Serum Fructosamine and Related Biochemical Parameters in Pregnant Women with Pre-Eclampsia

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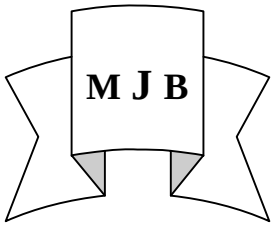

Abstract

The objective of this study is to determine the pattern of change in serum fructosamine and related biochemical parameters in normally pregnant women and to compare them with pregnant women complicated with pre-eclampsia and also to examine the influence of serum albumin or total protein on fructosamine concentrations with the need for correcting their values accordingly.

Fasting plasma glucose (FPG) and serum fructosamine (FAm), albumin and total protein were measured in three groups of women who were attending Al-Batool antenatal clinic and Al-Khansa antenatal clinic in Mosul during the period of 7 months from 1st November 2000 to 31st May 2001. These included 250 non-pregnant apparently healthy women (group 1), 242 normally pregnant women (group 2) and 53 pregnant women complicated by pre-eclamptic toxaemia (group 3).

The distribution of serum FAm in group 1 showed a normal (Gaussian] pattern with reference range calculated as mean ± 2SD of 1.37-2.49 mmol/L. In group 2, a significantly lower FAm values were observed ($Z=4.1$, $p<0.001$) with no significant correlation between FAm and albumin or total protein. There was a downward trend in FAm values with the progress of pregnancy as their means were (1.82, 1.80, 1.77 mmol/L) in the early, middle and late stages respectively which were significantly lower than the mean value of 1.93 mmol/L in group 1. Serum albumin and total protein showed also a trend to decrease during the gestational stages with a highly significant difference from group 1 ($p<0.001$). FPG showed no significant difference in all stages from group 1. Correction of FAm for albumin or total protein is required in group 2 and comparison between corrected fructosamine (FAc) in group 1 and group 2 revealed no significant difference. In group 3, lower FAm values were obtained with mean ± SD of 1.60 ± 0.27 mmol/L, which is significantly ($p<0.001$) lower than group 1 ($Z=6.2$) and group 2 ($Z=3.4$). There was also a tendency for mean FAm to decrease with the increase in the degree of proteinuria. Following correction according to albumin or total protein, the difference was eliminated as compared with group 2, but in comparison with group 1 the difference was eliminated only when correction according to total protein was made.

These data highlight the importance of measuring serum fructosamine in women during pregnancy. Its concentration is affected by the changes in serum albumin and total protein during the different stages of gestation. In pregnancy complicated by pre-eclamptic toxaemia, correction of fructosamine according to albumin or total protein, is mandatory as the values differ from those in non-pregnant and normal pregnant women.

الخلاصة

لقياس أمين الفركتوز (البروتين المسكر) وبعض المعالم الكيماوي الحيوية ذات العلاقة في الحوامل المصابات بتسمم الحمل تم دراسة التغيير في امين الفركتوز ومعالم كيمياوية حيوية ذات العلاقة في مصل الدم خلال فترة الحمل الطبيعي والمصحوب بتسمم الحمل ، وتاثير تركيز الزلال أو البروتين الكلي في مصل الدم على تركيز أمين الفركتوز والحاجة لتصحيح قيمه وفق ذلك .

تم قياس تراكيز سكر العنب حال الصوم ، أمين الفركتوز والزلال والبروتين الكلي في ثلاثة مجاميع للنساء المراجعات لعيادة البتول ومستشفى الخنساء للولادة للفترة من 11/1/ 2000 إلى 2001/5/31 ، المجموعة الاولى : العينة الضابطة من النساء الاصحاء غير الحوامل كان تركيز سكر العنب حال الصوم عندهم اقل من 6.0 مليمول/لتر(العدد 250) ؛ المجموعة الثانية: نساء حوامل أصحاء (العدد 242] ؛ المجموعة الثالثة : الحوامل اللواتي ظهرت عليهن اعراض تسمم الحمل (العدد 53)

اظهر توزيع امين الفركتوز لدى المجموعة الاولى توزيعاً طبيعياً (نموذج كوزيان] ، لذا احتسب المجال المرجعي بالمعدل ± 2 (الانحراف القياسي] فكان مساوياً (1.37-2.49) مليمول/لتر . لقد اظهرت الدراسة ترابطاً معتداً شديداً (ر = 0.18 ، ب < 0.01] بين امين الفركتوز وزلال مصل الدم ، بينما اظهرت ترابطاً غير معتد بين أمين الفركتوز والبروتين الكلي لمصل الدم في هذه المجموعة . في المجموعة الثانية أظهر امين الفركتوز اختلافاً ذا معنى احصائي عن المجموعة الاولى (ب<0.001) مع وجود ترابط غير معتد بين تركيز امين الفركتوز والزلال أو البروتين الكلي لمصل الدم . كان هناك انخفاض في امين الفركتوز خلال مراحل الحمل بمعدل (1.82 ، 1.80 ، 1.77 مليمول/لتر] في مراحل الحمل الاولى ، الثانية والثالثة بالتتابع عن المعدل 1.93 مليمول/لتر في المجموعة الاولى . كما اظهر زلال مصل الدم والبروتين الكلي انخفاض خلال مراحل الحمل كان ذا مغزى احصائي مقارنة بالمجموعة الاولى (ب<0.001) . اظهر تركيز سكر العنب عدم وجود اختلاف معنوي خلال مراحل الحمل عن المجموعة الاولى . تصحيح امين الفركتوز طبقاً لزلال مصل الدم أو البروتين الكلي يعد ضرورياً في المجموعة الثانية حيث ارتفعت قيمته بعد التصحيح ليصبح غير مختلف احصائياً عن المجموعة الاولى . في المجموعة الثالثة وجد انخفاض في معدل امين الفركتوز والبروتين الكلي (ب<0.001) وكان انخفاض امين الفركتوز (معدل ± الانحراف المعياري] 1.60 ± 0.27 مليمول/لتر ، ذا مغزى احصائي مقارنة بالمجموعة الاولى (ز = 6.2 ، ب<0.001) وكذلك بالمجموعة الثانية (ز = 3.4 ، ب<0.001) . كما لوحظ انخفاض معدل امين الفركتوز مع زيادة طرح الزلال بالبول . بعد التصحيح اعتماداً على الزلال والبروتين الكلي في مصل الدم ارتفعت قيمة امين الفركتوز مع عدم وجود فرق معنوي ذا مغزى احصائي مقارنة بالمجموعة الثانية ولكن مقارنة بالمجموعة الاولى ارتفعت قيمة امين الفركتوز بعد التصحيح اعتماداً على البروتين الكلي فقط لتصبح غير مختلفة احصائياً عن المجموعة الاولى .

تبين من خلال هذه الدراسة بأن تركيزامين الفركتوز لمصل الدم يتأثر بالتغيرات المصحوبة بالحمل وخاصة تلك التي تتعلق بتركيز زلال مصل الدم والبروتين الكلي مما يبرز اهمية تصحيح قيمته وفق ذلك للاستفادة منه وان معادلة التصحيح اعتمادا على البروتين الكلي أفضل من الزلال .

Introduction

Measurement of glycated protein, albumin or haemoglobin have an established role in the assessment of glycaemic control in diabetic subjects including pregnant diabetics[1, 2]. Maintaining maternal glycaemic control within normal, as reflected by these glycated parameters, is an important objective in the management of diabetics that focused extra-importance in pregnant women complicated by diabetes mellitus[3, 4].

Fructosamine is the trivial name for 1-amino-1-deoxy-fructose which is a ketoamine derivative of the post-translational non-enzymatic modification of a sugar (usually glucose] and a protein (usually albumin). [2, 5]. However, it is until 1983 that the fructosamine assay was introduced into the clinical chemistry literatures by Johnson *et al.*[6] as a general term for ketoamine reflecting glycated albumin and protein. It is an intermediate control index that reflects the glycaemic state within the

prevailing 2-3 weeks. The assay is cheap, easy to perform, precise and can be automated with high throughput[7-11].

Measurement of serum fructose-amine reflects the glycation of different proteins which is increased in diabetics, and the glycaemic state as well as the state of serum protein and albumin influence its accurate assessment [12]. This is particularly important in patients with hypoproteinemia or with altered metabolism of protein [9]. Such alteration in serum protein profile sufficient to affect glycated protein may be considered as a potential limitation for the usefulness of glycated protein in diabetic care.

Since fructosamine concentration depends on the prevailing concentration of glucose and protein mainly albumin, it seems logical to correct its measured value for expression in term of constant albumin or total protein concentration. However, the need of this correction is controversial as glycation depends not only on protein and albumin concentrations but also on their half-life [12].

The aims of the current study are: 1. To establish the reference range for serum fructosamine in the apparently healthy non-pregnant women and normal pregnant women. 2. To examine the influence of serum albumin or total protein on fructosamine values during normal pregnancy, and the need for correcting fructosamine accordingly using different equations. 3. To assess the value of fructosamine in pregnant women complicated with pre-eclamptic toxaemia and the need for correction of fructosamine in this group.

Subjects and Methods

The study sample included pregnant women, who were attending Al-Batool antenatal clinic and Al-Khansa antenatal clinic in Mosul during the period of 7 months from 1st November 2000 to 31st May 2001. The control group included non-pregnant, apparently healthy women who were volunteered for comparison. A complete record of every pregnant woman was obtained including: age, gravity, parity, last menstrual period, history of diabetes or hypertension, and history of obstetric manifestations of diabetes mellitus. The subjects enrolled in this study were divided into 3 groups:

Group 1

This group included 250 apparently healthy, non-pregnant women who were not using contraceptive pills. The control subjects were selected from the teaching staff of Mosul Medical College and the relatives who were accompanying the inpatients in Al-Batool and Al-Khansa Maternity Hospitals. Their ages ranged from 23-45 years with mean ± SD of 31.4 ± 4.85 years. They had no history of diabetes mellitus, and they served as a control group.

Group 2

This group was initially composed of 257 pregnant women. Seven women were known to be diabetics and were instantly excluded and eight women showed, for the first time, a hyperglycemic state and were also excluded. The remaining 242 pregnant women were included in this group 2, their ages ranged from 18-46 years with a mean ± SD of 27.3 ± 6.48 years. They were subdivided according to their gestational age as:

Early stage (9-19 weeks) (referred to as group 2a, n=85);

Middle stage (20-29 weeks) (referred to as group 2b, n = 73);
Late stage (30-39 weeks) (referred to as group 2c, n = 84).

Group 3

This group involved 53 pregnant women who were diagnosed to have pre-eclamptic toxaemia (PET) according to the diagnostic criteria of this complication. Their mean ± SD of age was 29.7 ± 6.9 years (range 19-46 years]. The diagnosis includes the presence of at least one of the followings [13]: Hypertension (a systolic blood pressure > 150 mm Hg or a diastolic blood pressure > 90 mm Hg**)** with oedema, or hypertension with proteinuria, or oedema with proteinuria. Seven women were primigravida, 22 were multipara and 24 were grand multipara. This group was subdivided according to the stage of pregnancy into two subgroups:

- Group 3a included women with PET in the middle stage of pregnancy (20-29 weeks, n = 24).
- Group 3b included women with PET in the late stage of pregnancy (30-39 weeks, n = 29).

Further subgrouping according to the degree of proteinuria was also made:

- Group 3c included women with PET with trace of proteinuria (n = 16).
- Group 3d included women with PET with (+) proteinuria (n = 21).
- Group 3e included women with PET with (++) proteinuria (n =16).

Blood samples were obtained from all subjects involved in the study between 8-10 am in the fasting state. A sample of 2 ml blood was taken from each subject and divided into two containers: Fluoride-oxalate container for the estimation of plasma glucose concentration and plain tube container for the estimation of other serum biochemical parameters (fructosamine, albumin, and total protein).

Plasma glucose was estimated by glucose-oxidase-peroxidase method [14], using a kit supplied by Randox Ltd (England]. Serum fructosamine was determined using nitroblue tetrazolium colorimetric method [6], which is based on the reducing ability of fructosamine in alkaline buffer solution, using reagents from Sigma (USA). Serum total protein was determined by biuret method [15], and albumin was determined by bromocresol green dye binding method (16] using kits purchased from Randox Ltd (England]. Qualitative determination of proteinuria was done by turbidimetric method using 20% sulphosalicylic acid (SSA) [17]. The degree of proteinuria was assessed according to the degree of turbidity present in the sample after adding 1-2 drops of SSA to 1.0 ml of urine sample as trace, (+) or (++).

Calculation of corrected fructosamine (FAc] was done according to the following equations depending on serum albumin concentration (equations 1, 2, and 3) or total protein (equation 4):

1. FAc1 = FAm + 0.03 (40 - Albumin concentration g/l)[18]

2. FAc2 = a decrease or increase in serum albumin concentration of (1g/l) requires: addition or subtraction of (0.023) mmol/L of FAm for albumin < 40 g/l or > 40g/l respectively [19].

3. FAc3 = FAm x 40/albumin concentration (g/l)[20]

4. FAc4 = FAm x 70/ total protein (g/l)[21]

The statistical methods were used for the analysis of data included: standard statistical methods, the mean, median, standard deviation (SD), standard error

(SE), and skewness. Paired and unpaired student Z-test was used to compare results for various biochemical parameters among subjects of the same group and in the different groups respectively. Linear regression analysis was also performed for finding the relationship between the dependent and independent variables. Duncan's test was also used to identify group(s) responsible for statistical difference through comparison, following analysis of variance (ANOVA). All values quoted as the mean ± SD. Differences between observations are considered significant at $p \leq 0.05$[22].

Results

The results of data analysis are presented according to the grouping of subjects.

Group 1

This group was considered as a control group for the establishment of the reference range of serum fructosamine and for comparison with other groups during pregnancy. They were normoglycaemic since their FPG was 4.7 ± 0.6 mmol/L, mean ± SD, and median was 4.7 mmol/L with a range of 3.2-6.0 mmol/L. So all of them had normal FPG concentration of ≤ 6.0 mmol/L according to the criteria of American Diabetes Association (ADA) Expert Committee on Diabetes Mellitus [23].

The frequency distribution of fructosamine showed an essentially normal pattern (Gaussian distribution) with skewness to the right of 0.13; Fig 1. The results of FAm was 1.93 ± 0.28 mmol/L, and the median was 1.93 with a range of 1.27 - 2.58 mmol/L.

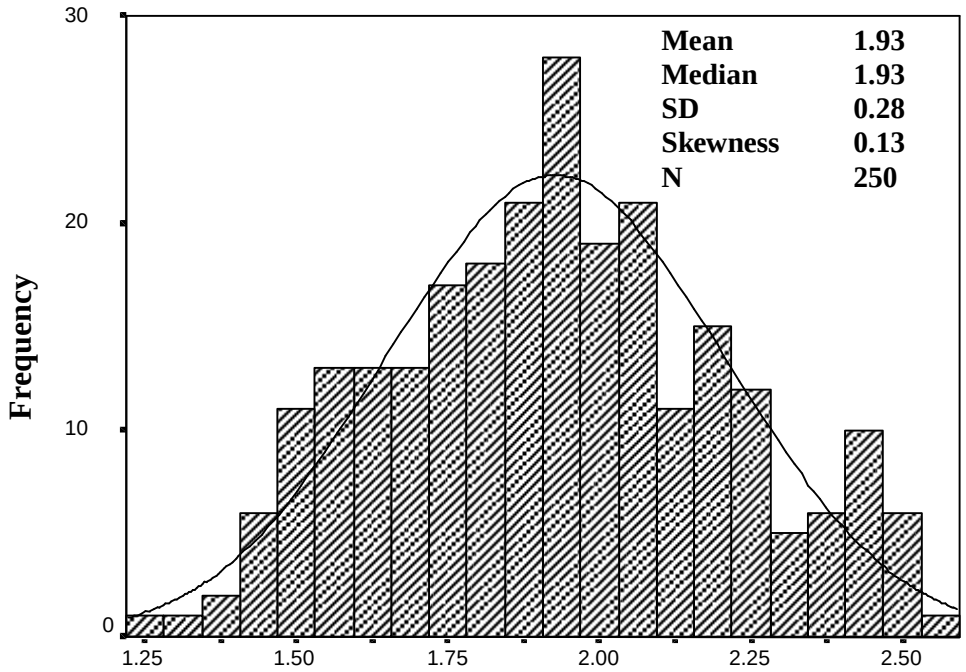

Fig. 1 Distribution of measured serum fructosamine (FAm) in group 1 (non-pregnant women).

The reference range (95th centile confidence limit) calculated as the mean ± 2SD was 1.37-2.49 mmol/L. Correction of FAm according to serum albumin or total protein concentration was done. The result of serum albumin was 41.4 ± 4.5 g/l, and the median was 41g/l with a range of 30-54g/l, serum total protein was 68.7 ± 7.8 g/l, and the median was 69 g/l with a range of 52-82 g/l.

Four equations for correction of measured serum fructosamine (three depending on serum albumin and one depending on serum total protein) were followed(70, 79-81). The results were as follow:

a- **Using Howey *et al*. equation**[18] **(FAc1)**

The FAc1 was 1.89 ± 0.29, and the reference range was 1.31-2.47 mmol/L.

b- **Using Vandieijen Visser *et al*. equation**[19] **(FAc2)**

The FAc2 was 1.89 ± 0.28, and the reference range was 1.33-2.45 mmol/L.

c- **Using Senecal *et al.* equation**[20] **(FAc3)**

The FAc3 was 1.88 ± 0.31, and the reference range was 1.26-2.50 mmol/L.

d- **Using Baker *et al*. equation**[21] **(FAc4)**

The FAc4 was 1.98 ± 0.35 and the reference range was 1.38-2.68 mmol/L.

The relationship between measured and corrected fructosamine was studied using linear regression analysis. A highly significant positive correlation was observed between FAm and FAc_1; FAm and FAc_2; FAm and FAc_3, Fam and Fac4 where r = 0.88, 0.93, 0.76 and 0.75 respectively ($p < 0.001$). The relationship between serum albumin and FAm was also studied, there was a significant correlation between them ($r = 0.18$, $p<0.01$). There was no significant correlation between FAm and total protein ($r = 0.11$, $p > 0.05$).

Group 2

The biochemical parameters in women with normal pregnancy showed the followings:

a. The FPG was 4.6 ± 0.65 mmol/L, range of 3.0-5.0, and median of 4.6 mmol/L.

b. Serum albumin was 39.1 ± 3.8 g /l, range of 29-50 g /l, and median of 39 g /l.

c. Serum total protein was 64.8 ± 5.25 , range of 50-82 and median of 66 g /l.

d. The FAm was 1.79 ± 0.33, range of 0.67-2.44 and median of 1.8 mmol/L. The data showed a Gaussian distribution with a skewness of -0.24, Fig 2. The reference range (95th centile confidence limit) calculated as mean ± 2SD was 1.13-2.45 mmol/L.

e. The FAc using different equations were FAc_1 1.83 ± 0.35, FAc_2 1.81 ± 0.35, FAc_3 1.85 ± 0.38 and FAc_4 1.95 ± 0.39.

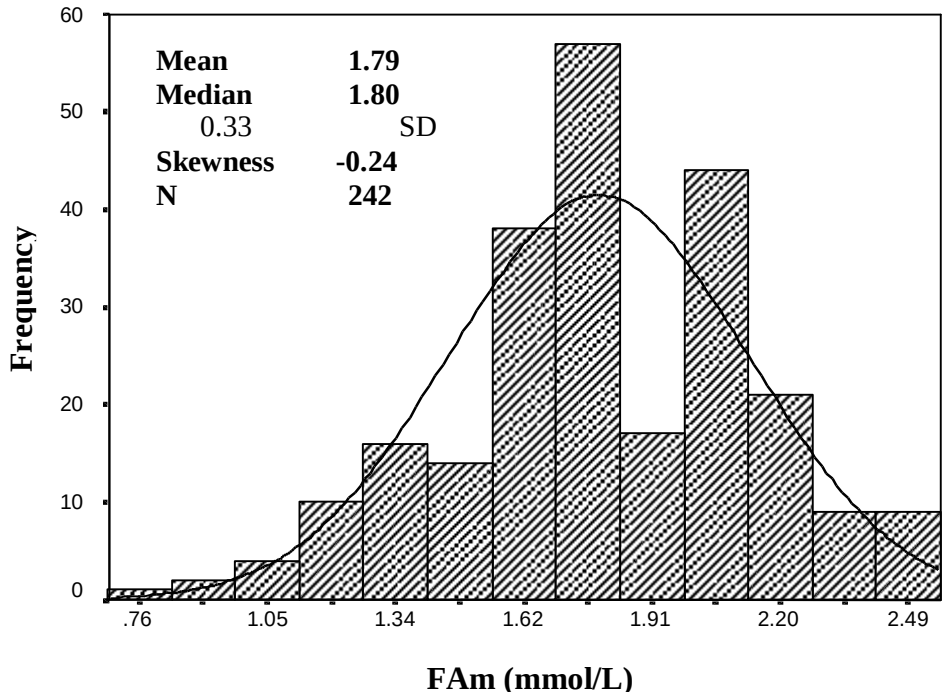

Fig. 2 Distribution of measured serum fructosamine (FAm) in group 2 (pregnant women).

When these values were compared with their corresponding results in the control group, there was no significant difference in FPG, while there was a highly significant difference ($p<0.001$) in serum albumin, total protein, and FAm, (table 1). Following correction of the FAm, this difference disappeared regarding FAc_1, FAc_2, FAc_3 and FAc_4 ($p>0.05$]. The relationship between FAm and FAc using each equation was studied and showed a highly significant positive correlation with $r_1=0.95$, $r_2=0.97$, $r_3=0.88$ and $r_4=0.91$ ($p<0.001$).

Table 1 Comparison between group 1 (non-pregnant) and group 2 (pregnant) in certain biochemical parameters (mean ± SD).

Parameters	Group 1 (n=250)	Group 2 (n=242)	Z	p
FPG (mmol/L)	4.7 ± 0.6	4.6 ± 0.65	1.6	NS
Albumin (g/l)	41.4 ± 4.5	39.1 ± 3.8	6.0	< 0.001
Total protein (g/l)	68.7 ± 7.8	64.8 ± 5.25	5.7	< 0.001
FAm (mmol/L)	1.93 ± 0.28	1.79 ± 0.33	4.1	< 0.001
FAc_1 (mmol/L)	1.89 ± 0.29	1.83 ± 0.35	1.5	NS
FAc_2 (mmol/L)	1.89 ± 0.28	1.81 ± 0.35	1.3	NS
FAc_3 (mmol/L)	1.88 ± 0.31	1.85 ± 0.38	0.2	NS
FAc_4 (mmol/L)	1.98 ± 0.35	1.95 ± 0.39	0.9	NS

According to the gestational age, this group was subdivided into three subgroups during early, middle, and late stages respectively. The results of

different parameters including FPG, albumin, total protein and fructosamine (measured and corrected) in the early (n=85), middle (n=73) and late (n=84) stages are presented in table (2).

Table 2 Biochemical parameters in group 2 in the different stages of pregnancy (mean ± SD).

Parameters	Early stage (n=85)	Middle stage (n=73)	Late stage (n=84)
FPG (mmol/L)	4.6 ± 0.63	4.6 ± 0.63	4.55 ± 0.66
Albumin (g/l)	39.6 ± 3.7	39.7 ± 3.7	38.0 ± 3.5
Total protein (g/l)	66.0 ± 5.5	66.0 ± 4.9	63.5 ± 4.4
FAm (mmol/L)	1.82 ± 0.35	1.80 ± 0.33	1.77 ± 0.33
FAc_1 (mmol/L)	1.84 ± 0.35	1.82 ± 0.32	1.83 ± 0.37
FAc_2 (mmol/L)	1.83 ± 0.35	1.80 ± 0.32	1.80 ± 0.37
FAc_3 (mmol/L)	1.85 ± 0.37	1.83 ± 0.33	1.88 ± 0.42
FAc_4 (mmol/L)	1.95 ± 0.40	1.93 ± 0.37	1.96 ± 0.41

There was no significant correlation between FAm and albumin in the three stages, where (r=0.12, 0.13, 0.06) respectively ($p > 0.05$). The relationship between measured and corrected fructosamine was also studied in each stage by Z-test. In the early stage, there was no significant difference between FAm and FRc_1, FAc_2 and FAc_3 ($p > 0.05$), while there was a highly significant difference between FAm and FAc_4 ($Z = 5.9$, $p<0.001$). In the middle stage, there was a highly significant difference between FAm and corrected fructosamine by the four equations where (Z = 7.53, 7.52, 7.5, 7.54) respectively ($p < 0.001$). In the late stage, there was also a highly significant difference between FAm and corrected fructosamine using the four equations where (Z = 8.1, 8.0, 8.06, 8.1] respectively ($p < 0.001$).

Group 3

This group included 53 pregnant women with signs or symptoms of PET after 20^{th} week of gestation. They were normoglycaemic since their FPG concentration was 4.6 ± 0.68 mmol/L with a range of 3.33-5.8 mmol/L. The results of the different parameters including FPG, albumin, total protein and fructosamine (measured and corrected) in comparison with groups 1 and 2 are presented in table (3).

Table 3 Comparison between group 3 and group 1; and between group 3 and group 2, (mean ± SD).

Parameters	Group 3 (n=53)	Group 1 (n=250)	Z	p	Group 2 (n=242)	Z	p
FPG (mmol/L)	4.6 ± 0.68	4.7 ± 0.61	0.6	NS	4.6 ± 0.65	0.5	NS
Albumin (g/l)	37.1 ± 2.9	41.4 ± 4.5	6.6	< 0.001	39.1 ± 3.8	3.4	< 0.001
Total protein (g/l)	60.8 ± 5.6	68.7 ± 7.8	6.7	< 0.001	64.8 ± 5.25	4.6	< 0.001
FAm (mmol/L)	1.6 ± 0.27	1.93 ± 0.28	6.2	< 0.001	1.79 ± 0.33	3.4	< 0.001
Fac_1 (mmol/L)	1.70 ± 0.29	1.88 ± 0.29	3.1	< 0.001	1.83 ± 0.35	1.9	NS
Fac_2 (mmol/L)	1.70 ± 0.28	1.88 ± 0.28	3.8	< 0.001	1.81 ± 0.35	1.8	NS
Fac_3 (mmol/L)	1.77 ± 0.32	1.88 ± 0.31	2.6	< 0.001	1.85 ± 0.38	1.03	NS
Fac_4 (mmol/L)	1.89 ± 0.32	1.98 ± 0.35	1.09	NS	1.95 ± 0.39	0.9	NS

This group showed a highly significant difference as compared with group 1 (non-pregnant) and with group 2 (normal pregnant) in albumin, total protein and FAm, while there was no significant difference in FPG. Corrected fructosamine showed a significant difference ($p<0.001$) in FAc_1, FAc_2 and FAc_3, while there was no significant difference in FAc_4 compared with group 1. However, no significant difference was noted in FAc using all equations when compared with group 2.

This group was then subdivided according to the gestational age into 2 groups; group 3a included women with PET in the middle stage and group 3b included women with PET in the late stage.

Group 3a showed FPG concentration of 4.4 ± 0.56 mmol/L, range 3.3-5.4 mmol/L with a median of 4.35 mmol/L; albumin concentration 37.3 ± 2.1 g/l, range 33-40 g/l with a median of 38 g/l, and total protein concentration 62.5 ± 5.3 g/l, range 54-73 g/l with a median of 63 g/l. The FAm was 1.69 ± 0.19 mmol/L, range of 1.2-1.95 mmol/L, with a median of 1.77 mmol/L.

Group 3b showed FPG concentration of 4.8 ± 0.71 mmol/L, range 3.3-5.8 mmol/L with a median of 4.8 mmol/L; albumin concentration 36.9 ± 3.6 g/l, range 29-42 g/l with a median of 38 g/l, and total protein concentration 59.4 ± 5.6 g/l, range 52-70 g/l with a median of 57 g/l. FAm was 1.57 ± 0.31 mmol/L, range 0.67-1.9 mmol/L with a median of 1.72 mmol/L.

Comparing group 3a with group 1 showed a significant difference in FPG ($p<0.01$), with a highly significant difference in albumin ($p<0.001$), total protein ($p< 0.001$) and FAm ($p< 0.001$). After correction, there was no significant difference between FAc_1, FAc_2, FAc_3 and FAc_4, (table 4).

Table 4 Comparison between group 3 (a and b) and group 1 (mean±SD).

Parameters	Group 1 (n=250)	Group 3a (n=24)	Z	p	Group 3b (n=29)	Z	p
FPG (mmol/L)	4.7 ± 0.6	4.4 ± 0.56	2.2	< 0.01	4.8 ± 0.71	1.2	NS
Albumin (g/l)	41.4 ± 4.5	37.3 ± 2.1	4.9	< 0.001	36.9 ± 3.6	4.9	< 0.001
Total protein (g/l)	68.7 ± 7.8	62.5 ± 5.3	4.0	< 0.001	59.4 ± 5.6	5.8	< 0.001
FAm (mmol/L)	1.93 ± 0.28	1.69 ± 0.19	3.9	< 0.001	1.57 ± 0.31	5.3	< 0.001
FAc_1 (mmol/L)	1.89 ± 0.29	1.78 ± 0.2	1.7	NS	1.67 ± 0.34	2.9	< 0.001
FAc_2 (mmol/L)	1.89 ± 0.28	1.76 ± 0.19	1.4	NS	1.65 ± 0.33	3.4	< 0.001
FAc_3 (mmol/L)	1.88 ± 0.31	1.83 ± 0.23	0.43	NS	1.73 ± 0.39	2.0	< 0.01
FAc_4 (mmol/L)	1.98 ± 0.35	1.91 ± 0.2	0.88	NS	1.88 ± 0.39	0.78	NS

Comparing group 3a with group 2 in the middle stage showed also a highly significant difference ($p<0.001$) in albumin and total protein, while there was no significant difference in FPG and FAm. Comparing FAc revealed no significant difference using all equations between these groups, (table 5).

Table 5 Comparison between group 3a and group 2 in the middle stage of pregnancy (mean ± SD).

Parameters	Group 3a (middle stage) (n=24)	Group 2 (middle stage) (n=73)	Z	P
FPG (mmol/L)	4.4 ± 0.56	4.6 ± 0.63	1.13	NS
Albumin (g/l)	37.3 ± 2.1	39.7 ± 3.7	3.1	< 0.001
Total protein (g/l)	62.5 ± 5.3	66.0 ± 4.9	2.8	< 0.001
FAm (mmol/L)	1.69 ± 0.19	1.80 ± 0.33	1.6	NS
FAc_1 (mmol/L)	1.78 ± 0.2	1.82 ± 0.32	0.7	NS
FAc_2 (mmol/L)	1.76 ± 0.19	1.80 ± 0.32	0.6	NS
FAc_3 (mmol/L)	1.83 ± 0.23	1.83 ± 0.33	0.2	NS
FAc_4 (mmol/L)	1.91 ± 0.2	1.93 ± 0.37	0.3	NS

Comparing group 3b with group 1 showed a highly significant difference ($p<0.001$) in FAm, albumin, and total protein but there was no significant difference in FPG. Following correction, comparison showed a highly significant difference in FAc_1, FAc_2, FAc_3, but there was no significant difference in FAc_4 (table 4). Comparing group 3b with group 2 in the late stage revealed a highly significant difference between them in total protein ($p<0.001$), FAm ($p< 0.001$), while there was no significant difference in FPG and

albumin. After correction of fructosamine the results showed no significant difference using all equations, (table 6).

Table 6 Comparison between group 3b and group 2 in the late stage of pregnancy (mean ± SD).

Parameters	Group 3b (late stage) (n=29)	Group 2 (late stage) (n=84)	Z	p
FPG (mmol/L)	4.8 ± 0.71	4.6 ± 0.66	1.98	NS
Albumin (g/l)	36.9 ± 3.6	38.0 ± 3.5	1.2	NS
Total protein (g/l)	59.4 ± 5.6	63.5 ± 4.4	3.5	< 0.001
FAm (mmol/L)	1.57 ± 0.35	1.77 ± 0.33	2.8	< 0.001
FAc_1 (mmol/L)	1.67 ± 0.34	1.83 ± 0.37	1.6	NS
FAc_2 (mmol/L)	1.65 ± 0.33	1.80 ± 0.37	1.2	NS
FAc_3 (mmol/L)	1.73 ± 0.4	1.88 ± 0.42	1.34	NS
FAc_4 (mmol/L)	1.88 ± 0.4	1.96 ± 0.41	0.75	NS

Further subgrouping of this group was also made according to the degree of proteinuria into three subgroups (3c with trace of proteinuria, 3d with [+] of proteinuria and 3e with [++] of proteinuria).Using ANOVA test revealed a significant difference between these subgroups in albumin and total protein (F = 4.6, 3.5 respectively, $p< 0.05$) while there was no significant difference in FPG (F=1.14, $p>0.05$) and FAm (F=1.5, $p>0.05$). Using Duncan's test for within group comparison showed a decrease in mean FAm, but it was still statistically not significant, while there was a significant difference between group 3c and group 3d and between group 3d and group 3e in serum albumin, and between group 3c and group 3e and also between group 3c and group 3d in total protein, (table 7). After correction, there was also no significant difference between these groups in FAc using all equations.

Table 7 Comparison between different biochemical parameters within group 3 (mean ± SE).

Parameters	Group 3c (n=16)	Group 3d (n=21)	Group 3e (n=16)
FPG (mmol/L)	4.5 ± 0.16 a	4.6 ± 0.16 a	4.8 ± 0.15 a
Albumin (g/l)	37.1 ± 0.46 ab	38.3 ± 0.44 a	35.5 ± 1.05 b
Total protein (g/l)	63.4 ± 1.5 a	60.7 ± 1.03 ab	58.3 ± 1.41 b
FAm (mmol/L)	1.69 ± 0.06 a	1.65 ± 0.05 a	1.54 ± 0.08 a
Fac_1 (mmol/L)	1.78 ± 0.06 a	1.70 ± 0.5 a	1.68 ± 0.10 a
Fac_2 (mmol/L)	1.76 ± 0.06 a	1.69 ± 0.05 a	1.64 ± 0.09 a
Fac_3 (mmol/L)	1.83 ± 0.07 a	1.73 ± 0.06 a	1.78 ± 0.11 a
Fac_4 (mmol/L)	1.88 ± 0.06 a	1.92 ± 0.07 a	1.87 ± 0.10 a

Different letters horizontally means significant difference at p = 0.05.

After correction of FAm, comparison was done between FAm and FAc in each subgroup (c, d, and e) using Duncan's test. The results revealed no significant difference between FAm and FAc using all equations in group 3c, while there was a significant difference only between FAm and FAc_4 in group 3d. In group 3e this difference is more obvious and involving FAc using all equations, (tables 8).

Table 8 Comparison between measured and corrected fructosamine using all equations within group 3 classified according to the degree of proteinuria, (mean ± SE).

Groups	FAm (mmol/L)	FAc_1 (mmol/L)	FAc_2 (mmol/L)	FAc_3 (mmol/L)	FAc_4 (mmol/L)
4c	1.69 ± 0.06 a	1.78 ± 0.06 a	1.76 ± 0.06 a	1.83 ± 0.07 a	1.88 ± 0.06 a
4d	1.65 ± 0.05 a	1.7 ± 0.05 a	1.69 ± 0.05 a	1.73 ± 0.06 a	1.92 ± 0.07 b
4e	1.54 ± 0.08 a	1.68 ± 0.1 ab	1.64 ± 0.09 ab	1.78 ± 0.11 ab	1.87 ± 0.1 ab

Same letters horizontally mean no significant difference at p = 0.05.

Discussion

In this study, the distribution of FAm results in non-pregnant females (group 1] and pregnant women (group 2) showed a Gaussian pattern with the reference range (95th centile confidence limit) was 1.37-2.49 mmol/L and 1.13-2.45 mmol/L respectively ($p<0.001$). Compared with other studies, the reference range established from 109 normal subjects was 1.53-2.33 mmol/L[24]. The reference range obtained from 100 healthy adults volunteers in Mosul was 1.25-2.50 mmol/L[25], and a recent study that included screening of 910 non-diabetic subjects also in Mosul showed a reference range of 1.30-2.65 mmol/L[26]. Since fructosamine method is dependent upon a variety of factors including pH, temperature, incubation time, and reagent concentration[6], therefore, alteration of these reaction conditions may lead to variation in the reported reference values. Another explanation is related to the origin and concentration of fructosamine standard used in the calibration of the analysis [6]. Therefore, some investigators did not recommend the use of a primary standard and the use of a secondary calibrator or other glycated protein preparation as glycated polylysine for fructosamine assay has been advised [27]. However, this difference may be of little importance as long as each laboratory establishes its own reference range and standardizes the calibration of the assay.

In non-pregnant women (group 1), there was a significant correlation between FAm and albumin, but not with total protein. This is comparable with other studies which reported significant correlation between albumin and fructosamine [20, 28], but in contrary to others which showed no significant correlation [7, 26]. A significant correlation was also observed between measured and corrected fructosamine

using all four equations where (r =0.88, 0.93, 0.76 and 0.90] ($p<0.001$). Correction of FAm, therefore, is not recommended in non-pregnant women when their serum albumin or total protein being within the reference ranges. This finding is also supported by a study done on 1114 non-diabetics of both sexes including children[29]. Another study done on 83 healthy non-diabetics showed also that fructosamine does not depend on albumin or total protein provided that albumin remains >30 g/l[7]. However, other investigators(12,18,30] recommended correction, a conclusion that was made by others as well[19, 20, 28].

In normal pregnant women (group 2) no significant difference in FPG level was noted as also reported by other investigators who observed no change in mean FPG throughout the entire duration of pregnancy[31]. Others showed a significant rise in FPG between 28th and 36th weeks of gestation[32, 33]. This may result from enhanced basal hepatic production of glucose. Catalano *et al.*(34] reported a 30% increase in basal hepatic glucose production by the late gestation. Another study has reported relative fasting hypoglycaemia during late pregnancy, a study that included lean individuals[35]. Also, a significant decrease in mean serum albumin, total protein and FAm, particularly during the middle and late stages, in comparison with non-pregnant women was noted. Serum FAm had been reported to decrease with the decrease in serum albumin and total protein through the progress of the pregnancy[36]. This may be explained by the redistribution of body water during pregnancy[4].

In this study, there was a decrease in mean FAm during the progress of pregnancy throughout early, middle and late stages of pregnancy; with a highly significant difference from the values in non-pregnant women. This is in agreement with Gunter *et al*[37] who showed that FAm values were significantly lower in the three trimesters corresponding to non-pregnant women. Abe *et al*.[36] reported that serum total protein and albumin decreased slightly but significantly in the middle and late stages of pregnancy suggesting an apparent decrease in fructosamine concentration. Comparing the different stages of pregnancy, it appears, therefore, that there is a downward trend in FAm value with the progress of pregnancy that is associated with significant decrease in albumin and total protein.

Many workers had studied the relationship between fructosamine and albumin in diabetics and non-diabetics with different formulae for correction of fructosamine[18-21]. In this study, correction of FAm for albumin and total protein in the early stage of pregnancy eliminated the differences from the control group. In the middle and late stages, correction for albumin was not sufficient to eliminate this difference. Hence, correction of fructosamine for albumin or total protein is indicated during pregnancy particularly middle and late stages. Achievement of correction using equation 4 that is based on total protein is more advantageous than the other three equations that are based on albumin. Globulins may, therefore, have a contributing role in fructosamine correction. However, correction of fructosamine for albumin or total protein may not completely eliminate the discrepancies between measured and corrected values. In vitro kinetic and in vivo studies have shown that glycation is also dependent on the

rate of turnover of plasma protein[7]. The decrease in plasma albumin may diminish its catabolism leading to prolongation in its half life resulting in greater extent of glycation on molar basis[38]. This is in agreement with the work of Sengy and Staly[39], Schleicher *et al.*(12] , Mula-Abed and Hanna[28] and Lim and Staley[40] who proved that serum fructosamine is more dependent on half-life of serum proteins than their concentrations.

In group 3 (pregnant women complicated with PET), the values of FAm showed lower levels than both normal pregnant and non-pregnant women with also lower values in albumin, total protein but not in FPG. When sub-grouping according to the gestational stage was done, a significant difference in FPG, albumin, total protein and FAm was noted in middle stage (group 3a) compared with control group 1. Following correction, the FAc values were elevated and as compared to control group, there was no significant difference observed in FAc according to albumin or total protein. As compared with the middle stage of normal pregnant women, there is no significant difference observed in FAc using all equations from corrected values in normal pregnant women. In pre-eclamptic women in the late stage of pregnancy (group 3b), FAm showed also low values and following correction there was no improvement in these values by all equations, when compared with non-pregnant women. However, as compared with values in normal pregnant women in their late stage the differences disappeared when using all equations.

When sub-grouping of pregnant women with PET according to the degree of proteinuria was done, a downward trend in FAm values was obtained with the increase in the degree of proteinuria. In pregnant women with (trace] of proteinuria (group 3c), FAm values were not statistically different and following correction using all equations there was still no difference. However, in those having (+) proteinuria (group 3d], correction according to albumin showed no difference while correction according to total protein resulted in significant elevation in FA values. In pregnant women with (++) proteinuria (group 3e), corrected FA using all equations showed significant difference from FAm. So correction of fructosamine in pre-eclamptic women seems to be mandatory as such correction for low protein or albumin increases the value of the test.

In conclusion: during pregnancy, there is a decline in serum fructosamine, albumin and total protein with the progress of pregnancy. There is a need for correcting fructosamine according to albumin or total protein especially in the middle and late stages of gestation. Correction of serum fructosamine for total protein concentration is superior to that for albumin concentration. In pregnancy complicated by pre-eclamptic toxaemia, correction of fructosamine according to albumin or total protein, is mandatory as the values differ from those in non-pregnant and normal pregnant women.

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