Oral Glucose Tolerance Test, Hemoglobin Glycate and Fructosamine Blood Levels in Pregnancy

Marzuki Suryaatmadja, dr*; Anthony Susanto, dr*; Suzanna Immanuel, dr*; Husna Amelz, dr**; Gulardi H. Wiknjosastro, dr**

Abstrak

Pada kehamilan terjadi beberapa perubahan metabolisme karbohidrat untuk menunjang pertumbuhan dan perkembangan janin. Adanya diabetes gestasional akan meningkatkan angka penyulit baik bagi ibu maupun janinnya. Tujuan penelitian ini adalah untuk mendapatkan angka rujukan tes toleransi glukosa oral (TTGO), kadar hemoglobin glikat (HbA1) dan fruktosamin dalam darah dan perubahannya selama kehamilan trimester kedua dan ketiga. Sebagai kelompok studi telah diperiksa 53 wanita hamil, usia 20-30 tahun yang melakukan kunjungan untuk mendapatkan perawatan antenatal dan memenuhi kriteria. Pemeriksaan darah dilakukan 2 kali yaitu pada trimester kedua dan ketiga. Duapuluh lima wanita tidak hamil yang berusia sama diperiksa sebagai kelompok kontrol. TTGO dilakukan dengan beban 75 gram glukose dan kadar glukosa darah diperiksa dengan cara kalium ferisianida. Kadar HbA1 diukur dengan cara kromatografi mikrokolom dan kadar fruktosamin diukur dengan cara nitroblue tetrazolium tereduksi. Hasil pada kehamilan trimester kedua adalah : kadar glukosa puasa, 1 dan 2 jam setelah beban glukosa adalah masing-masing 2.56-4.73 (3.65), 6.22-7.52 (6.87) dan 5.23-6.31 (5.77). Kadar HbA1 adalah 3.7-5.2 (4.4)% dan kadar fruktosamin adalah 182.9-254.7 (218.8) µmol/l. Hasil pada kehamilan trimester ketiga adalah : kadar glukosa puasa, 1 dan 2 jam setelah beban glukosa masing-masing 2.56-5.52 (3.54), 7.02-8.24 (7.63) dan 5.78-7.39 (6.58). Kadar HbA1 4.8-6.7 (5.8)% dan kadar fruktosamin163.4-243.2 (203.3) µmol/l. Tidak ada perbedaan bermakna antara kadar glukosa darah puasa pada kehamilan trimester ke 2 dan ke 3. Pada kehamilan kadar glukosa darah, hemoglobin glikat dan fruktosamin lebih rendah dibandingkan kontrol tidak hamil; toleransi glukosa menurun, yang lebih nyata pada trimester ketiga.

Abstract

In pregnancy there are changes in carbohydrate metabolism; the presence of gestational diabetes will increase the complication rate for both mothers and fetuses. The objective of this study was to obtain reference values of the oral glucose tolerance test (OGTT), hemoglobin glycate (HbA1) and fructosamine blood levels and their changes during the second and third trimesters of pregnancy. Fifty-three pregnant women, 20 to 30 years of age, who made visits for antenatal care and fulfilled the criteria were chosen as the study group. Blood examination was carried out twice, i.e. in the 2nd and 3rd trimester of pregnancy. Twenty-five non pregnant women in the same age range were examined as a control group. OGTT were performed with a 75 gram glucose load, and blood glucose levels were measured using the potassium ferrycyanide reducing method with the Technicon Auto Analyzer-I. HbA1 levels were measured by microcolumn chromatography, and fructosamine levels were determined by the modified reducing nitroblue tetrazolium method. The results in the 2nd trimester of pregnancy were as follows: fasting blood glucoses 1 and 2 hours after glucose load were 2.56-4.73 (3.65), 6.22-7.52 (6.87) and 5.23-6.31 (5.77) mmol/l respectively. HbA1 levels were 3.7-5.2 (4.4%) and fructosamine levels were 182.9-254.7 (218.8) µmol/l. The results in the 3rd trimester of pregnancy were as follows: fasting blood glucoses 1 and 2 hours after glucose load were 2.56-5.52 (3.54), 7.02-8.24 (7.63) and 5.78- 7.39 (6.58) mmol/l respectively. HbA1 levels were 4.8-6.7 (5.8) % and fructosamine levels were 163.4-243.2 (203.3) µmol/l. There were no significant difference between fasting blood glucose levels in the 2nd and 3rd trimesters, and both values were lower than in the control group. There was a decrease of glucose tolerance during pregnancy, which was more obvious during the 3rd trimester. HbA1 and fructosamine levels were lower in pregnant women than in the control group. There were low correlations among HbA1, fructosamine and glucose values measured in the same period of pregnancy.

Keywords *:Oral glucose tolerance test, Hemoglobin glycate, Fructosamine, Pregnancy*

* *Department of Clinical Pathology, Faculty of Medicine, University of Indonesia / Dr Cipto Mangunkusumo Hospital, Jakarta, Indonesia*

** *Department of Obstetrics and Gynecology, Faculty of Medicine, University of Indonesia / Dr Cipto Mangunkusumo Hospital, Jakarta, Indonesia*

INTRODUCTION

In pregnancy, carbohydrate metabolism changes to support fetal growth and development and to prepare the fetus for the neonatal phase.[1,2] Some studies have shown that the presence of gestational diabetes will increase the complication rate for both the mother and the fetus.[1,3,4,5,6] To prevent complications, early detection of gestational diabetes is necessary.[7] The oral glucose tolerance test (OGTT) can be used as a tool to determine whether there are carbohydrate metabolism changes in pregnant as well as non-pregnant women.[2,4,8,9,10,11]

According to the WHO classification of diabetes mellitus (DM) in 1980, gestational diabetes together with DM dan impaired glucose tolerance (IGT) were included in the clinical group. Diagnostic criteria for gestational diabetes were made similar to those for DM in non-pregnant adult women.[12]

In 1964, Sullivan and Mahan suggested screening for gestational diabetes by evaluating the blood glucose level one hour afte 50 gram oral glucose load followed by OGTT with 100 g glucose load if the former test revealed a level above 140 mg/dl.[6,13,14,15,16,17] O'Sullivan as cited by Abell mentioned that with a 50 gram glucose load only 79% of gestational diabetic cases could be detected.[3,17]

At present the Department of Obstetrics and Gynecology of the Faculty of Medicine of the University of Indonesia and Dr Cipto Mangunkusumo Hospital is still using fasting and postprandial blood glucose as a screening method without defining the calorie load; this method may not detect all cases of diabetes. Mestman et al found 10,9% incidence of abnormal OGTT with normal fasting blood glucose in 558 pregnant women.[9] Besides that, there is no uniform understanding about the proper time for conducting the blood glucose evaluation on pregnant women to diagnose the presence of gestational diabetes.[2,10] At present the OGTT is administered only to doubtful cases or cases at risk.[3] Robert et al state that if OGTT is administered to only suspected cases, only 62% of gestational diabetic cases would be detected.[17] Therefore, the best way to detect all gestational diabetic cases is to perform OGTT to all pregnant women.[3]

It is inconvenient for patients to undergo the OGTT due to the fasting, the drinking of glucose, and the multiple venapunctures over 2 to 3 hours. Therefore other parameters to support a diagnosis of DM and to monitor patients during treatment are still being looked for[4,17]. Hemoglobin glycate (HbA1) and fructosamine tests have been developed. These tests are based on the fact that hyperglycemia will cause protein to undergo a non enzymatic glycosylation process[10,18]. This process correlates well with the elevation of the blood glucose level. Glycosylation of Hb and albumin will produce Hb glycate (HbA1) and fructosamine respectively. Both of them have a good correlation with blood glucose levels within a period of 6 to 8 weeks; consequently, they can be used to determine the glycemic state of diabetic patients.[19] It was also proven that 85% of gestational diabetic women have a higher fructosamine level than non-diabetic pregnant women.[17] Therefore, these two new tests might be useful as screening tests for diagnosing gestational diabetes.[10,17,19]

At present in our organization there is no standard method for diagnosing gestational diabetes. Nor are there criteria for this purpose nor reference values for the oral glucose tolerance test. There are also as yet no standard values for hemoglobin glycate and fructosamine in pregnancy. The objective of this study was to establish proper parameters to diagnose gestational diabetes. The special objective was to obtain reference values for the OGTT as well as hemoglobin glycate (HbA1) and fructosamine blood levels and to measure changes in these values during the second and third trimesters of pregnancy.

The first hypothesis was that in pregnant women the fasting blood glucose level would be lower than in non-pregnant women, and glucose tolerance would also be lower. The second hypothesis was that fructosamine and HbA1 levels would also be lower in pregnancy.

MATERIALS AND METHODS

Materials

This study was conducted from April through October 1990 at the Department of Obstetrics and Gynecology and the Department of Clinical Pathology of the Faculty of Medicine of the University of Indonesia and Dr Cipto Mangunkusumo Hospital in Jakarta.

Subjects consisted of study and control groups. The first group was made up of pregnant women who visited for antenatal care. The second group were non-pregnant women who were workers in one company. Specimens were serum and EDTA blood.

Inclusion criteria for the study group were pregnancy, age 20 - 30, gestational of 16-24 weeks, normal results on physical and laboratory examinations, and higher than 10 g/dl hemoglobin level. Inclusion criteria for the control group were similar except for non-pregnancy and a hemoglobin level higher than 12 g/dl.

Rejection criteria for both groups were diabetes mellitus (DM) i.e. fasting blood glucose level > 140 mg/dl or 2 hours after 75 gram glucose load blood glucose level > 200 mg/dl, IGT i.e. 2 hours after 75 gram glucose load blood glucose level 140-200 mg/dl; or history of DM, family history of DM, obstetrical history for DM manifestation, e.g. giving birth to a baby with > 4 kg body weight, intrauterine fetal death, prematurity, or congenital disorders.

Methods

Precision and accuracy of all tests were monitored within run as well as day to day.

Autonorm control serum (E.Merck) batch No.K-79 was used for precision testing, and 150 mg/dl glucose standard solution was used for accuracy testing of blood glucose determination, 5 times on the first day within run and once daily for 10 consecutive days for day to day testing.

Precision and accuracy of HbA1 determination were monitored by using Normal Glycohemoglobin Controls (Human) (7.3% ± 0.7%) and Abnormal Glycohemoglobin Controls (Human) (12.6% ± 1.9%), 5 times on the first day and once daily for 5 consecutive days.

Tests for fructosamine determination were done using Precinorm control serum (Boehringer Mannheim) Lot No.163751 (291 ± 43 µmol/l).

Sample taking

Specimens were taken twice from each patient in the study group, first at 16-24 weeks (3rd trimester). Biodata as well as obstetrical and family histories were noted. Then physical and laboratory examinations (routine hemotological tests including blood smear, HbA1, fasting blood glucose, total protein, albumin, ASt, ALT, total and direct bilirubin, urea and creatinine) were performed. Blood collection were carried out at 08.00 am after 10 hours of fasting. While the patient was in sitting position, 8 ml of blood was drawn from the cubiti vein. Two ml of blood was directly mixed with 2 mg Na2EDTA anticoagulant for routine hematological tests and HbA1. The EDTA blood was kept stable at 4°C for 1 week for HbA1 measurement. The remaining 6 ml of blood was allowed to clot and then centrifuged to obtain the serum. Two ml of serum was directly measured for the fasting blood glucose level, urea, and creatinine. The rest of the serum was frozen at-20°C for 2 weeks for fructosamine measurement.

Soon after fasting blood was taken, patients were given 75 g glucose diluted in 200 ml of water to be taken orally within less than 5 minutes. After 1 and 2 hours another venapuncture was performed to get 2 ml of blood each. The blood was left to clot and then centrifuged to obtain the serum. The blood glucose level of the serum was determined directly.

If the results showed that the patients belonged to the IGT or DM groups according to the WHO criteria, they were rejected for further study. Patients who fulfilled the criteria for this study were re-examined after 3 months i.e. at 32-38 weeks gestation age (3rd trimester).

The control group were examined using the same procedures as those used for the study group.

Blood glucose measurement

Blood glucose levels were measured using potassium ferrycyanide reducing method by Technicon Auto Analyzer I. The principle of the test is that yellow ferrycyanide is reduced by glucose to colourless ferrocyanide. The colour change is measured photometrically at 420 nm. The glucose level is obtained by comparing the absorbances to the standard curve. The dialysis membrane separate out most non glucose reducing substances, yielding a nearly true glucose value. Interpretation was made with reference to the WHO criteria.

HbA1 measurement

HbA1 levels were measured by microcolumn chromatography using Human's kit (cat No.10658). The kit contained ion exchange resin, lysing reagent, lyophilised standard, control solution (Cat No. 10259) containing normal (Lot No. 1465, 7.3% ± 0.7%) and abnormal (Lot No. 087C, 12.6% ± 1.9% level of HbA1). Instruments were the Clinicon 4010 photometer, vortex mixer, and pipettes.

The principle of the test is the ion exchange chromatography method. HbA1 values between 4.5 -7% were considered non DM or well controlled DM and any value higher than 8.5% was considered uncontrolled DM.

Fructosamine measurement

Fructosamine levels were determined by a modified reducing nitroblue tetrazolium method using Boehringer Mannheim's kit cat no.1054684. Reagents were carbonate buffer solution 200 mmol/l, (pH 10.3),

NBT, Precimat Fructosamine lot no. 61894301 (344 µmol/l) and Precinorm Fructosamine lot no. 163751 (291 µmol/l; 248-334 µmol/l) as standard and control. Instruments were the 37°C thermostated Clinicon 4010 photometer, semiautomatic pipettes and stop watch.

The principle of the test was colorimetry. Fructosamine reduce NBT to form formazan. Fructosamine concentration can be determined by comparing colour intensity to a standard. Fructosamine levels less than 285 µmol/l were considered normal.

Data processing

For the precision tests mean values ($\overline{X}$), standard deviations (SD) and coefficient of variations (CV) were calculated mathematically. For accuracy tests the means and deviation from target value were calculated.

Each data group was evaluated as to whether it represented Gaussian distribution by the Kolmogorov Smirnov (KS) test. If it was proven Gaussian then mean values, SD and mean $\pm$ 2 SD were calculated.

Significant differences were shown by Student's t test for non-related data with p = 0,01. Student's t for related data with p = 0,01 was used to check the differences between 2nd and 3rd trimesters data. Correlation was determined by Pearson's r product moment correlation test.

RESULTS

Precision and accuracy

Within run precision for blood glucose level testing showed 1.9% CV, while accuracy showed 2.0% deviation. Day to day precision and accuracy showed 2.3% CV and 2.3% deviation, respectively.

Within run precision and accuracy for blood HbA1 level testing showed 2.7% and 2.7% deviation while day to day precision and accuracy showed 3.3% CV and 4.4% deviation, respectively.

Within run precision and accuracy for blood fructosamine level testing showed 2.3% CV and 2.1% deviation, while day to day precision and accuracy showed 3.1% CV and 3.2% deviation.

Control group

KS test for blood glucose (fasting, 1 and 2 hours after 75 gram glucose load), HbA1 and fructosamine levels from 25 control patients showed Gaussian distribution with D max values of 0.02, 0.03, 0.01, 0.02 dan 0.02, respectively. Mean, SD dan $\overline{X} \pm 2SD$ values were then calculated. Fasting, 1 and 2 hours after glucose load blood glucose were 4.11 (range 2.92-5.3), 6.99 (6.27-7.7) and 4.61 (3.49-5.73) mmol/dl, respectively. HbA1 levels were 6.4 (5.8-7.1)%. Fructosamine levels were 242.2 (205.8-278.6) µmol/l.

Second trimester pregnant women

KS test for blood glucose (fasting, 1 hour and 2 hours after 75 gram glucose load), HbA1 and fructosamine levels from 53 2nd trimester pregnant women showed Gaussian distribution with D max values of 0.07, 0.07, 0.09, 0.02 dan 0.01, respectively. Mean, SD and $\overline{X} \pm 2SD$ values were then calculated.

Fasting, 1 and 2 hours after glucose load blood glucose were 3.65 (2.56-4.73), 6.87 (6.22-7.52) and 5.77 (5.23-6.31) mmol/dl, respectively. HbA1 levels were 4.4 (3.7-5.2) % and fructosamine levels were 218.8 (182.9-254.7) µmol/l.

Third trimester pregnant women

KS test for blood glucose (fasting, 1 hour and 2 hours after 75 gram glucose load), HbA1 and fructosamine levels from 53 3rd trimester pregnant women showed Gaussian distribution with D max values of 0.03, 0.08, 0.11, 0.03 dan 0.01, respectively. Mean, SD and $\overline{X} \pm 2SD$ values were then calculated.

Fasting, 1 and 2 hours after glucose load blood glucose were 3.54 (2.56-4.52), 7.63 (7.02-8.24) and 6.58 (5.78-7.39) mmol/dl, respectively. HbA1 levels were 5.8 (4.8-6.7) % and fructosamine levels were 203.3 (163.4-243.2) µmol/l.

Comparison of OGTT between control, 2nd and 3rd trimester pregnant women

There was no significant difference for 1 hour after glucose load blood glucose between control group and 2nd trimester pregnant women; but there was a significant difference between the control group and 3rd trimester pregnant women.

The blood glucose level at 2 hours after glucose load in the control group differed significantly from those of the 2nd and 3rd trimester pregnant women. The blood glucose levels of 2nd and 3rd trimester pregnant women after fasting showed no significant difference, while the difference was significant 1 hour and 2 hours after glucose load.

Comparison of HbA1 level between control, 2nd and 3rd trimester pregnant women

Student's t test of HbA1 levels showed significant difference between control group, 2nd and 3rd trimesters pregnant women.

Comparison of fructosamine level between control, 2nd and 3rd trimester pregnant women

Student's t test of fructosamine levels showed significant difference between control group, 2nd and 3rd trimesters pregnant women.

Correlation and regression

In the 2nd and 3rd trimester of pregnancy blood glucose levels (fasting, 1 and 2 hours after glucose load) showed low correlation to HbA1 levels (r = -0.090, 0.010, 0.002 and -0.100, - 0.008 and -0.006, respectively). They also showed low correlation to fructosamine levels (r = -0.070, 0.012, 0.004 and - 0.060, 0.003 and 0.001, respectively).

HbA1 levels showed low correlation to fructosamine levels in the 2nd and 3rd trimester of pregnancy (r = 0.05, and 0.03, respectively).

DISCUSSION

Precision and accuracy

Within run and day to day precision for glucose determination fulfilled WHO criteria (CV should be less than 7,7%).[20] Precision and accuracy for normal and abnormal levels of HbA1 showed an acceptable value under standards suggested by Boehringer Mannheim and Helena Laboratories, both were HbA1 kit producers. Within run and day-to-day precision and accuracy for fructosamine showed acceptable values in terms of the findings of Riet et al and Williams .[21,22]

Sample taking

Screening tests were performed to exclude conditions that could disturb measurement of glucose, HbA1, and fructosamine. Routine hematological examination, urinary urobilinogen and serum indirect bilirubin tests were conducted to detect hemolytic anemia that could influence HbA1 results. Serum total protein and albumin were measured to detect hypoproteinemia and hypoalbuminemia that could influence measurement of fructosamine levels. Serum creatinin was measured because elevated creatinine levels could give a false high blood glucose level. AST, ALT, total and direct bilirubin tests were performed to detect liver dysfunction that could influence OGTT results.

OGTT results

OGTT results showed significant lower values of fasting blood glucose levels in the 2nd and 3rd trimester of pregnancy, comparable to Forest et al.[1] Freinkel also found similar data and mentioned that the decrement of blood glucose levels was due to accelerated starvation[23] (Table 1).

Table 1. Oral glucose tolerance test results in non pregnant and pregnant women.

	75 gram glucose load			100 gram glucose load[1]		
	Fasting	1hour	2hour	Fasting	1hour	2hour
Control	4.11	6.99	4.61			
2nd T pregnancy	3.65	6.87	5.77	3.78	7.28	6.27
3rd T pregnancy	3.54	7.63	6.58	3.72	8.44	7.33

(mmol/l)

Glucose tolerance decreased and marked by a higher increment from the fasting to peak levels and a slower return to the fasting level compared with the control subjects. This decrement was more obvious and significant in 3rd trimester of pregnancy. These data were comparable to those of Forest et al although our blood glucose levels 1 and 2 hours after glucose load were lower. That might have been due to a lower glucose load. According to Nobel et al. differences in glucose load will cause differences in OGTT results especially in decreased glucose tolerance conditions.[11]

According to O'Sullivan & Mahan, the diagnostic criterion for gestasional diabetes on OGTT with 100 gram glucose load is 2 or more values higher than 105 5.83, 10.56, 9.17 and 8.06 mmol/l for fasting, 1, 2, and 3 hours after glucose load.[13] Our study's mean $\pm$ 2 SD values for blood glucose are lower than O'Sullivan & Mahan's values. The difference may be due to the different glucose loads used in both studies. (Table 2)

Table 2. Comparison of OGTT results from 2nd and 3rd trimesters of pregnancy to O'Sullivan criteria

	2nd trimester $\bar{X}$	2nd trimester $\bar{X}$±2SD	3rd trimester $\bar{X}$	3rd trimester $\bar{X}$±2SD	O'Sullivan criteria
Fasting	3.67	4.72	3.56	4.5	5.83
1 hour	6.89	7.5	7.61	8.22	10.56
2 hour	5.78	6.33	6.56	7.39	9.17

(mmol/l)

WHO recommends the same criteria for gestational diabetes and diabetes in non-pregnant women[15] using a glucose load of 75 grams, the amount also given in this study. In the 3rd trimester of pregnancy, blood glucose levels 2 hours after glucose load in our study showed mean ± 2SD value of 7.39 mmol/l, which is close to the WHO criteria for IGT. Therefore, that value might be used as reference value for OGTT in 2nd and 3rd trimesters of pregnancy using a 75 gram glucose load.

Application of values for diagnosing gestational diabetes still needs further study.

HbA1 result

HbA1 levels in the 2nd and 3rd trimesters of pregnancy were significantly lower than in the control group, while HbA1 levels in 3rd trimester were significantly higher than the 2nd trimester value. According to Phelps et al. there is a biphasic alteration of HbA1 value in pregnancy; first the level decreases to its lowest point at 24th weeks followed by a slow increase to peak at term, all these values being considered within the normal range. This phenomenon was proved by them with a 50 gram glucose load. They concluded that the alteration was due to blood glucose changes occurring about 4 weeks earlier than HbA1 change. Our study found higher values than theirs. This might be due to methodical differences; we measured total HbA1 instead of HbA1c[23] (Table 3)

Correlations among HbA1 and blood glucose levels in the 2nd and 3rd trimesters were low. Phelps et al. found that in IDDM there was a good correlation between HbA1c and fasting blood glucose levels some weeks earlier but not when both were measured at the same time.[24]

Table 3. Comparison of hemoglobin glycate levels from this study (Total HbA1) to Phelps result (HbA1c).

	non-pregnant	24wks pregnant	38-41wks pregnant
Phelps	4.75 + 0.13	4.33 + 0.19	4.87 + 0.17
This study	6.4 + 0.6	4.4 + 0.8	5.8 + 1.0

(%)

Our data might be used as reference values, mean ± 2SD 5.2% and 6.7% for the 2nd and 3rd trimesters of pregnancy respectively. HbA1 value could be affected by hemoglobinopathy e.g. thalassemia, Hb C or Hb S diseases. Since the prevalence of hemoglobinopathy is relatively high in Indonesia, these conditions should be taken into consideration in the interpretation of HbA1 results.[25]

Fructosamine result

Fructosamine levels in the 2nd and 3rd trimesters of pregnancy showed significantly lower levels than those of the controls. In the 3rd trimester, frustosamine levels were much lower than in the 2nd trimester. Roberts and Schlebusch et al. also found significant lowering of fructosamine levels through out the gestational period. This phenomenon can be explained by carbohydrate metabolism changes and lowering of serum total protein and albumin levels, especially in the 3rd trimester.[17] Robert et al. used different units of measurement. Our data were slightly lower than those of Schlebusch et al. This difference might be due to nutritional state factor.[26] (Table 4)

Table 4. Fructosamine levels in non-pregnant and pregnant women

	nonpregnant	pregnant	
		2nd trimester	3rd trimester
Robert (mmol/l)	2.42 + 0.16	2.41 + 0.14	2.34 + 0.14
Schlebusch (μmol/l)	225	215	
This study (μmol/l)	242 (206-279)	219 (183-255)	203 (163-243)

Robert et al. found that 8 out of 9 gestational diabetes cases showed much higher than 95 percentile fructosamine level. Our data $\bar{X} \pm 2SD$ 254.7µmol/l and 243.2 µmol/l might be used as reference values for the 2nd and 3rd trimesters of pregnancy, respectively.

Fructosamine levels showed low correlation with blood glucose levels in the 2nd and 3rd trimesters of pregnancy. This can be understood, since the fructosamine level mirrors the glycemic state of the period 2-3 weeks earlier, not at the time the sample is taken.

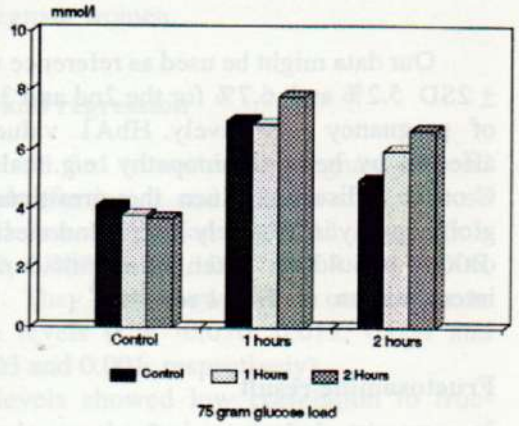
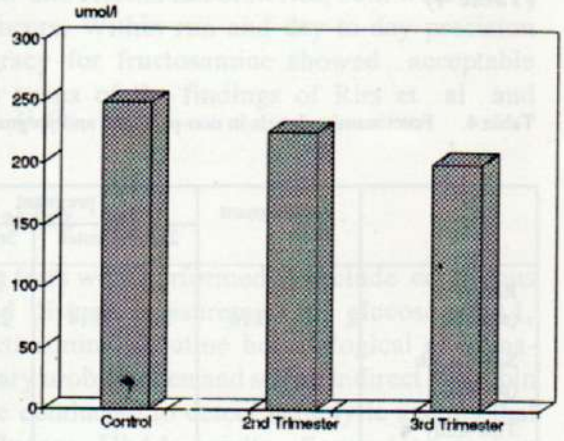


Figure 2. HbA1 level in control, 2nd and 3rd trimester of pregnancy



Correlation and regression

HbA1 and fructosamine levels showed low correlations in the 2nd and 3rd trimesters of pregnancy (r= 0.05 and 0.03). This showed that the two parameters reveal glycemic states at different periods of time, HbA1 6-8 weeks and fructosamine 2-3 weeks earlier. There is no evidence about the correlation of these parameters in normoglycemic states of pregnant women.

Figure 1. OGTT in control, 2nd and 3rd trimester of pregnancy

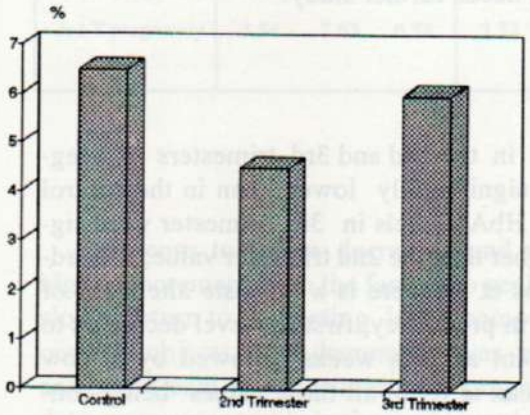


Figure 3. Fructosamine level in control, 2nd and 3rd trimester of pregnancy

CONCLUSION AND SUGGESTION

In 2nd trimester of pregnancy, OGTT results were as follows: fasting, 1 and 2 hours after glucose load blood glucoses were 2.56-4.73 (mean=3.65), 6.22-7.52 (mean=6.87) and 5.23-6.31 (mean=5.77) mmol/l respectively. HbA1 levels were 3.7-5.2 (mean=4.4) %, and fructosamine levels were 182.9-224.7 (mean=218.8) µmol/l.

The results for the 3rd trimester of pregnancy were as follows: fasting, 1 and 2 hours after glucose load blood glucoses were 2.56-4.52 (mean=3.54), 7.02-8.24 (mean-7.63) and 5.78- 7.39 (mean=6.58) mmol/l respectively. hbA1 levels were 4.8-6.7 (mean=5.8) % and fructosamine levels were 163.4-243.2 (mean=203.3) µmol/l.

There were no significant differences between fasting blood glucose levels in the 2nd and 3rd trimesters, but both values were lower than the control group. There was a decrease of glucose tolerance during pregnancy, which was more obvious during the 3rd trimester.

HbA1 and fructosamine levels were lower in pregnant women than in the control group. The 3rd trimester HbA1 values were slightly higher than the 2nd trimester values, while the 3rd trimester fructosamine values were slightly lower than the 2nd trimester values.

There were low correlations among HbA1, fructosamine, and glucose values measured at the same period of pregnancy.

In order to get more representative reference values for OGTT, HbA1 and fructosamine in pregnant women it is suggested to study a larger and more heterogenous sample from the standpoint of age, ethnicity, and nutritional as well as socio-economic conditions. It is also suggested to study pregnant women with gestational diabetes.

REFERENCES

1. Forest JC, Russo MG, Lemay A, Carrier R, Dube JL. Reference Values for the Oral Glucose Tolerance Test at Each Trimester of Pregnancy, Am J Clin Path 1980; 80: 828-31.
2. Baird JD. Some Aspects of the Metabolic and Hormonal Adaptation to Pregnancy. Acta Endocrinol 1986; 277: 11-8.
3. Abell DA, Beischer NA. Evaluation of the Three Hour Oral Glucose Tolerance Test in Detection of Significant Hyperglycaemia and Hypoglycaemia in Pregnancy, Diabetes 1975; 24: 874-80.
4. Abell DA, Beisher NA, Papas AJ, Willis MM. The Association Between Abnormal Glucose Tolerance Hyperglycemia and Hypoglycemia and Estriol Excretion in Pregnancy. Am J Obstet Gynecol. 1975; 121: 388-92.
5. Hollingsworth DR. Alterations of Maternal Metabolism in Normal and Diabetic Pregnancies : Differences in Insulin Dependent, Non Insulin Dependent and Gestational Diabetes. Am J Obstet Gynecol 1985; 4: 417-29.
6. O'Sullivan JB, Charles D, Mahan CM, Dandrow RV. Gestational Diabetes and Perinatal Mortality Rate. Am J Obstet Gynecol 1973; 116: 901-4.
7. Mestman JH, Anderson GV, Barton P. Carbohydrate Metabolism in Pregnancy. Am J Obstet Gynecol 1971; 109: 41-5.
8. Hofmann HMH, Weiss PAM, Purstner P, Josef H, Gmoser G. Serum Fructosamine and Amniotic Fluid Insulin Levels in Patients with Gestational Diabetes and Healthy Control Subjects. Am J Obstet Gynecol 1990; 162: 1174-80.
9. Berkus MD, Stern MP, Mitchell BD, Abashawl A, Langer O. Relationships Between Glucose Levels and Insulin Secretion During a Glucose Challenge tests. Am J Obstet Gynecol 1990; 163: 1818-22.
10. Baker JR, O'Connor JP, Metcalf PA, Lawson MR, Johnson RN. Clinical Usefulness of Estimation of Serum Fructosamine Concentration as a Screening Test for Diabetes Mellitus. Br J Med 1983; 287: 863-67.
11. Nobel ED, Laar AV. The Size of the Loading Dose as an Important Determinant of the Results of the Oral Glucose Tolerance Test. Diabetes 1977; 27: 42-8.
12. Anonymous. Diabetes Mellitus, WHO Technical Report Series 727, WHO, Geneva 1985: 7-33, 99-103.
13. O'Sullivan JB, Mahan CM, Charles D, Dandrow R. Screening Criteria for High Risk Gestational Diabetic Patients. Am J Obstet Gynecol 1973; 116: 895-900.
14. Phelps RL, Metzger B, Freinkel N. Carbohydrate Metabolism in Pregnancy. Am J Obstet Gynecol 1981; 140: 730-6.
15. Green JR, Pauson IG, Schumacher LB, Perry J, Kretchmer N. Glucose Tolerance in Pregnancy: Ethnic Variation and Influence of Body Habitus. Am J Obstet Gynecol 1990; 163: 86- 92.
16. Berkus MD, Stern MP, Mitchell BD, Newton ER, Langer O. Does Fasting Interval Affect the Glucose Challenge Test, Am J Obstet Gynecol 1990; 163: 1812-17.
17. Robert AB, Baker JR. Serum Fructosamine: a Screening test for Diabetes in Pregnancy. Am J Obstet Gynecol 1986; 15: 1027- 30.
18. Hindle EJ, Rostron GM, Gatt JA. The Estimation of Serum Fructosamine: an Alternative Measurement to Glycated Haemoglobin. Ann Clin Biochem 1985; 22: 84-9.
19. Bunn HF. Evaluation of Glycosylated Hemoglobin in Diabetic Patients 1981; 30: 613-7.
20. Anonymous. International External Quality Assessment Scheme. WHO Collaborating Centre for Research and Reference Services. Birmingham, 1983.
21. Rietz P, Eisenwiener HG, Morger F, Heerspink W. Automated Analysis of Glycated Protein by Fructosamine Assay on Cobas System. Basle: F Hoffman - La Roche & Co, 1986.
22. Willms B, Lehmann P. A New Fruktosamine Test as a Routine Parameter in Diabetes Monitoring. In: Kraupp O, Sinzinger H. Wiener Klinische Wochenschrift. Vienna: Springer Verlag, 1990: 5-10.

23. Buchanan TA, Metzger BE, Freinkel N. Accelerated Starvation in Late Pregnancy: A Comparison Between Obese Women With and Without Gestational Diabetes Mellitus. Am J Obstet Gynecol 1990; 164: 1015-20.
24. Phelps RL, Honig GR, Green D, Metzger BE, Frederiksen MC, Freinkel R. Biphasic Changes in Hemoglobin A1c Concentration During Normal Human Pregnancy. Am J Obstet Gynecol 1983; 6: 651-3.
25. Wahidiyat I, Penelitian Thalassemia di Jakarta, Jakarta : Inter Mega, 1979; 192-201.
26. Schlebusch H, Sorger M, Liappis N, Week C, Paffenholz I. Fructosamine Reference Ranges for Pregnant Women, Children and Adolescents determined by an Improved NBT Method. In: Kraupp O,Sinzinger H. Wiener Klinische Wochenschrift. Vienna: Springer Verlag, 1990: 51-6.