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Study of Immunological Dysregulation and *Helicobacter Pylori* in Gestational Diabetes Mellitus patients

A Thesis

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيم

﴿ يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ ﴾

صَدَقَ اللهُ العَلي العَظيم

المجادلة: (11)

Certification

We certify that this thesis entitled "**Study Immunological dysregulation and Gram negative bacteria Isolated from Different clinical Specimens** "was prepared by **Hajar Dawood Salman AL-Shujiry** under our supervision at the college of Medicine, University of Babylon, as a partial requirement for the degree of Master in **Medical Microbiology.**

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Dedication

Dear father, please know that I miss you, and that I have been sending you prayers and love ever since you passed away. My God provide you eternal peace in heaven. It was you and the way I remembered you that helped shape the kind, caring person I am today, I dedicate this work to your soul, and to my mother.

Hajar AL-Shujíry (2023)

Acknowledgment

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I would like to introduce my full gratefulness to my supervisor, **Dr. Ifad Kerim Al-Shibly** for his guidance and support throughout my graduate and my study experience. I also thank supervisor professor **Dr. Huda H. Al-Hasnawy** for his advice and kindness. I would like to thank head department professor **Dr. Hayam K. Al-Masoudi**

I would like to thank every person help me; I would like to thank my friends**.** I would like to thank patients **from them the samples were taken** for their real help in finalizing this work at excellent manner.

Hajar AL-Shujiry (2023)

Summary

In a normal immune system, immune cells recognize and fight invaders, like infections. A dysregulated immune disorder occurs when the body can't control or restrain an immune response. The body either Underreacts to foreign invaders, this can cause infections to spread quickly or Overreacts to foreign invaders, this causes the immune cells to attack healthy cells, tissues, and organs.

Gestational diabetes usually reveals itself in the last half of pregnancy and is determined by intolerance to variable-intensity carbohydrates. Hyperglycemia develops during pregnancy due to the secretion of placental hormones, which cause insulin resistance. *Helicobacter pylori* is a common type of bacteria that grows in the digestive tract. The maternal immune dysregulation during gestational diabetes mellitus can induce the infection with *H. pylori*.

Eighty-nine individuals were enrolled in this study (case-control). Whole blood samples were collected from twenty-nine pregnant women apparently healthy control subjects whereas whole blood samples were collected from sixty patients with gestational diabetes mellitus (GDM) who were attended to the outpatient clinic of Gynecology department in Al-Mahaweel Hospital/ Babylon/ Iraq and Al-Imam Al-Sadiq Hospital/ Babylon/ Iraq and from some private clinics during a period extended from August 2022 to December 2022. Patients were diagnosed by Gynecology consultant surgeon and were examined by several examinations such as (Fasting Blood Sugar, Oral Glucose Tolerance Test, Hemoglobin A1c, Complete Blood Count and C-Reactive Protein test).Venous blood samples were divided into two aliquots; one for chemical tests and the other separate the serum for immunological tests [IgG and IgM *Helicobacter pylori* tests

and interleukine-1beta, interleukine-18 and NOD-like receptor protein 3 (NLRP3) concentration tests].

The Study has reached the following results: The age range of patients with GDM was (25-47) years; therefore, we chose the age range of control was (23-45) years. The mean age of patients was (34.83 ±0.80 years) and of control was (28.45 ±1.16 years). There was highly significant difference between patients and control groups (P≤0.01). The Bod mass index range of patients was (22-41) Kg/m^2; therefore, of control was (20-40) Kg/m^2.The mean BMI of patients was (34.67 ±0.70) and of control was (31.38 ±1.13). There was significant difference between patients and control groups (P≤0.05).The mean fasting blood sugar of patients with GDM was (132.60 ± 3.52 mg/dl) and of control was (66.31 ± 1.86 mg/dl); there was highly significant difference between patients and control group (*P*≤0.01). The mean Oral Glucose Tolerance Test of patients was (208.78 ± 5.96 mg/dl) and of control was (85.68 ± 1.26mg/dl); there was highly significant difference (*P*≤0.01) between patients and control groups. Hemoglobin A1c of patients was (7.41 ±0.06 %) and of control was (4.45 ±0.08 %), there was highly significant difference between patients and control groups (*P*≤0.01). Lymphocyte of patients was (34.43 ±1.09 %) and control was (27.45 ±1.15 %), there was highly significant difference between patients and control groups (*P*≤0.01). Neutrophil of patients was (61.60 ±1.15) % and control was (59.24 ±0.83) %. There was Non-significant difference between patients and control groups (*p* =0.182). In the present study, there was significant difference between patients and control group (P≤0.05) with C- reactive protein. In the present study, there was significant difference between patients and control group (P≤0.05) in physical activity. Family history of

GDM is show significant difference between patients and control groups (P≤0.05), also the family history of DM between patients with GDM and control is shown significant difference between patients and control groups (P≤0.05).

Immunological study reveds to: the mean of IL-1ß of patients with GDM was (38.68 ±5.02) and control was (6.12 ±0.37), there was highly-significant difference between patients and control groups (P≤0.01).The mean of IL-18 of patients was (58.87 ±4.79) and control was (20.13 ±1.06). There was highly-significant difference between patients and control groups (P≤0.01).The mean of NLRP3 of patients was (76.39 ±6.71) and the control was (41.15 ±2.66). There was highly-significant difference between patients and control group (P≤0.01).

In present study showed that GDM significantly increased the incidence of *H. pylori* infection (*P*≤0.01). In Addition the infection rates of *H. pylori* IgM and IgG were significantly higher in the GDM group and Control group (*P*≤0.01).

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List of Abbreviations

Abbreviation	Full Name
ADA	American Diabetes Association
APCs	Antigen presenting cells
B cells	Bone marrow cells
BMI	Body mass index
Cag A	Cytotoxic-associated gene A
CARD	caspase recruitment domain
CBC	Complete blood cells count
CD4	Cluster of differentiation 4
CRP	C-reactive protein
DAMPs	Danger-associated molecular patterns
DCs	Dendritic cells
DCs	Dendritic cells
DM	Diabetes Mellitus
EDTA	Ethylene diamine tetra acetic acid
ELISA	Enzyme linked immune sorbent assay
FBS	Fasting blood sugar
FIA	Fluorescence Immunoassay
g\dl	grams per deciliter
GCC	Gulf Cooperation Council

GCT	Glucose Challenge Test
GDM	Gestational diabetes mellitus
H .pylori	*Helicobacter pylori*
HAPO	Hyperglycaemia and Adverse Pregnancy Outcome
Hb	Haemoglobin
HbA1c	Hemoglobin A1c
HOMA-IR	Homeostasis Model Assessment of Insulin Resistance
HPL	Human Placental Lactogen
HRP	Horseradish Peroxidase
IDF	International Diabetes Federation
IFN- γ	Interferon gamma
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-18	Interleukin- 18
IL-1b	Interleukin-1 beta
IR	Insulin Resistance
IU	International unit
LPS	Lipopolysaccharides
LRR	Leucine-rich repeat
MCH	Mean corpuscular haemoglobin
MHC II	Major histocompatibility complex class II
MPS	Mononuclear phagocyte system

NET	neutrophil extracellular trap
NK	Natural killer
NKT	Natural killer T
NLR	Neutrophil to Lymphocyte Ratio
NLRP3	Nod-Like Receptor protein 3
OD	Optical density
OGTT	Oral glucose tolerance test
PAMPs	pathogen-associated molecular patterns
PAPCs	Professional antigen presenting cells
PCOS	Polycystic Ovary Syndrome
PE	pre-eclampsia
PRRs	pattern recognition receptors
RBC	Red blood cells
SFA	Saturated Fatty Acids
T cells	Thymus cells
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
Th	T-helper type cells
Th1	T-helper type 1 cells
Th17	T-helper type 17 cells
Th2	T-helper type 2 cells
TLR	Toll- Like receptors
TNF-α	Tumor necrosis factor alpha

Treg cells	Regulatory T cells
US	United states
UTI	urinary tract infections
VacA	Vacuolating cytotoxin A
VEGF	Vascular endothelial growth factor
WBC	White blood cell
WHO	Word healthy organization

Chapter One

Introduction and Literatures Review

1.1 Introduction

Gestational diabetes mellitus (GDM), referred to any level of glucose intolerance with beginning or first recognition throughout pregnancy, is a serious obstetrical complications characterized by an inadequate insulin response [Hyperglycemia] to make up the resistance to insulin state of the pregnancy (McElwain *et al*., 2021).

It has been estimated that hyperglycemia throughout pregnancy affected 21.3 million births (16.2%) worldwide, with GDM accounting for 86.4% of these cases (Nigatu *et al*., 2022).

Gestational diabetes mellitus has been linked to a higher risk of a variety of problems in pregnant women's and fetuses throughout birth and later in lifetime. GDM complications consist of preeclampsia, a cesarean delivery, and a higher possibility of developing type 2 Diabetes mellitus (T2DM) in later daily life. Macrosomia, congenital abnormalities, birth trauma, respiratory difficulty, and hypoglycemia and jaundice are among the child's problems (Darbandi *et al*., 2021).

Hyperglycemia has been shown to impair immune function, impairing neutrophil chemotaxis, the macrophage function, and phagocytic responses, making diabetic patients more vulnerable to infections and related comorbidities. In addition the imbalance in innate and adaptive immune responses during the pregnancy, a condition previously established by immunological modifications, will result in additional risks to health, as evidenced by the increased risk of hypertension and pre-eclampsia (PE), macrosomia, which premature delivery, and a stillbirth with GDM detection (Mouton *et al*., 2020).

In GDM, hyperglycemia is linked to elevated placental inflammation.in which excess glucose can activate the pyrin domain-containing protein 3 (NLRP3) inflammasomes in lymphocytes, resulting in the production of inflammatory cytokines such as IL-1β _ and IL-18 (Wu *et al*., 2022).

The Human placenta and obese tissue are now known to produce a variety of pro-inflammatory factors, including the cytokines such as Interleukin-1 beta (IL-1β) (Zarezadeh *et al*., 2022).

Interleukin 1b (IL-1β), which a pro-inflammatory cytokine, was recently implicated in the development of glucotoxicity and decreased insulin production in diabetes mellitus, and it increases in non-diabetic the pregnancy at pre-term labor and in the process of thermogenesis among individuals with heart failure (Alfadul *et al*., 2022).

Studies in GDM patients have shown a link between NLRP3 inflammasome activation and insulin resistance. When compared to normal and low glucose levels, higher levels of glucose increase NLRP3 activation (Zhou and Zhang , 2021).

Individuals with GDM had significantly greater IL-18 level than healthy pregnant womens, yet, after changing for glucose, insulin, and BMI values, IL-18 has no significant variation (Zhao *et al*., 2018).

Interleukin 18 (IL-18) is a cytokine that is produced by [macrophages, epithelial cells, dendritic cells, keratinocyte, osteoblasts, and adrenal cortex cells] and plays a significant part in inflammation diseases (Hirooka *et al*., 2021).

The immune response includes a variety of processes which effect on innate as well as adaptive immunity, resulting in inflammatory and anti-inflammatory reactions that allow numerous *H. Pylori* infections to survive (Niu *et al*., 2020).

H. pylori bacteria are gram-negative, microaerophilic, and spiral-bacilli. The body contains between two and six flagella, and flagella mobility provides and allows for rapid motion in viscous fluids, like the mucus membrane of the stomach epithelial cell .It avoids innate immune receptors in the body but virulence factors activate innate immunity, resulting in increased stomach inflammation (Kim *et al*., 2021).

Aim of Study

The aim of this study was to evaluate the interaction between maternal immune dysregulation and *H. pylori* infection as an immunological-bacteriological study among GDM women in Iraq.

This study has been carried out to achieve the following objectives:

1. To measure serum Interleukin 1 β (IL-1β).
2. To measure serum Interleukin 18 (IL-18).
3. To measure serum pyrin domain-containing protein 3 (NLRP3).
4. To measure serum *H. pylori* IgG
5. To measure serum *H. pylori* IgM

6. To determine the levels of FBS, OGTT, HbA1c, C- reactive protein (CRP), Lymphocyte and Neutrophils counts.
7. Statistical analysis.

1.2 Literatures Review

1.2.1 Gestational Diabetes Mellitus (GDM)

1.2.1.1 Definition of GDM

Gestational diabetes mellitus (GDM), referred to any level of glucose intolerance with beginning or first recognition throughout pregnancy, is a serious obstetrical complications characterized by an inadequate insulin response [Hyperglycemia] to make up the resistance to insulin state of the pregnancy (McElwain *et al*., 2021).

Is a temporary metabolic state during pregnancy that involves carbohydrate intolerance, hyperglycemia, periphery insulin resistance, inadequate the production of insulin or action, dysfunctional endothelial cells, beta-pancreatic disorder, immunological disorder, and low-grade inflammation (Olmos *et al*., 2021).

In the year 2021, that was estimated that hyperglycemia during gestation will impact 21.3 million newborns (16.2%) worldwide, with GDM accounting for 86.4% of these instances. The incidence of GDM in a population reflects the incidence of T2DM in that group, thus, ethnic and racial groups with an elevated incidence of T2DM are more likely to develop GDM (Hong *et al*., 2022).

The maternal peripheral resistance to insulin is a critical event in the development of GDM. There is a transitory and physiological condition of impaired insulin sensitivity during normal pregnancy, which is required to take precedence fetal glucose uptake. In reaction, cells grow and produce

more insulin as a means of combating insulin resistance and promoting euglycemia (Giordano *et al*., 2020).

The increased energy requirements of the mother, as well as the expanding fetus and placenta, cause significant alterations in metabolism during GDM (Salazar *et al*., 2022).Pregnancy causes changes in the regulation of glucose metabolism due to the actions of different hormones that include placental lactogenic; placental hormone growth production, and additional substances that interfere with the actions of insulin, resulting in an outcome of in relation insulin resistance as the pregnancy progresses (Sferruzzi *et al*., 2020).

Recent research has also revealed that the human placenta or adipose tissue release a variety of pro- and anti-inflammatory cytokines, including Interleukin-1 beta (IL-1β) (Shahcheragh *et al*., 2023). IL-1 beta is an essential inflammatory mediator that has been demonstrated to be elevated in the placentas of obese mothers and in GDM patients (Melton *et al*., 2021).

Both plasma and placental levels of the inflammatory cytokine (IL-1 β) elevated under all hyperglycemic circumstances, and NLRP3 was activated in all hyperglycemic groups (Guo *et al*., 2023).

Studies in GDM patients have shown a link between NLRP3 inflammasome activation and insulin resistance (Zhang *et al*., 2019). When compared to normal and low glucose levels, higher levels of glucose enhance NLRP3 activation. It is well recognized that hyperglycemia and diabetes while pregnant can activate the inflammasome NLRP3 and cause the release of several inflammatory cytokines, resulting in serious pregnancy problems (Wu *et al*., 2022).

Cytokines have both pro-inflammatory and anti-inflammatory properties. Diabetes, which is glucose intolerance, and insulin resistance are all linked to a rise in the production the cytokines that are pro-inflammatory including IL_18 (Burhans *et al*., 2018) (Figure 1.1)

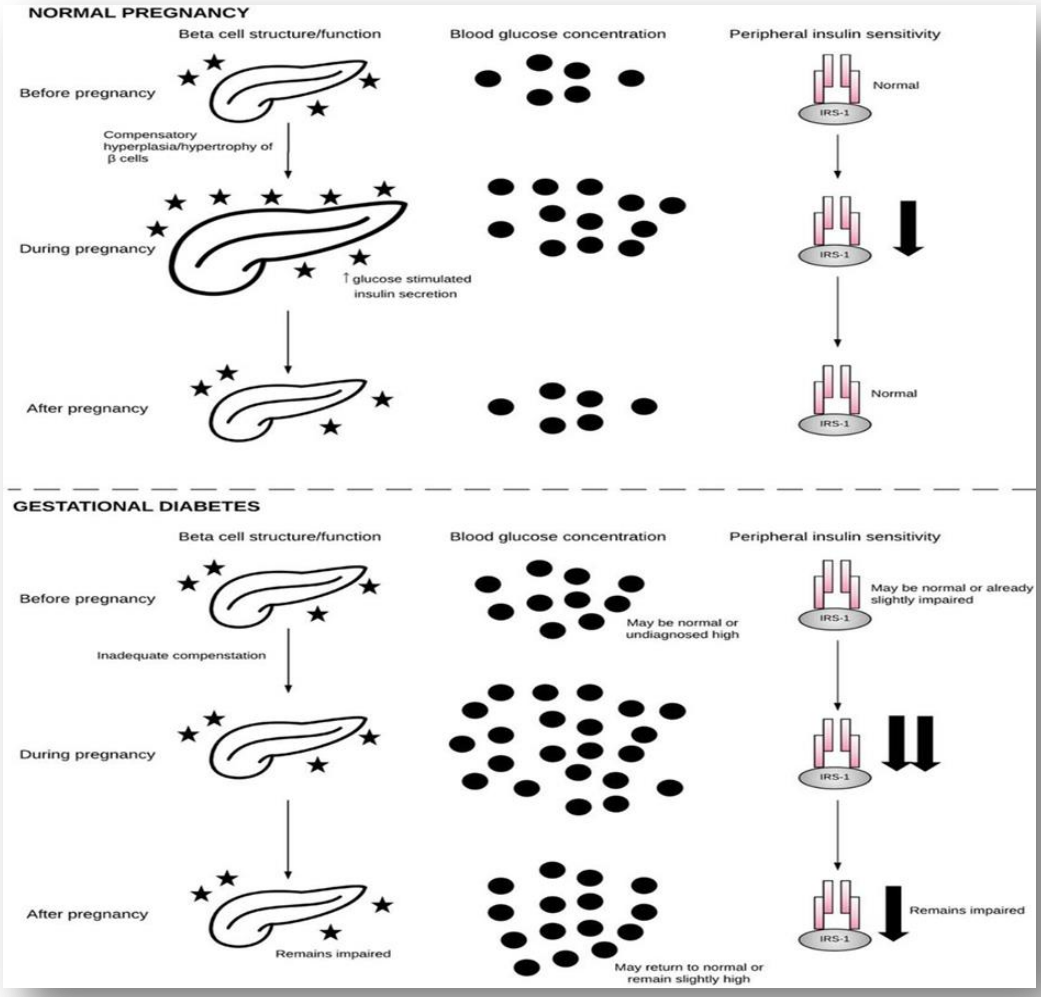

Figure (1.1): The relationship between β-cell dysfunction, insulin resistance, and GDM (Plows *et al*., 2018).

1.2.1.2 Prevalence

Gestational diabetes is defined as hyperglycemia, or blood glucose levels that are higher than normal but lower than those required diagnosing diabetes. Gestational diabetes is kinds of diabetes that develops during pregnancy. Women who have gestational diabetes are more likely to have difficulties during pregnancy and delivery. These mothers, as well as their children, are at a greater risk of developing type 2 diabetes during later life (Crocetti *et al*., 2012).

The worldwide prevalence is estimated to range between 1 to 14%, based on the subject of the research population, method employed, and time of diagnosis (Melchior *et al*., 2017), this show in (figure 1.2).

According to the Arab world, a study conducted in the countries of the Gulf Cooperation Council (GCC) found that prevalence rates of GDM varied by 4.2% in Oman, 10.1% in Bahrain, 16.3% in Qatar, and 2.7 - 12.5% in Saudi Arabia. These disparities in prevalence, despite similarities in native ethnic groups across the GCC, may be attributed to a variety of factors, including variations in screening and diagnosis criteria, as well as rising obesity rates in those nations (Al-Rifai *et al*., 2021). Meanwhile, the total incidence of GDM in Yemen is 5% lower than in most GCC nations, where rates range from 4.2 to 25%. In Egypt, the frequency of GDM with pregnant women is roughly 8% (Agarwal , 2020).

The prevalence of GDM in Jordan is substantially greater, at roughly 13.5%.10. In Jordan, a cross-sectional investigation on 644 singleton pregnancies that were screened for GDM using 75-g, 2-h OGTT at 24-28 weeks of gestation was undertaken. Lebanon continuous observational

research showed 13 GDM cases among 79 pregnant women's, i.e., 16.5%. (Al Subhi *et al*., 2021).

According to a localized study conducted in Gaza-Palestine to assess the frequency and sociodemographic aspects of GDM in Gaza. By 24-28 weeks of gestation of pregnancy, all pregnant women who visited UNRWA general health care facilities were routinely screened for GDM using WHO criteria. The prevalence of GDM was reported to be 1.8% in 200 out of 11241 postpartum women. The study's data was gathered from UNRWA's general health care, while the WHO criteria were used (Naser *et al*., 2022).

In the United Kingdom, as much as five percent of women delivering birth every year have already existing diabetes mellitus, also known as GDM. GDM is prevalent in Thailand at around 7%, which is comparable to the US. GDM frequency in an ethnically diverse California community ranged from approximately five percent in non-Hispanic White women to 8.5% in Asian women, with Black and Hispanic women at intermediate risk, according to a study from the United States (US) (Cade *et al*., 2019).

The estimated incidence in South Europe is roughly 6%. in other areas of Europe, like Ireland it is 10%, and in Finland is 10-11%. On the other hand, in the Northern seaboard portions of Europe, the frequency of GDM was lower. In a cohort investigation of pregnant in Germany, which was the general incidence of GDM was 13.2% (Crocetti *et al*., 2012).

Total live births to women aged 20-49 years	131.4 million
Hyperglycaemia in pregnancy	
Global prevalence	16.2%
Number of live births affected	21.3 million
Proportion of cases due to GDM	86.4%
Proportion of cases due to other types of diabetes first defected in pregnancy	7.4%
Proportion of cases due to diabetes detected prior to pregnancy	6.2%

Figure (1.2): Worldwide hyperglycemia estimates in pregnancy, 2017. (Jain *et al* ., 2019).

1.2.1.3 Pathogenesis & Risk Factors

1.2.1.3.1 Pathogenesis

GDM is a special physiologic state distinct with the normal changes in metabolism caused by pregnancy, comparing to a normal pregnancy. The GDM can be identified by higher insulin resistance (Nair *et al*., 2021).

The Insulin resistance is characterized as decrease in the tissue response to insulin action for the metabolism of glucose, which includes decreased glucose absorption in adipose tissue and muscles, reduced liver glycogen syntheses and higher liver glucose production (Petersen *et al*., 2018).

GDM has been associated to peripheral insulin resistance. [In women with GDM, decreased insulin activated glucose activity precedes the development of low insulin sensitivity and leads to the development of cell dysfunction, which results in hyperglycemia] (Yin *et al*., 2022).

Insulin resistance is defined as a reduction in tissue responsiveness to insulin activity. This influences glucose metabolism and uptake in several tissues, including adipose tissues and muscles, resulting in reduced glucose absorption, decreased production of glycogen, and enhanced glucose synthesizing, accordingly (James *et al*., 2021).

Hormonal alterations in pregnant women increase the resistance to insulin due to impaired insulin sensitivity; pregnancy is a diabetogenic state, (Dipla *et al*., 2021).

The body's response to insulin remains unchanged as insulin output rises early in pregnancy. Later, at around 20 weeks of pregnancy, there is a gradual decline in the sensitivity to insulin, which becomes even more pronounced in the third trimester, and GDM resolves right before delivery, (Saedi *et al*., 2023).

During pregnancy, maternal tissue becomes insulin resistant. This is due to placenta lactogenic hormone as well as other hormones such as hormone cortisol progesterone, and growth hormone. Tissue insulin resistance causes parental hyperglycemia and induces fetal hyperinsulinemia disease, (Hannan *et al*., 2023).

Pregnancy can be accompanied by a condition of poor-quality systemic inflammation; during normal pregnancy, the maternal innate

immune system is boosted, whilst the adaptive immune system is generally inhibited. These alterations in the immune system's adaptive capacity are intended to avoid fetal allograft rejection, (Andreoli *et al*., 2022).

The immune system is involved in the pathogenesis of GDM, (Xu *et al*., 2022). During female placental lactogenic and placental hormone growth synthesis, the placenta is implicated in the formation of resistance to insulin and fetal development during both non-diabetic pregnancy and pregnancy affected by GDM. It is suspected that cytokine-induced inflammation may be linked to increased resistance to insulin in gestational diabetes, (Al-Suhaimi *et al*., 2022).

The placental and adipose tissue was found to generate a variety of pro-inflammatory substances, include cytokines like Interleukin-1 beta (IL-1 β), (Zarezadeh *et al*., 2022). IL-18 has been related to the development of T2DM and metabolic syndrome, and its physiological activities have been claimed to induce autoimmunity pancreatic cell death resulting in type-1 diabetes, (Rasouli *et al*., 2023).

Studies in GDM patients have shown a link between NLRP3 inflammasome activation and insulin resistance. When compared to normal low and elevated glucose levels, high glucose levels enhance NLRP3 activation, (Alfadul *et al*., 2023) (Figure 1.3).

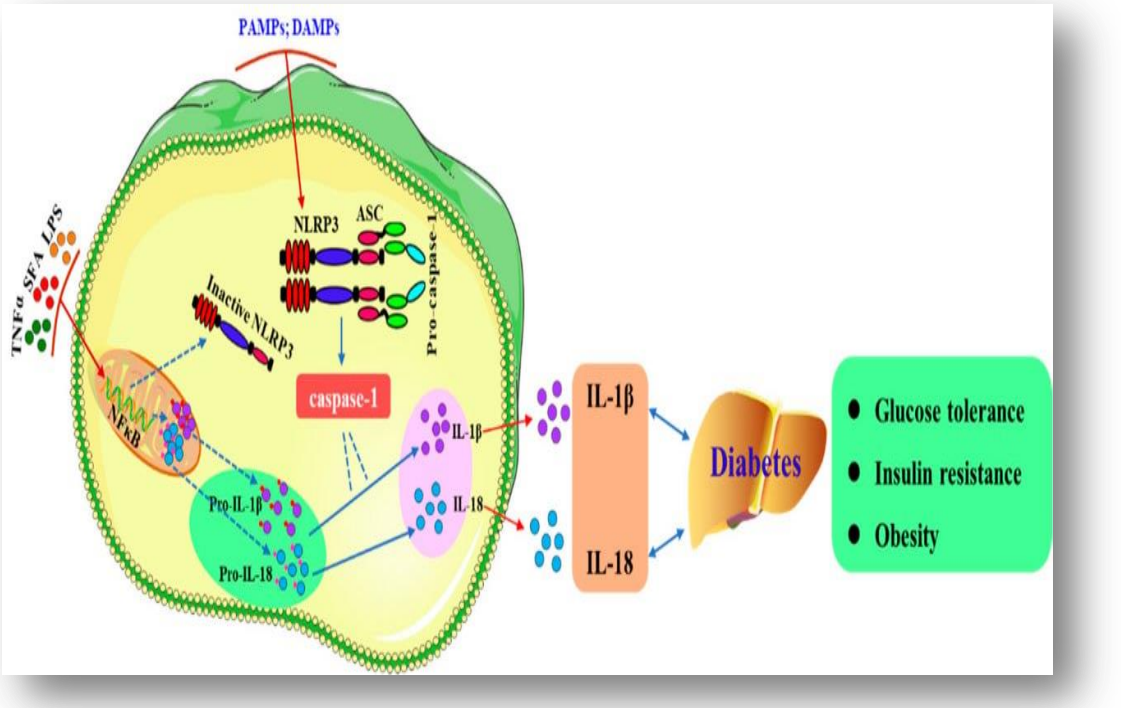

Figure (1.3): NLRP3 activation and IL-1 and IL-18 release (Ding *et al* ., 2019).

1.2.1.3.2 Risk Factors

Several studies have found a relationship between numerous risk factors of GDM for pregnant women, including (Andreoli *et al*., 2022):

1. Pre-Pregnancy Body Mass Index (>25 kg/m2).
2. Pre-Eclampsia.
3. Maternal Age (>25-45).
4. Genetics and Family History of Hyperglycemia.
5. Metabolic Syndrome and Nutritional Diet.
6. Polycystic Ovary Syndrome (PCOS).
7. Gravidity and Parity.

1.2.1.4 Diagnosis

Women with GDM are frequently asymptomatic, screening is critical for detecting any reduction in glucose tolerance. Because the Immune Response looks to be at high risk during the second trimester, screening for GDM during (24-28) weeks of pregnancy is recommended (Urbanová *et al*., 2020).

Screening for GDM is critical to prevent maternal and neonatal risk factors which can contribute to various issues for the mother and fetus throughout pregnancy and after birth (Farahvar *et al*., 2019), GDM can be diagnosed using the following methods:

A. Oral glucose tolerance test (OGTT)

The (OGTT) is a one-step method for diagnosing GDM .Between 24 and 28 weeks of pregnancy, using a (75 g) of glucose for (OGTT) testing. The following levels are considered adequate to diagnose GDM in a pregnant woman (Urbanová *et al*., 2020) (Figure 1.4).

1) Fasting blood glucose (FBG) (≥ 95 mg/dL)

2) 1-hour plasma glucose level (≥ 180 mg/dL)

3) 2-hour plasma glucose (≥ 155 mg/dL).

4) 3-houre plasma glucose (≥ 140 mg/dL).

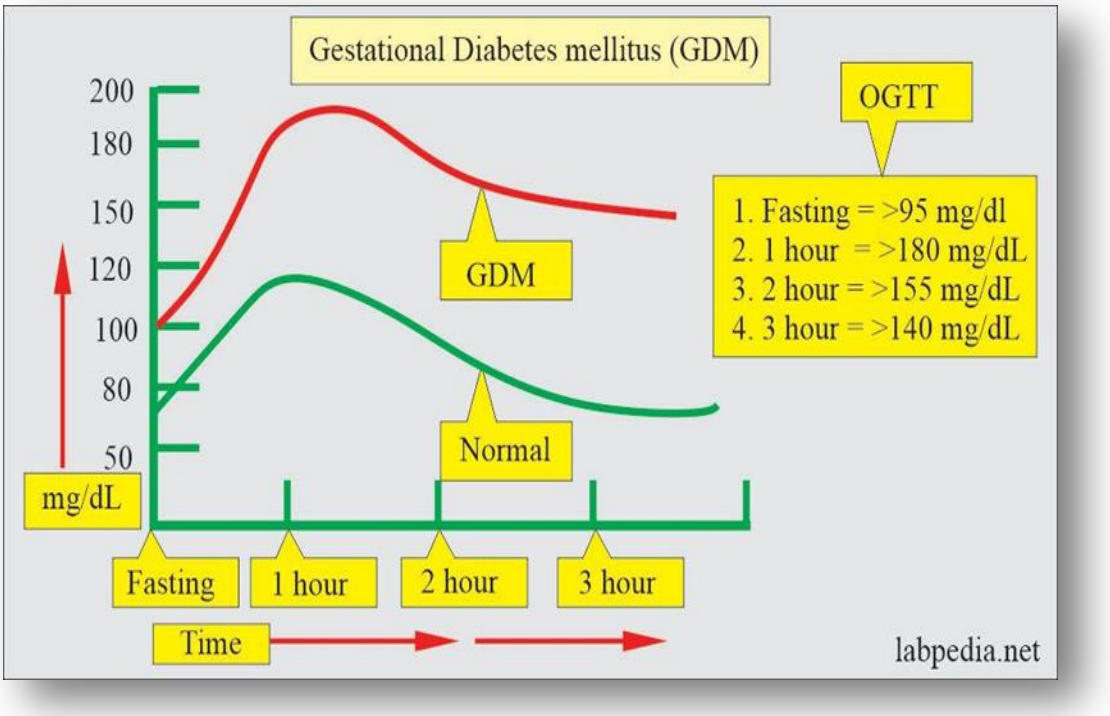

Figure (1.4): Screening of GDM during OGTT method (Urbanová *et al*., 2020).

B. Glycated hemoglobin A1C (HbA1C).

Glycated hemoglobin (HbA1C) is simple examination, fewer time consuming, doesn't need specific patient preparation, and is considered easy compared with the OGTT test , Several studies revealed that the combination of (HbA1c) with (OGTT) may be suitable in diagnosis of GDM,; HbA1c 5.8% has good specificity in diagnosing GDM when OGTT is used as a reference. The HbA1c cut-off (6.5%) has been established for determination of GDM (Khalafallah *et al*., 2016).

1.2.1.5 Complication of GDM

According to (WHO), the diabetes disease could be a particularly common metabolic complication on both the mother and their fetus (McIntyre *et al*., 2019).

1.2.1.5.1 Fetal complications

A-Unexplained stillbirth

It is typically connected with poor glycemic management, since hyperglycemia causes a prolonged decrease in oxygen levels; hence, fetal metabolite could be accountable for this unexplained stillbirth (Facchinetti *et al*., 2023).

B- Spontaneous abortion

When HbA1c levels exceed 12%, it is associated with poor glycemic control. The most prevalent defect in diabetic pregnant women is heart malformation, which accounts for 11% of the caudal regression related with diabetes (ElSayed *et al*., 2023).

C- Altered fetal growth

It is linked to congenital malformations and progressive vascular problems (Peyvandi *et al*., 2023).

D- Macrosomia

In diabetic pregnant women, the incidence is 25%-40%, and the estimated fetal weight is >4-4.5 kg or greater than the 90th percentile depending on gestational age (Dunne *et al*., 2003).

1.2.1.5.2 Neonatal complications

Preterm delivery, along with necrotizing enterocolitis, is one of the most serious consequences of gestational diabetes; however respiratory distress syndrome is usually related to gestational diabetes (Babović *et al*., 2022).

Birth weight that is low, especially among women who delivered birth during 24 and 33 weeks at a time hypoglycemia of the baby caused by hyperplasia of islet cells produced by persistent hyperglycemia in mom, and polycythemia are further problems (Ali and Nori, 2021).

1.2.1.5.3 Maternal complications

A- Infection

In diabetic pregnancies, infections that include vulvovaginal candidiasis, respiratory infections, urinary tract infections (UTI) and pelvis sepsis are more common (Johnson *et al*., 2021).

B-Polyhydramnios

Polyhydramnios affects 3%-30% of diabetic women and is caused by fetal glucose loading with polyuria, which gastrointestinal obstruction, fetal

swallowing impairment, and excessive sugar in the amniotic membranes (Preda *et al*., 2022).

C- Preterm delivery

Preterm birth affects regarding 605 diabetic pregnant women and is considered as serious obstetrical complications (Dinsmoor *et al*., 2023).

D-Preeclampsia

The obstetric issue that results in premature birth in diabetes pregnancies (Daskalakis *et al*., 2023).

1.2.2 *Helicobacter Pylori* infection during GDM.

H. pylori bacteria are gram-negative, microaerophilic, spiral-bacilli. The body contains 2 - 6 flagella, and flagella mobility imparts and permits fast movement in viscous fluids, like the mucus membrane of the stomach epithelial cell (Azad *et al*., 2022).

Helicobacter pylori, unlike numerous other gastrointestinal tract bacteria, lack fimbriae attachments. Temperatures ranging from 34 to 40° C are optimal for development. Despite its native home of the acidic gastric mucous membrane. The bacteria survives pH <4 exposure, and growth happens only in a very limited pH range that varies from 5.5 – 8.0, with neutral pH being ideal (Ahmed *et al*., 2023).

H. pylori infections are often acquired throughout childhood, mostly by interpersonal transmission. These types of transmission include gastro-

oral; oral-oral, and fecal-oral transmission. Furthermore, *H. pylori* transmission by food and water that is contaminated has been recorded (Edrees *et al*., 2023).

It evolved for survival within acidic and harsh conditions of the stomach, and it has the ability to change the pH of the stomach so that bacteria can survive. The spiral shape of bacteria allows bacteria to penetrate the mucus layer and protect bacteria from the immune response, which leads to colonization of the stomach and causes an inflammatory and anti-inflammatory response (Aleman *et al*., 2023).

1.2.2.1 Prevalence

Two-thirds of the human population worldwide is assumed to be infected with *H. pylori*, which is more frequent in underdeveloped nations, and more than (80%) of those affected are asymptomatic, but signs and symptoms include vomiting, nausea, indigestion, and bloated in general (Agarwal *et al*., 2020).

Although *H. pylori* are found all around, its occurrence rate differs depending on ethnicity, country differences, economic status situations, lifestyles, or personal hygiene. As indicated by numerous previous monitoring researches, the incidence of infection with *H. pylori* differs from one country for another, from ethnic background to ethnic origin, and even within the same country. The incidence of *H. pylori* re-infection is limited in wealthy countries but high in underdeveloped countries (Agi *et al*., 2022).

Helicobacter pylori have previously been identified as a pathogen associated with ulcers of the stomach and gastric cancer. Some studies verified that pregnant women infected to *H. pylori* had poor pregnancy outcomes, suggesting that the effects extend beyond the gastrointestinal tract. An infection with *H. pylori* can interfere with trace element intake and metabolic processes, particularly during pregnancy, raising morbidity. *H. pylori* seroprevalence were 62% within pregnant women, notably in the 20-24 year group of women, and 32.5% of individuals experiencing epigastric discomfort, vomiting, nausea, flatulence, and stomach burning (Zanzal *et al*., 2022).

The health, of a pregnant woman is critical, and several tests are required to identify the health issues that influence the pregnant woman or the baby. Local data on the association *of H. pylori* infection in pregnancy was inadequate, particularly in Iraq and poor nations (Yisak *et al*., 2022).

1.2.2.2 Immunopathology

In recent years, research of *H. pylori* have gotten more broad, and the links between *H. pylori* and different extra gastric disorders, including pregnancy-related diseases, have steadily been described .Pregnant women are among the most sensitive to *H. pylori* (Xie *et al*., 2023).

The complex interaction between *H. pylori* virulence factors, environmental factors, and host mediates *H. pylori* causes and disease consequences (Fiorani *et al*., 2023). Once *H. pylori* enter the stomach host,

four steps are required for *H. pylori* to achieve successful invasion, persistent infection, and pathogenesis (Nabavi-Rad *et al*., 2023).

(1) Resist the acidity of the stomach.

(2) Ability to motile toward and attach to epithelium cells.

(3) Attachment to host cells by adhesions/receptors interaction.

(4) Causing mucous layer damage by releasing enzymes and toxins.

Gestational diabetes mellitus [GDM] is linked to inflammation and inappropriate immune cell action. During pregnancy, the placenta performs multiple essential roles, including transferring waste products and nutrients between the mother and the fetus, producing and supplying hormones, and keeping up pregnancy-friendly immune surroundings. It is thus critical to determine whether or not pregnancy-related diabetic conditions affect the placenta and immune system reactions (Gao and Wang, 2023).

Infection with *H. pylori* has been associated with toll-like receptor stimulation by lipopolysaccharides (LPS), resulting in metabolic alterations ending in resistance to insulin (Pachathundikandi *et al*., 2023).

H. pylori cytotoxic-related gene A (CagA) positive the serotypes are more common in pregnant women with serious symptoms of nausea and vomiting, anemia due to deficiencies in iron is connected with *H. pylori* infection regardless of the presence or absence of peptic ulcers (Rasin *et al*., 2022) (Figure 1.5).

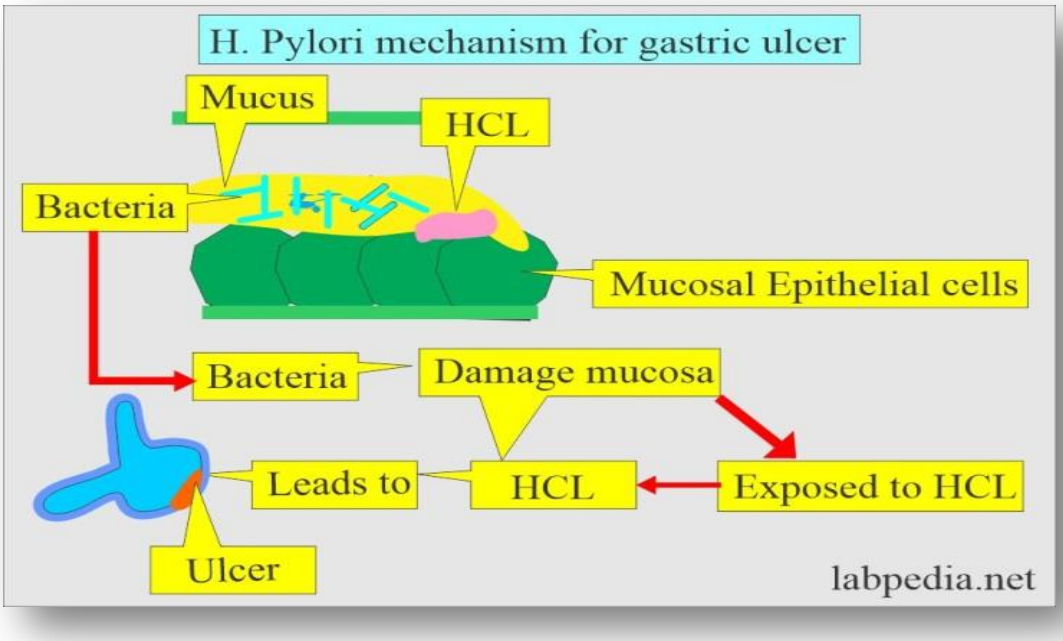

Figure (1.5): *H. Pylori* mechanism for gastric ulcer ((Kanungo *et al*., 2022).

1.2.2.3 Diagnosis

There are numerous diagnostic instruments available for the identification *of Helicobacter pylori* infections, including invasive and non-invasive procedures. (Invasive tests) including "culture, histopathology, and biopsy urease examination", need an endoscopic examination of stomach tissue (Maity *et al*., 2023).

Culture permits screening for antimicrobial susceptibility, as well as its sensitivity and specificity were (77-95% and 100 %,) respectively. The rapid urease assay is a qualitative urease identification assay with sensitivity and specificity of (89-98% and 93-98%), respectively. Under a light microscope, histopathology using hematoxylin-eosin and altered Giemsa stain is crucial for detecting *Helicobacter pylori*, with sensitivity and specificity of (93-98% and 95-98%), respectively .(Non-invasive tests)

include the '(expensive) urea breath test, serology tests, and stool antigen testing' (Kanungo *et al*., 2022).

Anti- *H. pylori* immunoglobulin G (IgG) and anti- *H. pylori* immunoglobulin M (IgM) levels were measured using an ELISA that has previously been validated and modified for use in American and Asian populations (Barzegar *et al*., 2023).This revolutionary quick test is based on feces and blood sample using monoclonal antibody immune chromatography. The test was found to be highly specific as well as sensitive. In the early stages of infection, the findings are positive, and they can be used to identify eradication following therapy. The Food and Drug Administration of the United States has approved direct fecal antigen for the identification of *Helicobacter pylori* for diagnostic and subsequent testing, (Shahaddeen *et al*., 2023) (Figure 1.6).

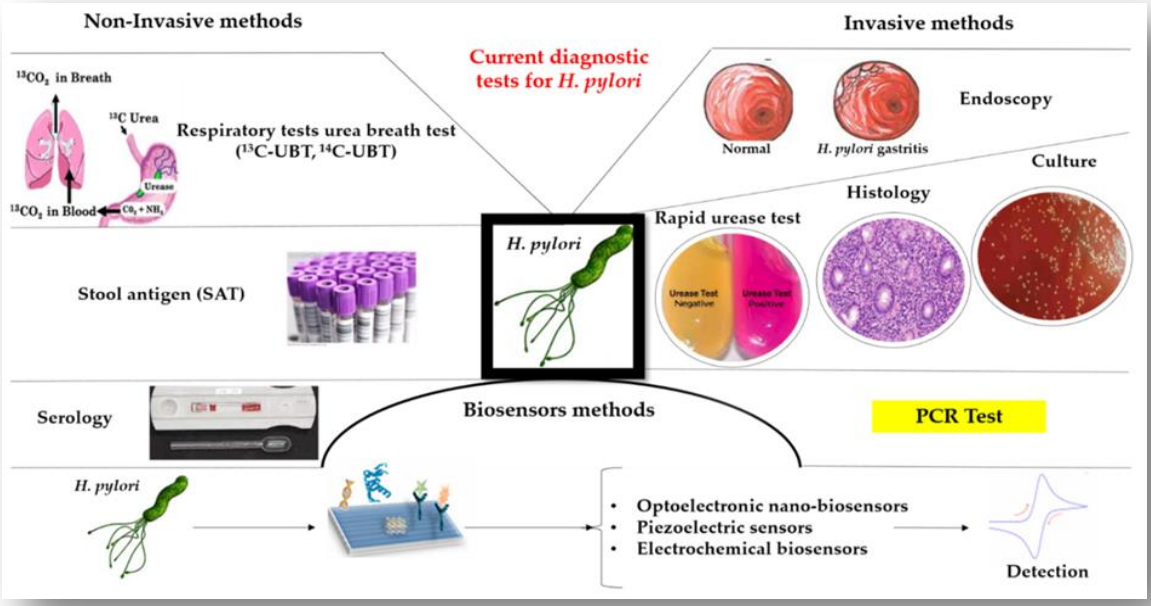

Figure (1.6): diagnosis of Helicobacter pylori infections (Pandey *et al*., 2023).

1.2.3 Role of Disturbance of Immune System in GDM.

The immune system protects its host from both exterior dangers such as bacterial infection and viral infection, as well as physical harm, and internal dangers like as malignant transformation. The immune system was once separated into two distinct parts [the adaptive immune system and innate immune system. They are divided for the purpose of description only and aren't in any way mutually exclusive (Pandey *et al*., 2023).

The maternal immunity faces significant challenges throughout pregnancy and fetal development. To avoid adverse pathology or pregnancy disruption, essential maternal immune-mediated mediators such as macrophages and natural killer (NK) cells, as well as regulatory T cells (Tregs) must be carefully balanced by the immune system of the mother from the initial stages of implanting and decidual formation to successful fetal delivery. The adaptation of numerous immune mediators to the stage of pregnancy will thus have an influence on both fetal and mother health outcomes (McElwain *et al*., 2021).

Hyperglycemia in GDM is related to elevated placental inflammatory processes, since excess glucose can activate the pyrin domain-containing protein 3 (NLRP3) an inflammasome in lymphocytes, resulting in the production of inflammatory cytokines such as IL-1β and IL-18 (Liyanagamage *et al*., 2020) (Figure 1.7).

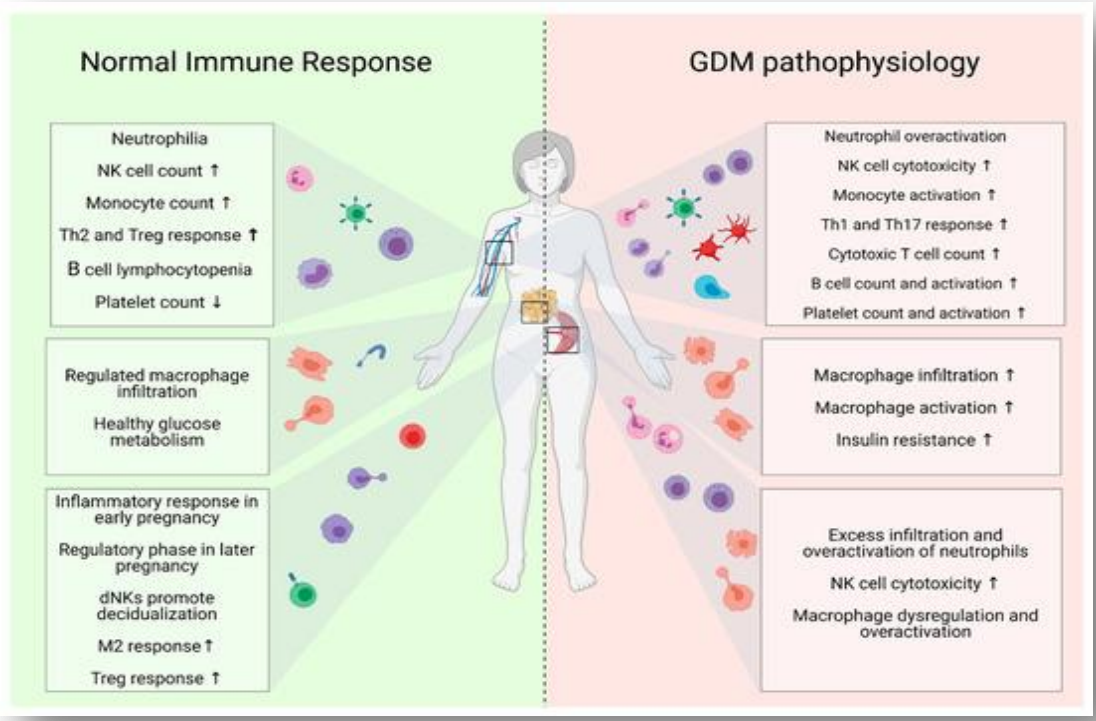

Figure (1.7): **A review of immune cell morphologies during maternal circulation, adipose tissue, and placental tissue in healthy simple pregnancy versus GDM-complicated pregnancy (McElwain *et al*., 2021).**

1.2.3.1 Innate Immune System.

Innate immunity is first line of defense against infections, and it can be induced via pattern recognition receptors (PRRs). PRRs identify [pathogen-associated molecular patterns (PAMPs)] and [danger-associated molecular patterns (DAMPs)]; there are various kinds of PRRs, including toll-like receptors and others (Kubelkova *et al*., 2023).

Innate immune activity entails direct recognition of pathogens via a limited number of germline-encoded pattern recognition receptors known as Toll-receptors, which are shared by all innate immune cells (Preissner *et al*., 2022).

1.2.3.1.1 Neutrophils

Neutrophils are polymorphic nuclear phagocytic leukocyte cells which acts as the immune system's first line of protection against pathogenic organisms that invade (Pandey *et al*., 2023).

Although the neutrophils are short-lived the cells with an acute role in innate immune response, they may have long-term effects, such as CD4+ and CD8+ T cell and B cell activation via presenting antigens in neutrophil extracellular trap (NET) formation, that could have a role in pathogen elimination(Ande *et al*., 2022).

In GDM neutrophil activation. Neutrophils appear to be highly active, resulting in a large number of neutrophil extracellular traps (NETs). Under normal conditions, this web-like structure acts as an extra innate immune system function that protects humans from invading germs (Andrés *et al*., 2022).

GDM occurrence, glycemic levels and homeostasis model assessment of insulin resistance (HOMA-IR) all rise when neutrophil count increases greater than. Similarly higher neutrophil counts were identified in GDM, implying a considerable rise in NLR in the second trimester as an indicator of inflammatory state and GDM (Naish *et al*., 2022).

When assessed during the third trimester in GDM women compared to simple pregnancies, relatively novel biomarkers of immature neutrophil counts with inflammation were additionally enhanced. Also, recent research has found that women with GDM have a different neutrophil activation profile than healthy controls (Ptasiewicz *et al*., 2022).

1.2.3.1.2 Natural Killer Cells (NKc).

Natural Killer Cells (NKc) were innate immune system cells that drive anti-tumor and anti-viral responses by robust cytolysis and account for 5-10% of human cells (Floerchinger *et al*., 2023).

Pregnancy causes significant changes in peripheral NK cells. The immunological response of circulating NK cells appears to be intensified during pregnancy, with a higher frequency of NK cells express CD107a, a cytolysis activity marker, and increased interferon (IFN)-expression (Esparvarinha *et al*., 2023).

Elevated production of CD38 and NKp46, surfaces indicators of activity and toxicity, is observed in pregnant women's peripheral NK cells compared to non-pregnant women, indicating an immune protective function in pregnancy (Liao, 2020)

Increased peripheral cytotoxicity NKCD16+56dim cell count in overweight GDM women were found to indicate a shift toward greater cytotoxic capability when compared with overweight women with simple pregnancy (Li *et al*., 2020).

1.2.3.1.3 Macrophages.

The macrophage is Antigen Presenting Cells (APCs) that are produced by monocyte differentiated and have separate phenotypic classifications into M1-like, pro-inflammatory and microbicide subtype or M2-like, anti-inflammatory and immune regulatory subtype. These subtypes

are distinguished by particular markers such as HLA-DR, CD11c, and CD86 on M1-like macrophages and CD163, CD204, CD206, and vascular endothelial growth factor (VEGF) on M2-like macrophages (Kuntzel *et al*., 2022).

Macrophages, which account for 20-30% of decidual leukocytes throughout pregnancy, play a crucial role in modulating tolerance across the maternal-fetal interaction (Lasch *et al*., 2022).

1.2.3.1.4 Dendritic Cells.

Dendritic cells (DCs) react with both exogenous and endogenous the antigens, resulting in T cell priming and activation, as well as cytokine release (Shui *et al*., 2023).

Dendritic cells (DCs), unlike monocytes and macrophages, DC is parts of mononuclear phagocyte system (MPS). Yet, their involvement in the immune system's reaction is geared toward antigen presentation (Blander *et al*., 2023).

Dendritic cells (DCs). Show high morphological and functional variation in simple healthy pregnancy, indicating their crucial capacity to adjust to the demands of pregnancy (Roquilly *et al*., 2022).

1.2.3.2 Adaptive Immune System.

The adaptive immune system's (second-line) is stimulated by the immune system's natural defenses and replies to antigen in which the microbe is already exposed, allowing for a more successful response (Sterling *et al*., 2023).

The adaptive response is significantly more refined, allowing for the particular identification of foreign particles as well as the selective proliferation of cells primed to attack specific diseases and acquire immunological memory. T cells and B cells make up the adaptive immune system. Both T and B lymphocytes are essential components of adaptive immune system reactions (Sies *et al*., 2022).

The adaptive part of the body's immune system aids in the identification of particular foreign pathogens and initiates optimized responses to target the infectious agent and create immunological memory via the activity of T and B cells. The adaptive immune system's functioning varies on a daily basis, in both natural processes (such as trafficking of lymphocytes and the formation of T lymphocytes subsets) and in reactions to a challenge (Pandey *et al*., 2023).

1.2.3.2.1 B Cells.

B cells are the most important cells in adaptive immunity. These cells are produced of the bone marrow as well as play an important role in disease etiology, autoimmunity, and overall immunity by presenting antigens and secreting cytokines (Kalkal *et al*., 2023).

During pregnancy, B cells can undergo a specific subtype shift toward a regulatory B cell role, offering anti-inflammatory and protective actions. Humans have dramatically decreased peripheral B cell counts within pregnancy, especially during the third trimester and soon after delivery, when compared with non-pregnant control (Muchamuel *et al*., 2023).

B cell types in gestational diabetes, which found that the number of B cells have risen in gestational diabetes and are favorably related with maternal resistance to insulin and expressing very significant amounts of immunoglobulin A. In obesity-related insulin resistance, elevated IgA levels have been associated to inflammation of adipose tissue and impaired glucose homeostasis (Milardi *et al*., 2023).

A similar trend has been discovered in patients with type 2 diabetes (T2DM), where hyperglycemia and hyperlipidemia were shown to be associated with an unbalanced pro-inflammatory peripherals B cell profile, (Liu *et al*., 2023).

Circulating immunological factors clearly have the potential to play a substantial role in the genesis of resistance to insulin and poor glycemic control during pregnancy. GDM-mediated immunological failure is visible in a variety of cell types (Caielli *et al*., 2023).

1.2.3.2.2 T Cells.

The T cells were lymphocyte that plays an important role in immunity through cell-mediated mechanisms, particularly in the response of adaptive immunity, where they protect the host against dangerous pathogens' cells, which originate in the thymus, have a particular TCR on their cell surface (Friedman *et al*., 2023).

T cell phenotypic including T-helper 1 and 2 [Th1, Th2] the cells, as well as additional functional subtypes such as Th17 cell, also known as Tregs, and cytotoxic T cell (Harker and Lloyd , 2023).

Maternal immune responses to of paternal-derived antigen through the initial stages of pregnancy, and the related immune response effects both mother's and baby's health effects. Effector CD4+ T cells produce a multitude of cytokines in response to antigen presentation, so the cytokine released by the cell population defines their categorization. T-helper cells, or CD4+ T cells, are mostly important for controlling efficient immune responses to infections, (Künzli and Masopust, 2023).

These cells need careful equilibrium during pregnancy in order to continue offering host protection while permitting fetal cell growth, without causing a negative reaction, (Feeney, 2020). Early on and during pregnancy, fetal-specific CD8+ T lymphocytes have been found in maternal blood, pointing to a possible function of the fetal derived immune system response in controlling the mother's physiological reaction to pregnancy, (Auriti *et al*., 2021).

Recent research reveals that GDM alters both the naive and memory Treg populations' growth and function, with suppressive Treg subtype expression being reduced, (Burt and McCune , 2023).

1.2.3.3 Role of Immunological markers:

1.2.3.3.1 Role of interleukin (IL-18) in GDM.

IL-18 is a cytokine that belongs to the IL-1 family and was once thought to induce interferon gamma (IFN-g). A collection of 11 cytokines that support the function of the innate immune system. In addition to macrophage, endothelial cells, vascular smooth muscle cells, dendritic cells, and Kupffer cells, the cytokine is also generated constitutively in a wide range of other cell types (Yasuda *et al*., 2019).

IL-18 production was first detected in Kupffer cells, which are liver-resident macrophages, even when they are not stimulated (Omoto *et al*., 2010).

However, several later studies found IL-18 synthesis in non-hematopoietic cells like epithelial cells in the intestines, the cells known as and endothelial cells was still occurring even in the steady state. IL-18 is distinguished by its distinct distribution and constitutive synthesis in a wide range of cell types and tissues, as well as its distinct cellular manufacturing pathway (Jarret *et al*., 2020).

Cytokines like IFN-γ and IL-4 are usually released when their respective genes are produced because their genes contain a signal peptide required for extracellular release from the endoplasmic reticulum to the Golgi (Das *et al*., 2022).

On the other hand, the IL18 genes, like the others in the IL-1 family, lack a signal peptide. IL-18 has been found to be kept in the cytoplasm of IL-18 generating cells. Additionally, IL-18 is generated as a physiologically

inactive precursor, comparable to IL-1 but unlike IL-1 or IL-33 (Dinarello , 2019).

Precursor IL-18 (pro-IL-18) requires post-translational processing to become active and released. As a result, various mechanisms govern the extracellular release of physiologically active IL-18, including regular transcriptional gene regulation, post-transcriptional gene regulation, and post-translational gene regulation (Chauhan *et al*., 2020).

Adipocytes also generate IL-18.although the main generator of IL-18 in adipose tissue has been shown to be non-adipocyte cells. The IL-1 family includes IL-18, which was formerly referred to as "IFN-inducing factor." It encourages Th1 and Th2 cell responses and increases the toxic capacity of NK cells (Dinarello , 2019).

A strong pro-inflammatory cytokine called interleukin-18 (IL-18) has a role in the host's defense against infections and controls the innate and adaptive immune system reaction. Both of hematopoietic & non-hematopoietic cells, such as mesenchymal cells, keratinocytes, macrophages, and monocytes, generate IL-18 (Ihim *et al*., 2022).

The innate immune system and the acquired immune system are stimulated by IL-18. It causes the production of interferon gamma (IFN-g) when IL-12 is present via acting on (T helper 1 Th1), macrophage, natural killer cells (NK cells), natural killer T (NKT) , B cells, dendritic cells (DCs), as well as non-polarized T cells, (Nguyen *et al*., 2019).

IL-18 has a pleiotropic effect depending on its cytokines milieu, indicating that it plays a key pathophysiological function in health and

illness. Because IL-18 is involved with both the adaptive and innate immune responses, it is linked to a number of inflammatory and autoimmune diseases, (Wang *et al*., 2023).

Autoimmunity may be induced by IL-18 through immune cell cytotoxicity and inflammation. Patients with certain immune-related disorders blood have been shown to contain its higher amounts in some cases (Makaremi *et al*., 2022) (Figure 1.8).

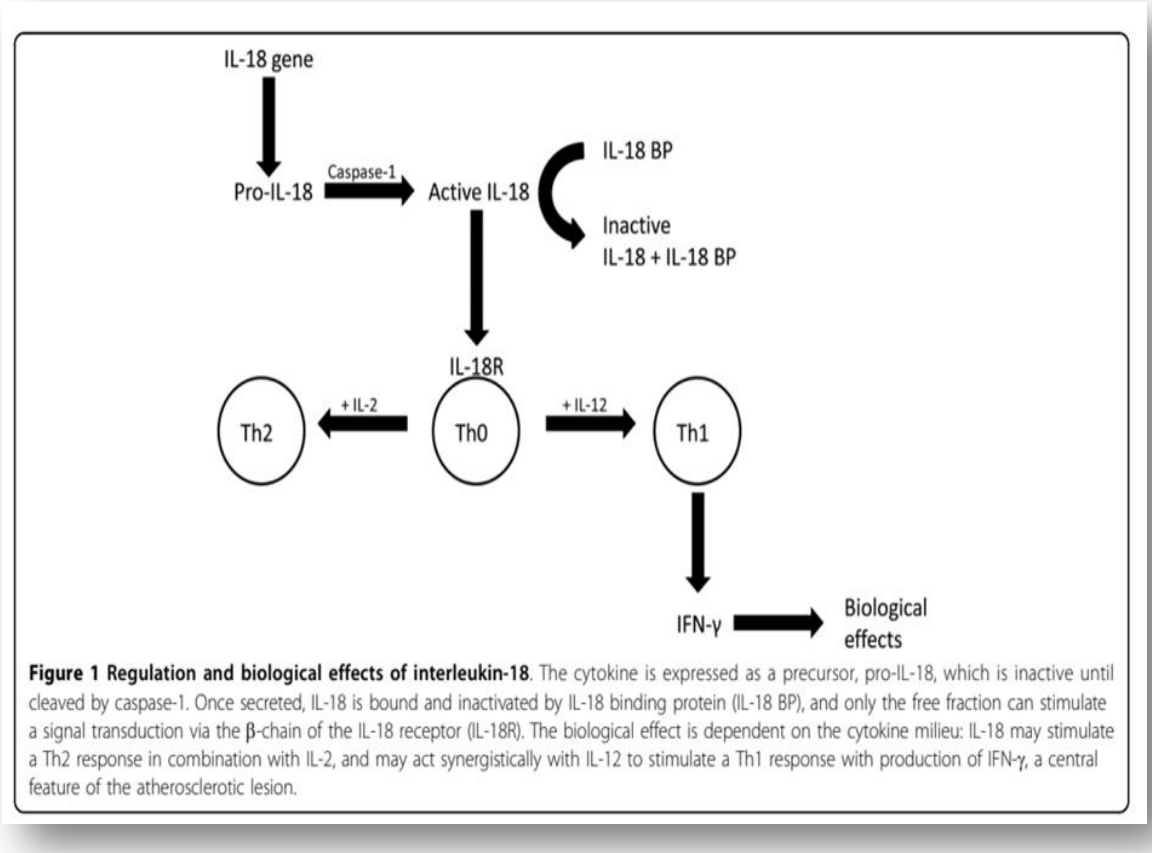

Figure 1 Regulation and biological effects of interleukin-18. The cytokine is expressed as a precursor, pro-IL-18, which is inactive until cleaved by caspase-1. Once secreted, IL-18 is bound and inactivated by IL-18 binding protein (IL-18 BP), and only the free fraction can stimulate a signal transduction via the β-chain of the IL-18 receptor (IL-18R). The biological effect is dependent on the cytokine milieu: IL-18 may stimulate a Th2 response in combination with IL-2, and may act synergistically with IL-12 to stimulate a Th1 response with production of IFN-γ, a central feature of the atherosclerotic lesion.

Figure (1.8): Regulation and biological effects of (interleukin- 18) (Trøseid and Arnesen , 2010).

1.2.3.3.2 Role of the NOD-like receptor protein 3 (NLRP3) inflammasome in GDM.

The NOD-like receptor protein 3 (NLRP3) inflammasome is a crucial regulator of the host's immunological response, and its activation has been associated to a variety of immune and metabolic diseases (Tartey and Kanneganti, 2019).

The NLRP3 inflammasome belongs to the leucine-rich repeat (LRR)-containing protein family with a nucleotide binding oligomerization domain. It has a nucleotide-binding and oligomerization domain in the center, an LRR domain at the C terminus, and a pyrin domain at the N terminus (Sharma and Kanneganti, 2021)

That must be activated in two phases. The initial the signal like lipopolysaccharide (LPS), promotes the production of NLRP3 with Pro-interleukin (IL-1ß) and (Pro-IL-18). Many activators, including as cholesterol, uric acid, and ATP, as well as external stimuli, such as asbestos, UV radiation, pathogenic bacteria, and their metabolites, are involved in the second activation signal (Tanase *et al*., 2023).

Pro-caspase-1 has been recruited in order to self-splice and produce active caspase-1. Caspase-1 activated divides pro-IL-1b as well as pro-IL-18 to mature IL-1b and IL-18, which are subsequently released by cells to stimulate the downstream inflammatory response (Makoni and Nichols, 2021).

During the pathophysiology of gestational diabetes mellitus (GDM), the placenta may play an important role towards activation of inflammatory

and the start of insulin resistance. Interleukin (IL-1) and (IL-18) are key inflammatory cytokines in the start of maternal IR during GDM and are controlled by the NLR family pyrin domain containing3 (NLRP3) inflammasome. Even so, the mechanism regulating the NLRP3 inflammasome in the placenta is unclear (Gogeneni , 2017).

The stimulation of inflammation in the placenta and adipose tissues is important in the pathophysiology of GDM. Several inflammatory cytokines generated from the placenta and adipose tissue contributes to inflammation activation and establishes or worsens IR during pregnancy (Nguyen-Ngo *et al*., 2019).

During pregnancy, the placenta is a highly specialized organ that produces cytokines and hormones and contributes to maternal. Because IR improves considerably in GDM women shortly after birth, it is hypothesized that the placenta plays a role in the activation of inflammatory and the onset of IR during GDM pathogenesis (Parrettini Ngo *et al*., 2020).

Interleukin (IL-1) and (IL-18) are inflammatory cytokines that have a role in the beginning of maternal IR through GDM. The inflammasome nucleotide binding and an oligomerization domain like receptor family pyrin domaincontaining 3 (NLRP3) regulates (IL-1) and (IL-18) production. The NLRP3 inflammasome is triggered by a variety of pathogen and cellular damages, culminating in the production of cleaved caspase1 and the production of Interleukin (IL-1) and (IL-18). Previous research has shown that the NLRP3 inflammasome is considerably activated in individuals with obesity, dyslipidemia, and diabetes (Musa, 2022).

In GDM patients have shown a link between NLRP3 inflammasome activation and insulin resistance. When compared to normal and low levels of glucose, high glucose levels enhance NLRP3 activation. It is well recognized that hyperglycemia and diabetes during gestation can activate the inflammasome NLRP3 and cause the release of several inflammatory cytokines, resulting in serious pregnancy problems (Kushtagi , 2021).

In individuals with GDM, the suppressed NLRP3 activation, decreased trophoblast release of inflammatory (IL-1ß) , caspase-1 action, reduced further pro-inflammatory as well as anti-angiogenic reactions to excess glucose (Gomez-Lopez *et al*., 2019).

1.2.3.3.3 Role of interleukin 1-beta (IL-1ß) in GDM.

Interleukin 1-beta (IL- 1ß) is a member of the interleukin- 1-related cytokine family and has been shown to play a role in immune-mediated illness and inflammation control (Mantovani *et al*., 2019).

Interleukin 1-beta (IL- 1 ß) a pro-inflammatory cytokine is implicated in the event of glucotoxicity and reduced secretion of insulin in diabetes mellitus, and it is raised in non-diabetic pregnancy at pre-term delivery. Human placental & adipose tissue have recently been discovered to generate a variety of pro-inflammatory substances, including cytokines like Interleukin-1 beta (Calabrese *et al*., 2022).

The developing fetus and placenta, as well as the mother have increased metabolic demands, cause significant alterations in metabolism throughout pregnancy. Pregnancy is related with changes in the control of

glucose metabolism produced by hormones that antagonize insulin's activities, resulting in a condition of resistance to insulin as pregnancy develops (Iznardo *et al*., 2021).

During human placenta lactogenic and placental hormone growth synthesis, the placenta is implicated in the occurrence of resistance to insulin and fetal development during both non-diabetic pregnancy and pregnancy affected by GDM (Stern *et al*., 2021).

Prior to pregnancy, there was sometimes acquired and partially hereditary insulin resistance in GDM. Even in women without GDM, partially acquired resistance to insulin rises throughout the second half of pregnancy but diminishes postpartum (Szlapinski *et al*., 2021).

The reduction in insulin sensitivity throughout pregnancy corresponds to the expansion of the fetal-placental unit and resolves once the placenta is delivered. GDM (Gestational Diabetes Mellitus) affects around 4-8% of pregnancies. Diabetes mellitus during pregnancy (GDM) is described as any degree of glucose resistance with onset and first identification during pregnancy (Sirico *et al*., 2021).

In normal human plasma, it is almost undetectable. When compared to a healthy individual, the production rises with the development of the auto inflammatory response and can exhibit a 5-10 times increase in level. It occurs in glucotoxicity and decreased insulin secretion by mediating an auto- inflammatory process that results in cell death. IL-1 levels in GDM women were substantially greater than in normal pregnant women (Sharma *et al*., 2022).

Chapter Two

Materials and Methods

2. Materials and Methods.

2.1 Materials.

2.1.1 Instruments.

Table (2-1): The instruments that used in this study.

No.	Instrument	Company	Country
1	Centrifuge	PLC Series	Taiwan
2	Deep Freezer -20° C	Rx	Italy
3	ELISA- Washer	Paramedical	USA
4	Hematology analyzer System	Heidolph	Germany
5	I Chroma	Boditech	Korea
6	Incubator	Memmert	Germany
7	Pharmacy machine	DHM-600A	China
8	Refrigerator	LG	USA
9	Shaker	Karl Kolb	English
10	Spectrophotometry	Abbott	USA
11	Spectrophotometry _ ELISA Reader	Biotech	USA
12	Water Bath	Karl Kolb	English

2.1.2 Equipment.

Table (2-2): The equipment that used in this study.

No.	Equipment	Company	Country
1	Automatic pipette	Slimed	Germany
2	Cotton	Al-Rawabi	Lebanon
3	Disposable Syringe 5ml	Luer lock	China
4	EDTA Tube 2ml	Ab Medical	Korea
5	Eppendorf Tube	Bioneer	Korea
6	Gel Tube 6ml	Ab Medical	Korea
7	Gloves	Al-Rawabi	Lebanon
8	Pipette Tips	American	USA
9	Plane Tube 5 ml	Ab Medical	Korea

2.1.3 Kits

Table (2-3): The laboratory kits that used in this study.

No.	Laboratory kits	Company	Country
1	Blood Glucose Content Assay Kit	Biozek medical	Holland
2	C-reactive protein assay Kit	Biozek medical	Holland

3	***H. pylori* (IgG) ELISA Kit**	**Sunlong Biotech**	**China**
4	***H. pylori* (IgM) ELISA Kit**	**Sunlong Biotech**	**China**
5	**Human Hemoglobin A1C assay Kit (HbA1C)**	**Boditech**	**South Korea**
6	**IL-1 beta ELISA Kit**	**Sunlong Biotech**	**China**
7	**IL-18 ELISA Kit**	**Sunlong Biotech**	**China**
8	**NLRP3 ELISA Kit**	**Sunlong Biotech**	**China**

2.2 Methods

2.2.1 Study groups

Samples were collected from sixty women with Gestational Diabetes Mellitus (GDM), and 29 healthy Pregnant Women, who were attended to Al-Mahaweel General Hospital/ Babylon during period extended from August 2022 to December 2022. Patients were diagnosed by specialist Gynecologist.

2.2.2 Study Design

A case-control study design included two groups: patients group included 60 patients with gestational diabetes and control group included 29 apparently healthy pregnant women were enrolled in this study.

2.2.3 Questionnaire

Questionnaire for general demographic data (Age, BMI, Family history of DM, Family history of GDM and the Physical activity of pregnant (Appendix 2).

2.2.4 Inclusion Criteria

For patients group: GDM, without any other diseases. Patients were diagnosed by a specialized Gynecologist depending on clinical finding and laboratory investigation.

2.2.5 Exclusion Criteria

Exclusion criteria of GDM patients were involved:

- Renal diseases.
- Abnormal liver function.
- Thyroid diseases.
- Chronic diabetes without pregnancy.
- Cases and controls aged fewer than 23 and above 47 years old.
- Cases and controls BMI fewer than 20 k and above 41k
- Patients with endocrine or chronic diseases.

2.2.6 Ethical Approval

All samples of the present study will be collected after obtaining verbal ethical clearance from the Ethics Committees of Babylon health office unit in Babylon province. Moreover, this study should be approved by the ethical research committee in the college of medicine, Babylon University, and Babylon health directorate. All of the patients and the healthy control subjects are counseled and vocally agreed on the inclusion in the study before participation

in the study. This study is performed & being facilitated with permission from Babylon university, College of Medicine, and the General Health Directorate of Babylon province (Appendix 1).

2.2.7 Collection of Sample

From each patient and healthy control subject (5 ml) of blood were obtained by vein puncture using 5 ml disposable syringes. The blood sample was divided into two aliquots. The first aliquot (2 ml) was transferred into EDTA tube for HbA1c determination and then used for CBC Test. The second aliquot (3 ml) of separated serum was used for assays of ELISA, fasting blood glucose (FBG), OGTT, and C - reactive protein (Figure 2.1).

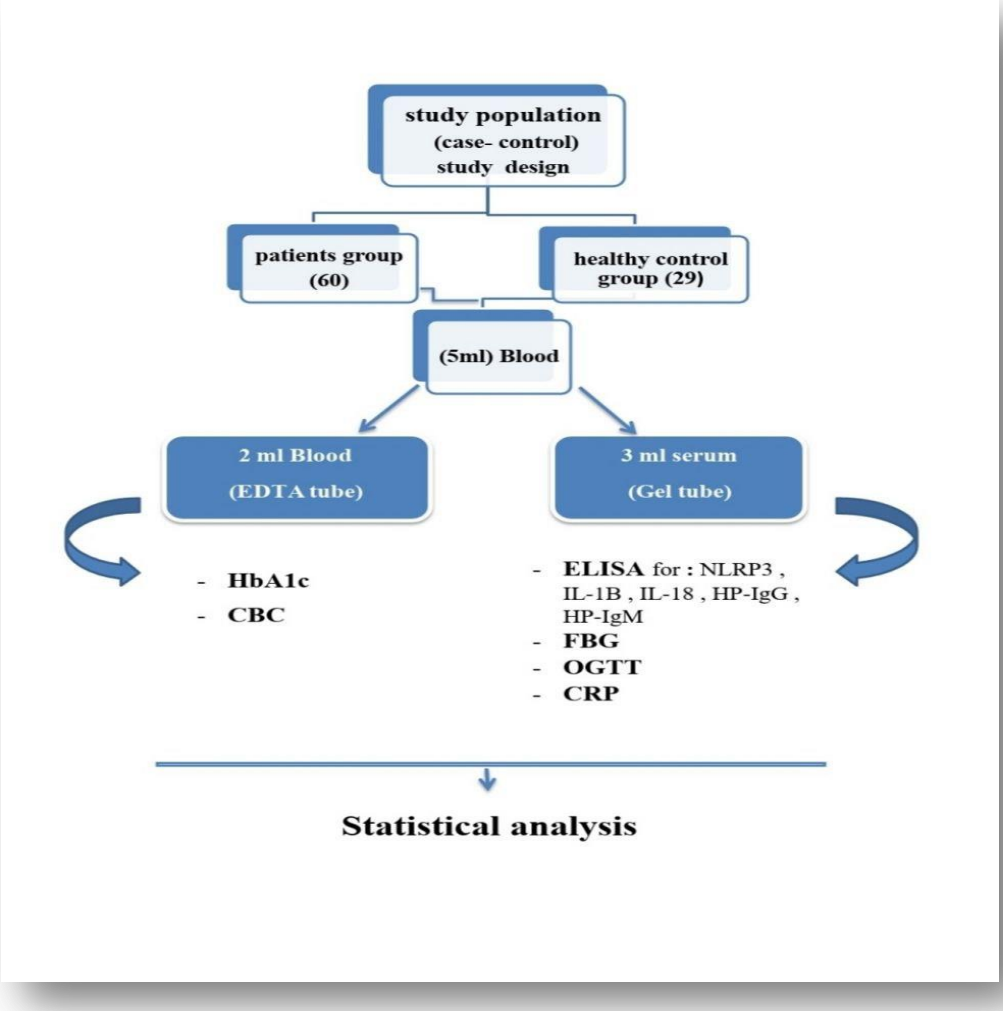

Figure (2.1): study scheme.

2.2.8. Calculation of Body Mass Index (BMI)

BMI was calculated by dividing a person's weight (kg) by length squared (M2). The weight was measured by electronic sensitive balance, while the height was measured in meters using standard metric bar (Buschur, 2018) (Table 2.4).

$$BMI = \text{weight (kg)} / \text{length}^2 \text{ (M)}^2$$

Table (2.4): The body mass index and weight status (Buschur, 2018)

Weight Status	BMI	2nd/3rd trimester rate of weight gain (kg/week)
Low	<19.8 kg/m2	1.0 (1-1.3 lb./week)
Normal	19.8-26 kg/m2	1.0 (0.8-1 lb./week)
High	>26-29 kg/m2	0.66 (0.5-0.7 lb./week)
Obese	>29 kg/m2	0.5 (0.4-0.6 lb./week)

2.2.9 ELISA Methods

2.2.9.1 Determination of Human Interleukin 18 (IL-18).

-Purpose

Our Human Interleukin 18 (IL-18) ELISA kit is to assay IL-18 levels in Human serum.

-Principle

This ELISA kit uses Sandwich-ELISA as the method. The Micro-Elisa plate provided in this kit has been pre-coated with an antibody specific to IL-18. Standards or samples are added to the appropriate Micro-Elisa plate wells and combined to the specific antibody. Then a Horseradish Peroxidase (HRP) - conjugated antibody specific for IL-18 is added to each Micro-Elisa plate well and incubated. Free components are washed away. The TMB substrate solution is added to each well. Only those wells that contain IL-18 and HRP conjugated IL-18 antibody will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of IL-18. You can calculate the concentration of IL-18 in the samples by comparing the OD of the samples to the standard curve.

-Materials provided with the kit

Table (2-5): Materials provided with the Human IL-18 ELISA kit.

	Materials provided with the kit	**96 determinations**	**Storage**
1	Chromogen Solution A	6ml×1 bottle	2-8℃
2	Chromogen Solution B	6ml×1 bottle	2-8℃
3	Stop Solution	6ml×1 bottle	2-8℃
4	wash solution	20ml ×1bottle	2-8℃

5	Standard：135 pg/ml	0.5ml×1 bottle	2-8℃
6	Standard diluent	1.5ml×1 bottle	2-8℃
7	HRP-Conjugate reagent	6ml×1 bottle	2-8℃
8	Sample diluent	6ml×1 bottle	2-8℃

-Serum preparation

After collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 10-20 minutes. Remove the clot by centrifuging at 2,000-3,000 rpm for 20 minutes. If precipitates appear during reservation, the sample should be centrifuged again.

- Procedure

1. Diluted the standard by small tubes first, then pipette the volume of 50ul from each tube to microplate well, each tube use two wells, total ten wells (figure 2.2).

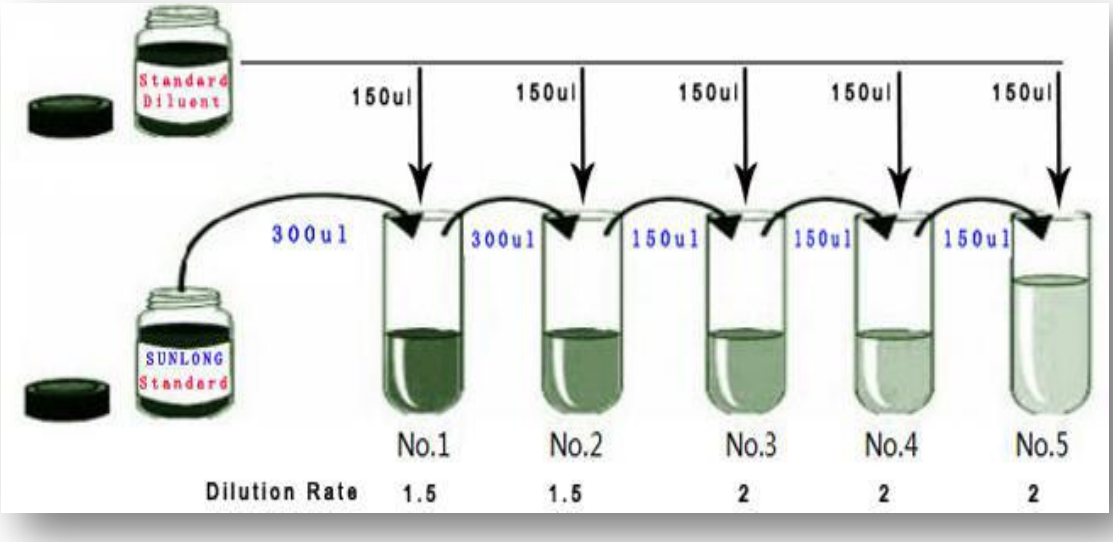

Figure (2.2): method of dilution of standards

2. In the Microplate, leave a well empty as blank control. In sample wells, 40µL Sample dilution buffer and 10µl sample are added (dilution factor is 5). Samples should be loaded onto the bottom without touching the well wall. Mix well with gentle shaking.

3. Incubated 30 min at 37℃ after sealed with Closure plate membrane.

4. Diluted the concentrated washing buffer with distilled water (30 times for 96T and 20 times for 48T).

5. Carefully peel off Closure plate membrane, aspirate and refill with the wash solution. Discard the wash solution after resting for 30 seconds. Repeat the washing procedure for 5 times.

6. Added 50µl HRP-Conjugate reagents to each well except the blank control well.

7. Incubated as described in Step 3.

8. Washed as described in Step 5.

9. Added 50 µl Chromogen Solution A and 50 µl Chromogen Solution B to each well, mix with gently shaking and incubate at 37℃ for 15 minutes. Please avoid light during coloring.

10. Added 50 µl stop solution to each well to terminate the reaction. The color in the well should change from blue to yellow.

11. Read absorbance O.D. at 450nm using a Microtiter Plate Reader. The OD value of the blank control well is set as zero. Assay should be carried out within 15 minutes after adding stop solution.

- Calculation of Results

Known concentrations of Human IL-18 Standard and its corresponding reading OD is plotted on the log scale (x-axis) and the log scale (y-axis) respectively. The concentration of Human IL-18 in sample is determined by plotting the sample's O.D. on the Y-axis. The original concentration is calculated by multiplying the dilution factor (figure 2.3).

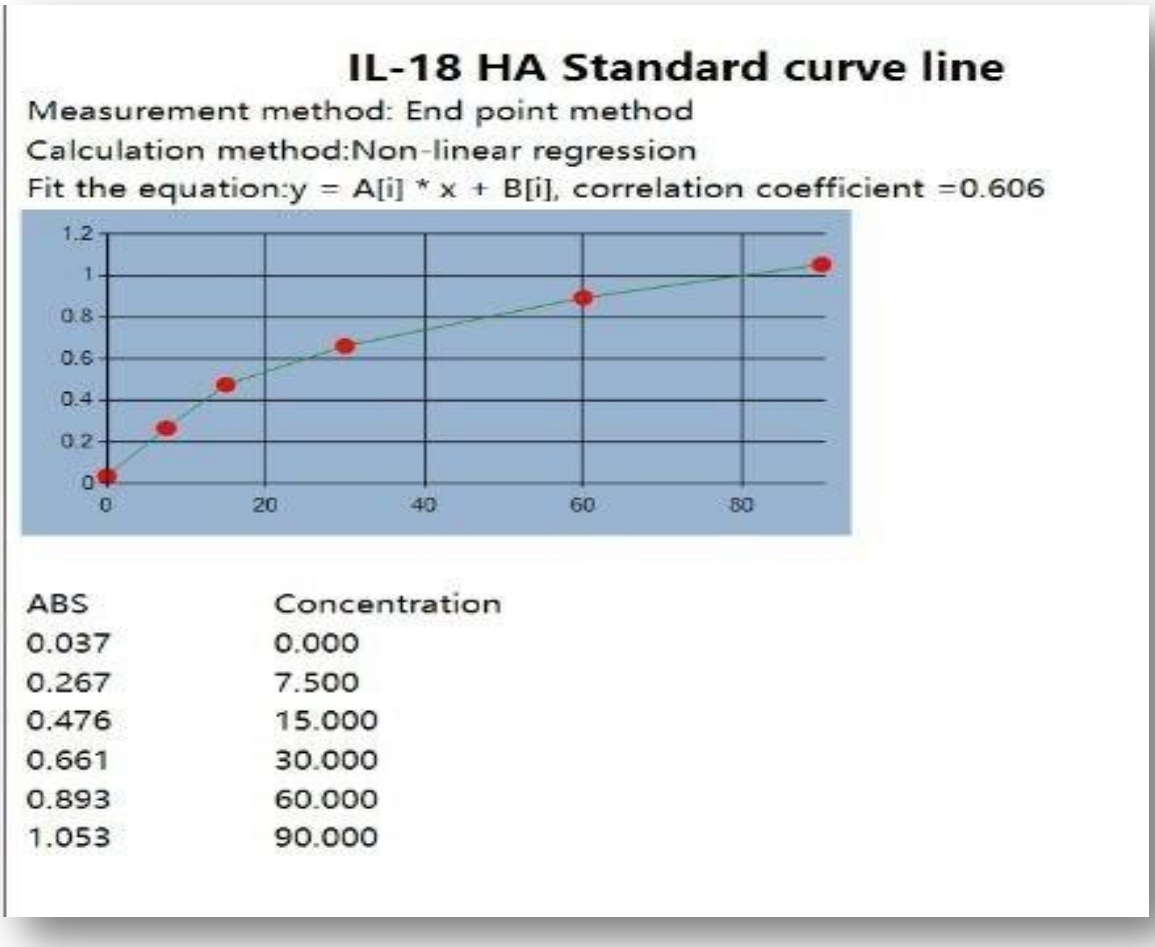

Figure (2.3): Human IL-18 Standard Curve that showed the Concentration (pg/ml) and optical density OD.

2.2.9.2 Determination of Human Interleukin 1beta (IL-1ß).

- Purpose

Our Human Interleukin 1beta, IL-1ß ELISA Kit is to assay IL-1ß levels in Human serum.

-principle, sample collection, material provide in this kit and this procedure are all similar IL-18 kit in (2.2.9.1).

- Calculation of Results

Known concentrations of Human IL-1ß Standard and its corresponding reading OD is plotted on the log scale (x-axis) and the log scale (y-axis) respectively. The concentration of Human IL-1beta in sample is determined by plotting the sample's O.D. on the Y-axis. The original concentration is calculated by multiplying the dilution factor (figure 2.4).

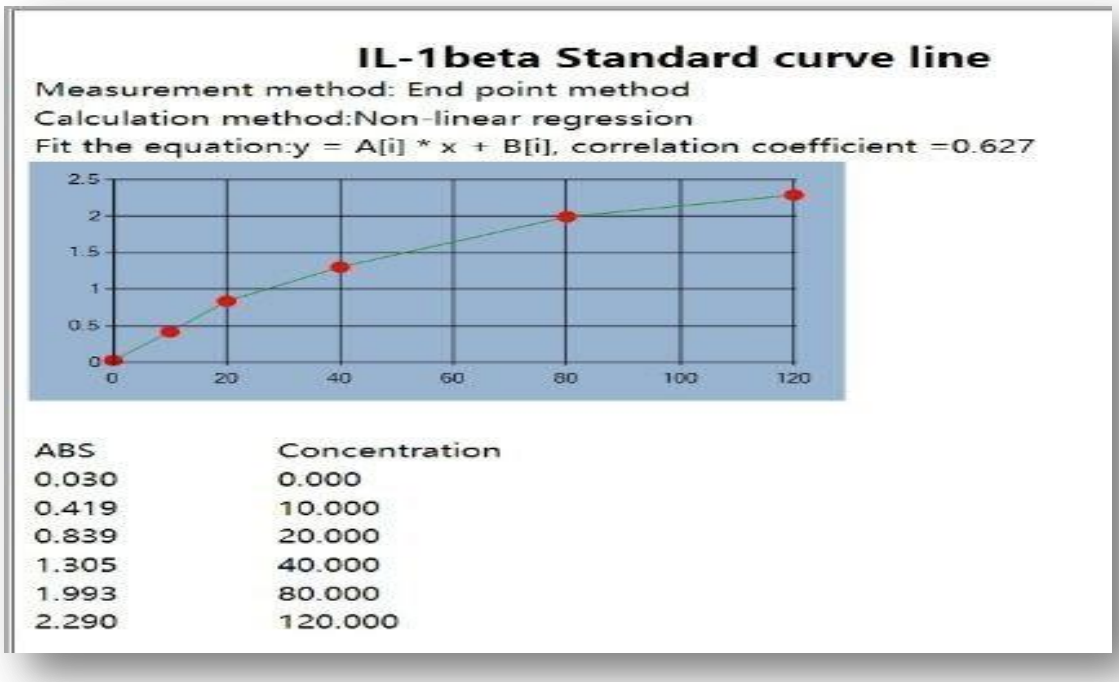

Figure (2-4): Human IL-1beta Standard Curve that showed the Concentration (pg/ml) and optical density OD.

2.2.9.3 Determination of Human NLR Family, Pyrin Domain Containing Protein 3 (NLRP3).

- Purpose

Our Human NLR Family, Pyrin Domain Containing Protein 3 (NLRP3) ELISA kit is to assay NLRP3 levels in Human serum.

-principle, sample collection, material provide in this kite and this procedure are all similar IL-18 kit in (2.2.9.1).

- Calculation of Results

Known concentrations of NLRP3 Standard and its corresponding reading OD is plotted on the log scale (x-axis) and the log scale (y-axis) respectively. The concentration of Human NLRP3 in sample is determined by plotting the sample's O.D. on the Y-axis. The original concentration is calculated by multiplying the dilution factor (figure 2.5).

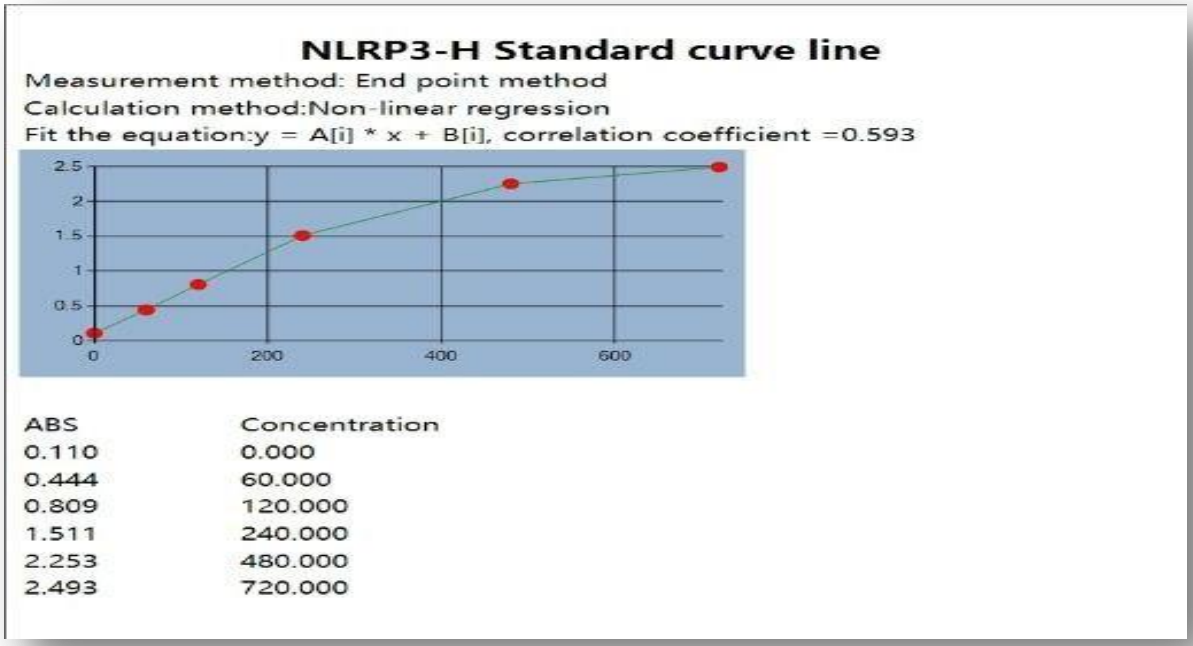

Figure (2.5): Human NLRP3 Standard Curve that showed the Concentration (pg/ml) and optical density OD.

2.2.9.4 Determination of Human *Helicobacter pylori* IgG (Hp-IgG).

- Purpose

Our Human Helicobacter pylori IgG (Hp-IgG) ELISA Kit is to for the qualitative determination of Hp-IgG in Human serum.

- Principle

The ELISA is based on the qualitative enzyme immunoassay technique. The Microplate provided in this kit has been pre-coated with an antigen specific to Hp-IgG, make it to solid-phase antigen. Samples are added to the Microplate wells and combined to the specific antigen. Then a Horseradish Peroxidase (HRP)-conjugated antigen specific for Hp-IgG is added to each Microplate well and incubated, so the antigen-antibody-Enzyme labeled antigen complex is formed. Following a wash to remove any unbound reagent, then the TMB substrate solution is added to each well. Only those wells that contain Hp-IgG and HRP conjugated Hp antigen will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The qualitative determination of Hp-IgG is determined by comparing with the CUTOFF value.

- Materials provided with the kit

Table (2-6): Materials provided with the Human (Hp-IgG) ELISA kit.

	Materials provided with the kit	96 determinations	Storage
1	Negative control	0.5ml×1 bottle	2-8℃
2	Positive control	0.5ml×1 bottle	2-8℃
3	HRP-Conjugate reagent	6ml×1 bottle	2-8℃
4	Sample diluent	6ml×1 bottle	2-8℃
5	Chromogen Solution A	6ml×1 bottle	2-8℃
6	Chromogen Solution B	6ml×1 bottle	2-8℃
7	Stop Solution	6ml×1 bottle	2-8℃
8	wash solution	20ml ×1bottle	2-8℃

-Procedure

1. In the Microplate, number the corresponding micro-pores of the sample in sequence, leave two wells as negative control, two wells as positive control and one empty well as blank control. (Blank control hole don't add samples and HRP-Conjugate reagent, the rest step operation are same)

2. Added samples: Negative and positive control in a volume of 50µl are added to the negative and positive control wells respectively. In sample wells, 40µl Sample dilution buffer and 10µl sample are added. Samples should be loaded onto the bottom without touching the well wall. Mix well with gentle shaking.

3. Incubated 30 min at 37℃ after sealed with Closure plate membrane.

4. Diluted the concentrated washing buffer with distilled water (30 times for 96T).

5. Carefully peel off Closure plate membrane, aspirate and refill with the wash solution. Discard the wash solution after resting for 30 seconds. Repeat the washing procedure for 5 times.

6. Added 50 µl HRP-Conjugate reagents to each well except the blank control well.

7. Incubated as described in Step 3.

8. Washed as described in Step 5.

9. Added 50 µl Chromogen Solution A and 50 µl Chromogen Solution B to each well, mix with gently shaking and incubate at 37℃ for 15 minutes. Please avoid light during coloring.

10. Added 50 µl stop solution to each well to terminate the reaction. The color in the well should change from blue to yellow.

11. Read absorbance O.D. at 450nm using a Microtiter Plate Reader. The OD value of the blank control well is set as zero. Assay should be carried out within 15 minutes after adding stop solution.

-Determine the result

Test effectiveness: the average value of positive control ≥1.00; the average value of negative control ≤0.10.

The critical value (CUT OFF) calculation: critical value = the average value of negative control + 0.15

Negative judgement: if the OD value< CUT OFF, the sample is Human Hp-IgG negative.

Positive judgement: if the OD value ≥CUT OFF, the sample is Human Hp-IgG positive.

2.2.9.5 Determination of Human *Helicobacter pylori* IgM (Hp-IgM).

- Purpose

Our Human *Helicobacter pylori* IgM (Hp-IgM) ELISA Kit is to for the qualitative determination of Hp-IgM in Human serum.

--principle, sample collection, material provide in this kite and this procedure are all similar Human Helicobacter pylori IgG (Hp-IgG) ELISA Kit in (2.2.9.4).

- Determine the result

Test effectiveness: the average value of positive control ≥1.00; the average value of negative control ≤0.10.

The critical value (CUT OFF) calculation: critical value = the average value of negative control + 0.15

Negative judgement: if the OD value< CUT OFF, the sample is Human Hp-IgM negative.

Positive judgement: if the OD value ≥CUT OFF, the sample is Human Hp-IgM positive.

2.2.10. Determination of C - reactive protein (CRP) test.

- CRP test is a blood test designed to measure the amount of CRP in the blood**.**

- Principle

The C - reactive protein test is based on the principle of the latex agglutination. When latex particles complexed human anti-CRP is mixed with a patient's serum containing C-reactive proteins, a visible agglutination reaction will take place within 2 minutes (According to the manufacturer's instruction).

- Procedure

1. Brought all reagents and serum sample to Room Temperature and mix latex reagent gently prior to use. Do not diluted the controls and serum.
2. Placed 1 drop of Serum, Positive control and Negative control on separate reaction circle on glass slide.
3. Then added 1 drop of CRP latex reagent to each of the circles.
4. Mixed with separate mixing sticks and spread the fluid over the entire area of the cell.
5. Tilted the slide back and forth slowly for 2 minutes observing preferably under artificial light.
6. Observed for visible agglutination.

-Result Interpretation of CRP Test

Positive: Agglutination of latex particles, indicating the presence of C – reactive protein at a significant and detectable level.

Negative: No Agglutination (Figure 2.6).

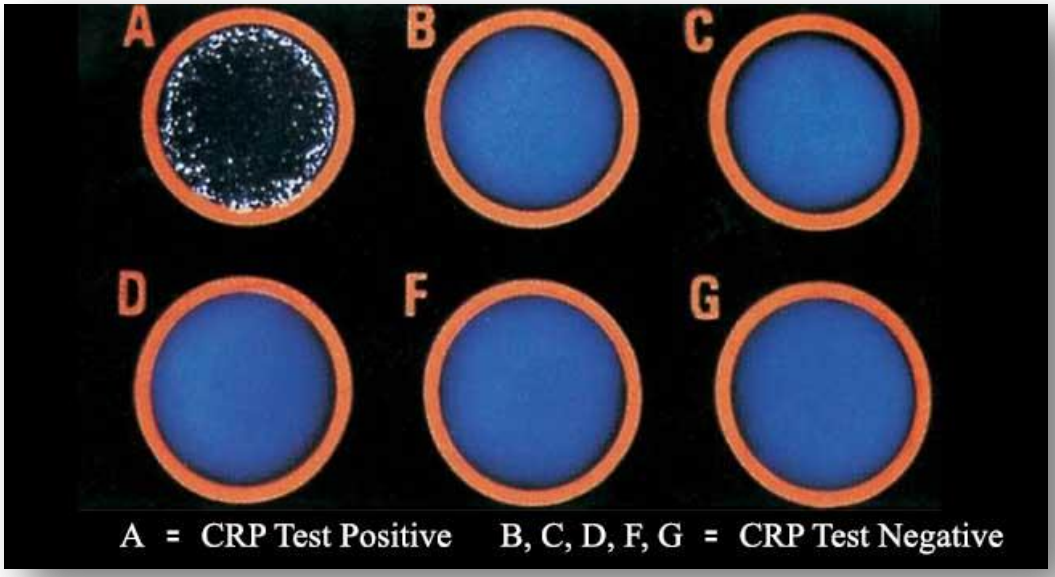

Figure (2.6): CRP test results

2.2.11. Determination of Human Hemoglobin A1C (HbA1C) tests.

-Intended Use

I Chroma™ HbA1c is a fluorescence Immunoassay (FIA) for the quantitative determination of HbA1c (Hemoglobin A1c) in human whole blood. It is useful as an aid in management and monitoring of the long-term glycemic status in patients with diabetes mellitus (According to the manufacturer's instruction).

-principle

The test uses a sandwich immunodetection method; the detector antibody in buffer binds to antigen in sample, forming antigen-antibody complexes, and migrates onto nitrocellulose matrix to be captured by the other immobilized-antibody on test strip. More antigens in sample form more antigen-antibody complex and leads to stronger intensity of fluorescence signal on detector

antibody. Instrument for i chrome™ tests displays the content of glycated hemoglobin in terms of percent of the total hemoglobin in blood.

-Components

I chrome™ HbA1c consists of Cartridges, Detection Buffer Tubes, Hemolysis Buffer Vial and an ID chip.

-Sample collection

The sample type for I Chroma™ HbA1c is human whole blood.

-procedure

1) Drew 100 µL of hemolysis buffer and transfer it into detection buffer tube.

2) Drew5 µL of fingertip blood or tube blood using 5 µL capillary tube and put the capillary tube into the detection buffer tube.

3) Closed the lid of the detection buffer tube and mix the sample thoroughly by shaking it about 15 times.

4) Took out the cartridge half form i-Chamber slot.

5) Pipetted out 75 µL of the sample mixture and load it into a sample well in the test cartridge.

6) Waited till the sample mixture flow appears in the windows. (about 10 seconds)

7) Inserted the cartridge into I-Chamber slot.

8) Left the cartridge in I-Chamber for 12 minutes before removing.

9) To scan the sample-loaded cartridge, insert it into the cartridge holder of the instrument for I Chroma™ tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow has been

marked on the cartridge especially for this purpose.

10) Pressed 'Select' button on the instrument for I Chroma™ tests to start the scanning process.

11) Instrument for I Chroma™ tests will start scanning the sample-loaded cartridge immediately.

12) Read the test result on the display screen of the instrument for I Chroma™ tests.

-Results

Table (2-7): Standard value of Human Hemoglobin A1C (HbA1C) test.

A1C value	ADA diagnosis
5.6% or less	Normal
5.7–6.4%	Prediabetes
6.5% or more	Diabetes

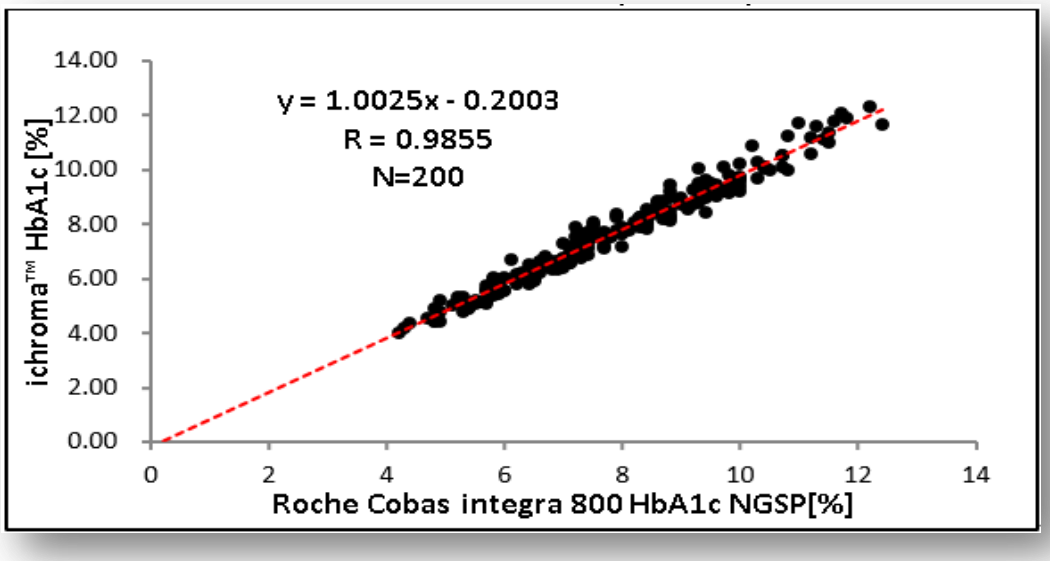

Figure (2-7): Standard curve of I Chroma HbA1C.

2.2.12. Determination of CBC (Neutrophils & Lymphocyte Count).

-principle

A complete blood count, or CBC, is an easy and very common test that screens for certain disorders that can affect your health. CBC determines if there are any increases or decreases in your blood cell counts. Normal values vary depending on age and gender. CBC can help diagnose a broad range of conditions, from anemia and infection to cancer (According to the manufacturer's instruction).

-Purpose

For counting Neutrophils and Lymphocyte cells in human blood.

-Sample collection

The sample type for CBC test is human whole blood.

-procedure

1. Collected the sample (whole blood) 3-4 ml and empty into EDTA tube.

2. Used shaker device for mixing the sample and remove blood clot for 1 min.

3. Filled the patient information in the device.

4. Opened the EDTA tube and put in needle of CBC device.

5. Ran the CBC device

6. Read the test result on the display screen of the **i**nstrument for CBC test.

2.2.13. Determination of Fasting Blood Sugar (FBS) test.

-Principle

Glucose-oxidase enzyme oxidizes glucose to gluconate and hydrogen peroxide, according to the following equation:

-purpose

A blood glucose test is used to find out if your blood sugar levels are in a healthy range. It is often used to help diagnose and monitor diabetes (According to the manufacturer's instruction).

-Procedure

1. For a fasting blood glucose test, you can't eat or drink anything except water for 8 hours before your test.

2. Collected the blood sample 1-2 ml from the vein or fingerprint from the patients.

3. Emptied the blood in plane tube and centrifuged by centrifuge device (4500, 5 min) for obtain blood serum.

4. Used 1000 ul from Glucose reagent with 10 ul from serum and mixing

5. Incubated the mixing for 10 min in 37 C by incubation device or 20 min at room temperature (15-25 C).

6. Useed spectrophotometry device (wavelength 505nm) for read the result.

-Result

A healthy (normal) fasting blood glucose level for someone without diabetes is 70 to 99 mg/dL (3.9 to5.5 mmol/L). Values between 50 and 70 mg/dL (2.8 to 3.9 mmol/L) for people without diabetes can be normal too.

2.2.14. Determination of Oral Glucose Tolerance Test (OGTT).

- The oral glucose tolerance test (OGTT) measures the body's ability to use a type of sugar, called glucose that is the body's main source of energy. An OGTT can be used to diagnose diabetes.

- Oral Glucose Tolerance Test (OGTT) test similar principle and procedure of FBG test except the OGTT test occur by fasting blood glucose test in 1h and then should be drinking fluid glucose (75 g), and measure blood sugar in deferent times (2-3 h).

2.2.15. Statistical Analysis

The Statistical Analysis System- SAS (2018) program was used to detect the effect of difference groups (patients and control) in study parameters. T-test was used to significant compare between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability). Estimate of correlation coefficient between variables in this study.

The level of significant was considered at $P \leq 0.05$. The level of high significant was considered at $P \leq 0.01$. NS was considered at Non significant

Chapter Three

Results and Discussion

3. Results and Discussion

3.1. Demographic Characteristics

3.1.1. Study Groups.

The current study included 60 patients with Gestational Diabetes Mellitus (GDM), and 29 Control without GDM. The frequency distribution of patients with GDM and control group is shown in table (3-1).

Table (3-1): Study Groups (GDM and Control group).

Groups	NO.	(%)
Patients	60	67.4
Control	29	32.6
Total	89	100

3.1.2. The Comparison of Age between Patients with GDM and Control group.

The comparison of mean age between patients with GDM and control is shown in table (3-2) .The age range of patients with GDM was (25-47) years; therefore, the age range of control was (23-45) years. The mean age of patients with GDM was 34.83 ±0.80 years and the mean age of control was 28.45 ±1.16 years. In the present study, there was highly significant difference in mean age between patients and control group ($P \leq 0.01$). A

histogram showing the frequency distribution of patients with GDM and control according to age has been conducted and is shown in figure (appendix 3).

Maternal age is the most important factor independently affecting the risk of gestational diabetes mellitus (GDM) .The incidence of GDM appeared to increase steadily with age pregnancy ($P < 0.05$). The incidence of GDM was high in older age were correlated with an increased risk of GDM, which was similar to our results, the diagnosis of GDM usually occurs after the age of 30 years (Yahaya *et al.,* 2020). Women aged <20 years had a significantly lower risk for GDM than those aged 20–24 years, There was no significant difference in GDM risk between Europid and Asian ethnicity in women aged <20 years compared with women aged 20–24 years ($p= 0.175$), but there was a higher GDM risk among Asian women in the other comparisons ($P< 0.001$), which was similar to our results. There was a higher percentage of women with GDM having age ≥35 years, Women aged 35 years and above had higher risk for GDM (Yong *et al*., 2020) which was similar to our results.

In present study we can proved the maternal age was higher in GDM than in healthy control group ($P\leq0.01$).

Table (3-2) : Comparison between patients and control groups in Age and BMI.

Group	Mean ± SE	
	Age (year)	BMI (kg/m^2)
Patients	34.83 ±0.80	34.67 ±0.70
Control	28.45 ±1.16	31.38 ±1.13
T-test	2.795 **	2.543 *
P-value	0.0001	0.0119
** ($P \leq 0.01$),		* ($P \leq 0.05$).

**P*-value significant at $p \leq 0.05$, ***P*-value highly significant at $P \leq 0.01$,

BMI: body mass index.

3.1.3. The Comparison of BMI between Patients GDM and Control group.

The comparison of mean BMI between patients with GDM and control is shown in table (3-2). The BMI range of patients with GDM was (22-41) Kg/m^2; therefore, we chose the BMI range of control was (20-40) Kg/m^2.The mean BMI of patients with GDM was 34.67 ±0.70 and the mean BMI of control was 31.38 ±1.13.In the present study, there was significant difference in mean BMI between patients and control group ($P \leq 0.05$). A histogram showing the frequency distribution of patients with GDM and

control according to BMI has been conducted and is shown in figure (appendix 4).

It is widely reported that obesity is a risk factor for DM and also GDM. The International Diabetes Federation (IDF) in 2021 stated that, around 10.9% of pregnant women in Europe suffered from GDM; having a high BMI as a risk factor for the development of GDM (International Diabetes Federation, 2021).

A pre-pregnancy BMI 30 kg/m2 had a stronger effect on GDM among those age 30–34 (Guoju *et al*., 2020) which were similar to our results. Also found in meta-analysis study that the risk of obese pregnant women to develop GDM is four times higher than non-obese women (Chu *et al*., 2007) which were similar to our results. The BMI was also significantly higher in women with GDM(P = 0.003) compared to controls (Sisay *et al*., 2020) .The National Institute for Health and Care Excellence recommends all obese pregnant women to be screened for GDM (Women's & Health, 2015). It is suggested that the rate of GDM presents to be twofold higher in obese Asian and Latina women than those in Caucasian and African- American (Shah *et al*., 2011) which was similar to our results. Moreover, applying BMI 30 Kg /m2 women with diabetes in pregnancy, this was similar to our results. In other study, BMI was significantly associated with GDM compared to normal pregnant. Increasing maternal BMI is a significant risk factor for the development of GDM (McIntyre *et al.*, 2018) which was similar to our results. Even though the association between BMI and GDM can still be used to counsel women about their risk of developing GDM, BMI as a screening tool does not have high enough sensitivity and

specificity to identify a group of women that should not receive GDM diagnostic testing.

This continues to support the notion for continuing universal screening programs for pregnant women rather than stratifying by BMI (Shah *et al.*, 2011) which was similar to our results. Also found who indicated that the high weight gain in early pregnancy, particularly during the 1st trimester, was associated with an increased risk of GDM (Erem *et al*., 2015).in addition The results also show that BMI was significantly higher in cases compared to controls ($P < 0.001$). Therefore, high BMI may be considered as a risk factor in pregnant women for GDM (Zainedeen, 2018) which was similar to our results.

In present study I can proved the maternal BMI was higher in GDM than in healthy control group ($P \leq 0.05$).

3.1.4. The Comparison of FBS between Patients GDM and Control group.

The comparison of mean FBS between patients with GDM and control is shown in table (3-3).The mean FBS of patients with GDM was (132.60 ±3.52 mg/dl) and the mean of control was (66.31 ±1.86 mg/dl), there was highly significant difference in mean FBS between patients and control group ($P \leq 0.01$).A histogram showing the frequency distribution of patients with GDM and control according to FBS has been conducted and is shown in figure (appendix 5).

FBS values tend to stay constant throughout the entire period of pregnancy. FBS values have less individual variation compared to other glucose values, therefore, abnormal FBS level is a significant indicator in diagnosing GDM. FBS is a good screening test for GDM with advantages such as simple procedure, reasonable cost, reproducibility, easy access, and acceptance (Cho *et al.,* 2016)) which was similar to our results.

Other studies have reported that abnormal FBS alone is capable of detecting 50% of pregnant women who had already been diagnosed with GDM with another screening method (Zawiejska *et al.,* 2014). The mean fasting plasma glucose and 2-hour plasma glucose (2hPG) were 4.37 ±0.51 mmol/l and 6.10 ± 1.41 mmol/l. About 13.1% of the women were diagnosed with GDM. GDM was diagnosed if either or both FPG was ≥ 5.6 mmol/l or 2hPG is ≥7.8 mmol/l according to the Ministry of Health Malaysia guideline (Yong *et al*,. 2020) which was similar to our results.in addition The FBG results were higher in GDM cases (105.8±15.8 mg/dl) in contrast to controls (66.5±8.1 mg/dl), the difference is also statistically significant (t =14.823, P < 0.001) (Zainedeen, 2018) which was similar to our results.

In present study I can proved the maternal FBS was higher in GDM than in healthy control group ($P \leq 0.01$).

Table (3-3) : Comparison between patients and control groups in FBS, OGTT, HbA1c, Lymphocyte and Neutrophil.

Group	Mean ± SE				
	FBS (mg/dl)	OGTT (mg/dl)	HbA1c (%)	Lymphocyte (%)	Neutrophil (%)
Patients	132.60 ±3.52	208.78 ±5.96	7.41 ±0.06	34.43 ±1.09	61.60 ±1.15
Control	66.31 ±1.86	85.68 ±1.26	4.45 ±0.08	27.45 ±1.15	59.24 ±0.83
T-test	10.417 **	17.196 **	0.223 **	3.505 **	3.489 NS
P-value	0.0001	0.0001	0.0001	0.0002	0.182
** ($P \leq 0.01$).					

NS: Non- significant, FBS: fasting blood sugar, OGTT: oral glucose tolerance test, HbA1C: hemoglobin A1C.

3.1.5. The Comparison of OGTT between Patients GDM and Control group.

The comparison of mean OGTT between patients with GDM and control is shown in table (3-3) .The mean OGTT of patients with GDM was (208.78 ±5.96 mg/dl) and the mean of control was (85.68 ±1.26mg/dl) , there was highly significant difference($P \leq 0.01$) in mean OGTT between

patients and control group .A histogram showing the frequency distribution of patients with GDM and control according to OGTT has been conducted and is shown in figure (appendix 6).

Oral Glucose Tolerance Test is a common test for potential GDM. It evaluates how the pregnant women process glucose. Therefore, the (WHO) as well as other approaches recommend the standardized, 75-g OGTT at 24 weeks of gestation or later. Accordingly, found a statistically significant difference between GDM group and control group regarding of using OGTT for predicting large gestational age newborns with p- value < 0.001 (Brankica *et al*., 2016) which was similar to our results.in addition both GDM during pregnancy and dysglycaemia at 4–6 years post-delivery were diagnosed using a 2-h (2 h) 75 g oral glucose tolerance test (OGTT) after an overnight fast (Chen *et al*., 2021). The average level of OGTT was higher in cases than controls which was also statistically significant (P < 0.001) (Zainedeen, 2018) which was similar to our results.

In present study I can proved the maternal OGTT was higher in GDM than in healthy control group ($P \leq 0.01$).

3.1.6. The Comparison of (HbA1c) between Patients GDM and Control group.

The comparison of HbA1c between patients with GDM and control is shown in table (3-3).The mean HbA1c of patients with GDM was (7.41 ±0.06 %) and the mean of control was (4.45 ±0.08 %), there was highly significant difference in mean HbA1c between patients and control group

($P \leq 0.01$) .A histogram showing the frequency distribution of patients with GDM and control according to HbA1c has been conducted and is shown in figure (appendix 7).

Glycated hemoglobin is a widely used marker in diagnosis of GDM. Consequently, some studies reported that using HbA1c can endorse diagnosis of GDM in third trimester gestation (Capula *et al*., 2013. However the HbA1c in some studies stay controversy tool as merely diagnostic marker during pregnancy especially in the first trimester. This is likely returned to certain conditions characterized pregnant women at early stages such as diversion of glucose toward developing fetus and also to reduce erythrocyte life span which results in lower timed exposure of new erythrocytes to glucose and thus lower glycation (Rajput *et al*., 2012).HbA1c test is currently considered to be the best measure and the gold standard for assessing glycemic control.

It measures the amount of glucose that is bound to hemoglobin molecule, reflects average plasma glucose over the previous 2-3 months in a single measure which can be performed at any time of the day and does not require any special preparation such as fasting (Delong& Burkhart, 2008). Found the average level of HbA1c in cases was higher compared to controls ($P < 0.001$), the higher levels of HbA1c in cases approve that there was a poor glycemic control in cases for at least the past 2 months (Zainedeen,2018) which was similar to our results. Also found the mean value of HbA1c% was obviously higher in GDM (6.52 ± 1.30) as compared with normal pregnant (5.06 ± 0.53), with p value (0.001) which was similar to our results. Also there is a significant statistical difference between cases

and controls regarding the means for HbA1c with P value <0.001 (Taher, 2018) which was similar to our results.

In present study I can proved the maternal HbA1c was higher in GDM than in healthy control group ($P \leq 0.01$).

3.1.7. The Comparison of Lymphocyte between Patients GDM and Control group.

The comparison of Lymphocyte between patients with GDM and control is shown in table (3-3).The mean Lymphocyte of patients with GDM was (34.43 ±1.09 %) and the mean of control was (27.45 ±1.15 %). there was highly significant difference in mean Lymphocyte between patients and control group ($P \leq 0.01$). A histogram showing the frequency distribution of patients with GDM and control according to Lymphocyte has been conducted and is shown in figure (appendix 8).

Neutrophil ratio, which can be easily calculated from complete blood count (CBC), is an inexpensive parameter with multiple clinical applications, for example GDM (Hai *et al*., 2020).

Pregnant females had higher total lymphocyte count in GDM. GDM was associated with significant in lymphocyte, found the mean lymphocyte in patients with GDM is (25.4 ± 1.1) and mean of lymphocyte in control group is (22.7 ± 0.8), in the study found highly significant difference in mean Lymphocyte between patients and control group (p =0.001) (Cinkajzlová *et al*., 2020) which was similar to our results. Also found Compared to the healthy pregnant women, the percentages of lymphocytes

were significantly different in women with GDM. The percentage of lymphocytes was positively related to insulin resistance. The number of 14.05% of lymphocytes was an optimal cutoff point that predicted the insulin resistance in women with GDM (Zhuang *et al*., 2019) which was similar to our results. Also Lymphocyte count was significantly ($p<0.05$) reduced in gestational diabetic women (45.78 ± 09.92%) when compared with the control subject (56.70 + 5.32%) (Hope *et al*., 2019) which was similar to our results.

In present study I can proved the maternal Lymphocyte count was higher in GDM than in healthy control group ($P\leq 0.01$).

3.1.8. The Comparison of Neutrophil between Patients GDM and Control group.

The comparison of Lymphocyte between patients with GDM and control is shown in table (3-3).The mean Neutrophil of patients with GDM was (61.60 ±1.15) % and the mean Neutrophil of control was (59.24 ±0.83) %. In the present study, there was Non-significant difference in mean Neutrophil between patients and control group (p =0.182). A histogram showing the frequency distribution of patients with GDM and control according to Neutrophil has been conducted and is shown in figure (appendix 9).

Neutrophils, which constitute the largest fraction of WBCs, have been found to be involved in chronic met inflammatory states such as diabetes, nonalcoholic fatty liver disease (Sun *et al*,.2020).also recent study found the

mean of neutrophil in control group is (6.03 6± 1.70×10^9 /L) but found the mean of neutrophil in patients with GDM group is (7.06 6± 1.76 3×10^9 /L), there was non- significant difference in mean Neutrophil between patients and control group (Sun *et al*,.2020) which was similar to our results. Also found the mean of neutrophil in control group is (71.1±7.0 %) but found the mean of neutrophil in patients with GDM group is (69.8±7.8), there was non- significant difference in mean Neutrophil (p=0.20) between patients and control group (Fashami *et al*., 2020) which was similar to our results. also found Neutrophil count was significantly (p<0.05) higher in gestational diabetic women (53.04 ± 9.99%) when compared to controls (43.60 ±6.24%) (Hope *et al*,. 2019) which was no similar to our results. Found Neutrophil count was non- significantly (p=0.18) higher in gestational diabetic women (7.2 ± 1.8 10^3/µl) when compared to controls (7.1 ±2.0510^3/µl) (Mertoglu *et al*., 2019) which was similar to our results.

In present study I can proved the maternal Neutrophil count was higher in GDM than in healthy control group ($P \leq 0.01$).

3.1.9. The Comparison of C- reactive protein between Patients GDM and Control group.

The comparison of CRP between patients with GDM and control is shown in table (3-4) .The Positive CRP of patients with GDM was (76.67%) and the negative CRP of patients with GDM was (23.33%), and the positive control was (10.34%) but the negative control was (89.66%). In the present study, there was significant difference between patients and control group ($P \leq 0.05$)

Increased levels of CRP are associated with the risk of gestational diabetes in most of the published studies. One of the recent cases for early diagnosis of GDM is the size of the C-reactive protein (CRP). Most of studies showed significant relationship between CRP with GDM. Blood levels of CRP could be used as a potential indicator for GDM. This results there is a statistically significant difference between cases and controls regarding CRP (P = 0.001), cases have higher physical activity in relation to controls (Amirian *et al*., 2020) which was similar to our results.

In present study I can proved the maternal CRP was higher in GDM than in healthy control group.

Table (3-4): Distribution of sample study according to CRP of GDM in patients and control.

Factor		Patients (No=60)	Control (No= 29)	P-value
CRP: No (%)	Positive	46 (76.67%)	3 (10.34%)	0.0001 **
	Negative	14 (23.33%)	26 (89.66%)	0.041 *
	P-value	0.0001 **	0.0001 **	---
* (P≤0.05), ** (P≤0.01).				

3.1.10. The Comparison of physical activity between Patients GDM and Control group.

The comparison of physical activity between patients with GDM and control is shown in table (3-5) .The result (yes) of physical activity of patients with GDM was (26.67%) and the result (No) of physical activity of patients with GDM was (63.33%) and the result Yes of control was (68.97%) but the result No of control was (31.03%). In the present study, there was significant difference between patients and control group ($P \leq 0.05$).

The benefits of physical activity on GDM, other benefits of physical activity in pregnant women have been found, e.g., the psychological benefits. Physical activity helps prevent GDM by improving glycemic control, insulin resistance, and pre-pregnancy weight gain. A study indicates that an increase of 100 min of moderate to vigorous physical activity per week could reduce the risk of GDM by 9% .However; the highest probability of preventing GDM by physical exercise is for pregnant women with morbid obesity (Artal *et al*., 2015). In previous study the results there is a statistically significant difference between cases and controls regarding physical activity (P= 0.485), cases have higher physical activity in relation to controls (Taher, 2018) which was similar to our results.

Table (3-5): Distribution of sample study according to Physical activity in patients and control.

Factor		Patients (No=60)	Control (No= 29)	P-value
Physical activity: No (%)	Yes	16 (26.67%)	20 (68.97%)	0.569 NS
	No	44 (63.33%)	9 (31.03%)	0.0001 **
	P-value	0.0001 **	0.0487 *	---
* (P≤0.05), ** (P≤0.01).				

3.1.11. The Comparison of family history of GDM between Patients GDM and Control group.

The comparison of family history of GDM between patients with GDM and control is shown in table (3-6) .The result (yes) of family history of GDM of patients with GDM was (53.33%) and the result (No) of family history of GDM of patients with GDM was (46.67%) and the result (Yes) of control was (31.03%) but the result (No) of control was (68.97%). In the present study, there was significant difference between patients and control group (P≤0.05).

Family history of GDM is statistically significant (P = 0.001) where the cases have higher family history of GDM as a risk factor for development of GDM in cases compared to the controls (Zainedeen, 2018), also This result are compatible with (El Sagheer *et al*., 2016), which was similar to our results. In addition the results there is a statistically significant difference between cases and controls regarding family history of GDM (P = 0.001), cases have higher family history of DM in relation to controls (Taher, 2018) which was similar to our results.

Table (3-6): Distribution of sample study according to Family history of GDM in patients and control.

Factor		Patients (No=60)	Control (No= 29)	P-value
Family history of GDM: No (%)	Yes	32 (53.33%)	9 (31.03%)	0.0001 **
	No	28 (46.67%)	20 (68.97%)	0.092 NS
	P-value	0.683 NS	0.0487 *	---
* (P≤0.05), ** (P≤0.01).				

3.1.12. The Comparison of family history of DM between Patients GDM and Control group.

The comparison of family history of DM between patients with GDM and control is shown in table (3-7) .The result (yes) of family history of DM of patients with GDM was (75.00%) and the result (No) of family history of DM of patients with GDM was (25.00%) and the result (Yes) of control was (31.03%) but the result (No) of control was (68.97%). In the present study, there was significant difference between patients and control group ($P \leq 0.05$ of patients), ($P \leq 0.01$ of control).

Women with a family history of diabetes make it more susceptible to develop GDM, which suggests that genes play a role. Family history of diabetes is observed as a risk factor for gestational glucose intolerance. Most screening policy involving DM family history as a sign of OGTT. However, few studies had evaluated the actual incidence of glucose intolerance in this group of women (Zainedeen, 2018).

In previous study show the results there is a statistically significant difference between cases and controls regarding family history of DM ($P = 0.003$), cases have higher family history of DM in relation to controls , also this result agree with those of (Tomedi *et al*., 2013) and which was similar to our results.in addition the results there is a statistically significant difference between cases and controls regarding family history of DM ($P = 0.030$), cases have higher family history of DM in relation to controls (Taher, 2018) which was similar to our results.

Table (3-7): Distribution of sample study according to Family history of DM in patients and control.

Factor		Patients (No=60)	Control (No= 29)	P-value
Family history of DM: No (%)	Yes	45 (75.00%)	9 (31.03%)	0.0001 **
	No	15 (25.00%)	20 (68.97%)	0.531 NS
	P-value	0.0001 **	0.0487 *	---
* (P≤0.05), ** (P≤0.01).				

3.2. Measure Immunological parameters for study groups.

3.2.1. Measure IL-1b Level in patients with GDM and control group.

The comparison of IL-1beta between patients with GDM and control is shown in table (3-8).The mean of IL-1beta of patients with GDM was (38.68 ±5.02 µl) and the mean IL-1beta of control was (6.12 ±0.37 µl). In the present study, there was highly-significant difference in mean IL-1beta between patients and control group (P≤0.01). A histogram showing the

frequency distribution of patients with GDM and control according to IL-1beta has been conducted and is shown in figure (appendix 10).

Gestational diabetes is a state of insulin resistance associated with altered levels of pro-inflammatory cytokines; increased IL-1β.these alterations might be attributed to placental pathology in pregnancies with GDM (Wu *et al*., 2021)

Maternal IL-1β levels at 2nd trimester of pregnancy in women with and without (normal) GDM found significant difference in mean IL-1beta between patients and control group (P≤0.001) (Vitoratos *et al*., 2008) which was similar to our results. In addition the serum IL-1β levels in GDM patients are significantly higher than those of healthy controls. These data indicate that the serum IL-1β level is involved in the occurrence and development of GDM ($^{*}P<0.05$), GDM vs Control (Liu *et al*., 2020). According to the results, we found the association between the IL1B susceptibility to GDM. The analysis of IL1B differences between patients with GDM and healthy controls. Differences were considered to be statistically significant when (P<0.05) (Laltlanzovi *et al*., 2022) which was similar to our results. . However, in previous study, serum IL-1beta played no significant role in the development of Gestational diabetes (P<0.076) which was no similar to our results. in other study found Serum IL-1b levels involvement in GDM development, and statistically significant when (P<0.05) in GDM patients than those of healthy controls (Lappas *et al*., 2014) which was similar to our results. In addition found the result indicate that circular levels of IL-1b is significantly (p< 0.611) in pregnant women with GDM (1.86 ± 0.66) or glucose intolerance compared with healthy controls (2.01 ±0.69), IL-1b may serve as potential biomarkers for

evaluating potential glucose intolerance or GDM with pregnancy (Zhao *et al*., 2018) which was similar to our results.

Table (3-8): Comparison between patients and control groups in Quantitative IL-1b and Quantitative IL-18.

Group	Mean ± SE	
	Quantitative IL-1b (µl)	Quantitative IL-18(µl)
Patients	38.68 ±5.02	58.87 ±4.79
Control	6.12 ±0.37	20.13 ±1.06
T-test	5.881 **	6.504 **
P-value	0.0001	0.0001
** (P≤0.01).		

3.2.2. Measure IL-18 Level in patients with GDM and control group.

The comparison of IL-18 between patients with GDM and control is shown in table (3-8).The mean of IL-18 of patients with GDM was (58.87 ±4.79 µl) and the mean IL-18 of control was (20.13 ±1.06 µl). In the present study, there was highly-significant difference in mean IL-1beta between patients and control group (P≤0.01). A histogram showing the frequency

distribution of patients with GDM and control according to IL-18 has been conducted and is shown in figure (appendix 11).

In previous study found the Levels of IL-18 higher in cases versus controls, respectively ($p<0.01$) this indicate the serum IL-18 level is development of GDM. High IL-18 levels are seen in GDM which may be associated with inflammation suggesting a role in development of insulin resistance (Fatima *et al*., 2017), which was similar to our results. Also the serum IL-18 levels in GDM patients are significantly than those of healthy controls. These data indicate that the serum IL-18 level is involved in the occurrence and development of GDM ($^{*}P<0.05$) (Wu *et al*,. 2021) which was similar to our results. In addition found the result indicate that circular levels of IL-18 is significantly ($p< 0.001$) up-regulated in pregnant women with GDM(52.2 ± 10.3) or glucose intolerance compared with healthy controls (22.4 ± 5.1), IL-18 may serve as potential biomarkers for evaluating potential glucose intolerance or GDM risk in Chinese women with pregnancy (Zhao *et al* ., 2018) which was similar to our results.in other study showed serum IL-18 level in pregnant women (87.86 ± 20.49)was significantly ($p<0.001$) in compared to the controls (138.30 ± 20.70),in this study found level IL-18 in patient lower than in control was no similar to our results (Jahromi *et al*., 2014). Also show (Kuzmicki *et al*., 2008) serum IL-18 level in pregnant women was significantly ($p<0.005$) in compared to the controls which was similar to our results.in addition found serum IL-18 level in pregnant women was significantly ($p<0.0049$) in compared to the controls which was similar to our results (Zieleniak *et al*., 2022).

3.2.3. Measure NLRP3 Level in patients with GDM and control group.

The comparison of NLRP3 between patients with GDM and control is shown in table (3-9) .The mean of NLRP3 of patients with GDM was (76.39 ±6.71 µl) and the mean NLRP3 of control was (41.15 ±2.66 µl). In the present study, there was highly-significant difference in mean NLRP3 between patients and control group ($P \leq 0.01$). A histogram showing the frequency distribution of patients with GDM and control according to NLRP3 has been conducted and is shown in figure (appendix 12).

The NOD-like receptor protein 3 (NLRP3) inflammasome is a key regulator of the host immune response, and many immune and metabolic disorders are linked to its activation .In patients with GDM, studies have proved a connection between activation of the NLRP3 inflammasome and insulin resistance. High glucose levels increase NLRP3 activation compared with those induced by normal and low glucose levels. It is known that hyperglycemia or diabetes during pregnancy can induce activation of the NLRP3 inflammasome and the secretion of many inflammatory cytokines, (Zhou *et al*., 2021).

The results of the previous study demonstrated that the expression levels of NLRP3 were elevated in the clinical placenta samples collected from pregnant women with GDM. Also the serum levels NLRP3 in GDM patients are significantly than those of healthy controls. These data indicate that the serum NLRP3 level is involved in the occurrence and development of GDM ($^{*}P<0.01$) (Wu *et al*., 2022) which was similar to our results.

Furthermore, the NLPR3 inflammasome was involved in the production of IL-1β and IL-18 in human placental cells. Known as a highly specialized organ during pregnancy, the placenta serves as the interface between maternal and fetal circulation. The key role of the placenta in the occurrence and development of GDM has been reported by multiple studies (Tsai *et al*., 2021). Currently, IR is the critical pathophysiological characteristic of GDM, which is also found during normal pregnancy. Placenta derived hormones, cytokines and gaseous signaling transmitter can induce IR by interfering with insulin receptor signal transduction. Furthermore, the dysregulation of hormones, cytokines and gaseous signaling transmitter in placenta may aggravate IR and trigger abnormal glucose metabolism (Simpson *et al*., 2018). Thus, the present study investigated the key molecules in the placenta responsible for the pathogenesis of GDM. The overactive inflammatory response may be the initiating factor for IR. Cytokines of the IL-1 family critically regulate the inflammatory response by controlling several inflammation processes (Dinarello, 2019). Both IL-1β and IL-18, which are classic pro-inflammatory cytokines of the IL-1 family, participate in the initiation of IR of GDM and type 2 DM (Liu *et al*., 2020).

The NLRP3 inflammasome complex is composed of NLRP3, ASC and pro caspase-1. Activation of the inflammasome recruits and cleaves pro caspase-1, which results in the formation of cleaved caspase-1. Subsequently, cleaved caspase-1 converts pro-IL-1β and pro-IL-18 into the mature forms, IL-1β and IL-18 (Wu *et al*., 2022), the expression levels of NLRP3 and cleaved caspase-1 are elevated in the placenta tissues of GDM mice. The results of the present study demonstrated that the expression

levels of NLRP3 and cleaved caspase-1 were elevated in the clinical placenta samples collected from pregnant women with GDM. Taken together, the results of the present study suggest that excessive activation of the NLRP3 inflammasome in the placenta may be involved in the development of GDM.

Table (3-9): Comparison between patients and control groups in NLRP3.

Group	Mean ± SE Quantitative NLRP3 (µl)
Patients	76.39 ±6.71
Control	41.15 ±2.66
T-test	11.759 **
P-value	0.0074
** (P≤0.01).	

3.3. Detection of *H. pylori* infection between the study groups.

3.3.1. The serum level of IgG concentration

The infection rates of *H. pylori* IgG were significantly difference in the GDM group and Control group (P≤0.01). Control positive, there were (0.00%) while control negative was (100%).but patients' positive subgroup, there were (10.00%), and while patients negative subgroup were (90.00%).The present study showed that IgG has a highly significant difference (P<0.01) between patients GDM group and healthy control group. Show in table (3-10).

In previous study showed that *H. pylori* infection significantly increased the incidence GDM (*P*≤0.01). *H. pylori* infection increased the incidence of GDM and preeclampsia and potentially reduced the incidence of uncomplicated pregnancy (Xia *et al*., 2020), Which was similar result.in other study (Tang *et* al., 2021).observed *H. pylori* infection positive during pregnancy was significantly related to higher rate of preeclampsia and gestational diabetes mellitus (p<0.01) Which was similar result.

There are many methods to detect *Helicobacter pylori*, including serological examination, breath test, and gastric mucosal biopsy and so on. The IgG antibody may reflect a current infection or prior exposure. The sensitivity and specificity of IgG antibody are reported to be 80–100% and 69–95%, respectively (Li *et al*., 2021). The main reasons for this are that DM causes damage to cellular and immune functions, and enhances sensitivity to infection (Koh *et al*., 2012). Furthermore, it can weaken gastrointestinal peristalsis and acid secretion, thus promoting the colonization and infection of pathogens in the intestine. Due to immune

adaptation during pregnancy, pregnant women are susceptible to *H. pylori* infection .When pregnancy is complicated with DM and the risk of infection further increases. Results of studies on the relationship between DM and adverse pregnancy outcomes have been relatively consistent (Li *et al*., 2021). High blood glucose can cause maternal and fetal complications, such as spontaneous abortion, fetal malformation, preeclampsia, stillbirth, macrosomia, neonatal hypoglycemia, and neonatal hyperbilirubinemia, which can seriously affect the health of the mother and infant. Infection might play a role in the etiology and pathogenesis of pregnancy complications and poor fetal development in DIP. In this study, the high infection rate of *H. pylori* in particular may have contributed to the higher incidences of GDM in pregnancy. We further compared the incidence rates of IgG between the *HP+* and *HP−* subgroups in each group (Patient and group). The results showed that *H. pylori* infection could increase the incidences of pregnancy-related GDM and hinder fetal development.

Our results have demonstrated that the double impact of high blood glucose and *H. pylori* infection in women with GDM significantly increases the risks of pregnancy-related diseases and poor fetal development compared to those in normal pregnant women and women with GDM or *H. pylori* infection alone

Table (3-10): Distribution of sample study according to *H. Pylori* IgG Qualitative in patients and control.

Factor		Patients (No=60)	Control (No= 29)	P-value
***H. Pylori* IgG Qualitative: No (%)**	Positive	6 (10.00%)	0 (0.00%)	0.097 NS
	Negative	54 (90.00%)	29 (100%)	0.0063 **
	P-value	0.0001 **	0.0001 **	---
** (P≤0.01).				

3.3.2. The serum level of IgM concentration

The infection rates of *H. pylori* IgM were significantly difference in the GDM group and Control group ($P \leq 0.01$). Control positive , there were (0.00%) while control negative was (100%).but patients' positive subgroup, there were (21.67%), and while patients negative subgroup were (78.33%).The present study showed that IgM has a highly significant difference ($P<0.01$) between patients GDM group and healthy control group. Show in table (3-11).

The IgM antibody may reflect a current infection and *H. pylori* infection is extremely common in diabetes in pregnancy. For women with

GDM, infection with *H. pylori* can increase the risks of pregnancy-related diseases and poor fetal development. *H. pylori* screening and eradication therapy before pregnancy may aid in preventing pregnancy-related diseases and improve fetal development. In previous study (Li *et al*., 2021) *H. pylori* infection rates in the GDM group were significantly higher than that in the control group (P=0.031) which was similar our result. Also Gestational diabetes mellitus (GDM) was the most common maternal complication during pregnancy (5.7%) in study population and was a statistically significantly higher percentage of *H. pylori*-positive pregnant women compared with women without GDM (P < 0.001) (Cardaropoli *et al*., 2015), which was similar our result.

Our findings indicated that *H. pylori* infection was extremely common in women with DIP, especially those with GDM-*H. pylori* infection. The influence of *H. pylori* on pregnancy-related diseases and fetal development was further aggravated by GDM. In normal pregnant women, *H. pylori* did not show an association with pregnancy-related diseases; however, it aggravated the incidences of pregnancy-related diseases in women with DIP. The harm caused by *H. pylori* to the fetuses and neonates of women with DIP was also evident, and it could lead to poor fetal and neonatal development.

Table (3- 11): Distribution of sample study according *to H. Pylori* IgM Qualitative in patients and control.

Factor		Patients (No=60)	Control (No= 29)	P-value
***H. Pylori* IgM Qualitative: No (%)**	Positive	13 (21.67%)	0 (0.00%)	0.0394 *
	Negative	47 (78.33%)	29 (100%)	0.0278 *
	P-value	0.0001 **	0.0001 **	---
* (P≤0.05), ** (P≤0.01).				

3.4. Correlation coefficient between variables in this study.

This table (3-12) shows correlation coefficient between immunological parameters (IL-1ß, IL-18 and NLRP3) with the variables in this study (Age, BMI, FBG, OGTT, HbA1c, Lymphocyte and Neutrophil). There was Non- significant difference between the immunological parameters and the variables in this study.

Table (3-12): Correlation coefficient between variables in this study.

Parameters	Age	BMI	FBG	OGTT	HbA1c	Lymphocyte	Neutrophil
Quantitative IL-1ß	0.03 NS	0.13 NS	-0.04 NS	0.02 NS	-0.13 NS	-0.02 NS	0.03 NS
Quantitative IL-18	-0.07 NS	-0.04 NS	-0.08 NS	-0.22 NS	-0.07 NS	-0.06 NS	-0.09 NS
Quantitative NLRP3	-0.06 NS	0.11 NS	-0.01 NS	-0.07 NS	-0.09 NS	0.06 NS	-0.008 NS
NS: Non-Significant.							

3.5. Correlation coefficient between Immunity variables in this study.

This table (3-13) shows correlation coefficient between Immunity variables in this study. There was Non- significant difference between the (Quantitative IL-1b & Quantitative IL-18), (Quantitative IL-1b & *H. Pylori* IgG test), (Quantitative IL-1b & *H. Pylori* IgM Test), (Quantitative IL-18 & Quantitative NLRP3), (Quantitative IL-18 & *H. Pylori* IgG test) and (Quantitative IL-18& *H. Pylori* IgM Test). There was a high significant difference between (Quantitative IL-1b & Quantitative NLRP3) but There was significant difference between (Quantitative NLRP3 & *H. Pylori* IgG test) and (Quantitative NLRP3 & *H. Pylori* IgM Test).

Table (3-13): Correlation coefficient between Immunity variables in this study.

Parameters	Correlation
Quantitative IL-1b & Quantitative IL-18	-0.09 NS
Quantitative IL-1b & Quantitative NLRP3	0.84 **
Quantitative IL-1b & *H. Pylori* IgG test	0.15 NS
Quantitative IL-1b & *H. Pylori* IgM Test	0.09 NS
Quantitative IL-18& Quantitative NLRP3	-0.04 NS
Quantitative IL-18 & *H. Pylori* IgG test	-0.07 NS
Quantitative IL-18& *H. Pylori* IgM Test	-0.06 NS
Quantitative NLRP3 & *H. Pylori* IgG test	0.30 *
Quantitative NLRP3 & *H. Pylori* IgM Test	0.32 *
* ($P \leq 0.05$), ** ($P \leq 0.01$), NS: Non-Significant.	

Conclusions and Recommendations

Conclusions

1. There was a significant relation between Family history with DM and GDM.
2. There was a significant relation between physical activity and GDM.
3. The mean levels of FBS, OGTT, HbA1c, C- reactive protein and Lymphocyte count were significantly higher in patients with GDM as compared to controls.
4. The mean level of BMI and Age was significantly higher in GDM women as compared to controls.
5. The mean levels of NLRP3, IL-1b, and IL-18 were significantly higher in GDM women as compared to controls.
6. There was a positive relationship between GDM and *H. pylori* infection as that *H. pylori* infection can raise the risk of GDM.
7. There was a significant correlation between IL-1b and NLRP3.
8. There was a significant correlation between *H. Pylori* IgG and NLRP3.
9. There was a significant correlation between *H. Pylori* IgM and NLRP3.

Recommendations

1. More detailed studies are needed about the relationship between gestational diabetes mellitus (GDM) and *H. pylori* infections focusing on the most important bacterial factors that modulate the disease outcome.
2. Higher sample sized- metacentric similar studies are recommended.
3. The present study recommends a genetic prospect regarding both the immunological and the bacterial aspect.
4. Observing the relation between IL-1b, IL-18, NLRP3 and bacterial infection in GDM patients.
5. We suggest routine testing of all pregnant women for possible H. Pylori infection, and treatment of women who are complained from *h. pylori* infection, may contribute to prevention of GDM later in pregnancy.
6. There should be educational awareness programs for pregnant women about Importance of healthy diet and increase physical activity.

References

References

Agarwal, M. M. (2020). Gestational diabetes in the Arab gulf countries: Sitting on a land-mine. International journal of environmental research and public health, 17(24), 9270.

Agi, V. N., Ollor, O. A., Azike, C. A., & Naziga, D. B. (2022). The prevalence rate of helicobacter pylori amongst patients presenting with presumptive gastritis in Rivers State, Nigeria using antigen detection method. Journal of Advances in Microbiology, 22(7), 1-12.

Ahmed, A. A. Q., Besio, R., Xiao, L., & Forlino, A. (2023). Outer Membrane Vesicles (OMVs) as Biomedical Tools and Their Relevance as Immune-Modulating Agents against H. pylori Infections: Current Status and Future Prospects. International Journal of Molecular Sciences, 24(10), 8542.

Al Subhi, S. K., Al Kindi, R. M., Al Rawahi, A., Al Seyabi, I. S., & Al Mukhaini, A. (2021). Prevalence of gestational diabetes mellitus using the latest world health organization diagnostic criteria among Omani women in Muscat, Oman. Oman Medical Journal, 36(1), e215.

Aleman, R. S., Paz, D., Cedillos, R., Tabora, M., Olson, D. W., & Aryana, K. (2023). Attributes of Culture Bacteria as Influenced by Ingredients That Help Treat Leaky Gut. Microorganisms, 11(4), 893.

Alfadul, H., Sabico, S., & Al-Daghri, N. M. (2022). The role of interleukin-1β in type 2 diabetes mellitus: A systematic review and meta-analysis. Frontiers in Endocrinology, 1694.

Ali, A. I., & Nori, W. (2021). Gestational diabetes mellitus: A narrative review. Medical Journal of Babylon, 18(3), 163.

Al-Rifai, R. H., Abdo, N. M., Paulo, M. S., Saha, S., & Ahmed, L. A. (2021). Prevalence of gestational diabetes mellitus in the middle east and North Africa, 2000–2019: A Systematic Review, Meta-Analysis, and Meta-Regression. Frontiers in endocrinology, 12, 668447.

Al-Suhaimi, E. A., & Khan, F. A. (2022). The pituitary gland: functional relationship with the hypothalamus, structure, and physiology. In Emerging Concepts in Endocrine Structure and Functions (pp. 73-131). Singapore: Springer Nature Singapore.

Amirian, A., Rahnemaei, F. A., & Abdi, F. (2020). Role of C-reactive Protein (CRP) or high-sensitivity CRP in predicting gestational diabetes Mellitus: Systematic review. Diabetes & Metabolic Syndrome: Clinical Research & Reviews, 14(3), 229-236.

Ande, S. N., Khade, A. S., Bakal, R. L., & Mohale, D. S. (2022). Anatomical architecture and molecular study of skin immune system: A brief review.

Andreoli, L., Chighizola, C. B., Iaccarino, L., Botta, A., Gerosa, M., Ramoni, V., ... & Tincani, A. (2022). Immunology of pregnancy and reproductive health in autoimmune rheumatic diseases. Update from the 11th International Conference on Reproduction, Pregnancy and Rheumatic Diseases. Autoimmunity reviews, 103259.

Andrés, C. M. C., Pérez de la Lastra, J. M., Juan, C. A., Plou, F. J., & Pérez-Lebeña, E. (2022). The Role of Reactive Species on Innate Immunity. Vaccines, 10(10), 1735.

Auriti, C., De Rose, D. U., Santisi, A., Martini, L., Piersigilli, F., Bersani, I., ... & Caforio, L. (2021). Pregnancy and viral infections: Mechanisms of fetal damage, diagnosis and prevention of neonatal adverse outcomes from cytomegalovirus to SARS-CoV-2 and Zika virus. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 1867(10), 166198.

Azad, K. N., Realegeno, S. E., Kagan, R. M., Schwab, D. A., Batterman, H. J., & Marlowe, E. M. (2022). An Easily Digestible Review of Helicobacter pylori Diagnostics. Clinical Microbiology Newsletter, 44(6), 51-61.

Babović, I. R., Dotlić, J., Sparić, R., Jovandaric, M. Z., Andjić, M., Marjanović Cvjetićanin, M., & Plesinac, J. (2022). Gestational Diabetes Mellitus and Antenatal Corticosteroid Therapy—A Narrative Review of Fetal and Neonatal Outcomes. Journal of Clinical Medicine, 12(1), 323.

Barzegar, M., Nouri, H., Mirmosayyeb, O., Motedayyen, H., Nehzat, N., & Shaygannejad, V. (2023). Association Between Helicobacter Pylori Infection and Seronegative Neuromyelitis Optica Spectrum Disorder. Caspian Journal of Neurological Sciences, 9(1), 9-14.

Blander, J. M. (2023, March). Different routes of MHC-I delivery to phagosomes and their consequences to CD8 T cell immunity. In Seminars in Immunology (Vol. 66, p. 101713). Academic Press.

Brankica, K., Valentina, V. N., Slagjana, S. K., & Sasha, J. M. (2016). Maternal 75-g OGTT glucose levels as predictive factors for large-for-gestational age newborns in women with gestational diabetes mellitus. Archives of endocrinology and metabolism, 60(1), 36-41.

Burhans, M. S., Hagman, D. K., Kuzma, J. N., Schmidt, K. A., & Kratz, M. (2018). Contribution of adipose tissue inflammation to the development of type 2 diabetes mellitus. Comprehensive Physiology, 9(1), 1.

Burt, T. D., & McCune, J. M. (2023). Human fetal T cells: Insights into developmental specialization and mechanisms of lineage transition. Immunological Reviews.

Cade, T. J., Polyakov, A., & Brennecke, S. P. (2019). Implications of the introduction of new criteria for the diagnosis of gestational diabetes: a health outcome and cost of care analysis. BMJ open, 9(1), e023293.

Caielli, S., Wan, Z., & Pascual, V. (2023). Systemic lupus erythematosus pathogenesis: interferon and beyond. Annual Review of Immunology, 41, 533-560.

Calabrese, L., Fiocco, Z., Satoh, T. K., Peris, K., & French, L. E. (2022). Therapeutic potential of targeting interleukin-1 family cytokines in chronic inflammatory skin diseases. British Journal of Dermatology, 186(6), 925-941.

Capula, C., Mazza, T., Vero, R., & Costante, G. (2013). HbA1c levels in patients with gestational diabetes mellitus: Relationship with pre- pregnancy BMI and pregnancy outcome. Journal of endocrinological investigation, 36(11), 1038-1045.

Cardaropoli, S., Giuffrida, D., Piazzese, A., & Todros, T. (2015). Helicobacter pylori seropositivity and pregnancy-related diseases: a prospective cohort study. Journal of reproductive immunology, 109, 41- 47.

Chauhan, D., Vande Walle, L., & Lamkanfi, M. (2020). Therapeutic modulation of inflammasome pathways. Immunological reviews, 297(1), 123-138.

Chen, L. W., Soh, S. E., Tint, M. T., Loy, S. L., Yap, F., Tan, K. H., ..& Chan, S. Y. (2021). Combined analysis of gestational diabetes and maternal weight status from pre-pregnancy through post-delivery in future development of type 2 diabetes. Scientific Reports, 11(1), 5021.

Cho, H. Y.; Jung, I. and Kim, S. J. (2016). The association between maternal hyperglycemia and perinatal outcomes in gestational diabetes mellitus patients: A retrospective cohort study. Medicine, 95(36).

Chu, S. Y., Callaghan, W. M., Kim, S. Y., Schmid, C. H., Lau, J., England, L. J., & Dietz,P. M. (2007). Maternal obesity and risk of gestational diabetes mellitus. Diabetes care, 30(8), 2070-2076.

Cinkajzlová, A., Anderlová, K., Šimják, P., Lacinová, Z., Kloučková, J., Kratochvílová, H., ... & Haluzík, M. (2020). Subclinical inflammation and adipose tissue lymphocytes in pregnant females with gestational diabetes mellitus. The Journal of Clinical Endocrinology & Metabolism, 105(11), e3892-e3902.

Crocetti, E., Trama, A., Stiller, C., Caldarella, A., Soffietti, R., Jaal, J.,... & RARECARE Working Group. (2012). Epidemiology of glial and non-

glial brain tumours in Europe. European journal of cancer, 48(10), 1532-1542.

Darbandi, M., Rezaeian, S., Dianatinasab, M., Yaghoobi, H., Soltani, M., Etemad, K., ... & Saeidi, R. (2021). Prevalence of gestational diabetes and its association with stillbirth, preterm birth, macrosomia, abortion and cesarean delivery: a national prevalence study of 11 provinces in Iran. Journal of Preventive Medicine and Hygiene, 62(4), E885.

Das, P. K., Sahoo, A., & Dasu, V. V. (2022). Current status, and the developments of hosts and expression systems for the production of recombinant human cytokines. Biotechnology Advances, 107969.

Delong, L. and Burkhart, N. (2008). Endocrine disorders. General and oral pathology pp 147-178, Philadelphia : lippincott Williams & Wilkins.

Dinarello, C. A. (2019). The IL-1 family of cytokines and receptors in rheumatic diseases. Nature Reviews Rheumatology, 15(10), 612-632.

Ding, S., Xu, S., Ma, Y., Liu, G., Jang, H., & Fang, J. (2019). Modulatory mechanisms of the NLRP3 inflammasomes in diabetes. Biomolecules, 9(12), 850.

Dinsmoor, M. J., Ugwu, L. G., Bailit, J. L., Reddy, U. M., Wapner, R. J., Varner, M. W., ... & Eunice Kennedy Shriver National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. (2023). Short-term neonatal outcomes of pregnancies complicated by maternal obesity. American Journal of Obstetrics & Gynecology MFM, 5(4), 100874.

Dipla, K., Zafeiridis, A., Mintziori, G., Boutou, A. K., Goulis, D. G., & Hackney, A. C. (2021). Exercise as a therapeutic intervention in gestational diabetes mellitus. Endocrines, 2(2), 65-78.

Dunne, F., Brydon, P., Smith, K., & Gee, H. (2003). Pregnancy in women with type 2 diabetes: 12 years outcome data 1990–2002. Diabetic Medicine, 20(9), 734-738.

Edrees, W. H. (2023). Seroprevalence, Knowledge, and Preventative Practices of Hepatitis A Virus and Helicobacter pylori Infections among Orphaned Children in Sana'a City, Yemen.

ElSayed, N. A., Aleppo, G., Aroda, V. R., Bannuru, R. R., Brown, F. M., Bruemmer, D., ... & Gabbay, R. A. (2023). 15. Management of Diabetes in Pregnancy: Standards of Care in Diabetes—2023. Diabetes Care, 46(Supplement_1), S254-S266.

Erem, C., Kuzu, U. B., Deger, O., & Can, G. (2015). Prevalence of gestational diabetes mellitus and associated risk factors in Turkish women: the Trabzon GDM Study. Archives of medical science: AMS, 11(4), 724-735.

Esparvarinha, M., Madadi, S., Aslanian-Kalkhoran, L., Nickho, H., Dolati, S., Pia, H., ... & Yousefi, M. (2023). Dominant immune cells in pregnancy and pregnancy complications: T helper cells (TH1/TH2, TH17/Treg cells), NK cells, MDSCs, and the immune checkpoints. Cell Biology International, 47(3), 507-519.

Facchinetti, F., D'Anna, R., & Hod, M. (2023). Inositol supplementation for preventing gestational diabetes mellitus. In A Clinical Guide to Inositols (pp. 123-150). Academic Press.

Farahvar, S., Walfisch, A., & Sheiner, E. (2019). Gestational diabetes risk factors and long-term consequences for both mother and offspring: a literature review. Expert review of endocrinology & metabolism, 14(1), 63-74.

Fashami, M. A., Hajian, S., Afrakhteh, M., & Khoob, M. K. (2020). Is there an association between platelet and blood inflammatory indices and the risk of gestational diabetes mellitus?. Obstetrics & gynecology science, 63(2), 133-140.

Fatima, S. S., Alam, F., Chaudhry, B., & Khan, T. A. (2017). Elevated levels of chemerin, leptin, and interleukin-18 in gestational diabetes mellitus. The Journal of Maternal-Fetal & Neonatal Medicine, 30(9), 1023-1028.

Feeney, M. E. (2020). The immune response to malaria in utero. Immunological reviews, 293(1), 216-229.

Fiorani, M., Tohumcu, E., Del Vecchio, L. E., Porcari, S., Cammarota, G., Gasbarrini, A., & Ianiro, G. (2023). The Influence of Helicobacter pylori on Human Gastric and Gut Microbiota. Antibiotics, 12(4), 765.

Floerchinger, A., Klein J. E., Finkbeiner, M. S., Schäfer, T. E., Fuchs, G., Doerner, J., ... & Engeland, C. E. (2023). A vector-encoded bispecific killer engager to harness virus-activated NK cells as anti-tumor effectors. Cell Death & Disease, 14(2), 104.

Friedman, M. J., Lee, H., Lee, J. Y., & Oh, S. (2023). Transcriptional and epigenetic regulation of context-dependent plasticity in T-helper lineages. Immune Network, 23(1).

Gao, S., & Wang, J. (2023). Maternal and infant microbiome: next-generation indicators and targets for inter-generational health and nutrition care. Protein & Cell, pwad029.

Giordano, M. D. C. P. P. (2020). Treatment Options for MODY Patients: A Systematic Review of Literature.

Gogeneni, H. (2017). Porphyromonas gingivalis infection in gestational diabetes mellitus and survival in tobacco smokers.

Gomez-Lopez, N., Motomura, K., Miller, D., Garcia-Flores, V., Galaz, J., & Romero, R. (2019). Inflammasomes: their role in normal and complicated pregnancies. The Journal of Immunology, 203(11), 2757- 2769.

Guo, J., Zhou, M., Zhao, M., Li, S., Fang, Z., Li, A., & Zhang, M. (2023). TIGAR deficiency induces caspase-1-dependent trophoblasts pyroptosis through NLRP3-ASC inflammasome. Frontiers in Immunology, 14.

Guoju, X., Yanbin, H., Qiang, Z., Jing, W., & Ming, L. (2020). Impact of cultivation on soil organic carbon and carbon sequestration potential in semiarid regions of China. *Soil Use and Management*, *36*(1), 83-92.

Guruge, S., Thomson, M. S., George, U., & Chaze, F. (2015). Social support, social conflict, and immigrant women's mental health in a C anadian context: a scoping review. *Journal of psychiatric and mental health nursing*, *22*(9), 655-667.

Hai, L., & Hu, Z. D. (2020). The clinical utility of neutrophil to lymphocyte ratio in pregnancy related complications: a mini- review. Journal of Laboratory and Precision Medicine, 5(1), 1-9.

Hannan, F. M., Elajnaf, T., Vandenberg, L. N., Kennedy, S. H., & Thakker, R. V. (2023). Hormonal regulation of mammary gland development and lactation. Nature Reviews Endocrinology, 19(1), 46-61.

Harker, J. A., & Lloyd, C. M. (2023). T helper 2 cells in asthma. Journal of Experimental Medicine, 220(6), e20221094.

Hirooka, Y., & Nozaki, Y. (2021). Interleukin-18 in inflammatory kidney disease. Frontiers in medicine, 8, 639103.

Hong, J. G. S., Mohd. Noor, A. F., & Tan, P. C. (2022). Three Days Compared to One Day Per Week of Self-Monitoring of Blood Glucose in Mild Gestational Diabetes: A Randomized Trial. Journal of Clinical Medicine, 11(13), 3770.

Hope, O., Ifeanyi, O. E., & Braxton, A. Q. (2019). Investigation of some haematological parameters in pregnant women with gestational diabetes at Federal Medical Center, Owerri, Imo State, Nigeria. Annals of Clinical and Laboratory Research, 2, 305.

Ihim, S. A., Abubakar, S. D., Zian, Z., Sasaki, T., Saffarioun, M., Maleknia, S., & Azizi, G. (2022). Interleukin-18 cytokine in immunity, inflammation, and autoimmunity: Biological role in induction, regulation, and treatment. Frontiers in Immunology, 4470.

Iznardo, H., & Puig, L. (2021). The interleukin-1 family cytokines in psoriasis: Pathogenetic role and therapeutic perspectives. Expert Review of Clinical Immunology, 17(2), 187-199.

Jahromi, A. S., Shojaei, M., & Ghobadifar, M. A. (2014). Insulin resistance and serum levels of interleukin-17 and interleukin-18 in normal pregnancy. Immune network, 14(3), 149-155.

Jain, R., Olejas, S., Goo, L. S., Bhavatharinin, N., Dengra, A. S., Shoghli, R., ... & Jain, R. (2019). Review of FIGO & ADA, WHO, IADPSG Guidelines for GDM for Low Resource Setting and Integration of DIPSI with MOHFW Govt of India, Guidelines. International Journal of Diabetes and Endocrinology, 4(3), 73.

James, D. E., Stöckli, J., & Birnbaum, M. J. (2021). The aetiology and molecular landscape of insulin resistance. Nature Reviews Molecular Cell Biology, 22(11), 751-771.

Jarret, A., Jackson, R., Duizer, C., Healy, M. E., Zhao, J., Rone, J. M.,... & Flavell, R. A. (2020). Enteric nervous system-derived IL-18 orchestrates mucosal barrier immunity. Cell, 180(1), 50-63.

Johnson, C. Y., Rocheleau, C. M., Howley, M. M., Chiu, S. K., Arnold,K. E., Ailes, E. C., & National Birth Defects Prevention Study. (2021). Characteristics of women with urinary tract infection in pregnancy. Journal of Women's Health, 30(11), 1556-1564.

Kalkal, M., & Das, J. (2023). Current understanding of the immune potential of B cell subsets in malaria pathogenesis. Frontiers in Microbiology, 14, 10.

Kanungo, S. D., Safwath, S. A., Nabi, M. A. U., Dey, S., Choudhury, N. A., & Islam, M. U. (2022). The Diagnostic Usefulness of Stool Antigen Test with Serum Helicobacter pylori Antibody and CLO Test in the Diagnosis of Helicobacter pylori Infection in Dyspeptic Patients. Saudi J Pathol Microbiol, 7(6), 245-253.

Khalafallah, A., Phuah, E., Al-Barazan, A. M., Nikakis, I., Radford, A., Clarkson, W., ... & Corbould, A. (2016). Glycosylated haemoglobin for screening and diagnosis of gestational diabetes mellitus. BMJ open, 6(4), e011059.

Kim, S. H., Chelliah, R., Ramakrishnan, S. R., Perumal, A. S., Bang, W. S., Rubab, M., ... & Oh, D. H. (2021). Review on stress tolerance in Campylobacter jejuni. Frontiers in cellular and infection microbiology, 10, 596570.

Koh GC, Peacock SJ, van der Poll T, et al. The impact of diabetes on the pathogenesis of sepsis. Eur J Clin Microbiol Infect Dis 2012;31:379-88.Kubelkova, K., Bostik, V., Joshi, L., & Macela, A. (2023). Innate Immune Recognition, Integrated Stress Response, Infection, and Tumorigenesis. Biology, 12(4), 499.

Kubelkova, K., Bostik, V., Joshi, L., & Macela, A. (2023). Innate Immune Recognition, Integrated Stress Response, Infection, and Tumorigenesis. Biology, 12(4), 499.

Kuntzel, T., & Bagnard, D. (2022). Manipulating Macrophage/Microglia Polarization to Treat Glioblastoma or Multiple Sclerosis. Pharmaceutics, 14(2), 344.

Künzli, M., & Masopust, D. (2023). CD4+ T cell memory. Nature Immunology, 1-12.

Kushtagi, P. (2021). Gestational Diabetes 7. High Risk Pregnancy & Delivery, 52.

Kuzmicki M, Telejko B, Zonenberg A, Szamatowicz J, Kretowski A,Nikolajuk A, Laudanski P, Gorska M: Circulating pro- and anti-inflammatory cytokines in Polish women with gestational diabetes. Horm Metab Res (2008); 40:556–560.

Laltlanzovi, C., Choudhury, M., Singh, R., Sharma, S., Raghunandan, C., & Hrahsel, L. (2022). Study of Serum Adiponectin and Interleukin-1β Levels in Women with Gestational Diabetes. Indian Journal of Endocrinology and Metabolism.

Lappas, M. (2014). Activation of inflammasomes in adipose tissue of women with gestational diabetes. Molecular and cellular endocrinology, 382(1), 74-83.

Lasch, M., Sudan, K., Paul, C., Schulz, C., Kolben, T., Dorp, J. V., ... & Meister, S. (2022). Isolation of decidual macrophages and Hofbauer cells from term placenta—comparison of the expression of CD163 and CD80. International Journal of Molecular Sciences, 23(11), 6113.

Li, G., Wei, T., Ni, W., Zhang, A., Zhang, J., Xing, Y., & Xing, Q. (2020). Incidence and risk factors of gestational diabetes mellitus: a prospective cohort study in Qingdao, China. Frontiers in Endocrinology, 11, 636.

Liao, J. S. (2020). DETERMINING THE EFFECT OF ARSENIC ON THE IMMUNE SYSTEM DURING PREGNANCY AND INFLUENZA RISK (Doctoral dissertation, Johns Hopkins University).

Liong, S., & Lappas, M. (2015). Endoplasmic reticulum stress is increased in adipose tissue of women with gestational diabetes. PloS one, 10(4), e0122633.

Liyanagamage, D. S. N. K., & Martinus, R. D. (2020). Role of mitochondrial stress protein HSP60 in diabetes-induced neuroinflammation. Mediators of inflammation, 2020.

Maity, A., Bhattacharya, S., Mahato, A. C., Chaudhuri, S., & Pradhan, M. (2023). A pattern-recognition-based clustering method for non- invasive diagnosis and classification of various gastric conditions. European Journal of Mass Spectrometry, 14690667231174350.

Makaremi, S., Asgarzadeh, A., Kianfar, H., Mohammadnia, A., Asghariazar, V., & Safarzadeh, E. (2022). The role of IL-1 family of cytokines and receptors in pathogenesis of COVID-19. Inflammation Research, 71(7-8), 923-947.

Makoni, N. J., & Nichols, M. R. (2021). The intricate biophysical puzzle of caspase-1 activation. Archives of biochemistry and biophysics, 699, 108753.

Mantovani, A., Dinarello, C. A., Molgora, M., & Garlanda, C. (2019). Interleukin-1 and related cytokines in the regulation of inflammation and immunity. Immunity, 50(4), 778-795.

McElwain, C. J., McCarthy, F. P., & McCarthy, C. M. (2021). Gestational diabetes mellitus and maternal immune dysregulation: what we know so far. International Journal of Molecular Sciences, 22(8), 4261.

McIntyre, H. D., Catalano, P., Zhang, C., Desoye, G., Mathiesen, E. R., & Damm, P. (2019). Gestational diabetes mellitus. Nature reviews Disease primers, 5(1), 47.

McIntyre, H. D.; Gibbons, K. S.; Lowe, J. and Oats, J. J. (2018). Development of a risk engine relating maternal glycemia and body mass index to pregnancy outcomes. Diabetes research and clinical practice, 139, 331-338.

Melchior, H., Kurch-Bek, D., & Mund, M. (2017). The prevalence of gestational diabetes: a population-based analysis of a nationwide screening program. Deutsches Ärzteblatt International, 114(24), 412.

Melton, E., & Qiu, H. (2021). Interleukin-1β in Multifactorial Hypertension: Inflammation, Vascular Smooth Muscle Cell and Extracellular Matrix Remodeling, and Non-Coding RNA Regulation. International Journal of Molecular Sciences, 22(16), 8639.

Meng, W., Muscat, R. A., McKee, M. L., Milnes, P. J., El-Sagheer, A. H., Bath, J., ... & Turberfield, A. J. (2016). An autonomous molecular assembler for programmable chemical synthesis. *Nature Chemistry*, *8*(6), 542-548.

Mertoglu, C., Gunay, M., Gungor, M., Kulhan, M., & Kulhan, N. G. (2019). A study of inflammatory markers in gestational diabetes mellitus. Gynecology obstetrics & reproductive medicine, 25(1), 7-11.

Milardi, G., & Lleo, A. (2023). Tumor-Infiltrating B Lymphocytes: Promising Immunotherapeutic Targets for Primary Liver Cancer Treatment. Cancers, 15(7), 2182.

Mouton, A. J., Li, X., Hall, M. E., & Hall, J. E. (2020). Obesity, hypertension, and cardiac dysfunction: novel roles of immunometabolism in macrophage activation and inflammation. Circulation research, 126(6), 789-806.

Muchamuel, T., Fan, R. A., Anderl, J. L., Bomba, D. J., Johnson, H.W., Lowe, E., ... & Kirk, C. J. (2023). Zetomipzomib (KZR-616) attenuates lupus in mice via modulation of innate and adaptive immune responses. Frontiers in Immunology, 14.

Mulligan, N., Jiajia, W. A. N. G., CAPPARELLI, E. V., Alice, S. T. E. K., Emily, B. A. R. R., BUSCHUR, S. L., ... & MIROCHNICK, M. (2018). Dolutegravir pharmacokinetics in pregnant and postpartum women living with HIV. *AIDS (London, England)*, *32*(6), 729.

Mur-Artal, R., Montiel, J. M. M., & Tardos, J. D. (2015). ORB-SLAM: a versatile and accurate monocular SLAM system. *IEEE transactions on robotics*, *31*(5), 1147-1163.

Musa, E. (2022). Role of obesity and gestational diabetes mellitus status on the expression of kisspeptin, inflammatory markers and other endocrine signals, and their correlation with foetal outcomes and placental structure.

Nabavi-Rad, A., Yadegar, A., Sadeghi, A., Aghdaei, H. A., Zali, M. R., Klionsky, D. J., & Yamaoka, Y. (2023). The interaction between

autophagy, Helicobacter pylori, and gut microbiota in gastric carcinogenesis. Trends in Microbiology.

Nair, S., Ormazabal, V., Lappas, M., McIntyre, H. D., & Salomon, C. (2021). Extracellular vesicles and their potential role inducing changes in maternal insulin sensitivity during gestational diabetes mellitus. American Journal of Reproductive Immunology, 85(2), e13361.

Naish, E., Wood, A. J., Stewart, A. P., Routledge, M., Morris, A. C., Chilvers, E. R., & Lodge, K. M. (2022). The formation and function of the neutrophil phagosome. Immunological Reviews.

Naser, I. A., Shaat, M. R., Taleb, M. H., & Najim, A. A. (2022). Nutritional assessment of birth outcomes of gestational diabetic mothers in Gaza Strip, Palestine: A retrospective case-control study. International Journal of Academic Medicine, 8(4), 205.

Nguyen-Ngo, C., Willcox, J. C., & Lappas, M. (2019). Anti-diabetic, anti-inflammatory, and anti-oxidant effects of naringenin in an in vitro human model and an in vivo murine model of gestational diabetes mellitus. Molecular nutrition & food research, 63(19), 1900224.

Nigatu, B., Workneh, T., Mekuria, T., Yifter, H., Mamuye, Y., & Gize, A. (2022). Prevalence of Gestational Diabetes Mellitus among pregnant women attending antenatal care clinic of St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia. Clinical diabetes and endocrinology, 8(1), 1-6.

Niu, Q., Zhu, J., Yu, X., Feng, T., Ji, H., Li, Y., ... & Hu, B. (2020). Immune response in H. pylori-associated gastritis and gastric cancer. Gastroenterology Research and Practice, 2020.

Olmos-Ortiz, A., Flores-Espinosa, P., Díaz, L., Velázquez, P., Ramírez-Isarraraz, C., & Zaga-Clavellina, V. (2021). Immunoendocrine dysregulation during gestational diabetes mellitus: the central role of the placenta. International Journal of Molecular Sciences, 22(15), 8087.

Omoto, Y., Yamanaka, K., Tokime, K., Kitano, S., Kakeda, M., Akeda, T., ... & Mizutani, H. (2010). Granzyme B is a novel interleukin-18 converting enzyme. Journal of dermatological science, 59(2), 129-135.

Pachathundikandi, S. K., Tegtmeyer, N., & Backert, S. (2023). Masking of typical TLR4 and TLR5 ligands modulates inflammation and resolution by Helicobacter pylori. Trends in Microbiology.

Pandey, V. K., Tripathi, A., Srivastava, S., Pandey, S., Dar, A. H., Singh, R., ... & Mukarram, S. A. (2023). A Systematic Review on Immunity Functionalities and Nutritional Food Recommendations to Develop Immunity against Viral Infection. Applied Food Research, 100291.

Parrettini, S., Caroli, A., & Torlone, E. (2020). Nutrition and metabolic adaptations in physiological and complicated pregnancy: focus on obesity and gestational diabetes. Frontiers in Endocrinology, 11, 611929.

Petersen, M. C., & Shulman, G. I. (2018). Mechanisms of insulin action and insulin resistance. Physiological reviews, 98(4), 2133-2223.

Peyvandi, S., & Rollins, C. (2023). Fetal brain development in congenital heart disease. Canadian Journal of Cardiology, 39(2), 115-122.

Plows, J. F., Stanley, J. L., Baker, P. N., Reynolds, C. M., & Vickers, M. H. (2018). The pathophysiology of gestational diabetes mellitus. International journal of molecular sciences, 19(11), 3342.

Preda, A., Ștefan, A. G., Preda, S. D., Comănescu, A. C., Forțofoiu, M. C., Vladu, M. I., ... & Moța, M. (2022). Transient Polyhydramnios during Pregnancy Complicated with Gestational Diabetes Mellitus: Case Report and Systematic Review. Diagnostics, 12(6), 1340.

Preissner, K. T., & Fischer, S. (2022). Functions and cellular signaling by ribosomal extracellular RNA (rexRNA): Facts and hypotheses on a non-typical DAMP. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research, 119408.

Ptasiewicz, M., Grywalska, E., Mertowska, P., Korona-Głowniak, I., Poniewierska-Baran, A., Niedźwiedzka-Rystwej, P., & Chałas, R. (2022). Armed to the teeth—the oral mucosa immunity system and microbiota. International Journal of Molecular Sciences, 23(2), 882.

Rajput, R., Rajput, M., & Nanda, S. (2012). Utility of HbA1c for diagnosis of gestational diabetes mellitus. Diabetes research and clinical practice, 98(1), 104-107.

Rasouli-Saravani, A., Jahankhani, K., Moradi, S., Gorgani, M., Shafaghat, Z., Mirsanei, Z., ... & Mirzaei, R. (2023). Role of microbiota short-chain fatty acid chains in the pathogenesis of autoimmune diseases. Biomedicine & Pharmacotherapy, 162, 114620.

Roquilly, A., Mintern, J. D., & Villadangos, J. A. (2022). Spatiotemporal adaptations of macrophage and dendritic cell development and function. Annual Review of Immunology, 40, 525-557.

Saedi, S., Watson, S. E., Young, J. L., Tan, Y., Wintergerst, K. A., & Cai, L. (2023). Does maternal low-dose cadmium exposure increase the risk of offspring to develop metabolic syndrome and/or type 2 diabetes?. Life Sciences, 121385.

Salazar-Petres, E. R., & Sferruzzi-Perri, A. N. (2022). Pregnancy-induced changes in β-cell function: what are the key players?. The Journal of Physiology, 600(5), 1089-1117.

SAS. 2018. Statistical Analysis System, User's Guide. Statistical. Version 9.6th ed. SAS. Inst. Inc. Cary. N.C. USA.

Sferruzzi-Perri, A. N., Lopez-Tello, J., Napso, T., & Yong, H. E. (2020). Exploring the causes and consequences of maternal metabolic maladaptations during pregnancy: Lessons from animal models. Placenta, 98, 43-51.

Shah, A., Stotland, N. E., Cheng, Y. W., Ramos, G. A., & Caughey, A.B. (2011). The association between body mass index and gestational diabetes mellitus varies by race/ethnicity. American journal of perinatology, 28(7), 515.

SHAHABDEEN, A., ROMEO, K., MODI, T., KUMAR, S., HAJONG, S. Y., & PHUSAM, M. Q. (2023). Helicobacter pylori Infection in Patients with Non Alcoholic Fatty Liver Disease: A Cross-sectional Study. Journal of Clinical & Diagnostic Research, 17(2).

Shahcheraghi, S. H., Salemi, F., Small, S., Syed, S., Salari, F., Alam, W., ... & Khan, H. (2023). Resveratrol regulates inflammation and improves oxidative stress via Nrf2 signaling pathway: Therapeutic and biotechnological prospects. Phytotherapy Research.

Sharma, B. R., & Kanneganti, T. D. (2021). NLRP3 inflammasome in cancer and metabolic diseases. Nature immunology, 22(5), 550-559.

Sharma, S., Banerjee, S., Krueger, P. M., & Blois, S. M. (2022). Immunobiology of gestational diabetes mellitus in post-medawar era. Frontiers in Immunology, 12, 5575.

Shen, D., Lu, Y., Li, G., Hu, M., Li, S., Ju, H., ... & Wang, X. (2021). Mechanism of neutrophil extracellular traps generation and their role in trophoblasts apoptosis in gestational diabetes mellitus. Cellular Signalling, 88, 110168.

Shui, Y., Hu, X., Hirano, H., Tsukamoto, H., Guo, W. Z., Hasumi, K.,... & Li, X. K. (2023). Combined phospholipids adjuvant augments anti- tumor immune responses through activated tumor-associated dendritic cells. Neoplasia, 39, 100893.

Sies, H., Belousov, V. V., Chandel, N. S., Davies, M. J., Jones, D. P., Mann, G. E., ... & Winterbourn, C. (2022). Defining roles of specific reactive oxygen species (ROS) in cell biology and physiology. Nature Reviews Molecular Cell Biology, 23(7), 499-515.

Simpson S, Smith L and Bowe J (2018): Placental peptides regulating islet adaptation to pregnancy: Clinical potential in gestational diabetes mellitus. Curr Opin Pharmacol 43: 59-65, 2018.

Sirico, A., Dell'Aquila, M., Tartaglione, L., Moresi, S., Farì, G., Pitocco, D., ... & Lanzone, A. (2021). PTH-rP and PTH-R1 expression in placentas from pregnancies complicated by gestational diabetes: New insights into the pathophysiology of hyperglycemia in pregnancy. Diagnostics, 11(8), 1356.

Sisay M, Edessa D, Ali T, Mekuria AN, Gebrie A (2020) The relationship between advanced glycation end products and gestational diabetes: A systematic review and meta-analysis. PLoS ONE 15(10): e0240382.

Sterling, D., & McClure, J. (2023). Protective Mechanisms of the Body. Anaesthesia & Intensive Care Medicine.

Stern, C., Schwarz, S., Moser, G., Cvitic, S., Jantscher-Krenn, E., Gauster, M., & Hiden, U. (2021). Placental endocrine activity: adaptation and disruption of maternal glucose metabolism in pregnancy and the influence of fetal sex. International Journal of Molecular Sciences, 22(23), 12722.

Sun, T., Meng, F., Zhao, H., Yang, M., Zhang, R., Yu, Z., ... & Zang, S. (2020). Elevated first-trimester neutrophil count is closely associated with the development of maternal gestational diabetes mellitus and adverse pregnancy outcomes. Diabetes, 69(7), 1401-1410.

Szlapinski, S. K., & Hill, D. J. (2021). Metabolic adaptations to pregnancy in healthy and gestational diabetic pregnancies: The pancreas- placenta axis. Current vascular pharmacology, 19(2), 141-153.

Taher, A. T., Weatherall, D. J., & Cappellini, M. D. (2018). Thalassaemia. *The Lancet*, *391*(10116), 155-167.

Tanase, D. M., Valasciuc, E., Gosav, E. M., Ouatu, A., Buliga-Finis, O. N., Floria, M., ... & Serban, I. L. (2023). Portrayal of NLRP3 Inflammasome in Atherosclerosis: Current Knowledge and Therapeutic Targets. International Journal of Molecular Sciences, 24(9), 8162.

Tang, Y., Yang, Y., & Lv, Z. (2021). Adverse pregnancy outcomes and Helicobacter pylori infection: A meta-analysis. International Journal of Clinical Practice, 75(10), e14588.

Tartey, S., & Kanneganti, T. D. (2019). Differential role of the NLRP 3 inflammasome in infection and tumorigenesis. Immunology, 156(4), 329-338.

Tomedi, L. E., Chang, C. C. H., Newby, P. K., Evans, R. W., Luther, J. F., Wisner, K. L., & Bodnar, L. M. (2013). Pre-pregnancy obesity and maternal nutritional biomarker status during pregnancy: a factor analysis. *Public health nutrition*, *16*(8), 1414-1418.

Tönnies, T., Rathmann, W., Hoyer, A., Brinks, R., & Kuss, O. (2021). Quantifying the underestimation of projected global diabetes prevalence by the International Diabetes Federation (IDF) Diabetes Atlas. *BMJ Open Diabetes Research and Care*, *9*(1), e002122.

Trøseid, M., Seljeflot, I., & Arnesen, H. (2010). The role of interleukin- 18 in the metabolic syndrome. Cardiovascular diabetology, 9(1), 1-8.

Tsai K, Tullis B, Jensen T, Graff T, Reynolds P and Arroyo J (2021): Differential expression of mTOR related molecules in the placenta from gestational diabetes mellitus (GDM), intrauterine growth restriction (IUGR) and preeclampsia patients. Reprod Biol 21: 100503, 2021.

Urbanová, J., Brunerová, L., Nunes, M., & Brož, J. (2020). Identification of MODY among patients screened for gestational diabetes: a clinician's guide. Archives of Gynecology and Obstetrics, 302, 305-314.

Vitoratos, N., Valsamakis, G., Mastorakos, G., Boutsiadis, A., Salakos, N., Kouskouni, E., & Creatsas, G. (2008). Pre-and early post-partum adiponectin and interleukin-1beta levels in women with and without gestational diabetes. Hormones, 7(3), 230-236.

Wang, X., Wang, L., Wen, X., Zhang, L., Jiang, X., & He, G. (2023). Interleukin-18 and IL-18BP in inflammatory dermatological diseases. Frontiers in Immunology, 14.

Wu, W., Tan, Q. Y., Xi, F. F., Ruan, Y., Wang, J., Luo, Q.,& Hu, T. X.(2022). NLRP3 inflammasome activation in gestational diabetes mellitus placentas is associated with hydrogen sulfide synthetase deficiency. Experimental and Therapeutic Medicine, 23(1), 1-8.

Xia, B., Wang, W., Lu, Y., & Chen, C. (2020). Helicobacter pylori infection increases the risk of metabolic syndrome in pregnancy: a cohort study. Annals of Translational Medicine, 8(14).

Xie, J., Cools, L., Van Imschoot, G., Van Wonterghem, E., Pauwels, M. J., Vlaeminck, I., ... & Vandenbroucke, R. E. (2023). Helicobacter pylori-derived outer membrane vesicles contribute to Alzheimer's disease pathogenesis via C3-C3aR signalling. Journal of Extracellular Vesicles, 12(2), 12306.

Xu, P., Dong, S., Wu, L., Bai, Y., Bi, X., Li, Y., & Shu, C. (2022). Maternal and Placental DNA Methylation Changes Associated with the Pathogenesis of Gestational Diabetes Mellitus. Nutrients, 15(1), 70.

Yahaya, T. O.; Salisu, T.; Abdulrahman, Y. B. and Umar, A. K. (2020). Update on the genetic and epigenetic etiology of gestational diabetes mellitus: a review. Egyptian Journal of Medical Human Genetics, 21(1), 1-13.

Yasuda, K., Nakanishi, K., & Tsutsui, H. (2019). Interleukin-18 in health and disease. International journal of molecular sciences, 20(3), 649.

Yin, Y., Pan, Y., He, J., Zhong, H., Wu, Y., Ji, C., ... & Cui, X. (2022). The mitochondrial-derived peptide MOTS-c relieves hyperglycemia and insulin resistance in gestational diabetes mellitus. Pharmacological research, 175, 105987.

Yisak, H., Belete, D., & Mahtsentu, Y. (2022). Helicobacter pylori infection and related factors among pregnant women at Debre Tabor General Hospital, Northwest Ethiopia, 2021: Anemia highly related with H. pylori. Women's Health, 18, 17455057221092266.

Yong, H. Y., Mohd Shariff, Z., Mohd Yusof, B. N., Rejali, Z., Tee, Y.Y. S., Bindels, J., & van Der Beek, E. M. (2020). Independent and combined effects of age, body mass index and gestational weight gain on the risk of gestational diabetes mellitus. Scientific Reports, 10(1), 8486.

Zainedeen, O., Al Haffar, I., Kochaji, N., & Wassouf, G. (2018). The efficacy of ultrasonography in monitoring the healing of jaw lesions. *Imaging science in dentistry*, *48*(3), 153-160.

Zanzal Ra'ad Al-dorri, A., Ibraheem Salih, N., & Saleh Khuder, H. (2022). Serological Detection of Helicobacter pylori Infection in Pregnant Women Related to ABO Blood Group. Archives of Razi Institute, 77(2), 591-597.

Zarezadeh Mehrabadi, A., Aghamohamadi, N., Khoshmirsafa, M., Aghamajidi, A., Pilehforoshha, M., Massoumi, R., & Falak, R. (2022). The roles of interleukin-1 receptor accessory protein in certain inflammatory conditions. Immunology, 166(1), 38-46.

Zawiejska, A.; Wender-Ozegowska, E.; Radzicka, S.; and Brazert, J. (2014). Maternal hyperglycemia according to IADPSG criteria as a predictor of perinatal complications in women with gestational diabetes: a retrospective observational study. The Journal of Maternal-Fetal & Neonatal Medicine, 27(15), 1526-1530.

Zhang, R., Zhang, X., Xing, B., Zhao, J., Zhang, P., Shi, D., & Yang, F. (2019). Astragaloside IV attenuates gestational diabetes mellitus via targeting NLRP3 inflammasome in genetic mice. Reproductive Biology and Endocrinology, 17, 1-8.

Zhao, X., Liu, J., Shen, L., Wang, A., & Wang, R. (2018). Correlation between inflammatory markers (hs-CRP, TNF-α, IL-1β, IL-6, IL-18), glucose intolerance, and gestational diabetes mellitus in pregnant women. Int J Clin Exp Med, 11(8), 8310-8316.

Zhou, F., Li, C., & Zhang, S. Y. (2021). NLRP3 inflammasome: a new therapeutic target for high-risk reproductive disorders?. Chinese medical journal, 134(01), 20-27.

Zhuang, Y., Zhang, J., Li, Y., Gu, H., Zhao, J., Sun, Y., ... & Xu, X. (2019). B lymphocytes are predictors of insulin resistance in women with gestational diabetes mellitus. Endocrine, Metabolic & Immune Disorders-Drug Targets (Formerly Current Drug Targets-Immune, Endocrine & Metabolic Disorders), 19(3), 358-366.

Zieleniak, A., Zurawska-Klis, M., Cypryk, K., Wozniak, L., & Wojcik,M. (2022). Transcriptomic Dysregulation of Inflammation-Related Genes in Leukocytes of Patients with Gestational Diabetes Mellitus (GDM) during and after Pregnancy: Identifying Potential Biomarkers Relevant to Glycemic Abnormality. International Journal of Molecular Sciences, 23(23), 14677.

Appendix

Appendix

Appendix (1): Approval of Ministry of health (MOH) for sample collection.

وزارة التعليم العالي والبحث العلمي
Ministry of Higher Education and Scientific Research
جمهورية العراق
جامعة بابل
كلية الطب
University of Babylon
College of Medicine
قسم الشؤون العلمية والدراسات العليا

العدد :
التاريخ:

الى/ دائرة صحة بابل

م/تسهيل مهمة

تحية طيبة:

يرجى تفضلكم بالموافقة على تسهيل مهمة طالبة الدراسات العليا **هاجر داود سلمان** / **ماجستير/ احياء مجهرية طبية** في كليتنا لغرض جمع العينات و اكمال متطلبات رسالة الماجستير.

مع التقدير

أ. م. د اشرف محمد علي حسين
م. العميد للشؤون العلمية
٢٠٢٢/١٢/٦

تسهيل المهمة والتعاون مع الاحترام
السيد/ة
احمد محسن علي
Ahmed M. Ali

نسخة منه الى//
م. المحاويل العام لنفس الغرض اعلاه مع التقدير
م. مرجان التعليمي لنفس الغرض اعلاه مع التقدير
م. الحلة التعليمي لنفس الغرض اعلاه مع التقدير
م. الامام الصادق التعليمي لنفس الغرض اعلاه مع التقدير
مركز صحي المحاويل لنفس الغرض اعلاه مع التقدير
مركز صحي حي الامام لنفس الغرض اعلاه مع التقدير
مركز صحي الشاوي لنفس الغرض اعلاه مع التقدير
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Appendix (2): Questionnaire sheet sample for (Case_ control) in this study.

اختي المواطنة ارجو مساعدتنا في اتمام هذه الدراسه والتي تهدف الى معرفه العلاقه بين سكري ألحمل وجرثومه ألمعده لدى الحوامل المصابات بسكري ألحمل (في كلية الطب – قسم الاحياء المجهرية)

أسم المريض ---------- رقم ألعينه ---------- ألعمر ----------

الطول ---------- الوزن ---------- مؤشر كتلة الجسم ----------

١- هل يوجد اقرباء من الدرجة الاولى مصابين بلسكر ؟	نعم ----	لا ------
٢- هل يوجد اقرباء من الدرجه الاولى مصابين بسكري الحمل؟	نعم ----	لا ------
٣- هل كان هناك اصابه سابقه بسكري الحمل ؟	نعم ----	لا ------
٤- النشاط البدني ؟	نعم ----	لا ------
٤- هل تعاني من الاصابه بجرثومه المعدة ؟	نعم ----	لا ------
٥- هل تعاني من امراض أخرى ؟	نعم ----	لا ------
٦- هل يتم مراقبة نسبة الكلوكوز في الدم بأنتظام ؟	نعم ----	لا ------

أنا موافقه على تعبئه هذا الاستبيان للمساعدة في اتمام الدراسه لديكم

التوقيع ----------

التاريخ ----------

Appendix (3): Comparison between patients and control groups in Age.

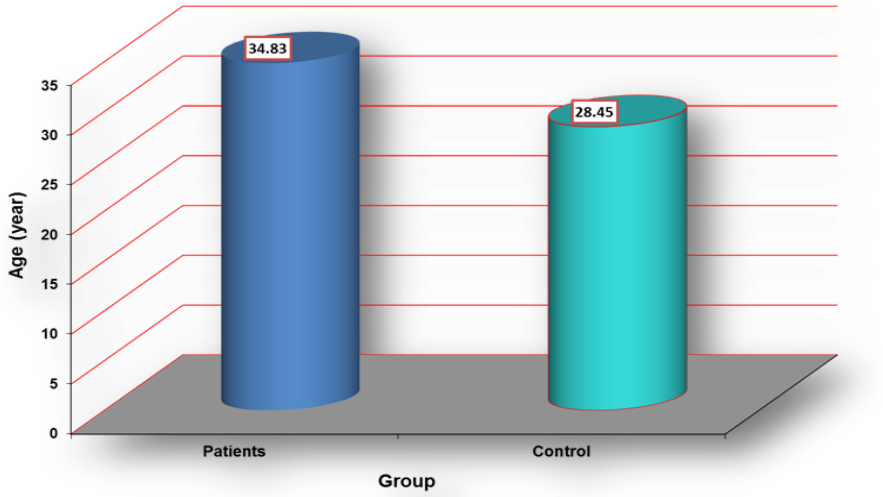

Figure 1. Comparison between patients and control groups in Age

Appendix (4): Comparison between patients and control groups in BMI.

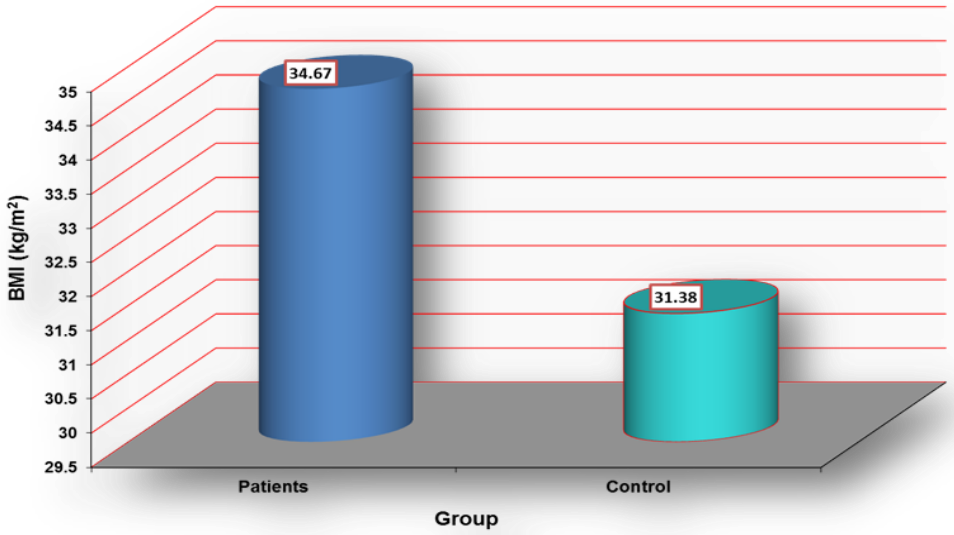

Figure 2. Comparison between patients and control groups in BMI

Appendix (5): Comparison between patients and control groups in FBS.

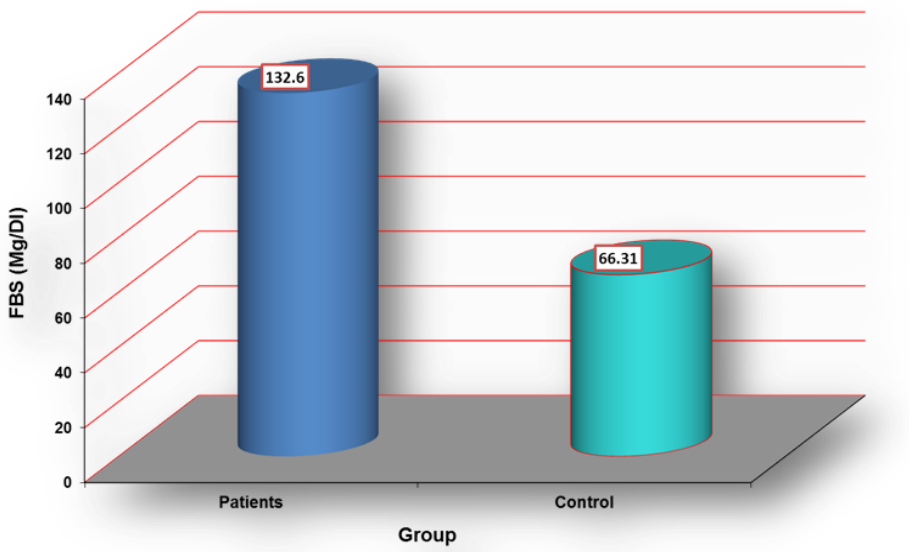

Figure 3. Comparison between patients and control groups in FBS

Appendix (6): Comparison between patients and control groups in OGTT.

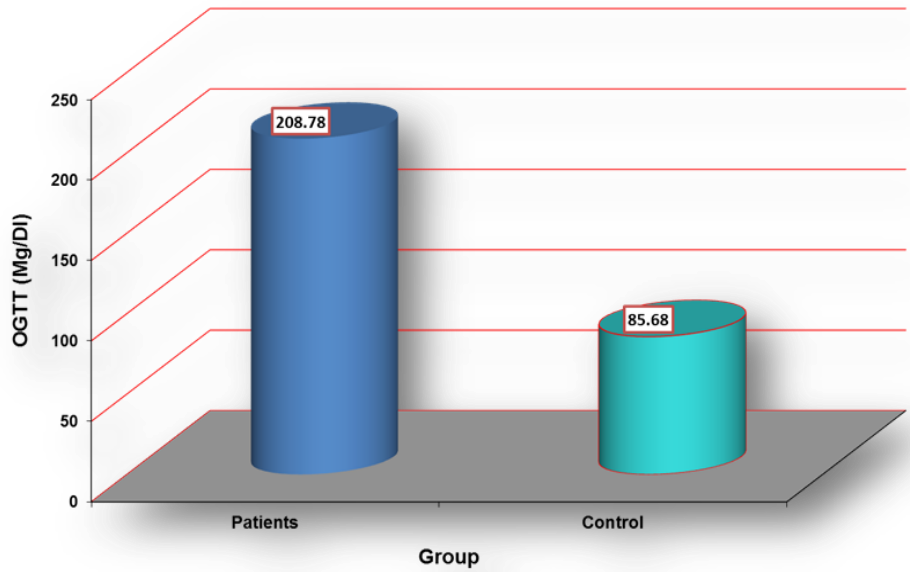

Figure 4. Comparison between patients and control groups in OGTT

Appendix (7): Comparison between patients and control groups in HbA1c.

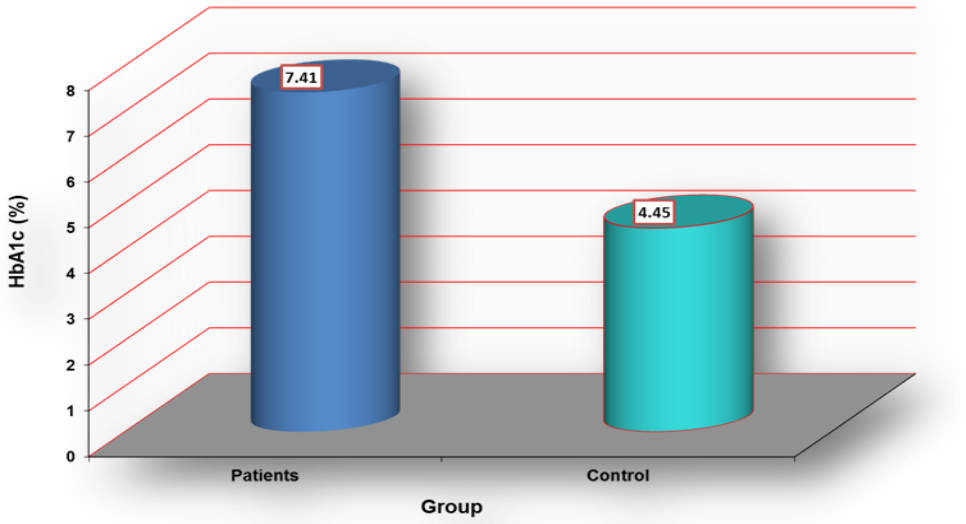

Figure 5. Comparison between patients and control groups in HbA1c

Appendix (8): Comparison between patients and control groups in lymphocyte.

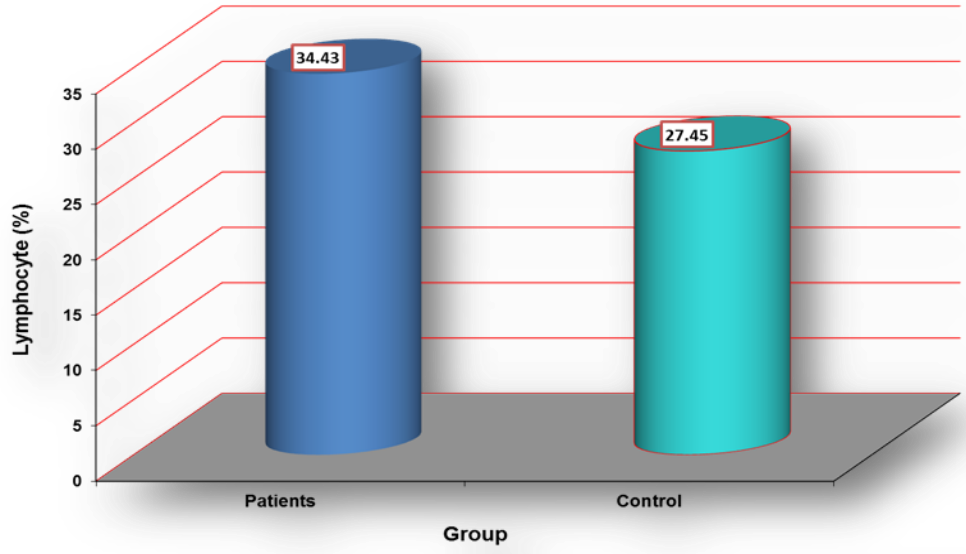

Figure 6. Comparison between patients and control groups in Lymphocyte (%)

Appendix (9): Comparison between patients and control groups in Neutrophil.

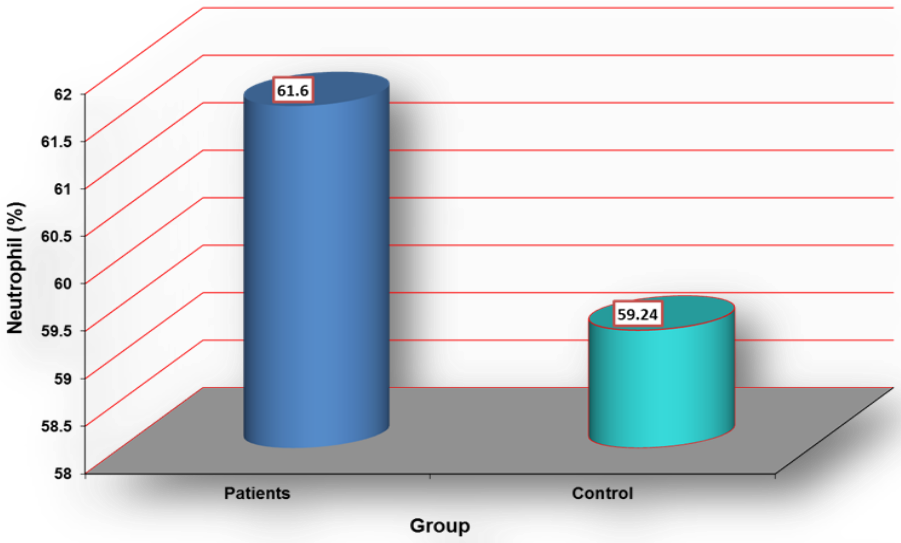

Figure 7. Comparison between patients and control groups in Neutrophil (%)

Appendix (10): Comparison between patients and control groups in IL-1b.

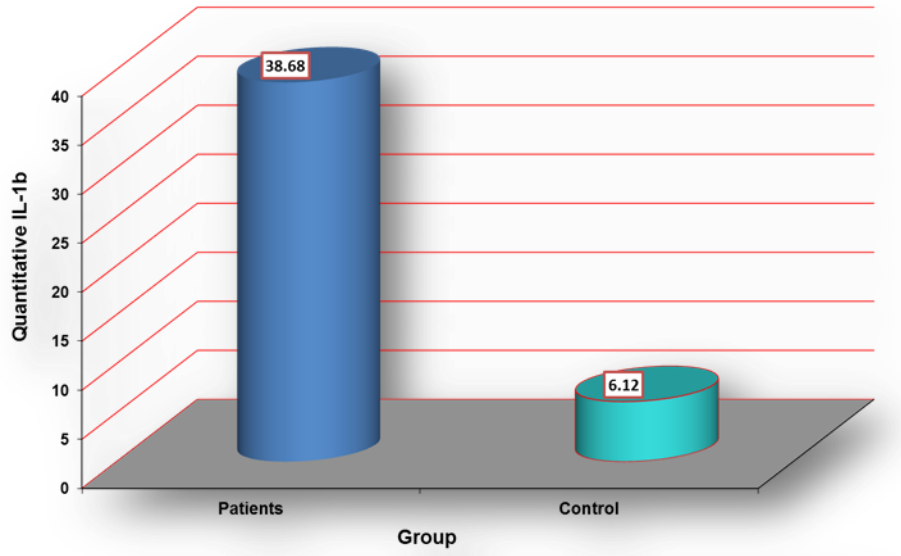

Figure 8. Comparison between patients and control groups in Quantitative IL-1b

Appendix (11): Comparison between patients and control groups in IL-18.

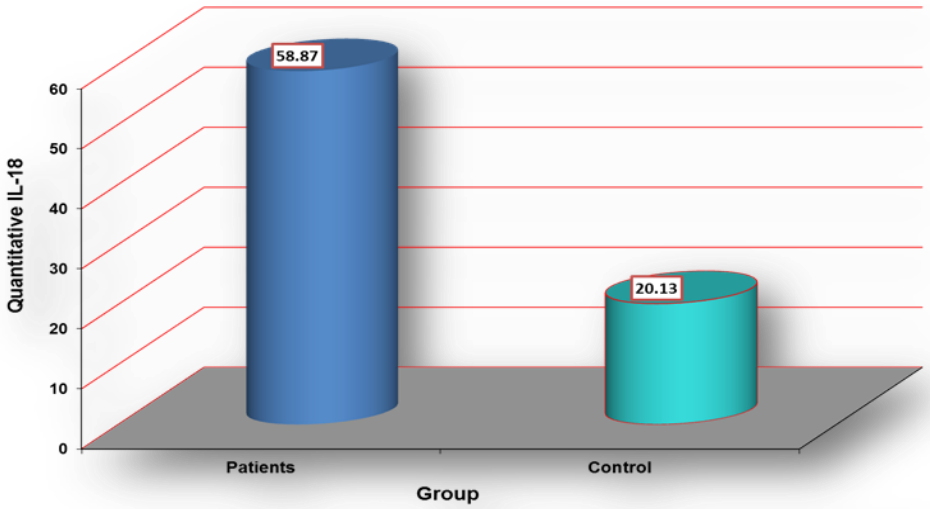

Figure 9. Comparison between patients and control groups in Quantitative IL-18

Appendix (12): Comparison between patients and control groups in NLRP3.

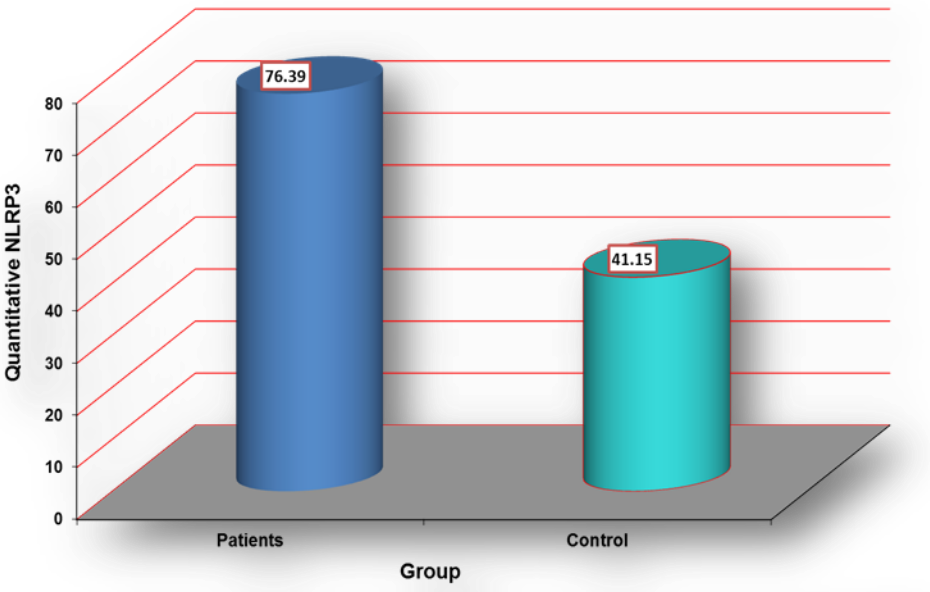

Figure 10. Comparison between patients and control groups in Quantitative NLRP3

الخلاصة

يظهر سكري الحمل عادة في النصف الأخير من الحمل ويتم تحديده من خلال عدم تحمل الكربوهيدرات متغيرة الشدة. يحدث ارتفاع السكر في الدم أثناء الحمل بسبب إفراز هرمونات المشيمة التي تسبب مقاومة الأنسولين. جرثومة ألمعدة هي نوع شائع من البكتيريا التي تنمو في الجهاز الهضمي. يؤدي خلل التنظيم المناعي للأم أثناء سكري الحمل إلى الإصابة ب *h. pylori*.

تم تسجيل تسعة وثمانين فردا في هذه الدراسة (الحالات المرضية والاصحاء). تم جمع عينات الدم الكاملة من تسعة وعشرين امرأة حامل على ما يبدو خاضعات للرقابة بينما تم جمع عينات دم كاملة من ستين مريضة مصابة بسكري الحمل (GDM) الذين حضروا إلى العيادة الخارجية لقسم أمراض النساء في مستشفى المحاويل / بابل / العراق ومستشفى الامام الصادق / بابل / العراق وبعض العيادات الخاصة خلال فترة امتدت من آب 2022 الى كانون الاول 2022. تم تشخيص المرضى من قبل استشاري امراض النساء و فحصهم بعدة فحوصات مثل (FBS، OGTT، HbA1c، CBC و CRP). تم تقسيم عينات الدم الوريدي إلى قسمين ؛ أحدهما للاختبارات الكيميائية والآخر للاختبارات المناعية [اختبارات IgG و IgM *Helicobacter pylori* , IL-18 ,IL-1ß ,NLRP3] ,

وقد توصلت الدراسة إلى النتائج التالية: - أن الفئة العمرية لمرضى GDM تراوحت بين (25-47) سنة. لذلك اخترنا أن الفئة العمرية للاصحاء كانت (23-45) سنة. كان متوسط عمر المرضى (34.83 ± 0.80 سنة) وكان الاصحاء (28.45 ± 1.16 سنة) و هناك فرق كبير ($P\leq0.01$) بين المرضى والاصحاء. كان مؤشر كتلة الجسم للمرضى (22-41) كجم/ م^2 لذلك كان الاصحاء (20-40) كجم/ م^2 ، ومتوسط مؤشر كتلة الجسم للمرضى 34.67 ± 0.70 والاصحاء(31.38 ± 1.13) هناك فرق معنوي بين المرضى و الاصحاء ($P\leq0.05$). كان متوسط FBS للمرضى الذين يعانون من GDM (3.52 ± 132.60 مجم/ ديسيلتر) والاصحاء (66.31 ± 1.86 مجم / ديسيلتر) و هناك فرق كبير بين المرضى والاصحاء ($P\leq0.01$). كان متوسط OGTT للمرضى (208.78 ± 5.96 مجم / ديسيلتر) وكان الاصحاء (85.68 ± 1.26 مجم / ديسيلتر) ، هناك فرق معنوي ($P\leq0.01$) بين المرضى والاصحاء . كان HbA1c للمرضى (7.41 ± 0.06٪) والاصحاء (4.45 ± 0.08٪) ، وهناك فرق كبير بين المرضى والاصحاء ($P\leq0.01$). كانت الخلايا الليمفاوية للمرضى (34.43 ± 1.09٪) والاصحاء

(27.45 ± 1.15٪) ، هناك فرق كبير بين المرضى والاصحاء (P≤0.01). كانت العدلات لدى المرضى (61.60 ± 1.15)٪ والاصحاء (59.24 ± 0.83)٪ و هناك فرق غير معنوي بين المرضى والاصحاء (0.182). في الدراسة الحالية كان هناك فرق معنوي بين المرضى والاصحاء (P≤0.05) مع بروتين سي التفاعلي. كان هناك فرق معنوي بين المرضى والاصحاء (P≤0.05) في النشاط البدني بين المرضى والاصحاء. يظهر التاريخ العائلي لسكري الحمل فرقًا كبيرًا بين المرضى ومجموعات المراقبة (P≤0.05). يظهر أيضًا التاريخ العائلي للداء السكري بين مرضى GDM والسيطرة فرقًا كبيرًا بين المرضى والاصحاء (P≤0.05). كان متوسط IL-1beta للمرضى الذين يعانون من GDM (38.68 ± 5.02) والاصحاء (6.12 ± 0.37) ، وكان هناك فرق كبير بين المرضى والاصحاء (P≤0.01). كان متوسط IL-18 للمرضى (58.87 ± 4.79) والاصحاء (20.13 ± 1.06). كان هناك فرق ذو دلالة إحصائية بين المرضى والاصحاء (P≤0.01) وكان متوسط NLRP3 للمرضى (76.39 ± 6.71) والاصحاء (41.15 ± 2.66). كان هناك فرق كبير بين المرضى والاصحاء (P≤0.01).

أظهرت الدراسة الحالية أن الاصابه ب GDM أدت إلى زيادة معنوية في حدوث *H. Pylori* (P≤0.01) . بالإضافة إلى ذلك ، كانت معدلات الإصابة بـ *H. pylori* IgM ,IgG أعلى بشكل ملحوظ في مجموعة GDM.

وزارة التعليم العالي والبحث العلمي

جامعه بـابـــل / كـليت الطـــب

فرع الأحياء المجهرية الطبية

دراسة الخلل التنظيم المناعي والاصابة بالبكتريا الحلزونية البوابية لدى مرضى سكري الحمل

رسالة مقدمة إلى

مجلس كلية الطب / جامعة بابل

وهي جزء من متطلبات نيل درجة الماجستير في العلوم / الاحياء المجهرية الطبية

من قبل

هاجر داود سلمان نحاري

بكالوريوس أحياء مجهرية / علوم حياة/ جامعة بابل (٢٠١٩)

بأشـــــراف

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