Predictive Value of Immunological Parameters in the Risk of Gestational Diabetes Mellitus: A Pilot Study

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***Title*:**

Predictive value of immunological parameters for the risk of gestational diabetes mellitus: a pilot study

Short running title: **Immune cells for predicting GDM**

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Abstract

Background: Immunological and biochemical parameters are gaining more and more importance in the prognosis of diabetes and its complications. Here we assessed the predictive power of immunological parameters correlated with biochemical ones in gestational diabetes mellitus (GDM). **Material and Methods**: 217 pregnant women were screened for GDM between the 2nd and the 3rd trimester of gestation, based on IAGDP methods in this cross-sectional descriptive study. Immunological and biochemical parameters were determined using appropriate methods. Receiver operating characteristics (ROC) curve analyses were conducted to assess the optimal cutoff and value of immunological to biochemical parameter ratios for predicting GDM. **Results**: 11.90% of pregnant women were diagnosed GDM positive. Serum glucose levels, total cholesterol, LDL-cholesterol, triglycerides and total proteins were significantly increased while HDL-cholesterol decreased in women with GDM compared to controls. The levels of glycosylated hemoglobin and creatinine, as well as transaminase (AST and ALT) activities did not significantly differ between GDM and pregnant controls. Total leucocytes (white blood cell), lymphocyte and platelet numbers were significantly higher in women with GDM than in pregnant controls. We also found that the lymphocyte:HDL-C, monocyte:HDL-C and granulocyte:HDL-C ratios were significantly higher in women with GDM than in pregnant controls ($p = 0.001$; $p = 0.009$ and $p = 0.004$ respectively). Women with a lymphocyte:HDL-C ratio greater than 3.66 had a 4-fold increased risk of developing GDM than those with lower ratios (odds ratio 4.00; 95% CI: 1.094 – 14.630; p=0.041). **Conclusion**: The lymphocyte:HDL-C, monocyte:HDL-C and granulocyte:HDL-C ratios may represent valuable makers, and the lymphocyte:HDL-C ratio in particular may have strong predictive power for GDM. This ratio can be easily assessed in patients.

Keywords: Immune cells; biochemical parameters; Gestational Diabetes mellitus, GDM prediction, Lymphocytes; HDL-cholesterol.

Background

A major concern of researchers is to find biological or clinical factors with prognostic or early diagnostic value for diseases in order to strengthen or improve prevention rather than cure. In this context, little is known about the utility of immunological and/or biochemical parameters in the prediction of gestational diabetes mellitus [1,2]. Very recently, we investigated the modulation of immune cell frequencies in gestational diabetes, and found that gestational diabetes mellitus (GDM) modulated the frequencies of total CD3+ and CD4+ T and B cells, suggesting that immune cells could play a specific role in the prognosis of this disease [3]. GDM is defined as glucose intolerance arising for the first time during pregnancy with or without remission after the end of pregnancy [4,5]. It is the most common metabolic disease during pregnancy and its incidence is increasing worldwide [4,5]. As we have recently reported, GDM is one of the major endocrine abnormalities that can induce disruption of several biochemical and immunological parameters [3, 6-7]. Recently, there has been renewed interest in immunological parameters including immune cell subpopulations and cytokines which are designated as predictors of endothelial dysfunction and inflammation [8]. In previous studies, we have reported that biochemical parameters, including glycaemia, triglycerides (TG), high density lipoprotein-cholesterol (HDL-C), total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), known as metabolic markers, are modulated during GDM and macrosomia [9–12]. Recently, we also demonstrated that immune cell frequencies, including neutrophils, eosinophils, monocytes, NK cells, and lymphocytes, can be modulated in type 1 diabetes and type 2 diabetes whether associated with pregnancy or not, suggesting that these cells can play important roles in the pathogenesis of this disease [3; 13,14]. Moreover, several studies have found that immune-hematological parameters, including white blood cell, red blood cell and platelet counts, lymphocytes, neutrophils, monocytes and the ratios between cells to cells and between cells to HDL-C, may be related to metabolic syndrome and atherosclerotic processes, as potential indicators of prothrombotic and pro-inflammatory states [15–18].

In addition, the global prevalence of GDM varies from 1 to 28% depending on population characteristics, screening methods, and diagnostic criteria [19-21] with a great percentage (87.6%) reported in low and middle-income countries, where access to maternal care is often limited [22]. Sedentary and modern lifestyle in developing countries contribute to the increased prevalence of GDM [23,24]. Early diagnosis of gestational diabetes, based on biochemical and immunological parameters, could be crucial to anticipate the care of pregnant diabetic women and thus prevent the wide range of adverse consequences on the offspring,

including macrosomia, fetal death, prematurity, birth trauma, respiratory distress syndrome, obesity, impaired glucose tolerance, and type 2 diabetes in adulthood [7,12,25].

Biochemical parameters can be easily determined in plasma and immune-hematological parameters can be easily measured from peripheral blood. Biochemical and immunological indicators, as discussed above, can be used as potential markers to predict GDM. Thus, the principal objective of this study is to determine whether immunological parameters could be correlated with biochemical ones to assess their predictive value for GDM, the prevalence of which was also determined in a southern Beninese population.

Material and methods

Study participants

Two hundred and forty-six (246) pregnant women were enrolled in this study by specialist clinicians of the department of obstetrics and gynecology of three national hospital centers in southern Benin. Based on inclusion criteria that included the absence of preexisting type 1 or type 2 diabetes, infectious diseases including hepatitis, HIV and malaria after blood sample tests, 210 pregnant women, aged from 19 to 43 years, were selected and then screened for GDM (see protocol below). Socio-demographic data and risk factors as well as family history associated with diabetes were recorded. Informed and written consent was obtained from each participant. The privacy rights of subjects were observed. The study was conducted in accordance with the Declaration of Helsinki (1964) (as revised in Edinburgh 2000) and was approved by the Ethics Committee on Research of the Institute of Applied Biomedical Sciences of Cotonou, Benin under the number Dec.n°100/CER/ISBA-2016.

Screening of gestational diabetes mellitus

Gestational diabetes mellitus was diagnosed in pregnant women following the protocol of International Association of Diabetes and Pregnancy Study Group [26]. Briefly, women between 24 and 28 weeks of gestation after overnight fasting were submitted to an oral glucose tolerance test (OGTT**)** and given 75g of glucose. Subjects were declared as positive for GDM when overnight fasting plasma glucose was ≥ 92 mg/dL (5.1 mmol/L), or 1-hour OGTT plasma glucose level was ≥ 180 mg/dL (10.0 mmol/L), or 2-hours OGTT plasma glucose level was ≥ 153 mg/dL (8.5 mmol/L). Consequently, two experimental groups of subjects were then established: a group of women positive for GDM and a group of women negative for GDM considered as the control group. Then, twenty-five (25) women with GDM and thirty-five (35) age-matched and body mass index-matched pregnant controls were selected and submitted for blood collection and biochemical and immunological assays.

Blood samples

Blood samples were collected from each selected participant in appropriate tubes and immediately transported to the laboratory for biological assays within 2 hours. Immune-hematological parameters and glycosylated hemoglobin (HbA1c) were determined in whole blood. Plasma samples were immediately used for glucose determination. Serum obtained by low-speed centrifugation was used for biochemical assays.

Biochemical assays

Plasma glucose, total cholesterol, HDL cholesterol, triglycerides were measured by colorimetric enzymatic method using ELITech reagents (ELITech Group, Puteaux, France) according to manufacturer's instructions. LDL-cholesterol was calculated using Friedewald method [27]. Total protein levels were determined by direct Biuret colorimetric method (ELITech Group, Puteaux, France). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymatic activity and creatinine levels were determined by enzymatic kinetic assay (DiaSys reagents, Diagnostic Système GmbH, Germany). HbA1c concentration was calculated using a percentage of total hemoglobin, according to the manufacturer's instructions (Reference 41190, Labkit Chemelex SA, Barcelona, Spain).

Determination of immune cells and hematological parameters

Immune cells and hematological parameters were determined through complete blood formula count using an automatic hematological analyzer (Cell Dyn 3500, Abbott, France). These parameters included total white blood cells (WBC), lymphocytes, monocytes, granulocytes, red blood cells (RBC), hemoglobin concentration (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin content (MCH), mean cell hemoglobin concentration (MCHC), red blood cell distribution width (RDW) and platelets (PLT).

Statistical analysis

Data analyses were performed using Graph Pad Prism 6.0 (Graph Pad Inc., CA, USA) and IBM® SPSS® Statistics (version 25.0). Values are means ± standard deviation or medians with interquartile ranges. Student's t-test, Mann–Whitney U test and Chi-squared ($\chi 2$) test were used when appropriate. Pearson and Spearman correlations were used to determine the association between immunological and biochemical parameters. Receiver Operating Characteristics (ROC) curve analysis was used to assess the value of immunological to biochemical parameter ratios for predicting gestational diabetes mellitus and to obtain the best cutoff value using Youden's index (sensitivity + specificity – 1). The odds ratios (ORs) are presented with 95% confidence intervals (CI). Differences were considered significant with a two-tailed p value < 0.05.

Results

Prevalence, clinical data and some factors associated with GDM

After GDM screening, our study revealed a prevalence of 11.90% of gestational diabetes (Table 1). Moreover, we observed that 60% of women with GDM were between 30 and 39 years old. Among 25 women with GDM, seventeen (68%) had moderate physical activity compared to eight (32%) who had inadequate physical activity ($p = 0.033$) (Table 1). The menstruation cycle was quite similar, either regular or irregular between both groups of women, even though a high rate of women (57% of pregnant controls and 60% of GDM) had irregular menstruation. 64% of women with GDM were multiparous (at least three children) compared to 21% of pregnant control women (Table 1). A significantly higher number of women with gestational diabetes had a history of miscarriages (48%) and preterm delivery (16%) compared to pregnant control women (24% and 4% respectively) (Table 1).

Biochemical parameters in women with GDM and pregnant controls

Biochemical parameters of women with GDM and pregnant controls are presented in Table 2. We observed that plasma fasting glucose ($p < 0.001$), total cholesterol ($p = 0.001$), LDL cholesterol ($p = 0.015$), triglyceride ($p = 0.035$) and total protein ($p = 0.005$) and HDL cholesterol ($p = 0.001$) levels significantly increased, while HDL-cholesterol level decreased in women with GDM compared to pregnant controls (Table 2). However, HbA1c and creatinine levels, and transaminase (AST and ALT) activities did not significantly differ between the two groups of women (Table 2).

Immunological and hematological parameters in women with GDM and pregnant controls

Immunological and hematological parameters of women with GDM and pregnant controls are shown in Table 3. We did not observe any significant difference in the number of red blood cells (RBC) and associated constants, including hemoglobin (Hb), hematocrit (Ht), mean cell volume (MCV) and mean cell hemoglobin concentration (MCHC) in women with GDM compared to pregnant controls (Table 3). However, total white blood cells ($p = 0.045$), total lymphocytes ($p = 0.015$) and platelet numbers ($p = 0.033$) were significantly higher in women with GDM than in pregnant controls. However, no significant difference was observed in the numbers of monocytes and granulocytes between both groups (Table 3).

Correlation between immunological and biochemical parameters

In pregnant control women, there was a positive correlation between blood glucose with lymphocytes (r = 0.89; p = 0.03) and between lymphocytes with total cholesterol (r = 0.50; p = 0.04) (Table 4). Moreover, a positive correlation was found between monocytes with triglycerides (r = 0.58; p = 0.04). In contrast, a negative correlation was observed between monocytes and HDL-cholesterol levels (r = − 0.68; p = 0.007) (Table 4).

In women with GDM, there was a positive correlation between glucose with white blood cells (r = 0.70; p = 0.03) on the one hand and between glucose with lymphocytes (r = 0.67; p = 0.02) on the other hand (Table 5). Also, we noticed a positive correlation between serum triglycerides with monocytes (r = 0.87; p = 0.045). In contrast, a negative correlation between granulocytes with HDL cholesterol was noted (r = − 0.90; p = 0.026). No correlations were found between other parameters analyzed in this study (Table 5).

Ratios of immunological to biochemical parameters for predicting GDM

Table 6 shows ratios between immunological to biochemical parameters in women with GDM and pregnant controls. We found that ratios between lymphocytes to HDL-C, monocytes to HDL-C and granulocytes to HDL-C were significantly higher in women with GDM than in pregnant controls (p = 0.001; p = 0.009 and p = 0.004 respectively) (Table 6).

As shown in Figure 1, a ROC curve analysis was used to assess the accuracy, sensitivity, specificity and value of the lymphocyte:HDL-C ratio, granulocyte:HDL-C ratio and monocyte:HDL-C ratio for prediction of GDM. The analysis showed that the lymphocyte:HDL-C ratio had a higher accuracy in predicting gestational diabetes mellitus (AUC = 0.859; p < 0.001; 95% CI: 0.752-0.966) than the granulocyte:HDL-C ratio (AUC=0.787; p < 0.01; 95% CI: 0.654-0.921) or the monocyte:HDL-C ratio (AUC=0.716; p < 0.01; 95% CI: 0.576-0.855) (Table 7). The optimal cutoff values of lymphocyte:HDL-C ratio, granulocyte:HDL-C ratio and monocyte:HDL-C ratio for predicting GDM were, respectively, 3.66 (sensitivity = 80.0%; specificity = 50.1%); 5.50 (sensitivity = 70.3%; specificity = 59.4%) and 1.56 (sensitivity = 60.9%; specificity = 50.0%) (Table 7). Odds ratios were used to assess the risk of GDM. We observed that pregnant women with a lymphocyte:HDL-C ratio greater than 3.66 had a 4-fold increased risk of developing GDM than those with lower ratios (odds ratio 4.00; 95% CI: 1.094 – 14.630; p = 0.041) (Table 8).

Discussion

Increasingly, the identification of biological parameters that can facilitate the prediction and prognosis of gestational diabetes has become a major concern for researchers. Given the

complications associated with GDM in mothers, fetuses, newborns and adult offspring, an early diagnosis of GDM could help anticipate the care of pregnant women and limit the adverse effects. Therefore, the aim of this study was to investigate whether immunological parameters, in association with biochemical data, could be used to predict the risk of GDM. Gestational diabetes mellitus is a glucose tolerance disorder that leads to different levels of hyperglycemia and is diagnosed for the first-time during pregnancy, with blood glucose levels above normal but still below the established thresholds for the diagnosis of diabetes [28]. Formerly considered as a rare disease in Africa, gestational diabetes is becoming more and more frequent, probably due to diet which goes together with a sedentary lifestyle and obesity [12,29]. Gestational diabetes screening was done according to the criteria of International Association of the Diabetes and Pregnancy Study Groups, using fasting glycaemia and OGTT test. In the present study, we observed that the prevalence of gestational diabetes was 11.90%. This result was higher than that reported by Ogoudjobi *et al*. [30] who reported a rate of 9% in Benin. Studies carried out in Africa have reported prevalences ranging from 5% to 17% [31,32]**.** The frequency of gestational diabetes varies by country, population studied and diagnostic criteria according to International Diabetes Federation 2013 [33]. The increasing rate of GDM might be due to the change in lifestyle of the population [6,12,34]. In the present study, it appeared that physical activity did not influence GDM onset. However, the majority of women, either GDM or controls, had moderate physical activity. Independently of the status of women, menstruation appeared similar, either regular or irregular, between women with GDM and pregnant controls. However, it is worth noting that a majority of women with GDM or pregnant controls exhibited irregular menstruation. Among 25 women with GDM, 12 (48%) had a history of miscarriages and 4 (16%) had gone through preterm birth. Similar results have been reported by Djagadou et *al.* [35], who showed that women with gestational diabetes had more frequently a history of spontaneous miscarriages and premature birth compared to control women. In the present study, most of those diagnosed with gestational diabetes were aged between 30 and 40 years. Our results are consistent with others showing that miscarriages, premature birth and gestational age are risk factors associated with gestational diabetes [36].

Diabetes is known to be associated with biochemical and metabolic disturbance [37]. In the present study, we observed that HbA1C levels were normal and did not significantly differ between both groups of women although glycaemia remained high in women with GDM compared to pregnant controls. The normal level of HbA1c may suggest that women with GDM were under adequate metabolic control [38,39]. However, the fact that their glycaemia remained high contradicted our assumption that women with GDM were on good glycemic control. This

discrepancy could be explained by the fact that these pregnant women are newly diagnosed for GDM and that they have not yet been subjected to any anti-diabetic treatment [38,39].

It is commonly reported that GDM is associated with the modulation of lipid profiles. Although the reports describing lipid profiles in pregnancy and GDM are various and extensive, results have been inconsistent [6, 9-12, 40,41]. The present study showed that serum TC, LDL-C and TG levels significantly increased, whereas HDL-C levels significantly decreased in women with GDM compared to pregnant controls. These results are in agreement with those reported by other investigators who have also reported a significant decrease in HDL levels in pregnant women with glucose intolerance compared to control women [42-44]. Other studies have, however, noted a significant elevation of all lipids including HDL-C in gestational diabetic women from the middle of the 2nd trimester of pregnancy to reach their peak at delivery [45]. A meta-analysis showed that serum TG was significantly increased in women with GDM across the 3rd trimester of pregnancy, meanwhile serum HDL-C levels were significantly lower in the 2nd and 3rd trimesters in women with GDM than in pregnant controls [40]. In fact, in normal pregnancy, circulating lipid concentrations markedly increase during pregnancy due to estrogen stimulation and insulin resistance [46]. It was also shown that during pregnancy, an increase in maternal fat accumulation was associated with both hyperphagia and increased lipogenesis and energy demand of the fetus, necessary for delivery and lactation preparation [37, 47–49]. In GDM, the situation seems to be similar as lipid levels increased during pregnancy. In fact, the increased levels of TG, TC and LDL-C observed in GDM in the present study, might result in an increase of lipid storage in gestational diabetic subjects due to a decrease of lipolytic clearance of TG and elevation of hepatic lipase activity which is thought to result in increased HDL catabolism [50,51].

There is evidence that gestational diabetes induces a profound variation of hematological parameters [8]. As immunological aspects, our very recent results demonstrated that GDM was associated with high frequencies of total CD3+ and CD4+ T cells and B cell expansion, suggesting a concomitant activation of cellular and humoral immunity associated with this disease [3]. In the present study, we found that total white blood cell and lymphocyte numbers significantly increased in women with GDM as compared to control pregnant. There was no significant difference in granulocyte and monocyte counts in both groups of women. These results are in agreement with previous work which reported that increased inflammatory cellular markers were associated with impaired glucose metabolism, insulin resistance and GDM [52-54]. In fact, the increased numbers of white blood cells (total leucocytes) and

lymphocytes in particular in women with GDM were consistent with the increase of a wide range of inflammatory metabolic markers such as TG, TC and LDL-cholesterol which together lead to insulin resistance [52-54].

These observations prompted us to investigate the correlations between both immunological and metabolic parameters during GDM. Indeed, we observed, in both pregnant controls as well as in women with GDM, a positive correlation between blood glucose and total lymphocytes, between TG and monocytes; between TC and lymphocytes in pregnant controls and between blood glucose and white blood cells in women with GDM on the one hand. On the other hand, we found a negative correlation between HDL-cholesterol and monocytes in pregnant controls and between HDL-cholesterol and granulocytes in women with GDM. All these correlations suggested that these parameters could be useful in GDM prediction.

In an attempt to determine whether both parameters could help in predicting GDM, we assessed the ratios between immunological (lymphocytes, granulocytes and monocytes) and biochemical parameters (glucose, TC, TG and LDL-C). Interestingly, we found that ratios between lymphocytes to HDL-C, monocytes to HDL-C, and granulocytes to HDL-C were significantly higher in women with GDM than in pregnant controls, suggesting that these ratios may certainly have significant value in GDM prediction. In fact, the odds ratio analysis indicated that only pregnant women with the ratio of lymphocytes to HDL-C greater than 3.66 have increased risk 4.0-fold higher for developing GDM than those with a lower ratio of lymphocytes to HDL-C (odds ratio 4.00; 95% CI: 1,094 - 14,630; $p = 0.041$).

Moreover, we would like to highlight the role of HDL-C in the present results as this lipoprotein seems to represent a central parameter to which immune cell frequencies could be added to more reliably determine the pathogenesis of GDM. In fact, HDL-C, as an anti-atherogenic lipoprotein, is recognized as a protective factor in atherosclerosis and inflammation [55,56]. It has also been reported that TG/HDL-C ratio is a better marker for evaluating insulin resistance and diabetes [57]. In addition, previous studies have shown that hematological parameters can be used as novel markers for predicting inflammation, metabolic syndromes, diabetes and atherosclerosis [58]. More specifically, immune-hematological parameters may serve as predictors of gestational diabetes mellitus. Indeed, Pattanathaiyanon *et al*. [59] demonstrated that increased leucocyte numbers in early pregnancy may lead to a significant risk of GDM. Wolf *et al*. [60] have also previously reported that leucocyte numbers greater than 9100 cells/µL in early pregnancy were significantly associated with a heightened risk of GDM.

To the best of our knowledge, our study is the first to analyze the ratios between lymphocyte/monocyte/granulocyte frequencies and HDL-C levels as novel markers of and their predictive value for GDM. In summary, a novel and independent relationship between biochemical and immunological parameters was found. The lymphocyte:HDL-C ratio may help in predicting the onset and development of GDM, and this ratio is easily assessed in patients. We nevertheless recognize some limitations of this study. Since it is cross-sectional in design, the analysis of the causative effect of the lymphocyte:HDL-C ratio on GDM is limited, and the sample size was relatively small. In addition, other inflammatory biomarkers such as cytokines may be evaluated in future investigations.

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Data availability

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki (1964) (as revised in Edinburgh 2000) and was approved by the Ethics Committee on Research of the Institute of Applied Biomedical Sciences of Cotonou, Benin under the number Dec.n°100/CER/ISBA-2016. Informed and written consent was obtained from each participant. The privacy rights of subjects were observed.

Consent for publication and disclosure statement

All authors give their consent for publication of the article in ***BMC Endocrine Disorders***. We confirm that neither the manuscript submitted nor any part of it has been published or is being considered for publication elsewhere.

Competing interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Authors' contributions

AF was in charge of major parts of technical aspects of work and participated in the manuscript writing. **SEMF** and **MPN** participated in the technical work and participated in the interpretation of data. **KM** read and approved the final manuscript. **AY** designed the study, supervised the work, wrote the manuscript and established the collaborative aspects.

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References

1. Wang C, Zhu W, Wei Y, Su R, Feng H, Lin L, Yang H. The Predictive Effects of Early Pregnancy Lipid Profiles and Fasting Glucose on the Risk of Gestational Diabetes Mellitus Stratified by Body Mass Index. J Diabetes Res 2016; 2016:1–8.

2. Kang X, Liang Y, Wang S, Hua T, Cui J, Zhang M, Ding Y, Chen L, Xiao J. Prediction model comparison for gestational diabetes mellitus with macrosomia based on risk factor investigation. J Matern Fetal Neonatal Med 2019:1–10.

3. Fagninou A, Nekoua MP, Sossou D, Moutairou K, Fievet N, Yessoufou A. Th2-Immune Polarizing and Anti-Inflammatory Properties of Insulin Are Not Effective in Type 2 Diabetic Pregnancy. J Immunol Res. 2020;2020:2038746. doi: 10.1155/2020/2038746.

4. Seshiah V, Das AK, Balaji V, Joshi SR, Parikh MN, Gupta S, Diabetes in Pregnancy Study Group. Gestational diabetes mellitus--guidelines. J Assoc Physicians India 2006; 54:622–628.

5. Kalra P, Kachhwaha CP, Singh HV. Prevalence of gestational diabetes mellitus and its outcome in western Rajasthan. Indian J Endocrinol Metab 2013; 17:677–680.

6. Ategbo J-M, Grissa O, Yessoufou A, Hichami A, Dramane KL, Moutairou K, Miled A, Grissa A, Jerbi M, Tabka Z, and Khan NA. Modulation of Adipokines and Cytokines in GestationalDiabetes and Macrosomia. J Clin Endocrinol Metab 2006;91(10):4137-4143.

7. Seck A, Hichami A, Doucouré S, Diallo Agne F, Bassène H, Ba A, Sokhna C, Khan NA, Samb A. Th1/Th2 Dichotomy in Obese Women with Gestational Diabetes and Their Macrosomic Babies. J Diabetes Res. 2018;2018:1-7. doi:10.1155/2018/8474617.

8. Hope O, Ifeanyi OE, N AQB. Investigation of Some Haematological Parameters in Pregnant Women with Gestational Diabetes at Federal Medical Center, Owerri, Imo State, Nigeria. Ann Clin Lab Res 2019; 7.

9. Guermouche B, Yessoufou A, Soulimane N, Merzouk H, Moutairou K, Hichami A, Khan NA. n-3 Fatty Acids Modulate T-Cell Calcium Signaling in Obese Macrosomic Rats. Obes Res 2004; 12:1744–1753.

10. Soulimane-Mokhtari NA, Guermouche B, Yessoufou A, Saker M, Moutairou K, Hichami A, Merzouk H, Khan NA. Modulation of lipid metabolism by n−3 polyunsaturated fatty acids in gestational diabetic rats and their macrosomic offspring. Clin Sci 2005; 109:287–295.

11. Yessoufou A, Soulaimann N, Merzouk SA, Moutairou K, Ahissou H, Prost J, Simonin AM, Merzouk H, Hichami A, Khan NA. N-3 Fatty acids modulate antioxidant status in diabetic rats and their macrosomic offspring. Int J Obes 2006; 30:739–750.

12. Yessoufou A, Moutairou K. Maternal Diabetes in Pregnancy: Early and Long-Term Outcomes on the Offspring and the Concept of "Metabolic Memory". Exp Diabetes Res. 2011;2011:218598. doi: 10.1155/2011/218598. Epub 2011 Nov 21. Review.

13. Nekoua MP, Fachinan R, Atchamou AK, Nouatin O, Amoussou-Guenou D, Amoussou-Guenou MK, Moutairou K, Yessoufou A. Modulation of immune cells and Th1/Th2 cytokines in insulin-treated type 2 diabetes mellitus. Afr Health Sci 2016; 16:712.

14. Nekoua MP, Fachinan R, Fagninou A, Alidjinou EK, Moutairou K, Hober D, Yessoufou A. Does control of glycemia regulate immunological parameters in insulin-treated persons with type 1 diabetes? Diabetes Res Clin Pract 2019; 157:107868.

15. Akboga MK, Canpolat U, Sahinarslan A, Alsancak Y, Nurkoc S, Aras D, Aydogdu S, Abaci A. Association of serum total bilirubin level with severity of coronary atherosclerosis is linked to systemic inflammation. Atherosclerosis 2015; 240:110–114.

16. Balci KG, Balci MM, Sen F, Canpolat U, Akboga MK, Unal S, Kara M, Maden O, Selcuk H, Selcuk T. The role of baseline indirect inflammatory markers in prediction of response to cardiac resynchronisation therapy. Kardiol Pol 2015:119–126.

17. Lim HJ, Seo MS, Shim JY, Kim KE, Shin YH, Lee YJ. The association between platelet count and metabolic syndrome in children and adolescents. Platelets 2015; 26:758–763.

18. Ertem AG, Yayla C, Acar B, Kirbas O, Unal S, Uzel Sener M, Akboga MK, Efe TH, Sivri S, Sen F, Gokaslan S, Topaloglu S. Relation between lymphocyte to monocyte ratio and short-term mortality in patients with acute pulmonary embolism. Clin Respir J 2018; 12:580–586.

19. Jiwani A, Marseille E, Lohse N, Damm P, Hod M, Kahn JG. Gestational diabetes mellitus: results from a survey of country prevalence and practices. J Matern Fetal Neonatal Med 2012; 25:600–610.

20. Eades CE, Cameron DM, Evans JMM. Prevalence of gestational diabetes mellitus in Europe: A meta-analysis. Diabetes Res Clin Pract 2017; 129:173–181.

21. Lee KW, Ching SM, Ramachandran V, Yee A, Hoo FK, Chia YC, Wan Sulaiman WA, Suppiah S, Mohamed MH, Veettil SK. Prevalence and risk factors of gestational diabetes mellitus in Asia: a systematic review and meta-analysis. BMC Pregnancy Childbirth 2018; 18.

22. Ogurtsova K, da Rocha Fernandes JD, Huang Y, Linnenkamp U, Guariguata L, Cho NH, Cavan D, Shaw JE, Makaroff LE. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. Diabetes Res Clin Pract 2017; 128:40–50.

23. England LJ, Dietz PM, Njoroge T, Callaghan WM, Bruce C, Buus RM, Williamson DF. Preventing type 2 diabetes: public health implications for women with a history of gestational diabetes mellitus. Am J Obstet Gynecol 2009; 200:365.e1-365.e8.

24. Yang H, Wei Y, Gao X, Xu X, Fan L, He J, Hu Y, Liu X, Chen X, Yang Z, Zhang C, on behalf of China National GDM Survey Working Group. Risk factors for gestational diabetes mellitus in Chinese women-a prospective study of 16 286 pregnant women in China. Diabet Med 2009; 26:1099–1104.

25. Opara PI, Jaja T, Onubogu UC. Morbidity and mortality amongst infants of diabetic mothers admitted into a special care baby unit in Port Harcourt, Nigeria. Ital J Pediatr 2010; 36:77.

26. IADPSG Consensus Panel. International association of diabetes and pregnancy study groups (IADPSG) recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes Care 2010; 33:676–682.

27. Benlian P, Béréziat G. Une nouvelle méthode de dosage direct du LDL cholestérol. Revue Française des Laboratoires 2001; 2001:68–69.

28. Pirson N, Maiter D, Alexopoulou O. Prise en charge du diabète gestationnel en 2016 : une revue de la littérature Louvain Med 2016; 135(10), 661-668.

29. Simmons D. Diabetes and obesity in pregnancy. Best Pract Res Clin Obstet Gynaecol 2011; 25:25–36.

30. Ogoudjobi OM, HS Lokossou MS, Tognifode V, Aboubakar M, Kerekou A, Tandjiekpon E, Denakpo JL, Perrin RX. Randomized Study Comparing Two Gestational Diabetes Screening Strategies in a Reference Maternity Hospital in Southern Benin. Clin Mother Child Health 2018; 15.

31. Touzet S, Rocher L, Dureau-Drevard E, Poncet B, Colin C, Orgiazzi J, Berland M. Étude d'observation des pratiques de dépistage du diabète gestationnel à partir d'une cohorte de 701 femmes. J Gynecol Obstet Biol Reprod 2002; 31, 248-255.

32. Damoune I, Damoune I, El Ouahabi H, Ajdi F. P51 Facteurs de risque du diabète gestationnel à propos de 100 cas. Diabetes Metab 2014; 40:A42.

33. International Diabetes Federation. IDF Diabetes atlas. Brussels: International Diabetes Federation, Executive Office; 2013.

34. Horsch A, Gross J, Jornayvaz FR, Lanzi S, Puder JJ. Gestational diabetes{what are the non-medical approaches? Revue medicale suisse 2016; 521:1089–91.

35. Djagadou KA, Tchamdja T, Némi KD, Balaka A, Djibril MA. Aspects diagnostiques, thérapeutiques et pronostiques du diabète gestationnel au Centre Hospitalier Universitaire Sylvanus Olympio. Pan Afr Med J 2019; 34:1–5.

36. Vambergue A, Fontaine P, Puech F. Physiopathologie du diabète gestationnel, J Gynecol Obstet Biol Reprod 2019; 31(6), 4S3-4S10.

37. Mankuta D, Elami-Suzin M, Elhayani A, Vinker S. Lipid profile in consecutive pregnancies. Lipids Health Dis 2010; 9:58.

38. Mahmoud F, Abul H, Omu A, Haines D. Lymphocyte Sub-populations in Gestational Diabetes. Am J Reprod Immunol 2005; 53:21–29.

39. Viardot A, Grey ST, Mackay F, Chisholm D. Potential Antiinflammatory Role of Insulin via the Preferential Polarization of Effector T Cells toward a T Helper 2 Phenotype. Endocrinology 2007; 148:346–353.

40. Ryckman K, Spracklen C, Smith C, Robinson J, Saftlas A. Maternal lipid levels during pregnancy and gestational diabetes: a systematic review and meta-analysis. BJOG Int J Obstet Gynaecol 2015; 122:643–651.

41. Shen H, Liu X, Chen Y, He B, Cheng W. Associations of lipid levels during gestation with hypertensive disorders of pregnancy and gestational diabetes mellitus: a prospective longitudinal cohort study. BMJ Open 2016; 6:e013509.

42. Amraei A, Azemati M. Metabolic status of women with gestational diabetes mellitus six months after delivery. Research Journal of Biological Sciences 2007; 2:104–107.

43. Aziz R, Mahboob T. Lipid profile and serum insulin levels in gestatio. Journal of the Dow University of Health Sciences 2008; 2:102–106.

44. Eadala Suresh, GBVVSV Prasad. Study of biochemical changes in gestational diabetes mellitus. International Journal of Applied Research 2017; 3:03–08.

45. Herrera Martínez A. Hyperlipidemia during gestational diabetes and its relation with maternal and offspring complications. Nutr Hosp 2018.

46. Butte NF. Carbohydrate and lipid metabolism in pregnancy: normal compared with gestational diabetes mellitus. Am J Clin Nutr 2000; 71:1256S-1261S.

47. Murphy SP, Abrams BF. Changes in energy intakes during pregnancy and lactation in a national sample of US women. Am J Public Health 1993; 83:1161–1163.

48. Di Cianni G, Miccoli R, Volpe L, Lencioni C, Ghio A, Giovannitti MG, Cuccuru I, Pellegrini G, Chatzianagnostou K, Boldrini A, Del Prato S. Maternal triglyceride levels and newborn weight in pregnant women with normal glucose tolerance. Diabet Med 2005; 22:21–25.

49. Ghio A, Bertolotto A, Resi V, Volpe L, Di Cianni G. Triglyceride metabolism in pregnancy. Advances in Clinical Chemistry, vol. 55. Elsevier; 2011:133–153.

50. Herrera E, Lasunción MA, Gomez-Coronado D, Aranda P, López-Luna P, Maier I. Role of lipoprotein lipase activity on lipoprotein metabolism and the fate of circulating triglycerides in pregnancy. Am J Obstet Gynecol 1988; 158:1575–1583.

51. Meyer BJ, Stewart FM, Brown EA, Cooney J, Nilsson S, Olivecrona G, Ramsay JE, Griffin BA, Caslake MJ, Freeman DJ. Maternal Obesity Is Associated With the Formation of Small Dense LDL and Hypoadiponectinemia in the Third Trimester. J Clin Endocrinol Metab 2013; 98:643–652.

52. Kim S, Nguon C, Guillard B, Duong S, Chy S, Sum S, Nhem S, Bouchier C, Tichit M, Christophel E, Taylor WRJ, Baird JK, et al. Performance of the CareStart^{TM} G6PD deficiency screening test, a point-of-care diagnostic for primaquine therapy screening. PloS One 2011; 6:e28357.

53. Demirtas L, Degirmenci H, Akbas EM, Ozcicek A, Timuroglu A, Gurel A, Ozcicek F. Association of hematological indicies with diabetes, impaired glucose regulation and microvascular complications of diabetes. Int J Clin Exp Med 2015; 8:11420–11427.

54. Yılmaz ZV, Yılmaz E, İçer B, Küçüközkan T. Association of Complete Blood Count Parameters with Gestational Diabetes Mellitus. Gynecol Obstet Reprod Med 2016; 23:1–5.

55. Murphy AJ, Woollard KJ, Hoang A, Mukhamedova N, Stirzaker RA, McCormick SPA, Remaley AT, Sviridov D, Chin-Dusting J. High-Density Lipoprotein Reduces the Human Monocyte Inflammatory Response. Arterioscler Thromb Vasc Biol 2008; 28:2071–2077.

56. Rohatgi A. High-Density Lipoprotein Function Measurement in Human Studies: Focus on Cholesterol Efflux Capacity. Prog Cardiovasc Dis 2015; 58:32–40.

57. Lin D, Qi Y, Huang C, Wu M, Wang C, Li F, Yang C, Yan L, Ren M, Sun K. Associations of lipid parameters with insulin resistance and diabetes: A population-based study. Clin Nutr 2018; 37:1423–1429.

58. Chen T, Chen H, Xiao H, Tang H, Xiang Z, Wang X, Wang X, Zou H. Comparison of the Value of Neutrophil to High-Density Lipoprotein Cholesterol Ratio and Lymphocyte to High-Density Lipoprotein Cholesterol Ratio for Predicting Metabolic Syndrome Among a Population in the Southern Coast of China. Diabetes Metab Syndr Obes Targets Ther 2020; Volume 13:597–605.

59. Pattanathaiyanon P, Phaloprakarn C, Tangjitgamol S. Comparison of gestational diabetes mellitus rates in women with increased and normal white blood cell counts in early pregnancy: White blood cells and gestational diabetes. J Obstet Gynaecol Res 2014; 40:976–982.

60. Wolf M, Sandler L, Hsu K, Vossen-Smirnakis K, Ecker JL, Thadhani R. First-Trimester C-Reactive Protein and Subsequent Gestational Diabetes. Diabetes Care 2003; 26:819–824.

Figure 1

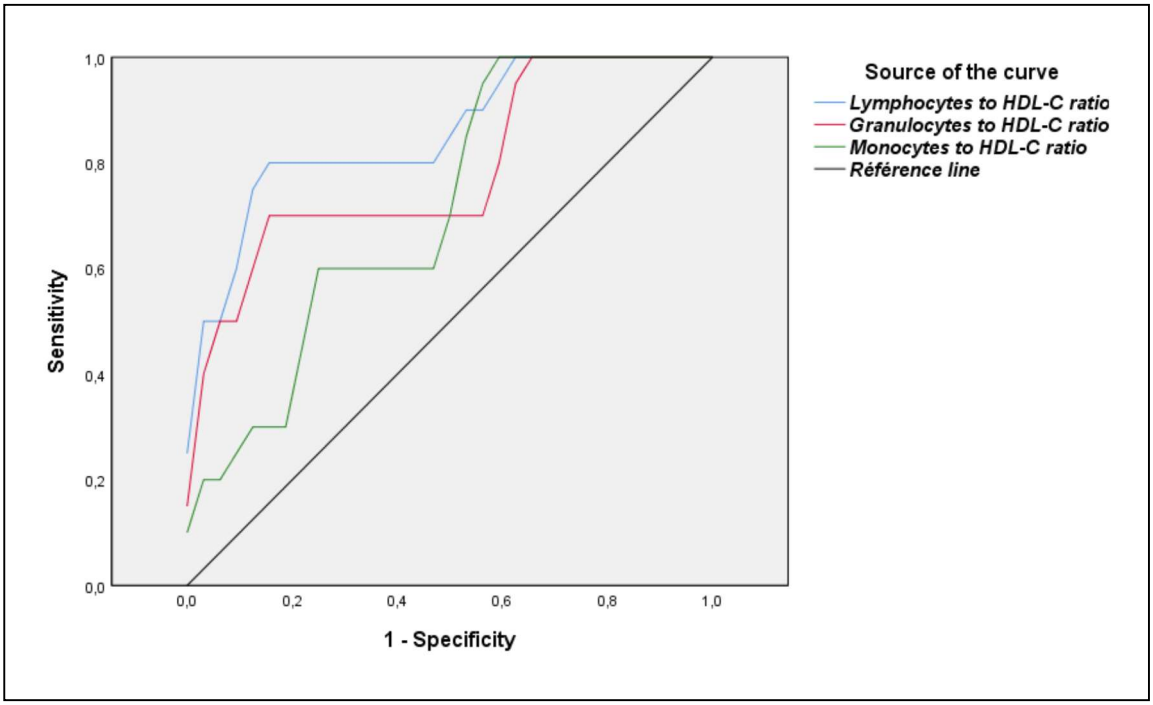

Figure 1: Receiver Operating Characteristics (ROC) curve analysis of the value of ratios, lymphocytes to HDL-C, granulocytes to HDL-C and monocytes to HDL-C for predicting gestational diabetes mellitus in pregnant women. HDL-C = high-density lipoprotein - cholesterol.

Table 1: Prevalence of GDM and socio-demographic profiles and various factors of women with GDM and pregnant controls

Characteristics	Pregnant control women		Women with GDM		Total
	Number	Percentage (%)	Number	Percentage (%)	
Number of subjects	185	88.10	25	11.90	210
Age (years)					
< 20	05	2.70	00	00	05
[20 ; 30[	104	56.22	09	36	113
[30 ; 40[	71	38.38	15	60	86
≥ 40	05	2.7	01	04	06
Physical activity					
Intense	00	00	00	00	00
Moderate	136	73.51	17	68	153
Inadequate	49	26.49	08	32	57
Menstruation					
Regular	80	43.24	10	40	90
Irregular	105	56.76	15	60	120
Number of children					
0	50	27.02	02	08	52
[1 ; 2]	97	52.44	07	28	104
≥ 3	38	20.54	16	64	54
Previous disturbances					
Miscarriage	44	23.78	12	48	56
Prematurity	07	3.78	04	16	11
Normal delivery	134	72.43	09	36	143

Gestational diabetes mellitus (GDM) was diagnosed in pregnant women following the protocol of International Association of Diabetes and Pregnancy Study Group (IADPSG). Using overnight fasting glucose and OGTT test in pregnant women between 24 and 28 weeks of gestation Subjects were declared as positive for GDM positive when overnight fasting plasma glucose was ≥ 92 mg/dL (5.1 mmol/L), or 1-hour OGTT plasma glucose level was ≥ 180 mg/dL (10.0 mmol/L), or 2-hours OGTT plasma glucose level was ≥ 153 mg/dL (8.5 mmol/L). Moderate physical activity = 30 minutes of physical activity per day; Inadequate physical activity = insufficient or no physical activity.

Table 2: Biochemical parameters in women with GDM and pregnant controls

Parameters	Pregnant control women (n = 35)	Women with GDM (n = 25)	p-value
Glucose (g/L)	0.81 ± 0.03	1.16 ± 0.04	**0.001**£
HbA_{1c} (%)	5.65 ± 0.23	6.47 ± 0.48	0.451£
TC (g/L)	1.53 ± 0.11	2.11 ± 0.31	**0.001**£
HDL-C (g/L)	1.21 ± 0.15	0.35 ± 0.10	**0.001**£
LDL-C (g/L)	0.91 (0.14-1.36)	1.53 (1.15-1.68)	**0.015**¥
TG (g/L)	1.30 ± 0.08	1.63 ± 0.18	**0.035**£
AST (UI/L)	26.63 ± 2.64	26.20 ± 6.46	0.951£
ALT (UI/L)	10.50 (9.75-15.25)	14.00 (12.00-20.00)	0.425¥
Creatinine (mg/L)	7.30 ± 0.45	8.92 ± 0.30	0.125£
Total proteins (g/L)	71.60 ± 1.585	82.23 ± 3.32	**0.005**£

Total Cholesterol (TC); HDL-Cholesterol (HDL-C); LDL-Cholesterol (LDL-C); Triglycerides (TG), Friedewald method was used to calculate LDL-cholesterol. LDL-C = CT – (HDL-C+TG/5) in g/l. This method is valid as the TG levels were under 4 g/l in the present study. Aspartate aminotransferase (ASAT); Alanine aminotransferase (ALAT). Statistical analyses were performed using the Student's t-test (£) or Mann-Whitney test (¥). *p* values < 0.05 indicate significant differences.

Table 3: Immunological and hematological parameters in women with GDM and pregnant controls

Parameters	**Pregnant control women** (n = 3**5)**	**Women with GDM (n = 25)**	**p-value**
RBC (10^{12}/L)	4.07 ± 0.08	4.00 ± 0.15	0.696£
Hb (g/dL)	11.38 ± 0.22	11.08 ± 0.35	0.552£
Ht (%)	33.00 (31.60-33.80)	34.50 (27.35-35.75)	0.263¥
MCV (fL)	83.21 ± 1.03	80.55 ± 1.81	0.393£
MCHC (%)	33.72 ± 0.25	34.48 ± 0.28	0.179£
MCH (pg)	28.07 ± 0.56	26.42 ± 1.28	0.220£
WBC (10^9/L)	5.84 ± 0.21	6.98 ± 0.80	**0.045**£
Lymphocytes (10^9/L)	1.78 ± 0.43	2.29 ± 0.34	**0.015**£
Monocytes (10^9/L)	0.37 ± 0.13	0.53 ± 0.12	0.459£
Granulocytes (10^9/L)	3.86 ± 0.75	4.48 ± 0.32	0.974£
Platelets (10^9/L)	206.07 ± 13.30	248.40 ± 25.53	**0.033**£

RBC (Red Blood Cell Count); Hb (Hemoglobin); Ht (Hematocrit); MCV (Mean Cell Volume); MCHC (Mean Cell Hemoglobin Concentration); MCH (Mean Cell Hemoglobin); WBC (White Blood Cell Count). Statistical analyses were performed using the Student's t-test (£) or Mann-Whitney test (¥). *p* values < 0.05 indicate significant differences.

Table 4: Correlations between immune cell subpopulations and metabolic parameters in pregnant control women

Immune cell subtypes	**Glucose**		**TC**		**HDL-C**		**LDL-C**		**TG**	
	r	p	*r*	p	*r*	p	*r*	p	*r*	p
White blood cells	-0.50	0.45	0.37	0.22	-0.19	0.46	-0.42	0.09	0.37	0.24
Lymphocytes	0.89	0.03*	0.50	0.04*	0.36	0.17	0.11	0.68	0.16	0.73
Monocytes	-0.45	0.40	-0.43	0.08	-0.68	0.007*	0.36	0.17	0.58	0.04*
Granulocytes	-0.81	0.07	-0.27	0.30	0.22	0.39	-0.25	0.35	-027	0.39
Platelets	0.68	0.08	-0.33	0.20	-0.36	0.18	0.20	0.45	0.33	0.27

Total cholesterol (TC); HDL cholesterol (HDL-C); LDL cholesterol (LDL-C) triglycerides (TG). Spearman or Pearson correlation tests were used when appropriate. * p values < 0.05 indicate significant differences.

Table 5: Correlations between immune cell subpopulations and metabolic parameters in women with GDM

Immune cell subtypes	Glucose		TC		HDL-C		LDL-C		TG	
	r	p	*r*	p	*r*	p	*r*	p	*r*	p
White blood cells	0.7	0.03*	0.20	0.76	0.66	0.26	0.61	0.30	0.21	0.76
Lymphocytes	0.6	0.02*	0.12	0.99	0.81	0.13	-0.12	0.95	0.16	0.95
Monocytes	-0.23	0.66	0.66	0.28	0.37	0.15	0.66	0.26	0.87	0.04*
Granulocytes	0.72	0.23	-0.48	0.5	-0.9	0.02*	-0.41	0.51	-0.2	0.78
Platelets	-0.51	0.45	0.73	0.23	0.8	0.13	0.70	0.23	0.11	0.97

Total cholesterol (TC); HDL cholesterol (HDL-C); LDL cholesterol (LDL-C) triglycerides (TG). Spearman or Pearson correlation tests were used when appropriate. * p values < 0.05 indicate significant differences.

Table 6: Ratios between immune cells to biochemical parameters in women with GDM and pregnant controls

Variables	**Pregnant control women** (n = 35**)**	**Women with GDM (n = 25)**	**p-value**
Lymphocytes to Glucose ratio	2.48 ± 0.43	2.03 ± 0.59	0.125
Lymphocytes to HDL-C ratio	1.65 ± 0.86	7.38 ± 3.21	**0.001***
Lymphocytes to LDL-C ratio	2.53 ± 0.58	1.62 ± 0.22	0.591
Lymphocytes to TG ratio	1.50 ± 0.27	1.25 ± 0.60	0.469
Monocytes to Glucose ratio	0.34 ± 0.09	0.47 ± 0.13	0.424
Monocytes to HDL-C ratio	0.39 ± 0.05	1.73 ± 0.49	**0.009***
Monocytes to LDL-C ratio	0.41 ± 0.27	0.39 ± 0.15	0.701
Monocytes to TG ratio	0.25 ± 0.07	0.34 ± 0.09	0.117
Granulocytes to Glucose ratio	5.13 ± 1.24	3.33 ± 0.73	0.082
Granulocytes to HDL-C ratio	3.55 ± 2.02	14.18 ± 5.70	**0.004***
Granulocytes to LDL-C ratio	5.16 ± 3.42	3.27 ± 0.95	0.657
Granulocytes to TG ratio	3.17 ± 0.61	2.91 ± 0.73	0.229

Total cholesterol (TC); HDL cholesterol (HDL-C); LDL cholesterol (LDL-C) triglycerides (TG). Spearman or Pearson correlation tests were used when appropriate. * p values < 0.05 indicate significant differences.

Table 7: Areas Under the ROC Curve (AUC), Sensitivity and Specificity by the Optimized Cutoff Points for lymphocytes to HDL-C ratio, granulocytes to HDL-C ratio and monocytes to HDL-C ratio in predicting GDM.

Risk factor	AUC (95% CI)	Cutoff According to Youden's Index	Sensitivity (%)	Specificity (%)
Lymphocytes to HDL-C ratio	0.859 (0.752-0.966)	3.66	80.0	50.1
Granulocytes to HDL-C ratio	0.787 (0.654-0.921)	5.50	70.3	59.4
Monocytes to HDL-C ratio	0.716 (0.576-0.855)	1.56	60.9	50.0

ROC: Receiver operating characteristics; AUC: Area under ROC curve; CI: Confidence interval.

Table 8: Odds ratio (OR) of independent predictors accessing risk of gestational diabetes mellitus in women with GDM and pregnant controls women.

Variable	**OR (95% CI)**	***p*-value**
Lymphocytes to HDL-C ratio	4.000 (1.094 – 14.630)	0.041
Granulocytes to HDL-C ratio	1.596 (0.486 to 5.241)	0.557
Monocytes to HDL-C ratio	1.500 (0.483 – 4.652)	0.572

Figures

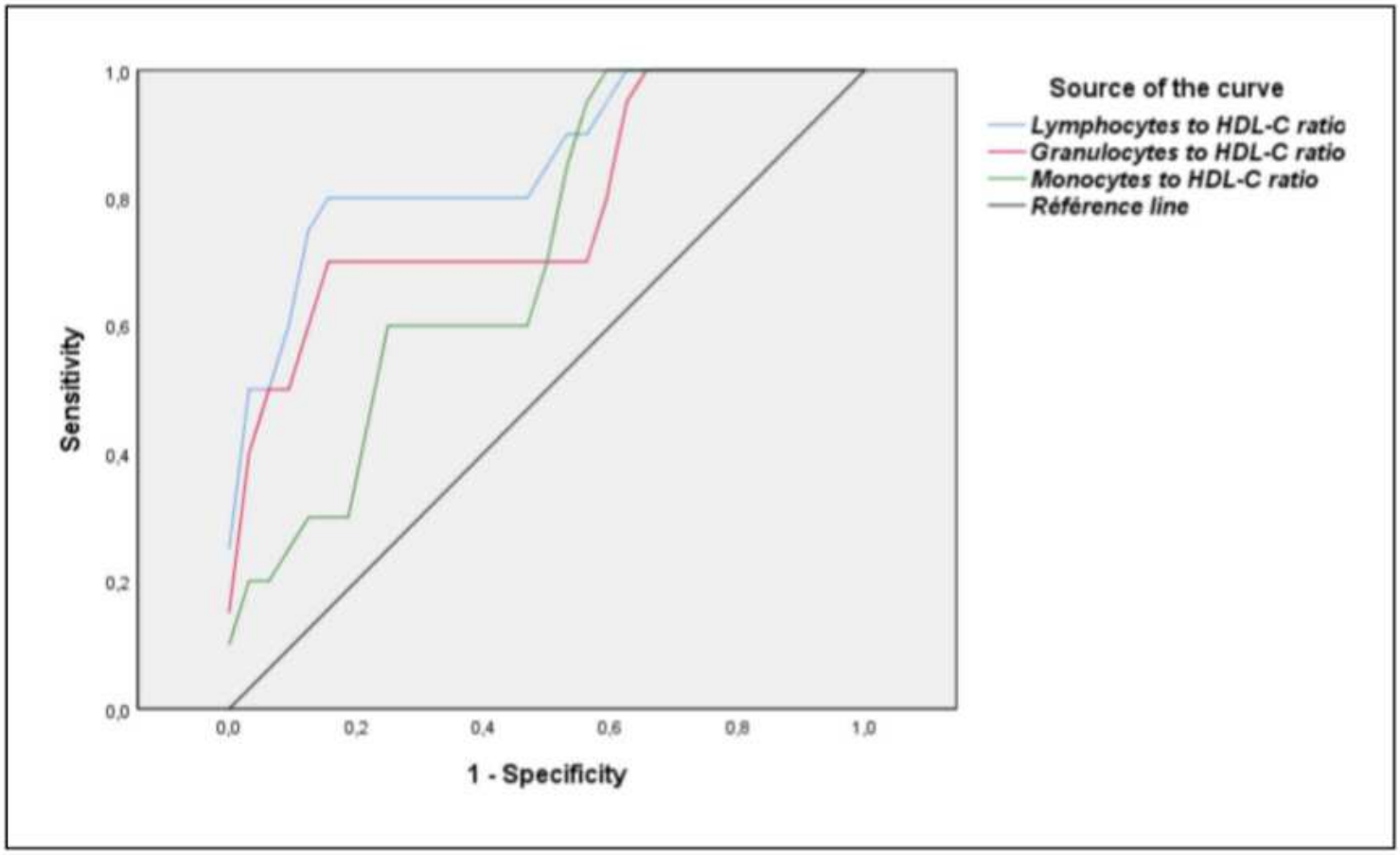

Figure 1

ROC curve analysis of the value of lymphocytes to high-density lipoprotein ratio, granulocytes to high-density lipoprotein ratio and monocytes to high-density lipoprotein ratio for predicting gestational diabetes mellitus in pregnant women.