible spectrophotometer. Briefly in test tubes, mix 1 ml of phosphate buffer (0.1 mHg, 0.1M, pH7.2), 0.975 ml of freshly prepared H_2O_2 (0.091M) and 0.025 ml of the enzyme source (homogenate). Absorption read at 560 nm every minute for 2 minutes (Aebi, 1984)

$$CAT(UI/gofpr) = \frac{\left(\left(\frac{2.3033}{T}\right)\times\left(\frac{\log A1}{\log A2}\right)\right)}{g\ of\ protein}$$

- **A1:**Absorbance at the first minute.
- **A2:**Absorbance at the second minute.
- **T:** Time interval in minutes.

I.2.5.6. Determination of Reduced glutathione (GSH) level:

The determination of the reduced glutathione concentration by measuring the optical density results from the formation of 2-nitro-5-mercocapturic acid from the reduction of dithio-bis-2-nitrobenzoic acid, which is called reagent of Ellman with SH groupings exist in GSH briefly, 800 µL of homogenate are added to 200 µL of salicylic acid (0.25%) and centrifuge at 1000 rpm for 5 minutes. 500 ml of supernatant are then mixed with 1000 µl of tris buffer (tris 0.4 mol, 0.02 molNaCl pH = 8.9) and 25 µL of DTNB (0.01 mol.L^{-1}). After 5 minutes of incubation, the absorbance is read at 412 nm (Weakbeker& Cory, 1988).

$$GSH(nM/mg\ ofPr\ ) = \frac{OD\times1\times1.525}{13133\ \times0.8\times0.5\times mg\ of\ Pr}$$

- **13133:** Absorption constant of SH groups at 412 nm.
- **OD:**The absorbance reader by the spectrophotometer.
- **1.525 ml:**Total volume of blend.
- **0.5 ml:**Volume of solution float.
- **1:**Volume of protein mixture.
- **0.8ml:**Volume of homogeneous solution without protein exists in 1ml.
- **GSH:** Concentration of glutathione.

I.2.5.7.Measuring of total antioxidant capacities (ORAC):

A. Principle:

The total antioxidant power of the serum, that is to say its capacity to absorb oxygen free radicals (ORAC: Oxygen Radical Absorbance Capacity) is estimated by the ability of red blood cells to resist free radical induced hemolysis in vitro in the presence of plasma according to the method of Blache & Prost. It is based on the time-dependent monitoring of red blood cell hemolysis induced by a free radical generator (Blache & Prost, 1992) (Annex 04).

B. Treatment of RBC:

- Centrifuge donor blood at 2000 rpm for 10 min and remove plasma.
- Wash gently 1 volume of the pellet with 2 volumes of physiological saline (without lysing the RBCs), then centrifuge again at 2000rpm for 5 min.

C. Operating mode:

- **Control tube:**

- To 1 ml of RBC add: 20 µl of $CuSO_4$ (2 mM), 20 µl of H_2O_2 (30%) and 2 ml of physiological saline, then stir gently.
- Incubate for 5 min at room temperature, centrifuge for 5 min at 2000 rpm.
- Read the OD at 450 nm from the supernatant, put it back in the tube.
- Repeat this operation every 10 minutes for 2 hours.

- **Standard tube:**

- To 1 ml of RBC are added: 20 µl of $CuSO_4$ (2 mM), 20 µl of H_2O_2 (30%) and 2 ml of physiological saline, and 20 µl of vitamin C (400 µM) and then gently stir.
- Incubate for 5 min at room temperature, centrifuge for 5 min at 2000 rpm.
- Read the OD at 450 nm from the supernatant and put it back in the tube and stir gently.
- Repeat this operation every 10 minutes for 2 hours.

- **Test tube:**

- To 1 ml of RBC are added: 20 µl of $CuSO_4$ (2 mM), 20 µl of H_2O_2 (30%) and 2 ml of physiological saline, and 20 µl of serum and then gently stir.
- Incubate for 5 min at room temperature, centrifuge for 5 min at 2000 rpm.

- Read the OD at 450 nm from the supernatant and put it back in the tube and stir gently.
- Repeat this operation every 10 min for 1 hour (t0, t10, t20, t30, t40, t50, t60, and average the latter:
- **ΣDO = Σ(t0, t10,t20, t30, t40, t50, t60)/7**
- To calculate the total antioxidant power using this method:

Calculated:

$$ORAC(UI) = \frac{\sum(\text{ODcontrol} - \text{ODsample})\Delta t}{\sum(\text{ODcontrol} - \text{ODstandard})\Delta t}$$

I.2.5.8. Malondialdehyde (MDA) assay:

MDA was measured according to the method described by (Yagi, 1976). Thiobarbituric acid 0.67% (w/v) was added to aliquots of the sample previously precipitated with 10% trichloroacetic acid (w/v). Then the mixture was centrifuged, and the supernatant was heated (100°C) for 15 min in a boiling water bath. Then cool in a cold water bath for 30 minutes, leaving the tubes open to allow evacuation of the gases formed during the reaction and the absorbance was measured at 532 nm using a spectrophotometer. The concentration of TBARS was determined using the molecular extinction coefficient of MDA ($a=1.53\ 10^5\ M^{-1}.Cm^{-1}$)

I.3. Statistical analysis:

Statistical analysis is performed by the SPSSV20.0 software results comparisons were carried out by using the Student T test to compare means among the groups, Correlation analysis was carried out using Pearson Correlation test and regression analysis was used for other analysis and statistical data. Differences were considered statically significant at $p < 0.05$.

Results & Discussion

I. Results:

I.1. Study of risk factors of gestational diabetes:

I.1.1. Description of study population:

Characteristics of the study population are shown in table 05. Women volunteers for this study from wilaya of El Oued. After a women agreement to participate in this study, the selected population reaches 70 control and 70 women with gestational diabetes a sample size large enough to maintain statistical power. The results obtained are homogeneous in both control and women with gestational diabetes, there is shown that no significant difference concerning mean age, number of children, body weight, job (worker and house wife), masse index and educational level. On the other hand, the blood types are different between the two groups studied.

Table 05: Description of study population**.**

		Control	Patients
Age		28.145±0.767	34.938±0.736
Number of children		2.297±0.269	3.615±0.217
Weight	**Pregnant(kg)**	72.66±2.56	89.30± 4.50
	Not pregnant(kg)	66.78±2.26	75.12 ±2.16
Mass index	**Pregnant(kg/m^2)**	26.985±0.863	28.69 ± 2.18
	Not pregnant(kg/m^2)	25.24±0.735	29.779± 0.901
Job	**Worker(%)**	12.85	20.96
	Housewife(%)	87.14	79.03
Educational Level	**Primary(%)**	12.85	3.51
	Medium(%)	38.57	33.33
	Secondry(%)	21.42	35.09
	High School(%)	25.71	28.07
Address(living)	**Inside El oued(%)**	41.42	72.86
	Outside El oued(%)	58.57	27.14
Blood type	**A(%)**	27.27	32.25
	B(%)	16.66	6.45
	AB(%)	3.03	6.45
	O(%)	53.03	54.8

I.1.2 Study of socioeconomic and clinic factors:

Odds ratio (OR) values for socioeconomic factors (table 06) and clinic pathological factors (table 07) show that passive smoke, social problems ,obesity, big baby , eating sugar and overweight are shown to be significant risk factors for gestational diabetes OR (1.99-5.41)with P<0.05. In addition disease in the first pregnancy, family history, chronic disease and taking contraceptive after 30 year also shown to be significant risk factors for gestational diabetes in the study population with the highest OR value (9.428-58.105) whith P<0.05. In contrast sport is protective factors against gestational diabetes in the study population (OR=0.330; P=0.004, OR=0.404; P=0.010) respectively. As that fast food, soda, canned food, spices, tap water, movement, nervousness, contraceptive pill , pills regularly,abortion,caesarean births,regular menstrual andpolycystic ovarian disease are not considered as predictors of gestational diabetes in our population since the OR values obtained are not significant (Annex 05).

Table 06: Comparison of the Socioeconomic features of gestational diabetes patients and control (N=140)

	Control(%)	Patient(%)	OR	CI 95%	P
Passive Smoke					
Positive	35.4	64.6	**3.065**	1.536-6.117	**0.001**
Negative	62.7	37.3			
Fast food					
Positive	46.7	53.3	1.185	0.528 -2.661	0.419
Negative	50.9	49.1			
Soda					
Positive	50	50	1.000	0.498-2.010	0.571
Negative	50	50			
Sugars					
Positive	38.1	61.9	**1.994**	4.17-0.952	**0.048**
Negative	55.1	44.9			
Sport					
Positive	69.2	30.8	**0.330**	0.150-0.723	**0.004**
Negative	42.6	57.4			
Canned food					
Positive	45.8	54.2	1.289	0.640-2.596	0.297
Negative	52.2	47.8			
Social problems					
Positive	32.7	67.3	**3.010**	1.452-6.240	**0.002**

Negative	59.3	40.7			
Spices					
Positive	48.9	51.1	1.135	0.565-2.283	0.429
Negative	52.1	47.9			
Tap water					
Positive	44.4	55.6	1.292	0.477-3.494	0.401
Negative	50.8	49.2			
Food additives					
Positive	64.6	35.4	0.404	0.196-0.830	**0.010**
Negative	42.4	57.6			
Obesity					
Positive	27.3	72.7	**3.536**	1.502-8.326	**0.002**
Negative	57	43			
Overweight					
Positive	36.8	63.2	**2.867**	1.444-5.693	**0.002**
Negative	62.5	37.5			
Movement/Activity					
Positive	51.6	48.4	0.596	0.217-1.639	0.225
Negative	38.9	61.1			
Nervousness					
Positive	49.5	50.5	1.066	0.529-2.151	0.500
Negative	51.1	48.9			

- **OR > 1** and **P < 0.05**indicate a risk factor**.**
- **OR< 1** and **P < 0.05** indicate a protective factor.

Table 07: Comparison of the clinic pathological of gestational diabetes patients and controls (N=140)

	Control (%)	**Patient (%)**	**OR**	**CI 95%**	**P**
Contraceptive pill					
Positive	47.9	52.1	1.135	0.565-2.283	0.429
Negative	51.1	48.9			
Contraceptive pill after 30 y	51.2	48.8	**9.428**	0.012-0.911	**0.018**
Positive	10.0	90.0			
Negative					
Pills regularly					
Positive	57.1	42.9	0.516	0.162-1.638	0.201
Negative	40.7	59.3			

First birth before 30 y Positive Negative	52.0 30.8	48.0 69.2	0.411	0.120-1.403	0.122
Caesaran births Positive Negative	48.8 50.5	51.2 49.5	1.071	0.517-2.219	0.500
Regular menstrual Positive Negative	47.3 45	52.7 55	0.913	0.441-1.888	0.476
Polycysticovarian disease Positive Negative	39.1 52.1	60.9 47.9	1.694	0.680-4.220	0.181
The fall of pregnant Positive Negative	50.8 49.4	49.2 50.6	0.944	0.485-1.837	0.500
Disease in the first pregnancy Positive Negative	5.3 57.0	94.7 43.0	**23.885**	-184.716 3.088	**0.000**
Family history Positive Negative	3 64.5	97 35.5	**58.105**	-442.132 7.636	**0.000**
Chronic disease Positive Negative	3.7 56.1	96.3 43.9	**33.222**	-252.653 4.369	**0.000**
Big baby Positive Negative	24.5 63.7	75.5 36.3	**5.419**	2.487-11.808	**0.000**
Others diseases Positive Negative	56.4 47.5	43.6 52.5	0.700	0.333-1.472	0.226

- **OR > 1** and **P < 0.05** indicate a risk factor.
- **OR< 1** and **P < 0.05** indicate a protective factor**.**

I.2.Study of biological markers and predictive factors:

I.2.1 Hematological markers:

According to the result of the table 08, this table is divided into two categories : pregnant women and not pregnant women , their results of the hematological analysis for not pregnant women show that HCT is significant decrease ($P < 0.05$) , and WBC , LYM are significantly increase ($P<0.01$) in the patients group as the control group and the reference values. And PLT, HGB and RBC are shown that no significant differences ($P>0.05$) in gestational diabetes women compared to the woman controls. The results for pregnant women show that HCT is significant decrease and WBC is significant increase ($P < 0.05$) and RBC, HGB are significant decrease and LYM is significantly increase ($P<0.001$) in the patients group as the control group and the reference values. And PLT show that no significant differences ($P > 0.05$) in gestational diabetes women compared to the woman controls.

Table 08: Hematological parameters in control women and women with gestational diabetes.

	Not pregnant			Pregnant		
Parameters	**Control**	**Patients**	**P**	**Control**	**Patients**	**P**
WBC (10^9/l)	4.348 ±0.269	5.87±0.449	**0.004**	5.373± 0.341	7.15 ± 0.585	**0.014**
RBC(10^12/l)	4.46± 0.0432	4.43± 0.0658	0.624	5.608 ± 0.241	3.48± 0.198	**0.000**
LYM (10^9/l)	1.61±0.0480	2.23 ± 0.179	**0.003**	1.130± 0.147	1.74± 0.119	**0.000**
HGB(g/dl)	12.08±0.301	12.05 ± 0.269	0.895	13.250 ± 0.506	10.56± 0.189	**0.000**
HCT(%)	35.69±0.741	33.59 ± 0.764	**0.015**	34.89± 1.90	31.48± 1.47	**0.041**
PLT (10^9/l)	209.71 ±7.32	194.4 ± 10.6	0.168	197.3 ±20.6	160.7± 18.0	0.067

I.2.2.Biochemical markers:

Concerning biochemical markers, our results in table 09 showed a significant increase in blood glucose level ($P<0.01$), cholesterol and TG concentration ($P<0.05$).and significant decrease in APL and amylase ($P<0.01$) in not pregnant women with diabetes as compared to that in the controls. Also, it showed that there is no significant changes in

urea, and creatinine (P>0.05). For those who are pregnant ,the results showed that a significant increase in blood glucose level (P<0.01) while there is a significant decrease in cholesterol (P<0.05) No significant changes in urea, creatinine, TG, ALP and amylase (P>0.05).

Table 09: Biochemical parameters in control and women with gestational diabetes.

	Not pregnant			Pregnant		
Parameter	**Control**	**Patient**	**P**	**Control**	**Patient**	**P**
Serum blood glucose(g/l)	0.951±0.017	1.473±0.167	**0.007**	0.861±0.046	1.041±0.05	**0.005**
Serum urea (g/l)	0.231±0.012	0.245±0.015	0.376	0.146±0.008	0.157±0.007	0.177
Serum creatinine (mg/l)	5.857±0.231	5.625±0.34	0.505	5.5±0.447	5±0.363	0.192
Serum cholesterol (g/l)	1.617±0.035	1.746±0.06	**0.049**	1.982±0.063	1.848±0.061	**0.048**
Serum TG(g/l)	0.791±0.059	1.134±0.149	**0.036**	1.223±0.085	1.144±0.095	0.425
Serum ALP (UI/l)	143.7±20	84.3±15.6	**0.002**	74.1±10	91.1±20.5	0.420
Serum amylase (UI/l)	61.57±2.67	49.5±3.94	**0.008**	47±4.15	45.71±2.39	0.600

I.2.3.Trace elements level:

For gestational diabetes not pregnant women, the results show a significant decrease in Zn , Cu and Fe concentration (P<0.05) ,also represent a significant increase in Zn/Cu (P<0.05) in women with GDM as compared to controls. We noted that result shows no significant change for serum Ca level (P>0.05) in GDM women withaw pregnancy case. However, for pregnancy GDM women, our result shows a significant increase in serum Zn ; Zn/Cu and serum Ca concentration (P<0.05) ,while serum Fe and serum Cu are decreased (P<0.05) compared to pregnancy control women.

Table 10: Trace elements in serum of control and women with GDM.

	Not pregnant			Pregnant		
Parameter	**Control**	**Patient**	**P**	**Control**	**Patient**	**P**
Serum Zn (µg/100ml)	285.6± 2.1	226.45± 14.35	**0.003**	173.55± 4.2	760.2±121.1	**0.001**
Serum Cu (µg/100ml)	98±1.05	89.25±0.01	**0.000**	115.15± 0.7	96.6+ 0.35	**0.000**
Zn/Cu	2.91±0.049	2.53± 0.076	**0.044**	1.501±0.015	7.87±0.64	**0.000**
Serum Ca(mg/l)	88.29±1.03	88.5±1.51	0.891	83.75±0.964	87.29±1.26	**0.015**
Serum Fe (mg/l)	0.638±0.055	0.55±0.038	**0.042**	0.566±0.085	0.25±0.061	**0.001**

I.2.4. Hormonal markers:

The results in table 11 are divided into two groups: For not pregnant women, the results show a significant increase in TSH ,T4 ($P<0.01$) and a significant decrease in T3, T3/T4($P<0.01$). In women with diabetes as compared to that in the controls. For pregnant women, the results obtained show a significant increase in TSH,T3 and T3/T4($P<0.01$). In women with diabetes as compared to that in the controls. No significant changes for T4($P>0.05$).

Table 11: Hormonal thyroid markers in control women and women with gestational diabetes.

	Not pregnant			Pregnant		
Parameters	**Control**	**Patient**	**P**	**Control**	**Patient**	P
Serum TSH(µIU/ml)	1.47±0.274	2.777±0.374	**0.005**	0.665±0.07	1.39±0.068	**0.000**
Serum T4(p mol/l)	12.89±0.259	15.473±0.389	**0.000**	11.3±0.311	11.053±0.426	0.547
Serum T3(p mol/l)	5.91±0.344	4.75±0.115	**0.000**	4.14±0.359	4.57±0.107	**0.002**
Serum T3/T4	0.45±0.02	0.3±0.009	**0.000**	0.355±0.025	0.41±0.008	**0.000**

I.2.5. Oxidative stress markers:

The results in table 12 show the analysis of blood oxidative stress parameters ,for not pregnant ,the results obtained show a significant increase in leukocyte MDA and catalase level($P<0.05$) ,and a significant decrease in leukocyte GSH ($P<0.01$) and ORAC level($P<0.001$) in women with diabetes as compared to that in the controls. No significant change in erythrocyte MDA and GSH($P>0.05$).For pregnant, our results showed a significant increase in leukocyte MDA and catalase level $P<0.05$ and a significant decrease in erythrocyte MDA and ORAC level ($P<0.05$) in women with diabetes as compared to that in the controls. Concerning leukocyte and erythrocyte GSH no significant change obtained($P>0.05$)**.**

Table 12: Oxidative stress parameters in the blood of control women and women with gestational diabetes.

		Not pregnant			Pregnant		
Parameter		**Control**	**Patient**	**P**	**control**	**Patient**	**p**
Leukocyte	**MDA (µmol/mg pr)**	2.986±0.603	4.825±0.791	**0.04**	4.516±0.205	10.83±0.82	**0.000**
	GSH (nmol/mg pr)	189.2±42.9	103±21.4	**0.001**	76.01±9.93	98.5±28.1	0.438

	CAT(UI/g pr)	8.08±1.1	10.459±0.92	**0.023**	5.741±0.268	10.511±0.654	**0.000**
Erythrocytes	**MDA (nmol/mgHb)**	6.7±2.88	5.44±1.95	0.539	5.12±2.45	2.64±0.43	**0.002**
	GSH (nmol/mg Hb)	15.52±1.04	11.2±2.15	0.063	3.75±1.43	6.4±1.77	0.161
Serum	**ORAC (UI/l)**	0.423±0.11	0.138±0.04	**0.000**	0.239±0.083	0.107±0.011	**0.000**

The results in table13 show that there are no significant changes in MDA, CAT and GSH in both of control and GDM patients women's saliva.

Table13 : Oxydative stress parameters in saliva of control women and women with gestational diabetes.

Parameters	Control	Patient	P
Saliva MDA(µmol/l)	2.083±0.345	2.093±0.256	0.971
Saliva CAT(UI/l)	2.202±0.015	2.148±0.031	0.121
Saliva GSH(µmol/l)	70±20	100±50	0.55

I.2.6. Correlation between biological markers:

- **In no pregnant women**:

The results represent the correlation between oxidative stress parameters (MDA WBC, MDA RBC, GSH RBC, and ORAC), glucose ,hormonal parameters (TSH, T3, T3/T4)and minerals (Zn;Cu Zn/Cu and iron) in group patients for not pregnant women. There was a positive correlation ($P<0.001$) between TSH and T3 ($P=0.000$, $R= 0.922$),RBC MDA and Zn($P=0.000$;$R=0.949$) T3 and T3/T4 ($P=0.000$, $R=0.656$).and negative correlation ($P<0.05$) between RBC MDA and RBC GSH($P=0.033$, $R= -0.232$), T3/T4 and RBC GSH ($P=0.015$ $R= -0.498$)T3/T4 and Zn/Cu($P=0.000$; $R=-0.975$) Cu and Zn/Cu($P=0.000$;$R=-0.959$)T3 and Zn/Cu($P=0.000$;$R=-0.968$). There was no correlation ($P>0.05$) between the rests of correlation test in patients groups.

Table 14: Correlation between biological markers for not pregnant women**.**

		MDA RBC	RBC GSH	Serum TSH	Serum T3	T3/T4	Serum Zn	Serum Cu	Zn/Cu
RBC MDA	P R	0 1	**0.033** -0.232	0.695 0.127	0.746 0.105	0.556 0.127-	**0.000** 0.949	0.077 -0.529	0.400 0.268
RBC GSH	P R	**0.033** -0.232	0 1	0.944 -0.023	0.489 -0.222	**0.013** -0.498	0.627 -0.157	0.719 -0.116	0.609 0.165
Serum TSH	P R	0.695 0.127	0.944 -0.023	0 1	**0.000** 0.922	0.138 0.312	0.417 -0.259	0.921 -0.032	0.738 0.108
Serum T 3	P R	0.746 0.105	0.489 -0.222	**0.000** 0.922	0 1	**0.000** 0.656	0.344 0.299	**0.000** 0.858	**0.000** -0.968
T3/T4	P R	0.556 -0.127	0.013 -0.498	0.138 0.312	**0.000** 0.656	0 1	0.586 -0.175	**0.000** 0.998	**0.000** -0.975
Serum Zn	P R	**0.000** 0.949	0.627 -0.157	0.417 -0.259	0.344 0.299	0.586 -0.175	0 1	0.464 -0.234	0.879 -0.049
Serum Cu	P R	0.077 -0.529	0.719 -0.116	0.921 -0.032	**0.000** 0.858	**0.000** 0.998	0.464 -0.234	0 1	**0.000** -0.959
Zn/Cu	P R	0.400 0.268	0.609 0.165	0.738 0.108	**0.000** -0.968	**0.000** -0.975	0.879 -0.049	**0.000** -0.959	0 1

- **<u>In Pregnant women</u>**:

The results represent the correlation between oxidative stress parameters (GSH RBC and ORAC), glucose and hormonal parameters (TSH, T3, T3/T4) and minerals(Zn;Cu Zn/Cu and iron)in group patients for pregnant women .There was a positive correlation (P<0.05) between T3 and TSH (P=0; R=0.972) ; GSH RBC and T3/T4(P=0.005;R=0.809) glucose and Cu(P=0.035;R=0.610) TSH and Zn/Cu(P=0;R=0.965) , Zn/Cu and Cu(P=0.021;R=0.654) and negative correlation between T3 and T3/T4(P = 0.049;R= -0.578).GSH RBC and Cu(P=0.003;R=-0.777)ORAC and Zn(P=0.000;R=-0.916)TSH and T3(P=0;R=-0.978)There was no correlation (P>0.05) between the rests of correlation test in patients groups.

Table 15: Correlation between biological markers for pregnant women.

		GSH RBC	ORAC	TSH	T3	T3/T4	Zn	Cu	Zn/Cu
RBC GSH	R	1	0.309	-0.12	-0.06	0.809	-0.33	-0.77	**-0.333**
	P	0	0.329	0.695	0.844	**0.005**	0.284	**0.003**	**0.290**
Serum ORAC	R	0.309	1	-0.00	-0.00	0.000	-0.91	0.000	**0.000**
	P	0.329	0	1	1	1	**0.000**	1	**1**
Serum T3	R	-0.06	-0.000	-0.97	1	-0.578	0.000	-0.21	**-0.877**
	P	0.844	1	0	0	**0.049**	1	0.515	0.000
T3/T4	R	0.809	0.000	0.371	-0.57	1	0.000	-0.67	**0.109**
	P	**0.005**	1	0.235	**0.049**	0	1	**0.015**	**0.725**
Serum Zn	R	-0.33	-0.916	0.000	0.000	0.000	1	0.00	**-0.0**
	P	0.284	**0.000**	1	1	1	0	1	**1**
Serum Cu	R	-0.77	0.000	0.431	-0.21	-0.678	0.00	1	**0.654**
	P	**0.003**	1	0.162	0.515	**0.015**	1	0	0.021
Zn/Cu	R	-0.33	0.000	0.965	-0.87	0.109	-0.0	0.654	**1**
	P	0.290	1	**0.000**	**0.000**	0.725	1	**0.021**	**0**

I.2.7. Predictive factors study:

The results obtained show that the catalase leucocyte, MDA level in erythrocyte and leucocyte as the highest percentage of specificity (62.5, 100, 87.5%) and important percentage of sensitivity (42.9, 42.9, 57.1 %)respectively for not pregnant women (Table 16and figure A).

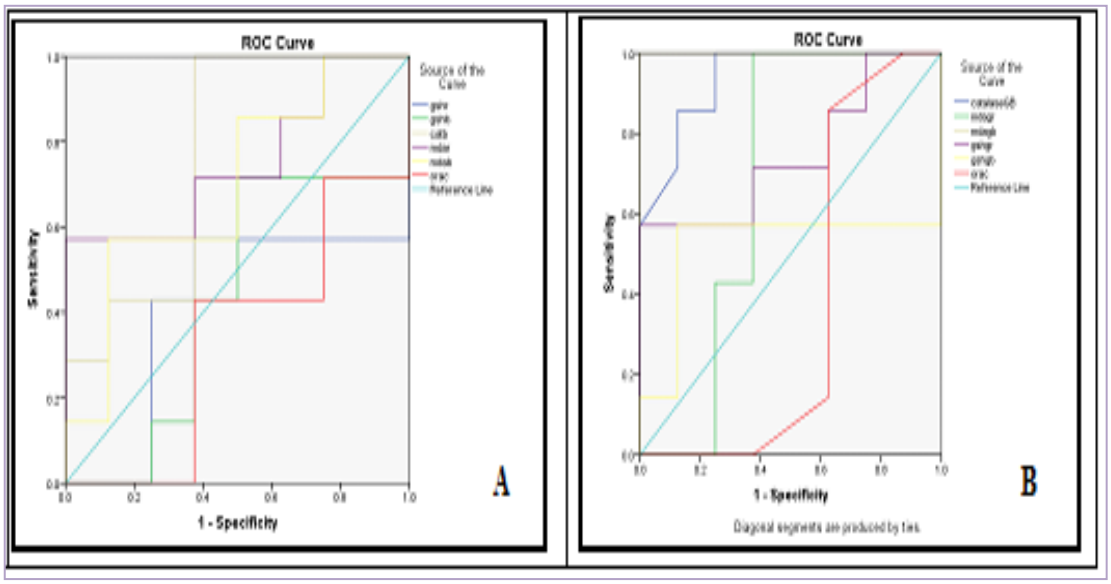

Figure 06: ROC Curve for oxidative stress markers in not pregnant women(A) and in pregnant women(B)

Table 16: Sensitivity, specificity and AUC values of some oxidative stress markers in no pregnant women.

Test Result Variable(s)	Sensitivity %	Specificity %	AUC %	SE	P value	Asymptotic 95% Confidence Interval	
						Lower Bound	Upper Bound
WBCCatalase	42.9	62.5	76.8	0.129	0.083	0.516	1.000
RBC MDA	42.9	100	75.0	0.135	0.105	0.486	1.000
WBC MDA	57.1	87.5	69.6	0.141	0.203	0.420	0.973
RBC GSH	57.1	0	41.1	0.163	0.563	0.091	0.730
WBC GSH	57.1	50	42.9	0.157	0.643	0.120	0.737
Serum ORAC	71.4	25	33.9	0.147	0.298	0.052	0.627

Concerning pregnant women, the results show that the catalase leucocyte, MDA level in leucocyte and erythrocyte and ORAC level in serum as the highest percentage of specificity (100, 100, 100 and 62.5 %) and important percentage of sensitivity (42.9, 100, 57.1,42.9%) respectively.(Table 17and figure B)

Table 17: Sensitivity, specificity and AUC values of some oxidative stress markers in pregnant women

Test Result Variable(s)	Sensitivity %	Specificity %	AUC %	SE	P value	Asymptotic 95% Confidence Interval	
						Lower Bound	Upper Bound
WBC Catalase	42.9	100	93.7	0.060	**0.005**	0.819	1.000
RBC MDA	100	100	67.9	0.156	0.247	0.372	0.985
WBC MDA	57.1	100	100.0	0.000	**0.001**	1.000	1.000
RBC GSH	57.1	37.5	75.0	0.135	0.105	0.486	1.000
WBC GSH	57.1	12.5	51.8	0.179	0.908	0.167	0.868
SerumORAC	42.9	62.5	37.5	0.163	0.418	0.055	0.695

II. Discussion:

Study of risk factors for gestational diabetes:

Our study showed that passive smoke was significantly associated with gestational diabetes risk. This result is in agreement with study of (Leng et *al*.,2010) who showed that passive smoking during pregnancy increased gestational diabetes mellitus (GDM) risk with pre-pregnancy obesity. Passive smoking or involuntary smoking or second-hand smoke (SHS) which is also called environmental tobacco smoke, is formed from the burning of cigarettes and other tobacco products and from smoke exhaled by the smoker (Öberg et *al.,* 2010; Bertrand, 2011)**,**passive smoke inhalation during pregnancy makes gestational diabetes more likely, bringing with it negative health consequences for the mother and her baby (Loeken,2017).Obesity is a well-established risk factor for GDM. Several studies have shown that passive smoking amplified the effect of maternal pre-pregnancy obesity on GDM risk to further increase the prevalence of GDM (Coughlan et *al*., 2004).

Our result showed that overweight and obesity was significantly associated with gestational diabetes risk. Other study confirmed that obesity is a strong risk factor for pregnancy complications and also overweight women increased risk for gestational diabetes (Marey, 2017;Meaghan et *al*., 2008). Obesity in pregnant women increases the risk of gestational diabetes mellitus (GDM).Maternal body mass index is positively correlated with systemic inflammation, as shown by the high levels of TNF-α, macrophages accumulate in the stroma of placental villi (Aye et *al* .,2014). This accumulation is associated with an increase in pro-inflammatory cytokines. These cytokines participate in the pathophysiology of this disease as well as oxidative stress causing an increase in gestational diabetes associated with insulin resistance (Gabory et *al*., 2016). Obesity and insulin resistance are central attributes of both GDM and the metabolic syndrome. These characteristics and dyslipidemia are associated with endothelial dysfunction, oxidative stress and over expression of inflammatory responses (Marshall & Carpenter, 2007).Our study showed that eating sugars a lot during pregnancy and the big weight of baby were significantly associated with gestational diabetes risk. During pregnancy, some of the hormones produced by the placenta reduce the action of insulin. The pancreas then needs to produce extra insulin to keep blood glucose levels in the normal range (Ellie, 2017). If the pancreas is unable to produce enough insulin, blood

glucose levels rise and gestational diabetes develop (Luc et *al*., 2016). If blood glucose levels are high during pregnancy, glucose passes through the placenta to the baby, who then makes extra insulin. This can make the baby grow too big, which can cause problems during labor, and increase the risk of early delivery or the need for a caesarean section (Debra , 2017).

The results of our study showed that women at first pregnancy and the use of contraceptive pill after the age 30 years are prone to gestational diabetes risk .Our results is in agreement with the study of (Buchanan et *al*., 2012) which showed that women in the first baby have a GDM risk and with the results of Rebecca et *al* (2013) show that the use contraceptive pill, after the age 40 years is a risk factor for gestational diabetes. The use of oral contraceptives has a direct effect on the regulation of blood glucose by promoting a disruption of the glucose control system in the body (Manuel & Andrea, 2014). It is in agreement with the study of Catherine et *al* (2002), which show that the use of contraceptive increased glucose and insulin levels and risk of diabetes.

Social problem is a significant risk for GDM according to our results. It may represent in depression. Depression during pregnancy is associated with poorer maternal health, increased likelihood of obstetric complications, preterm birth, and neonatal complications (Byrn & Penckofer, 2013), depression is linked to obesity and insulin resistance (Oladeji & Gureje, 2013), women with depression who become pregnant should be carefully monitored for impaired glucose tolerance. In addition, certain antidepressant and centrally acting antipsychotic medications may increase the risk of type 2 diabetes. This relationship is attributable to several mechanisms, both associated with and independent of weight gain (Barnard et *al*.,2013), and a similar relationship may exist for GDM.

Our results show that family history is a risk factor for GDM. There are studies that have examined the familial clustering of GDM and type 1and type 2 diabetes. Studies of Dorner et *al* (1987) who showed an increase in familial aggregation of diabetes on the maternal side of offspring with type 1 diabetes whose mothers had GDM. Similarly, there is evidence for clustering of type 2 diabetes and impaired glucose tolerance in families with a GDM (McLellan,1995) and evidence for higher prevalence of type 2 diabetes in mothers of women with GDM (Martin, 1985).

Disposition index is a measure of β-cell compensation (Bergman, 1981), and this association is consistent with the known biologic function of *HNF4A* in the pancreatic β-cells .Thus, variation in *HNF4A* may contribute to the β-cell dysfunction observed in GDM (Odom ,2004).

Biological marker study:

The obtained results show a significant increase in blood glucose level in patient group as compared to control. Blood glucose is a very important marker for confirmation and control of diabetes disease. This allows evaluation of the metabolic impairment associated with diabetes hyperglycemia (ADA, 2009).There are many potential mechanisms whereby excess glucose metabolites traveling along these pathways might promote the development of complications of diabetes mellitus and cause pancreatic cell damage. However, all these pathways have in common in the formation of ROS, that in excess and over time, causes chronic oxidative stress, which in turn causes defective insulin gene expression and insulin secretion as well as increased apoptosis (Yang et *al*., 2011).

On the other hand, we found a significant change in cholesterol level ,cholesterol metabolism is altered in diabetic states. Three main mechanisms seem to be involved in these alterations: an increased glycation of cholesterol-rich lipoproteins, an insulin-resistant state which is mainly present in overweight type 2 diabetic patients, and changes in insulin secretion which depends on the clinical type of diabetes(Carolyn,1984).In poorly controlled and/or in insulin resistant diabetic patients, both VLDL cholesterol production and cholesterogenesis are increased, mainly as a consequence of the insulin-resistant state. The excessive glycation of LDL results in a diminution of their catabolism and therefore an increase of their plasma concentrations (Monnier et *al* .,1995).

In our experimental study, the results show a significant increase in TG levels. High TG's signals insulin resistance; that's when you have excess insulin and blood sugar isn't responding in normal ways to insulin. This results in higher than normal blood sugar levels (Naomi, 2015). Fat in the bloodstream can build up inside the muscle cell and create toxic fatty breakdown products and free radicals that can block the insulin signaling process. When that happens, no matter how much insulin we have in our blood, it won't be able to open the glucose gates. That causes blood sugar levels to build up in the blood (Michael, 2016).

Our results showed that WBC and LYM are significantly increase in both groups pregnant and non-pregnant patients in GDM than control women. This result is in agreement with the study of Akif et *al* (2016), who showed that the leukocyte and lymphocyte counts were significantly higher in women with gestational diabetes compared than the control group. A high leukocyte count is a marker of inflammation, and it is thought that cytokine-induced insulin resistance is a central mechanism that underlies the relationship between inflammation and GDM (Akif et et *al.*, 2016).In our study, the results show a significant change in thyroid hormones (TSH, T3 and T3 / T4) in women with gestational diabetes compared to control. This in agreement with Other studies showed that thyroid disorders for TSH,T3, T4 in pregnant women with gestational diabetes were significantly increased than control group (Carlos et et *al*.,2000).Thyroid hormones contribute to the regulation of carbohydrate metabolism and pancreatic function, which may be the origin of change in biochemical parameters during gestational diabetes. On the other hand, gestational diabetes affects thyroid hormone to variable extents (Syeda, 2015), as an example the Terence& Chin- Peng (2002) study that found TSH is significant increase in GDM patients compared to control women, which may be the cause of complications in this disease. Change level of thyroid hormone associated with GDM can be compensated by increasing the placenta availability of T3 / T4 by increasing the activity of thyroid hormone carriers and / or reducing the diodines in the placental circulation leading to be significant increase to pregnant women (James et *al*., 2016). Our results show also that a significant correlation between T3/T4 activity and RBC GSH level, which is present the relationship between dysthyroidism and oxidative stress. Thyroid hormones are the most significant regulator of the basal metabolic state and oxidative stress (Vikram,2017) .Thyroid hormones play particularly important role that operate on the antioxidant balance, since both hyperthyroidism and hypothyroidism have been shown to be associated with oxidative stress (Mancini et *al.,* 2016).Also hyperthyroidism is a hyper metabolic state accompanied by increased oxygen utilization, increased production of reactive oxygen species and consequently measurable changes in anti-oxidative factors (Derouiche et *al*., 2018)**.**

In our study, we found a significant decrease in HCT level ($P < 0.05$)in the diabetic groups compared with controls, hematocrit (HCT) level were associated with insulin resistance and incident type 2 diabetes. Hematocrit is positively correlated with hyperinsulinemia and risk factors associated with insulin resistance, hematocrit is a major

determinant of blood viscosity. Increased blood viscosity also contributes to the development of insulin resistance (Tamariz et *al*., 2008).

In our results we found a significant decrease in hemoglobine; RBC;HTC in pregnant gestational diabetes patients as compared to controls.

Low hemoglobin is anemia. When pregnant, the amount of blood produced by the body increases by up to 30 percent, meaning that the body requires more iron in order to produce sufficient hemoglobin. If the body does not receive a sufficient amount of iron to do this, anemia can result when there is a low hemoglobin level, there is often a low red blood cell count and a low hematocrit, too (Rossing ,2004).

Anemia is relatively common in patients with diabetes mellitus, and low hemoglobin concentration contributes to many clinical aspects of diabetes mellitus or its progression. Low hemoglobin concentration in patients with diabetes mellitus is associated with a more rapid decline in glomerular filtration rate than that of other kidney diseases .Diabetic nephropathy and diabetic retinopathy result in increased susceptibility to low hemoglobin level (Ranil,2010).

Our study showed that iron was significantly decreased for both groups pregnant and non-pregnant patients compared to control women. This result is in agreement with the study of (Terence & Lai-Fong , 2004) ,who showed that iron deficiency anemia significantly decreased associated with gestational diabetes patients than control women but the study of (Afkhami & Rashidi ,2007) showed that iron is significantly increased in gestational diabetes patients than in control women, there was this hypothesis that high iron stores in GDM women could be due to nutritional improvement in pregnant women. Another study found that iron concentration in study group was significantly lower in patients gestational diabetes than control women. Akhlaghi *et al*(2012), showed that are required to prove the causal relationship between iron level and gestational diabetes. In our study, we have also shown that calcuim is significantly increase in gestational diabetes patients than controls, the study of Citlalli et *al* (2017), suggest that higher levels of calcium intake are associated with lower GDM risk. Calcium, the most abundant mineral in the body, performs a number of basic functions including maintaining insulin secretion, during pregnancy, calcium absorption increases to meet fetal bone mineralization requirements increased calcium intake. Whether dietary calcium

intake might modify GDM risk through direct (insulin release) or indirect effects (Citlalli et *al*., 2017).

Our results show that the level of serum Zn/Cu is significant decrease in GDM patients as compared to controls, our results are in agreement with the study of Yinsong et *al* (2002) showed the increase of Cu levels and decrease of Zn levels in the serum of diabetic patients, in the other hand, according to the study of Mariana et *al* (2016), zinc serum levels in pregnant women (24-28 weeks of gestational) with abnormal glucose tolerance test is lower than in normal pregnancy group. Lower zinc level in our results can be explain bay the copper which is able to reduce serum and tissue levels of zinc through competition at fixation in the intestine , copper prevents absorption of zinc and thus affect their bioavailability in the body. In addition, excess copper can lead to deregulation of the homeostasis of molecular Zn (Derouiche , 2016).

Our results show a strong correlation between serum Zn/Cu variation and thyroid hormones(TH).Zn deficiency may play a role in the biosynthesis or release of thyroid hormones that have important effects on insulin activity, to increase the body fat deposition, and thyroid hormones conversion (T4 to T3). Zinc concentration was inversely related to T3 level in diabetics, zinc deficiency impairs the metabolism of thyroid hormones, androgens, and above all growth hormones (Arreola et *al*., 1993).

Alkaline phosphatase (ALP), an enzyme presented primarily in bone and liver, in our study, results showed a significant decrease of this enzyme in diabetics group compared to controls which is confirmed by the study of Derouiche (2016). Alkaline phosphatase was the first zinc enzyme to be discovered in which three closely spaced metal ions (two Zn ions and one Mg ion) are present at the activity center (Joseph,2019). Zn and Mg deficiency are the most important causes of low ALP (Ray et *al*.,2017). One of the causes or the development of diabetes associated complications can be attributed to the low levels of Zn as there are many enzymes which contain zinc (Jyothirmayi & Vasantha, 2015) therefore zinc deficiency associated with diabetes may be the cause of decreased ALP activity in GDM peoples.

Our results show that amylase activity is decreased the GDM group as compared to control. Amylase is a metalloenzyme at a catalytic site subdivided into several sub sites. The specificities of amylolytic enzymes could also be explained by the existence of zinc

ions in their structure (Derouiche et *al*., 2013), so the reduction of zinc during GDM explains its consequence on amylase activity that are decreasing during GDM.

- **Study of oxidative stress markers**

The results of the oxidative stress study showed that MDA in RBC are significantly increased in both groups pregnant and non-pregnant gestational diabetes patients compared to control women. The results showed that MDA in RBC has a high specificity in ROC statistic, which showed the importance of MDA in the prognostic of gestational diabetes. The results found were similar to those observed in study of Peuchant et *al* (2004) showed that erythrocyte MDA levels were significantly higher in gestational diabetes women than in controls. They illustrate that the increased oxidative stress must demonstrate in pregnant women with GDM should be monitored by strictly controlling blood glucose during pregnancy with stringent recommendations and perhaps antioxidant supplementation (Peuchant et *al*.,2004).The studies of Arribas et *al* (2016) showed that maternal MDA level in serum and plasma that had been to be significantly higher in GDM patients compared to control women. Our results show that WBC MDA is significantly decreased in GDM for pregnant patients compared to control women, with high specificity in ROC statistic test, which showed the importance of this parameter in the identification of the disease. The decreased ability of pregnant women with GDM to compensate for oxidative stress was manifested as increased insulin resistance, reduced insulin sensitivity and β-cell dysfunction, all of which may play important roles in GDM (Zhu et *al* .,2015).

Our study showed that catalase level in leucocyte in both groups pregnant and non-pregnant women was significant increase in GDM patients than controls women with highest specificity of the disease study, which is in agreement with the study of Tarnai et *al* (2007), who showed that blood catalase activity is significantly decreased in GDM patients compared to control women. Catalase is the main regulator of hydrogen peroxide metabolism, which is associated with diabetes mechanisms such as Glut 4 expression, insulin secretion, insulin signaling, protein tyrosine phosphatase regulation, and glucose transport stimulation (Laszlo et *al.*,2005). The decreased of blood catalase activities in gestational diabetic patients may explain by a lower antioxidant capacity against the hydrogen peroxide, which increases in diabetes mellitus (Tarnai et *al.*, 2007).Women with previous GDM have high catalase levels, which correlate positively with glucose intolerance, indicating the potential effect of oxidative stress (Rodríguez et *al* .,2014).

The significant decrease in total antioxidant power ORAC in the serum and erythrocytes of pregnant women, especially during the third trimester of pregnancy clearly shows the evolution of the state of stress oxidative in pregnant women. So pregnancy is a physiological state characterized by oxidative disturbance that contributes to the initiation and progression of complications associated with pregnancy (Derouiche et *al*.,2018) including GDM.

Conclusion

Conclusion

Gestational diabetes mellitus (GDM) has emerged as a global public health problem, pregnant woman in which maintaining glucose homeostasis is of upmost importance. When hyperglycemia is detected in the pregnant mother, this is referred to as GDM, although controversy remains over diagnostic criteria . Several risk factors have been studied to find out which one is really involved in gestational diabetes.

The results of our study showed that passive smoke, social problems ,obesity, big baby, eating sugar, overweight, disease in the first pregnancy, family history, chronic disease, taking contraceptive after 30 year are proven to be major risk factors for gestational diabetes, which indicates the importance of social behavior and the clinical factor in causing gestational diabetes. In contrast, sport is protective factors against gestational diabetes in the study population.

Our study showed that the results of hematological markers exhibit low level of HCT, RBC and HGB and high level of WBC and LYM which are indicated in Gestational diabetes, concerning biochemical markers our results show that high level of blood glucose, cholesterol , TG concentration, copper, calcium and Zn/Cu and low level of ALP, amylase, deficiency of iron and zinc have a significant association with Gestational diabetes. So hematological and biochemical parameters are an important markers of diagnosis and therapeutic follow-up of the disease.

The results demonstrated the high level of MDA and catalase in leucocyte and low level of GSH in leucocyte and serum ORAC is indicated in GDM. The increased oxidative stress and reduction in antioxidant defense mechanisms occurs in the circulation and placenta of women that causing GDM. Since these imbalances can lead to maternal complications during pregnancies. Therefore, we propose to include antioxidants for the list of special drugs for GDM women or for pregnant women as protection from this disease.

The results demonstrated the high level of thyroid hormone with a strong relationship with serum copper and zinc level, which clearly showed the importance of these factors in the control of the disease and suggest supplementing specific treatments to regulate these factors to protect women against GDM and its complications.

On the other hand , for not pregnant women our result showed that the level of MDA and catalase in leucocyte represented the highest percentage of sensitivity and important percentage of specificity and AUC ,and for pregnant women showed that the level of MDA in leucocyte and erythrocyte, catalase activity in leucocyte, and serum ORAC represented the highest percentage of sensitivity and important percentage of specificity and AUC . Therefore, our results indicate these parameters are new reliable markers for diagnostic and predictive against gestational diabetes in women.

- **Prospects:**

Given the importance of these results, they open experimental perspectives and other in- depth studies that should allow us to clearly identify to the determine other factors associated with Gestational diabetes risk, evaluation of diagnostic marker variation in Gestational diabetes women and women healing, determine risk factor of development GDM to type 2 diabetes and we hope that some studies focus on the development of treatments that eliminate it after delivery.

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Annexes

Annexes

- **Annex 01:**

Age:…… Address:……..

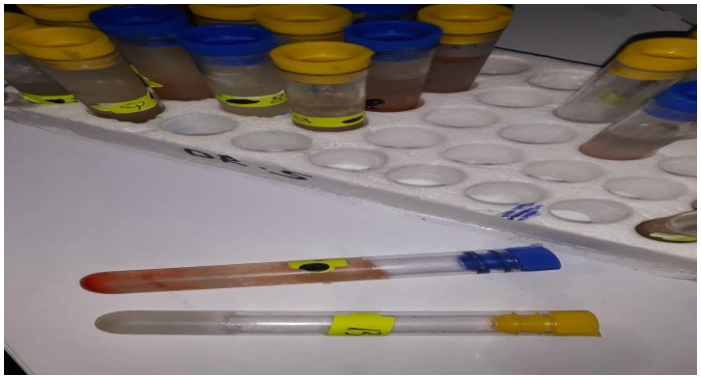
Number of children:……. Education level:…….

Blood type:…. Length:…….

Job:…… weight:…….

N°	Question	Yes	No	Notes
01	Is there any smoker in your family?			
02	Are you addicted to fast foods or eating out of the house?			
03	Are you addicted to soft drinks?			
04	Do you eat a lot of sugars in your pregnancy carvings?			
05	Are you exposed to chemicals in your daily life?			
06	Do you practice sports in your daily life?			
07	Did anyone in your family have the same disease?			
08	Do you suffer from other disease? Mention it			
09	Do you often eat canned food?			
10	Are you facing problems permanently?			
11	Do you use spices a lot?			
12	Do you drink tap water?			
13	Do you use food additives?			
14	Did you, or do you suffer from obesity?			
15	Do you suffer from overweight?			
16	Does the weight of your previous baby was big?			
17	Are you active in your daily life?			
18	Do you use contraceptive pills?			
19	Did you suffer from cyst in the womb or ovary?			
20	Are you nervous in your daily life?			
21	Do you use contraceptive pills regularly or sporadically?			
22	When did you start eating pills? Before 20 years / between 20and30 years / after 30years			
23	When was your first born?			
24	Did you have a cesarean section before?			
25	Does the disease repeat in each pregnancy?			
26	Is your menstrual cycle regular or intermittent?			
27	Have you ever had an abortion?			
28	In which pregnancy did this disease appear?			
29	Has the disease become : chronic /ended after birth ?			
30	Is there any consanguinity between you and your husband?			

- **Annex 02:**



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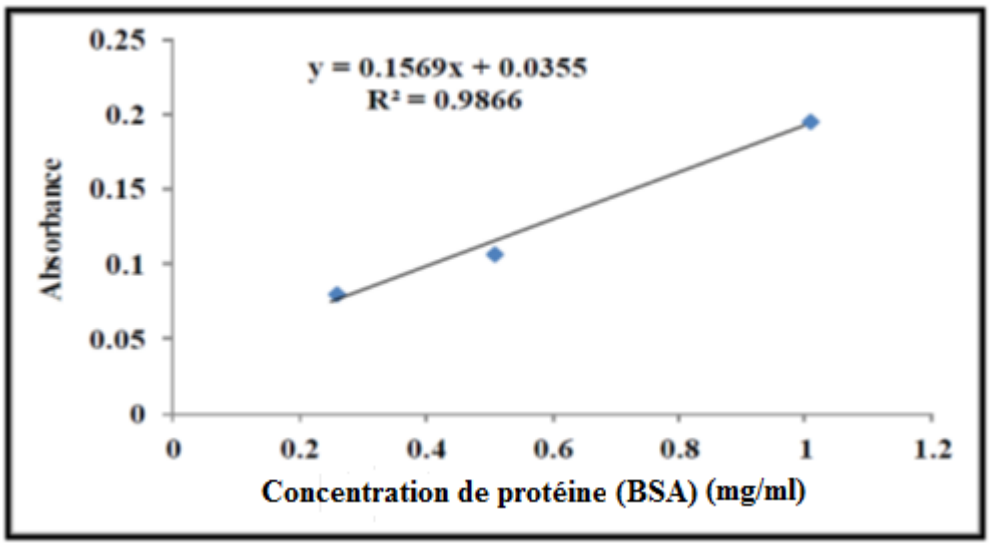
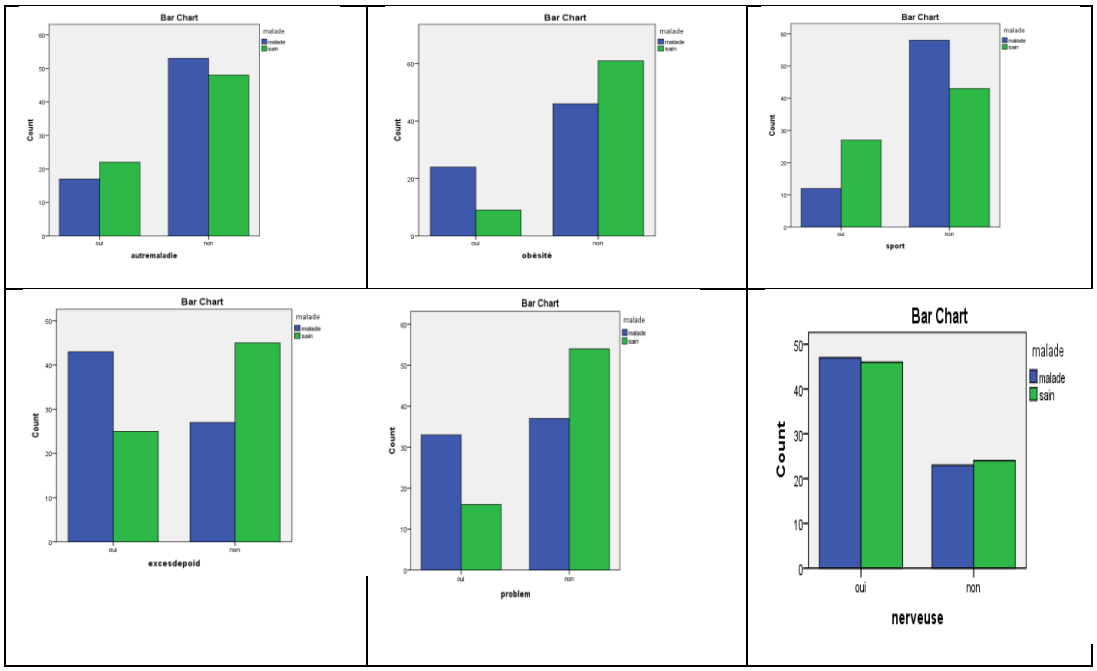


Figure : Courbe d'étalonnage de BSA pour le dosages des protéines

- **Annex 04:**

- **Annex 05:**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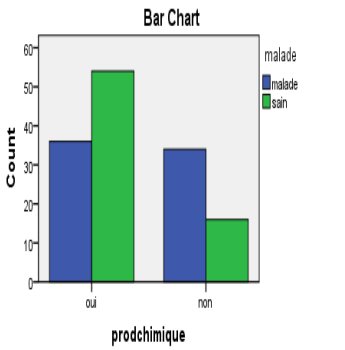
Annexes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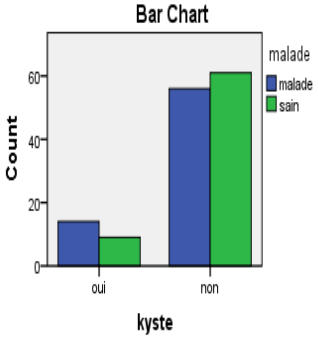
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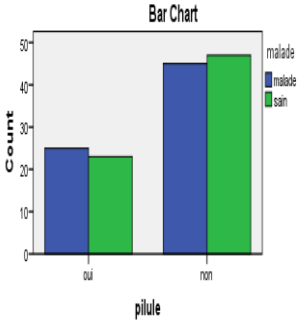
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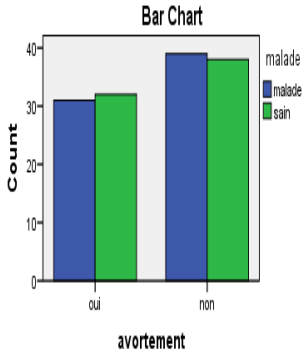
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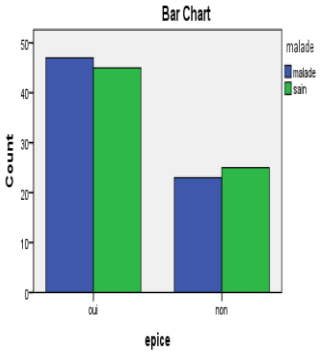
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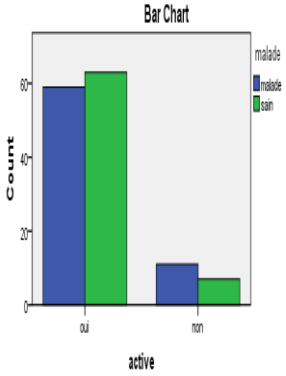
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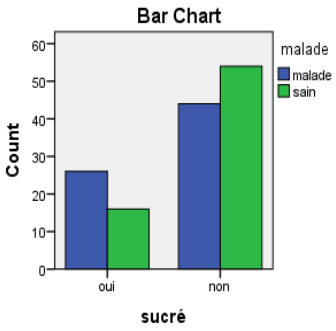
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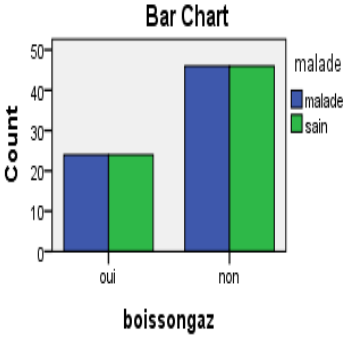
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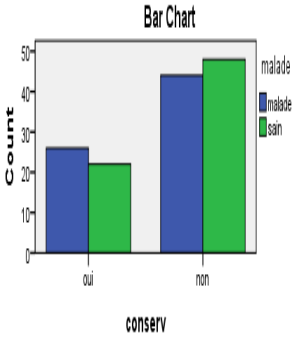
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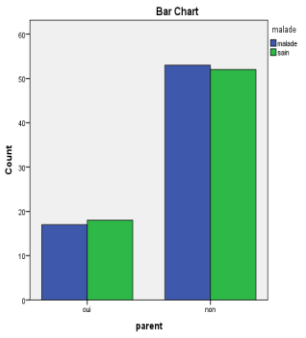
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