

# Impact of smoking on Galectin-3 and GDF-15 among pregnant women

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**Abstract:** In order to shed an understanding of the complicated mechanisms behind the health implications of tobacco use during pregnancy, this study examines the complex interplay between smoking and pregnancy on the levels of the biomarkers Galectin-3 and GDF-15, and to provide a comprehensive analysis, facilitating a deeper understanding and offering potential pathways for targeted interventions to mitigate health risks. Pregnant smokers had higher levels of hemoglobin and white blood cell counts, while both pregnant groups had lower platelet counts. Additionally, pregnant smokers had higher levels of total cholesterol, LDL, triglycerides, liver enzymes (ALT and AST), and C-reactive protein. These significant changes in hematological and metabolic parameters were found in a cross-sectional analysis involving 90 female participants who were divided into three groups: pregnant smokers, pregnant non-smokers, and non-pregnant non-smokers. Although there were slight variations in the levels of GDF-15 and Galectin-3 between the groups, they did not reach statistical significance. These results highlight the extensive and harmful metabolic alterations brought on by smoking during pregnancy, highlighting the need for focused actions to protect the health of both the mother and the fetus even in the face of largely stable biomarker levels.

**Keywords:** pregnancy; Galectin-3; GDF-15; biomarkers; haemoglobin; LDL; C-reactiveprotein.



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## 1. Introduction

It is commonly known that smoking during pregnancy increases the chance of several unfavourable outcomes for the health of both the mother and the fetus. Although there has been an attempt to lower the smoking rates among expectant mothers, it is still unclear how smoking causes harm to the fetus as well as the mother. These impacts entail intricate adjustments to the body's immune system, metabolic processes, and signaling systems. Two intriguing proteins that may serve as markers of these physiological alterations are growth differentiation factor-15 (GDF-15) and galectin-3, based on recent study [1,2]. As GDF-15 is well-known for its involvement in maintaining immunological and metabolic homeostasis [3], which is essential during physiological stress, galectin-3 has several functions in a variety of processes, both physiological and pathological, involving inflammatory and tissue repair. According to early studies, smoking may have an effect on the levels of the two substances Galectin-3 and GDF-15, which may have an effect on the course of pregnancy. There is, however, a dearth of particular research on how smoking affects these biomarkers. Furthermore, it's critical to understand how various smoking

patterns—from chronic to sporadic—may impact these indicators and if there are acceptable smoking thresholds during pregnancy. Knowing the relationship between smoking, Galectin-3, and GDF-15 may result in feasible therapeutic measures and enhanced advice and assistance for expectant mothers [4], stressing not only the overall risks of smoking during pregnancy but also the particular physiological alterations it causes. Even though smoking has been connected to risks during pregnancy, there is still a need for additional studies to improve diagnosis and create targeted interventions for improved maternal and fetal health. This is because there is currently a lack of comprehensive understanding of the exact biochemical mechanisms and the implications of changes in Galectin-3 and GDF-15 levels in pregnant smokers [5].

### 1.1. Literature review

Smoking during pregnancy is a significant public health concern, often labeled as one of the single most avoidable causes of adverse pregnancy outcomes. Numerous studies have consistently shown that tobacco consumption during pregnancy results in both short and long-term detrimental effects for both the mother and the unborn child [1,2]. This behavior is pervasive across various geographies, be it the developed or the developing world, making it the first major environmental risk factor an unborn child encounter. In comparison to other risk factors during the perinatal period, the exposure to tobacco smoke stands out as particularly detrimental [6]. The combustion byproducts are believed to inflict more harm on the fetus than the nicotine itself. However, pinpointing the exact toxic effects remains complex due to the myriad of harmful substances present in tobacco smoke [7].

An intriguing feature of GAL-3 is its versatile localization within cells [8]. While it's primarily found in the cytoplasm, it can transition to the nucleus and is also secreted externally, marking its presence in various biofluids [9]. These varied localizations signify its multifunctional role in cellular processes. For instance, within the cytoplasm, galectin-3's interactions with proteins such as Bcl-2 and GTP-bound K-Ras are known to support cell survival [10]. In the nucleus, it takes on roles such as enhancing pre-mRNA splicing and gene transcription [11]. Externally, it influences interactions between cells, especially between epithelial cells and the extracellular matrix [12]. Cumulatively, GAL-3 is involved in a myriad of biological processes, ranging from cell growth, differentiation, inflammation, and fibrogenesis to angiogenesis and host defence [10]. Its influence also extends to critical areas of health; for instance, it has been associated with cardiovascular restructuring and various autoimmune and inflammatory reactions [13].

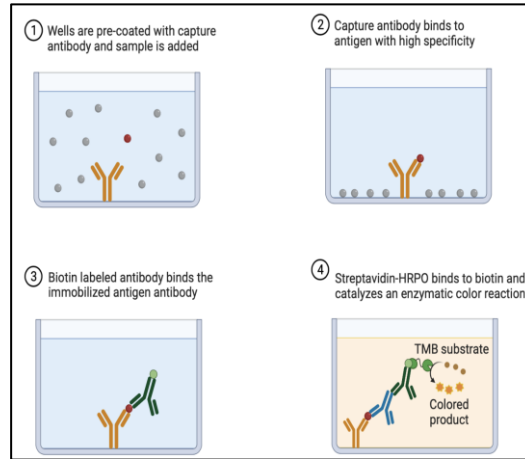
### 1.2. Study objectives

The current study aims to:

1. Investigate GDF-15 Concentrations: Systematically assess the disparities in GDF-15 serum concentrations between pregnant smokers and their non-smoking counterparts.
2. Examine Galectin-3 Levels: Rigorously determine the differences in serum Galectin-3 levels among pregnant women who smoke compared to those who do not.
3. Analyze the Biomarker-Smoking Nexus: Establish the correlation between smoking behaviour and the serum levels of both Galectin-3 and GDF-15 biomarkers in pregnant women -Detection of serum levels of Galectin-3 (GAL-3) and Growth differentiation factor-15 (GDF-15) using ELISA technique.

The serum levels of the Galectin-3 (GAL-3) and Growth differentiation factor-15 (GDF-15) were measured using Enzyme-Linked Immunosorbent Assay (ELISA) methods. ELISA is a sensitive technique used for detecting and quantifying substances such as peptides, proteins, antibodies, and hormones. The measurement of GAL-3 and GDF-15 enzymes in the plasma followed the Sandwich-ELISA principle (figure 1). The "sandwich" in the Sandwich ELISA refers to the format of layering the capture antibody, antigen, and

detection antibody – effectively creating a sandwich with the antigen in the middle. The strength of this method lies in its specificity. Using two antibodies targeting different epitopes on the antigen ensures that only the specific antigen of interest will be detected and quantified.

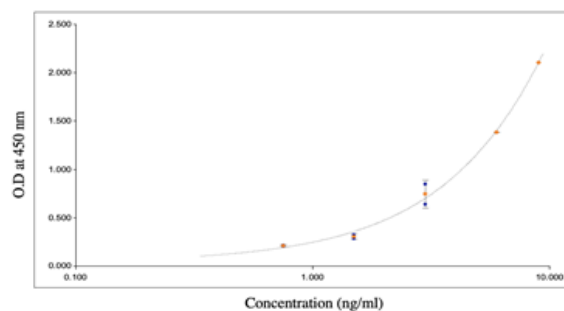


**Figure 1.** A schematic representation of the sandwich-ELISA principle employed for the detection of Galactin-3 (GAL-3) and Growth differentiation factor-15 (GDF-15).

## 2. Material and method

### 2.1. Detection of Galectin-3 (GAL-3) levels

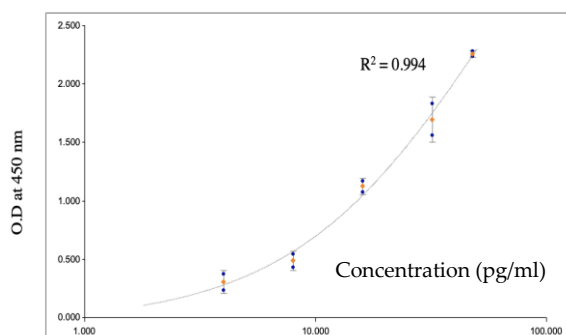
With participant plasma, GAL-3 was detected using a human Galectin-3 ELISA kit. In conclusion, a pre-coated Microelisa stripplate was filled with serum samples and standards, and then the incubation and washing processes were carried out. After that, HRP-conjugated antibody was added and allowed to incubate. After applying a mixture of chromogen solutions to allow the formation of a yellow colour, stop solution was added. By measuring the absorbance (O.D.) at 450 nm and comparing the outcomes to a standard curve, the concentration of GAL-3 in plasma samples was determined (Figure 2).



**Figure 2.** This is a figure. Schemes follow the same formatting.

### 2.2. Detection of Growth differentiation factor-15 (GDF-15) levels

A human GDF-15 ELISA kit was used to detect GDF-15 in plasma. A pre-coated Microelisa stripplate with the appropriate antibody was filled with standard samples and serum samples. This was followed by incubation, washing, and the addition of an HRP-conjugated antibody. For colored development, chromogen solutions were added, and a stop solution was used to end the reaction. A plate reader was used to measure the absorbance at 450 nm, and a normative curve was compared to sample readings to determine the GDF-15 concentration (figure 3).



**Figure 3.** The standard curve of GDF-15.

### 2.3. Ethical approval

Before starting blood samples collection, the ethical approval was obtained from the ethical writing and scientific committee of the Faculty of Allied Medical Sciences, Al-Ahliyya Amman University (IRB: AAU/4/7/2022-2023) (Appendix II). A consent form was obtained from all participating subjects.

### 2.4. Statistical analysis

Data were represented as mean  $\pm$  standard deviation (SD). T-test was used to analyse the mean difference to determine which group differed significantly. P-value of 0.05 or lower was interpreted as statistically significant. Statistical analyses were performed using SPSS software version 28. The artistic images were created using BioRender software.

## 3. Results

### 3.1. Subject declaration

There were 130 participants in this study, who were divided into three groups: non-smokers who were not pregnant, pregnant smokers, and pregnant smokers. Pregnant smokers had an average age of  $28.5 \pm 4.2$  years, which was significantly younger than that of pregnant non-smokers ( $31.7 \pm 3.8$  years) and non-pregnant non-smokers ( $30.6 \pm 4.1$  years).

Compared to pregnant non-smokers and non-pregnant non-smokers, pregnant smokers had lower average height ( $165 \pm 5$  cm), weight ( $65 \pm 7.8$  kg), and BMI ( $23.87 \pm 3.2$ ). Non-smokers who were pregnant were slightly taller, heavier, and had a higher BMI. Nonsmokers who were not pregnant shared comparable physical characteristics.

Of those who smoked while pregnant, 40% had smoked for five years or less, 35% for six to ten years, and 25% for more than ten years. The different levels of long-term smoking exposure within this group are reflected in the information (Table 1).

These results support the theory that smoking and nicotine can cause weight reduction because pregnant smokers have lower body weight and BMI than non-smokers. Nicotine's effects on appetite suppression and increased energy expenditure could be the cause of this. Despite these noted variations, it's important to take into account the larger health risks caused by smoking.

**Table 1.** Demographic characteristics of study participants'

Criteria	Pregnant Smokers (n=30)	Pregnant Non Smokers (n=30)	Non-Pregnant Non Smokers (n=30)
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Age (years)	28.5 ± 4.2	31.7 ± 3.8	30.6 ± 4.1
Height (cm)	165 ± 5	167 ± 6	166 ± 5
Weight (kg)	65 ± 7.8	70 ± 8.1	72 ± 7.7
BMI (kg/cm <sup>2</sup> )	23.87 ± 3.2	25 ± 2.1	26.2 ± 2.0
Duration of Smoking		N/A	N/A
5 years or less	40% (12)		
6-10 years	35% (11)		
More than 10 years	25% (8)		

### 3.2. Complete blood count (CBC) analysis

The Complete Blood Count (CBC) analysis was the primary goal of this study since it is an essential tool for determining disorders and diagnosing overall health. The study examined how smoking and pregnancy together affected hematological parameters. Blood parameters usually undergo physiological changes during pregnancy, and smoking introduces different compounds that can affect these parameters.

Pregnant smokers had significantly higher hemoglobin levels (15.8 ± 1.2 g/dL) than non-smokers (13.5 ± 1.1 g/dL) and non-pregnant smokers (13.7 ± 1.7 g/dL). These findings are consistent with other studies that have shown smoking raises hemoglobin levels in response to decreased oxygen delivery brought on by carbon monoxide exposure. In addition, pregnant smokers had an elevated hematocrit level (46 ± 3%), which could indicate a decrease in plasma volume or an increase in red cell mass.

Later examination demonstrated that pregnant smokers had larger red blood cells (macrocytosis), as evidenced by their Mean Corpuscular Volume (MCV), and their mean corpuscular haemoglobin (MCH) content was higher per red blood cell.

One remarkable finding was that pregnant smokers had the highest platelet counts (310 ± 85 x10<sup>9</sup>/L), most likely as a result of smoking's pro-thrombotic effects. Yet, platelet counts dropped in both pregnant groups – particularly in the non-smoking group – possibly as a result of higher platelet use during pregnancy (Table 2).

Pregnant smokers exhibited the highest white blood cell counts, which may be attributed to the combination of their mild neutrophilia and the inflammatory response caused by smoking. Unaffected by these factors, non-smokers who were not pregnant had the lowest count. This work provides important new understandings of the ways in which smoking and pregnancy interact to influence blood test results.

**Table 2.** Table 2. CBC parameters in participant group.

Variable	Pregnant Smokers (n=30)	Pregnant Non-Smokers (n=30)	Non-Pregnant Non-Smokers (n=30)	P value
Hemoglobin (g/dL)	15.8 ± 1.2	13.5 ± 1.1	13.7 ± 1.7	< 0.001
Hematocrit (%)	46 ± 3	41 ± 2.5	42 ± 2.7	0.0052
MCV* (fL)	98.45±2.56	92.34±5.7	87±4.1	< 0.001
MCH* (pg)	31.5±2.4	29.53±1.7	26.6±2.2	0.0155
MCHC* (g/dL)	35.73±1.55	32.24±1.42	30.23±2.5	0.0172
Platelets (x10 <sup>9</sup> /L)	310 ± 85	210±58	173±61	< 0.001
WBCs (x10 <sup>9</sup> /L)	11.9 ± 2.2	10.7±2.1	8.64 ± 3.4	< 0.001

Abbreviations: MCV\*, Mean Corpuscular Volume; MCH\*, Mean Corpuscular Hemoglobin; MCHC\* Mean Corpuscular Hemoglobin Concentration; WBC, White Blood Cells.

### 3.3. Lipid profile

The level of lipids is important as mentioned before. here are the results (Table 3). Total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides were all measured as part of the lipid profile analysis.

Pregnant Smokers had significantly higher total cholesterol (TC) levels (1900 ± 21.4 mg/dL) than Pregnant Non-Smokers (173 ± 18.4 mg/dL) and Non-Pregnant Non-Smokers (122 ± 12.5 mg/dL), highlighting the combined impact of smoking and pregnancy on TC (p<0.001).

There were notable variations between the groups (p<0.001) in the LDL cholesterol levels, with Pregnant Smokers having the highest levels (93.45 ± 5.2 mg/dL), followed by Pregnant Non-Smokers (79.93 ± 4.84 mg/dL) and Non-Pregnant Non-Smokers (61.6 ± 6.23 mg/dL).

HDL cholesterol, also known as "good cholesterol," was found to be lower in pregnant smokers (45.9 ± 4.43 mg/dL) when compared to non-smokers (56 ± 4.5 mg/dL) and pregnant non-smokers (52.2 ± 6.7 mg/dL). This indicates that smoking negatively affects HDL-C and counteracts the normal elevation that comes with pregnancy (p<0.01).

All pregnant cohorts showed a consistent increase in triglyceride (TG) levels; however, pregnant smokers had the highest elevation (181 ± 17.4 mg/dL) compared to pregnant non-smokers (157 ± 13.5 mg/dL) and non-pregnant non-smokers (97 ± 12.1 mg/dL). This suggests that smoking and pregnancy have a synergistic effect on triglyceride metabolism (p<0.001). These results highlight the significant effects of smoking and pregnancy on lipid profiles and the possible consequences for cardiovascular health.

**Table 3.** Lipid profile analysis in participant groups.

Variable	Pregnant Smokers (n=30)	Pregnant Non-Smokers (n=30)	Non-Pregnant Non-Smokers (n=30)	P value
Total Cholesterol (mg/dL)	190 ± 21.4	173 ± 18.4	122 ± 12.5	< 0.001
LDL* (mg/dL)	123.45 ± 5.2	109.93 ± 4.84	91.4 ± 6.23	< 0.001
HDL* (mg/dL)	45.9 ± 4.43	55.2 ± 6.7	62 ± 4.5	0.003
Triglyceride (mg/dL)	231 ± 17.4	207 ± 13.5	162 ± 12.1	< 0.001

Abbreviations: LDL\*, Low Density Lipoprotein; HDL\*, High Density Lipoprotein

### 3.4. Liver function test

This study evaluated the effects of smoking and pregnancy on liver enzyme profiles, particularly those of the liver function markers AST (aspartate aminotransferase) and ALT (alanine aminotransferase) (Table 4).

The highest liver enzyme concentrations were found in pregnant smokers, with AST levels peaking at 37.8 U/L and ALT levels averaging 35.5 U/L. When compared to pregnant non-smokers, these values were significantly higher ( $p < 0.01$  for both enzymes). The ALT (24.8 U/L) and AST (27.4 U/L) of pregnant non-smokers were marginally higher than those of non-pregnant non-smokers.

The ALT and AST levels of the non-pregnant, non-smoking control group were within normal ranges, at about 22.4 and 26.73 U/L, respectively. The statistical significance of the differences in ALT and AST between the groups ( $p < 0.001$  for both enzymes) underscores the significant influence of smoking and pregnancy on liver enzyme profiles. These results raise the possibility of hepatic strain or damage, especially in smokers who are pregnant.

**Table 4.** Liver function test analysis in participant groups.

Variable	Pregnant Smokers (n=30)	Pregnant Non-Smokers (n=30)	Non-Pregnant Non-Smokers (n=30)	p value
ALT* (U/L)	35.5 ± 7.23	24.8 ± 4.4	22.4 ± 5.3	< 0.001
AST* (U/L)	37.8 ± 10.2	27.4 ± 6.2	26.73 ± 3.2	< 0.001

Abbreviations: ALT\*, Alanine Transaminase; AST\*, Aspartate Transaminase.

### 3.5. C-reactive protein levels

In order to comprehend the relationship between smoking and pregnancy on inflammation, the study examined C-reactive protein (CRP) levels, an indicator of systemic inflammation (Table 5).

With an average CRP of 12.3 mg/L, pregnant smokers had the highest levels, and this elevation was statistically significant ( $p < 0.001$ ). This supports the theory that smoking increases the effects of inflammation during pregnancy. Although not as high as those observed in pregnant smokers, pregnant non-smokers also had elevated levels of CRP (7.5 mg/L), most likely because of the innate inflammation associated with pregnancy. The control group, which consisted of non-smokers who were not pregnant, had CRP levels that were closest to usual standards, averaging 4.2 mg/L.

These major variations in CRP levels among the groups emphasize the negative effects of smoking during pregnancy and stress the significance of encouraging quitting smoking during this crucial time.

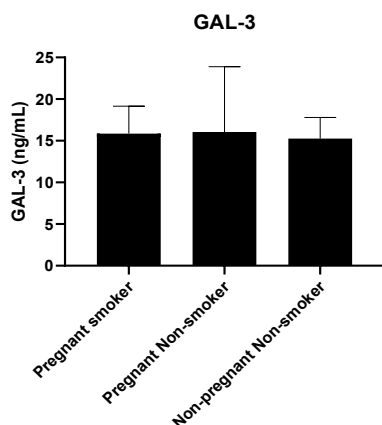
**Table 5.** C-reactive protein analysis in participant groups.

Variable	Pregnant Smokers (n=30)	Pregnant Non-Smokers (n=30)	Non-Pregnant Non-Smokers (n=30)	P value
CRP* (mg/L)	12.3 ± 3.5	7.2 ± 2.7	4.2 ± 1.8	< 0.001

Abbreviations: CRP\*, C-reactive protein.

### 3.6. Evaluating the plasma levels of Galectin-3

The levels of galectin-3 (Gal-3) were examined in three groups in this study: non-smokers who weren't pregnant, pregnant smokers, and pregnant smokers. Pregnant smokers had an average level of 15.86 ng/mL, pregnant non-smokers 16.06 ng/mL, and non-pregnant non-smokers 15.73 ng/mL. The Gal-3 levels indicated little variation between the groups. Statistical analysis, however, showed that these variations were not remarkable. Consequently, it seems that in the cohorts under study, neither smoking nor pregnancy had a significant impact on Gal-3 levels (figure 4).



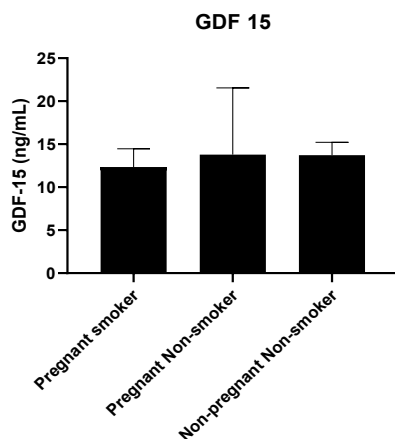
**Figure 4.** The serum levels of GAL-3 in participants groups.

### 3.7. Evaluating the serum levels of GDF-15

GDF-15 is known to be associated with various pathological conditions. Pregnant smokers averaged 12.35 ng/mL, pregnant non-smokers 13.8 ng/mL, and non-pregnant



non-smokers 13.73 ng/mL. The GDF-15 concentrations revealed subtle differences between the groups. These differences, statistical analysis showed, were not statistically significant. This implies that, for the purposes of this investigation, GDF-15 levels were not significantly impacted by smoking or pregnancy. (figure 5)



**Figure 5.** The serum levels of GDF-15 in participants groups.

#### 4. Discussion

The relationship between smoking and pregnancy and how it affects different physiological parameters are the main topics of discussion.

##### 4.1. Smoking during pregnancy and age:

Pregnancy-related smoking is more common in younger smokers, perhaps as a result of psychological or socioeconomic pressures [14,15,16].

##### 4.2. Body Mass Index (BMI)

Smokers who are pregnant have lower body weights and BMIs because nicotine suppresses appetite. Benefits from a lower BMI are overshadowed by illnesses, particularly during pregnancy [17,18].

##### 4.3. Timing of Smoking

Given that some pregnant smokers have smoked for a shorter period of time, interventions should take into account both long-term and recent smokers [19,18,20].

##### 4.4. Hematological Dimensions

Pregnancy and smoking both individually and together have complex effects on hematological parameters. Because smoking reduces oxygen delivery, pregnant smokers exhibit greater hemoglobin levels.

Smoking increases platelet counts, but pregnancy lowers them, leading to a tug-of-war effect. Smoking-induced inflammation and pregnancy-related variables both contribute to increased white blood cell counts in smokers [21,22,23].

##### 4.5. Fat-Based Metabolism

Smoking during pregnancy alters lipid metabolism, which may have an impact on cardiovascular markers. During pregnancy, cholesterol in general increases, and smoking can make this increase more [24] also may increase the potential for atherogenicity of LDL by enhancing its oxidation. In addition, it can affect how HDL is metabolized, which lowers

HDL levels. Pregnancy raises triglyceride levels, which may be further elevated by smoking [25].

#### 4.6. Hepatic Enzymes

##### 4.6. Hepatic Enzymes

Due to physiological changes, there are slight elevations in liver enzymes during pregnancy [26]. Smoking exposes a person to hepatotoxic chemicals, which causes pregnant smokers' ALT and AST levels to rise noticeably [27].

#### 4.7. Aggravation

Mild systemic inflammation brought on by pregnancy raises CRP levels. Smoking increases inflammation and causes pregnant smokers' CRP levels to rise noticeably. Growth Differentiation Factor-15 (GDF-15) and Galectin-3 (GAL-3) There are no discernible changes in GAL-3 levels in response to smoking or pregnancy. Additionally, there is no clear correlation between GDF-15 concentrations and smoking or pregnancy; instead, it appears that other factors may be more important.

The study, in summary, examines the intricate relationships between smoking and pregnancy on a range of physiological parameters, emphasising the need for more investigation to fully grasp the subtleties of these effects [28].

## 5. Conclusions

In our comprehensive study analyzing the interplay of pregnancy and smoking on various physiological markers, the results painted a nuanced picture. On one hand, clear and significant elevations were observed in markers like CRP, LDL cholesterol, and liver enzymes among pregnant smokers, signaling the compounded impacts of smoking and pregnancy. However, other biomarkers, such as Galectin-3 and GDF-15, exhibited subtle fluctuations that lacked statistical significance, suggesting that the effects of pregnancy and smoking on these parameters are less straightforward. Overall, our research contributes valuable insights to the intricate physiological responses triggered by pregnancy and smoking, shedding light on potential health risks and underscoring the imperative need for health interventions, especially for pregnant smokers.

## 6. Limitations

1. Sample size: Our cohort, though diverse, might not be representative of the broader population. A larger sample size could potentially capture more subtle effects and offer stronger statistical power.

2. Confounding variables: Factors like age, dietary habits, pre-existing health conditions, and genetic predispositions, among others, were not controlled for and could potentially influence the results.

3. Cross-sectional design: The nature of our study design captures a snapshot in time, potentially missing the dynamic changes that could occur over a longer duration or different stages of pregnancy.

4. Lack of longitudinal data: Without follow-up data, it's challenging to determine any long-term impacts of smoking during pregnancy on the markers studied.

Measurement Constraints: While our assays and methods are standardized, inherent errors or biases in measurement techniques could influence results.

## 7. Future Work

1. Longitudinal studies: Future research could employ a longitudinal design to track changes over different trimesters of pregnancy and postpartum, offering insights into temporal variations.

2. Inclusion of other biomarkers: Expanding the scope to include more inflammatory, metabolic, or cardiovascular markers can provide a more holistic understanding.

3. Dietary and Lifestyle Correlations: Investigating the influence of diet and lifestyle on the studied markers could offer more comprehensive insights into their modulation.

4. Genetic Studies: Understanding genetic predispositions that might make certain individuals more susceptible to the effects of smoking during pregnancy could be pivotal.

5. Interventional Studies: Future research could explore the efficacy of different health interventions, especially smoking cessation programs, in modulating these biomarkers during pregnancy.

6. Global Datasets: Collaborating with international cohorts can allow for the investigation of ethnically diverse populations, providing a global perspective on the impact of pregnancy and smoking.

**Author Contributions:** Conceptualization, A.A.I, Z.Z, L.A.I and Kh.A.; methodology, A.A.I, Z.Z and L.A.I.; software, L.A.I and Kh.A.; validation, A.A.I, Z.Z, L.A.I and Kh.A.; formal analysis, A.A.I and Z.Z.; resources, Kh.A.; data curation, Z.Z, L.A.I and Kh.A.; writing—original draft preparation, A.A.I and Z.Z.; writing—review and editing, L.A.I and Kh.A.; visualization, Z.Z.; supervision, A.A.I, Z.Z, L.A.I and Kh.A.

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Conflicts of Interest:** No potential conflict of interest was reported by the authors.

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