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Establishing Reference Intervals for HBA1C in all three Trimesters of Pregnancy; A Cross-Sectional Study on Healthy Pregnant Women of Quetta, Baluchistan

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ABSTRACT

Objective: To establish the reference intervals in healthy pregnant females of Quetta, Baluchistan, for Glycosylated Hemoglobin (HbA1c) in all three trimesters of pregnancy.

Study Design: Cross-sectional study.

Place and Duration of Study: Pathology Department Combined Military Hospital, Quetta Pakistan, from Jun 2018 to Jun 2019.

Methodology: Blood samples of healthy pregnant females were drawn for Glycosylated haemoglobin (HbA1c). Fasting plasma glucose and haemoglobin were also measured to rule out hyperglycemia and anaemia. Samples for Glycated haemoglobin (HbA1c) were analyzed by Turbidimetric Immuno-inhibition (TINIA) method. For all the trimesters, 5th and 95th percentiles were taken as reference intervals for Glycosylated haemoglobin (HbA1c) and compared for each trimester.

Results: A total of 388 samples were taken, of which 136(35.05%) females were from the first trimester, 128(32.98%) and 124(31.97%) from the second and third trimesters, respectively. The mean age of the study population was 25.1±3.7 years in the first trimester, 26.7±4.5 years in the second-trimester while and the third trimester it was 26.8±4.8 years. In the first, second, and third trimesters, the reference intervals for Glycosylated haemoglobin (HbA1c) were 3.8-5.2%, 4.1-5.4%, and 4.2-5.7%, respectively.

Conclusion: For the exact diagnosis of hyperglycemia in pregnancy, each laboratory should establish its reference intervals of Glycated haemoglobin (HbA1c) for each trimester as it varies from trimester to trimester.

Keywords: Glycated hemoglobin (HbA1c), Pregnancy, Reference interval.

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INTRODUCTION

Diabetes in pregnancy is linked with a high risk of complications both in mother and fetus, such as congenital malformations, macrosomic and large-for-date babies, hypertensive disorders, stillbirths, increased rate of cesarean births, and neonatal morbidity.[1] According to the International Diabetes Federation (IDF), in 2019, an estimated 223 million females were living with diabetes. Hyperglycemia affects 1 out of 6 live births during pregnancy.[2] A previous study reported a high incidence of maternal and neonatal complications in women having gestational diabetes mellitus.[3] Adverse perinatal and maternal outcomes may be reduced by having strict glycemic control during pregnancy.[4]

American Diabetes Association (ADA) recommends the use of self-blood glucose monitoring (BGS) and HbA1c both by patients and health care providers to monitor glycemic control during pregnancy.[5] Levels of HbA1c have been found to change throughout normal pregnancy. Moreover, many physiological changes in pregnancy should be considered when interpreting HbA1c results.[6] For example, decreased erythrocytes half-life and increased red cell turnover during pregnancy cause decreased HbA1c levels.[7] HbA1c values also decrease due to a decrease in mean blood glucose value pre and postprandially, early in the pregnancy.[6] While the higher pre and postprandial values of mean blood glucose in the third trimester cause increase in HbA1c values during the third trimester.[8]

HbA1c can be used during pregnancy to monitor hyperglycemia. Both long-term glycemic control and complications can be monitored by measuring HbA1c, which correlates well with chronic hyperglycemia. Furthermore, it is also an easy-to-perform laboratory test, as a fasting state is not required. Trimester-specific HbA1c reference intervals are sensitive for screening of hyperglycemia in pregnancy.[9] In this study, we aimed to establish reference intervals for HbA1c during the three trimesters of pregnancy in the healthy pregnant female population of Quetta, Baluchistan.

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METHODOLOGY

The cross-sectional study was conducted at the Department of Pathology, Combined Military Hospital, Quetta Pakistan for one year, (June 2018 to June 2019) after approval from the Ethical Review Committee (QTA IRB Approval#037). Non-probability consecutive sampling technique was used for the selection of subjects. Open Epi software was used to calculate the sample size of 303 (Confidence level=95%, and reference population=27%,[10] but we included all 388 patients who volunteered to be enrolled in our study.

Inclusion Criteria: All healthy pregnant women having fasting plasma glucose <5.1 mmol/l and with single intrauterine gestation confirmed on Ultrasonography were enrolled in the study.

Exclusion Criteria: Pregnant females having diabetes mellitus, other co-morbid conditions including hypertension, chronic kidney, liver disease, pulmonary or cardiac pathology and females with first degree relatives having diabetes mellitus were excluded from our study.

Gestational age was confirmed by the date of the last menstrual period (LMP) along with confirmation on Ultrasonography. After taking written informed consent, blood samples were taken by an experienced laboratory technician for HbA1c and haemoglobin estimation in the EDTA tube. In addition, a fasting plasma glucose sample was taken in a sodium fluoride tube. Both HbA1c and plasma glucose levels were analyzed on Cobas 501. The Hexokinase method was used for plasma glucose analysis, while HbA1c was analyzed by the Turbidimetric Immuno-inhibition method. Haemoglobin levels were determined to exclude anaemia which was performed on Sysmex XP 100 by Cyanide free Sodium Lauryl Sulphate method.

Data were analyzed using Statistical Package for Social Sciences (SPSS) version 21 and Microsoft Excel. Data were expressed as Mean±SD and Median (Interquartile range). Reference intervals were taken as 5th & 95th percentiles in each trimester of pregnancy.

RESULTS

Out of the total 388 pregnant women, 136(35.0%) were in their first trimester, 128(32.98%) were in the second, and 124(31.9%) were in the third trimester. The mean age of the study population was 25.1±3.7 years in the first trimester, 26.7±4.5 years in the second-trimester while and the third trimester it was 26.8±4.8 years. In the first, second, and third trimesters, the reference intervals for Glycosylated haemoglobin (HbA1c) were 3.8-5.2%, 4.1-5.4%, and 4.2-5.7%, respectively. HbA1c, fasting plasma glucose levels, haemoglobin levels, and age of the study subjects in each trimester were shown in Table-I. The upper reference value was 5.2 % in the first trimester, 5.4 in the second and 5.7 % in the third trimester (Table-II).

Table I: Characteristics of the Study Population (n=388)

Characteristics	First Trimester	Second Trimester	Third Trimester
Age (years)			
(Mean± SD)	25.1±3.7	26.7±4.5	26.8±4.8
Hemoglobin(g/dl) (Mean± SD)	12.4±0.9	12.0±0.6	11.7±0.8
HbA1c (%) (Mean± SD)	4.5±0.4	4.7±0.3	4.9±0.4
Fasting Plasma Glucose (mmol/l) (Mean± SD)	4.4±0.5	4.7±0.4	4.8±0.4

Table-II: Values of HbA1c in three Trimesters of Pregnancy (n=388)

Trimester	Mean ±SD	Median (Inter Quartile Range)	5th Percentile	95th Percentile	Reference Intervals
First	4.5±0.4	4.7(0.6)	3.8	5.2	3.8-5.2
Second	4.7±0.3	4.7(0.5)	4.1	5.4	4.1-5.4
Third	4.9±0.4	4.9(0.5)	4.2	5.7	4.2-5.7

DISCUSSION

Hyperglycemia during pregnancy is an established risk factor for the development of Diabetes Mellitus after pregnancy and the development of gestational Diabetes Mellitus in future pregnancy.[11] Moreover, hyperglycemia in gestation also affects the fetal outcome and may cause post-natal complications e.g. decreased birth weight and increased need and duration for neonatal intensive care unit (NICU) admission.[12] Adverse pregnancy outcomes might be predicted using HbA1c as a helpful screening tool for patients at increased risk (Selective Screening).[13] Hinkle *et al.* reported that pregnant women with higher values of HbA1c in their first trimester developed gestational diabetes mellitus in the second or third trimester, which suggested impaired glucose metabolism before conception or in their early pregnancy.[14] Several studies have reported that by maintaining tight control over blood glucose, the complications caused by gestational hyperglycemia can be reduced, and outcomes can be quite similar to normoglycemic pregnant women.[15-17] Radder *et al.* recommended

keeping the HbA1c levels at less than 5% in the first trimester and less than 6% in the third trimester to reduce fetal complications.[18] In our study, the lower value of HbA1c was significantly less in pregnant women, i.e. 3.8 % in the first trimester, 4.1% in the second trimester and 4.2 % in the third trimester.

Upper reference intervals for HbA1c in our study population were 5.2%, 5.4%, and 5.7% in the first, second, and third trimesters respectively. HbA1c increased from the first trimester to the second trimester and was highest in the third trimester. Versantvoort *et al*. reported a significant increase of HbA1c from the first to the third trimester, which is similar to our study.[19] However, Hiramatsu *et al*. reported a decrease in HbA1c in the second trimester.[20]. Mosca *et al*. compared levels of HbA1c between pregnant women (n=445) and the control group (n=384). They reported lower values of the upper reference limit of HbA1c in pregnant women compared to the non-pregnant control group. In their study, HbA1c results at different gestational periods for non-diabetic pregnant women at 15-24, 25-27, and 28-36 weeks were 3.8-5.5%, 4.0-5.5% and 4.4-5.5%, respectively.[21] Shobha *et al.* performed a study to measure HbA1c levels in non-diabetic pregnant women and reported a reference range of 4.5% to 6% HbA1c values in the third trimester of pregnancy.[22] Ismail *et al*. compared HbA1c in healthy pregnant and non-pregnant Sudanese women and documented that the mean concentration of the HbA1c in the pregnant women's group was (4.407±1.044) % in the first trimester (4.797±0.621) % in the second trimester and (4.823±0.616) % in the third trimester compared to (5.660±0.461%) in the non-pregnant women group with a *P* value of 0.00.[23] González *et al*. carried out a cross-sectional study on disease-free pregnant Mexican women. They reported 5.6% and 5.5% as upper reference limits for HbA1c for the first and second trimesters, respectively, which were higher than the values reported in our study. He reported 5.6% as the upper reference limit of HbA1c for the third trimester, which is lower than our third-trimester upper reference value.[24] O'Connor *et al*. from Ireland, reported an upper reference limit of HbA1c as 5.4% in the first and second trimesters and 5.7% during the third trimester of pregnancy which is very close to our reported upper reference limit for this trimesters.[25] A comparison of reference Intervals for HbA1c reported in our study with other studies in the literature was shown in Table-III.

Table-III: Comparison of Reference Intervals for HbA1c (%) with literature

	First Trimester	Second Trimester	Third Trimester
Present Study (Quetta, Balochistan)	3.8-5.2	4.1-5.4	4.2-5.7
González *et al*.[Error! Bookmark not defined.] (Mexican)	4.5–5.6	4.4–5.5	4.5–5.6
O'Connor *et al*.[Error! Bookmark not defined.] (Ireland)	4.3–5.4	4.4–5.4	4.7–5.7

LIMITATIONS OF STUDY

There were a few limitations to our study. We were not able to report the BMI of the study population. Non-diabetic status of the study group was not assessed by using OGTT (gold standard diagnostic test for Gestational Diabetes Mellitus), and the fasting blood glucose test was used instead. The strength of our study is the limited availability of similar literature for the population of Quetta, Balochistan. The results reported in our study can be considered during managing hyperglycemia in pregnant patients of Quetta and Balochistan in general.

CONCLUSION

Our study suggested that the upper reference limit for HbA1c in pregnancy might be lesser than the values used for the non-pregnant female population. It suggests that the diagnostic threshold for hyperglycemia in pregnancy must be lowered. Moreover, these reference values vary throughout pregnancy. Therefore, it is recommended to establish local trimester-specific reference intervals for HbA1c to prevent maternal and fetal complications.

Conflict of Interest: None.

Author's Contribution

Following authors have made substantial contributions to the manuscript as under:

AK & NA: Study design, drafting the manuscript, data interpretation, critical review, approval of the final version to be published.

RU & TAK: Data acquisition, data analysis, data interpretation, concept, approval of the final version to be published.

AK & SSM: Critical review, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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