Serum Fructosamine Measurement as an Index of Glycaemic State in Iraqi Diabetics

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Objective: To determine the usefulness of measuring serum fructosamine (glycosylated protein) as an index of assessment of glycaemic state.

Design: Prospective study of the glycaemic state of diabetic patients over a period of 6 months from 1st January 1996 to 30th June 1996.

Setting: Diabetic Clinic of Mosul Teaching Hospital.

Participants: A total of 340 individuals including 60 presumably type 1 diabetics (41 males, 19 females, aged 12-51 years), 180 type 2 diabetics (85 males, 95 females, aged 28-65 years) and 100 healthy subjects (59 males, 41 females, aged 19-60 years)

Main outcome measures: Measures were fasting plasma glucose and serum fructosamine levels. Correction of fructosamine concentration according to albumin level was performed. Student's unpaired t-test and linear regression analysis were used for data evaluation.

Main Results: The distribution of fructosamine in the control group showed a normal Gaussian pattern with the reference range calculated as mean ± 2SD was 1.50-2.50 mmol/l. Serum fructosamine was significantly higher in type 1 and type 2 diabetics in comparison with control group (P <0.001). A significant correlation was observed between plasma glucose and serum fructosamine in type I (r = 0.718), type 2 (r = 0.868) and control group (r = 0.814)(P <0.001). Agreement between glucose and fructosamine was noted in 81.7% of cases with type 1 and 90.5% of 'cases with type 2 diabetes. When measured fructosamine was corrected according to serum albumin, a highly significant correlation was observed between measured and corrected values in diabetics with type 1 (r =0.996), type 2 (r = 0.995) and non-diabetics (r = 0.95 1)(P <0.001).

Conclusion: Serum fructosamine presents a suitable index for the evaluation and monitoring of glycaemic control particularly in those with unstable diabetes. Correction of measured values for albumin concentration seems to be unnecessary particularly in those with normal protein status.

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Diabetes mellitus is a life-long disease that requires continuous monitoring to avoid uncontrolled glycaemic state and to minimize long term complications[1]. For years, this control has been evaluated by traditional biochemical measurements that include mainly urine and blood glucose analyses[2]. However, despite their common use, both tests are variable during the day, and may be affected by a variety of conditions[3,4].

Measurement of glycosylated haemoglobin (HbA1) has been widely used as an index of long term control since the mid 1970's[5]. Its major disadvantage is the period (2-3 months) during which it reflects the glycaemic state, thus fluctuating hyperglycaemia may not be assessed by HbA1 due to its long life[6]. Also, conditions associated with altered life span of RBC may result in disproportionate HbA1 levels compared to actual glycaemic state[7]. However, since 1979, the existence and usefulness of other glycated proteins has been recognised[8]. Serum glycosylated protein reflects glycaemic control during reasonable shorter period (2-3 weeks) and may be of value as an intermediate control index[9].

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Several methods are available for the measurement of these glycosylated indices that include colorimetric techniques, electrophoresis, affinity and high pressure liquid chromatography[10-12]. However, some of these procedures are expensive, labor intensive or may require technical skill or special instruments that can't be recommended for routine laboratory service.

A novel approach for estimating serum glycosylated protein has been described by Johnson et al[13] in 1983. The assay is based on the ability of ketoamine (which the authors termed fructosamine) to reduce nitroblue tetrazolium (NBT) in alkaline buffer[14]. It is a valuable intermediate term index of glycaemic state that seems to have the advantages of technical simplicity, low cost, reagent stability and its possible adoption to numerous automated analysers[15-17]. Fructosamine measurement may be of benefit mainly to those whose blood glucose control may be difficult to evaluate as in patients with type 1 diabetes because of the wide fluctuation during the day[18]. It may also be useful in diabetic children because of the difficulty in achieving a strict dietary control and the non-interference with HbF[19]. It is also suggested that serum fructosamine provides a useful screening test for diabetes mellitus[20,21], including gestational diabetes[22].

The aim of this study was to determine the usefulness of measuring serum fructosamine (glycosylated protein) as an index of assessment of glycaemic state in patients with different types of diabetes mellitus.

METHODS

The subjects enrolled in the current study included 240 diabetics who were attending the Diabetic Clinic of Mosul Teaching Hospital. The study period extended for 6 months from 1st January 1996 to 30th June 1996. The subjects were divided into:

Group 1: constituted 60 patients presumably with type 1 diabetes mellitus (41 males, 19 females, aged 12-51 years) who were insulin treated, at least most of them having IDDM, and were on different regimes of insulin therapy besides diet restriction.

Group 2 : constituted 180 patients with type 2 diabetes mellitus (85 males, 95 females, aged 28-65 years), who were using oral hypoglycaemic drugs (sulphonylureas or biguanides) besides diet restriction.

Group 3: constituted 100 apparently healthy subjects (59 males, 41 females, aged 19-60 years) and they served as a control group. Half of this group were students and teaching staff of Mosul Medical College, and the other half were blood donors attending Blood Bank of Mosul Teaching Hospital.

Blood samples were obtained from every subject in the fasting state and divided into 2 aliquots, one into fluoride-oxalate tube for plasma glucose and the other into plain tube for serum fructosamine and albumin measurements. Plasma glucose was measured by glucose oxidase-peroxidase method[23], and serum albumin by bromocresol green dye binding procedure[24]. Fructosamine was assayed manually using NBT colorimetric method. This is based on the reducing ability of ketoamine in alkaline solution at 37° C. The absorbance change during 5 min is measured between 10 and 15 min at 530 nm and compared with standard of Amadori rearrangement product (1.deoxy.1.morpholino. D.fructose; DMF), containing human albumin 40 g/l treated in an identical manner. The DMF was synthesized by the method of Hodge and Rist[25].

The statistical methods used included unpaired t-test and linear regression analysis. All values are quoted as mean 1 SD with difference between observations being considered not significant at $P > 0.05$.

RESULTS

The frequency distribution of serum fructosamine in the control group 3 showed an essentially normal (Gaussian) pattern with skewness of 0.36. The reference range of fructosamine was then established, calculated as mean ± 2SD, which was 1.50-2.50 mmol/1. The results of glycaemic indices in the different groups are presented in table 1.

Table 1: **Glycaemic indices in diabetic and control subjects. (Values are presented as mean ± SD)**

Glycaemic Index	*Group 1 (Type 1) n = 60*	*Group 2 (Type 2) n = 180*	*Group 3 (Type 3) n = 100*
Glucose (mg/dl)	215 ± 129.8	197 ± 79.7	84 ± 10.2
Fructosamine (mmol/l)	3.47 ± 1.15	3.11 ± 0.76	1.98 ± 0.25
Corrected Fructosamine (mmol/l)	3.52 ± 1.14	3.15 ± 0.76	1.98 ± 0.26

Group 1 had fasting plasma glucose of 215 ± 129.8 mg/dl, mean ± SD which is significantly higher than control group ($t = 9.96$, $P < 0.001$) and serum

fructosamine was 3.47 ± 1.15 mmol/l which is also significantly higher than control (t= 12.76, P <0.001). A significant correlation between these indices was obtained, where (r = 0.718, P <0.001 and y = 2.10 + 0.00637X) Fig 1.

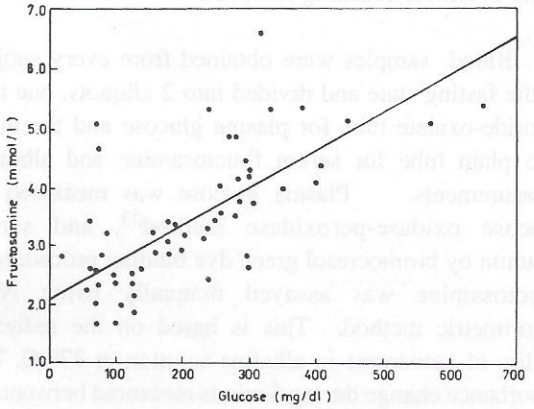

Figure 1. Correlation between plasma glucose and serum fructosamine concentrations in IDDM group (r = 0.72; P<0.001; n = 60; y = 2.10 + 0.00637X)

Group 2 had fasting plasma glucose of 197 ± 79.7 mg/dl which is significantly higher than control (t = 14.1, P <0.001) and serum fructosamine was 3.11 ± 0.76 mmol/l which is also significantly higher than control (t = 14.8, P <0.001). A significant correlation was also obtained between these parameters where (r = 0.868, P <0.001 and y = 1.48 + 0.00837X) Fig 2.

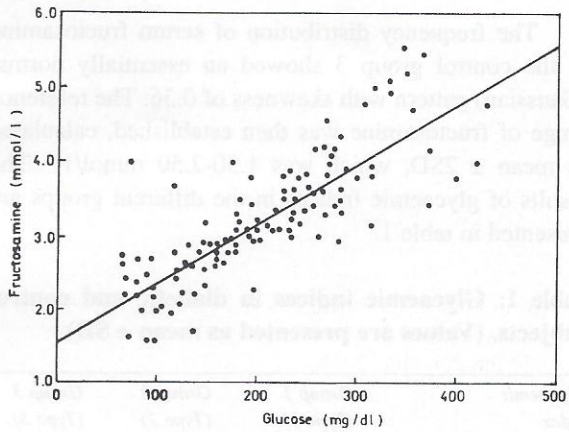

Figure 2. Correlation between plasma glucose and serum fructosamine Concentrations in NIDDM group (r = 0.87; P<0.001; n = 180; y = 1.48 + 0.00831)

Agreement between glucose and fructosamine in groups 1 and 2

The degree of agreement between plasma glucose and serum fructosamine levels in the 240 diabetics was studied. For this purpose, fasting glucose of 110 mg/dl and fructosamine of 2.50 mmol/l were taken as the cut off point with values higher than these being regarded as elevated.

In group 1 with type 1 diabetes; 38 (63.4%) patients had both glucose and fructosamine elevated, 11 (18.3%) had both normal, 3 (5%) had elevated glucose with normal fructosamine and 8 (13.3%) had normal glucose with elevated fructosamine. In group 2 with type 2 diabetes; 136 (75.5%) had elevated glucose and fructosamine, 27 (15%) had both normal, 13 (7.3%) had elevated glucose with normal fructosamine and 4 (2.2%) had normal glucose with elevated fructosamine.

Relationship between measured (FAm) and corrected fructosamine (FAc)

Correction of measured fructosamine for albumin, since it is the most predominant protein that undergoes glycosylation[10], is calculated as proposed by Howey et al[23]

FAc = [(FAm) + 0.03 X (40 - serum albumin in g/l)]

The findings revealed that serum fructosamine did not significantly correlate with albumin in groups 1, 2 and 3 (r = 0.156, 0.028, 0.051, PNS) respectively. Also, both corrected (FAc) and measured (FAm) fructosamine were very highly significantly correlated with each other (r = 0.996, 0.995, 0.951, P < 0.00 I) in groups 1, 2 and 3 respectively.

DISCUSSION

Different studies have reported some variation in the reference range for serum fructosamine. In this study, the reference range was 1.50-2.50 mmol/l which is comparable with the range of (1.87-2.87) mmol/l that was reported in 502 non-diabetics by Baker et al[27]. Other investigators obtained different ranges varying from as low as (0.46-0.80) mmol/l in 40 non-diabetics[28], to as high as (2.10-2.80) mmol/l in 133 healthy subjects[29]. Although population difference is an important factor, variation in these ranges may be further attributed to analytical factors particularly the change in buffer pH of the assay from 10.80, in the original work[13], to 10.35 later on; a modification that was disputed during the development of the assay[30]. Moreover, the subtraction of the absorbance change of zero standard (containing only albumin without DMF) from the standard curve, which was not followed in the early studies, also resulted in low reference

values[13,28,31]. Altered source of DMF standard and its binding with variable preparation and concentrations of protein included in the standard solutions can also be blamed[13,28,32,33] .

In type 1 diabetics, serum fructosamine reflected the glycaemic state in the majority of patients as evidenced by the significant correlation (r = 0.718, P <0.001, Fig 1) between glucose and fructosamine. The result is consistent with the report of other researchers[13-15,18]. Diabetics who were not properly controlled with high glucose also had high fructosamine as observed in 63.4% of patients, and those with normal glucose also had normal fructosamine as noticed in 18.3% of patients. We expected to obtain a higher degree of correlation but the normal values of glucose with high fructosamine in the 13.3% of the diabetics reflected an attempt by the patients to improve their control shortly before their visits to the clinic, or the patients may have received their insulin therapy shortly before attending the diabetic clinic. Furthermore, glycaemic variation in such diabetics renders single blood glucose estimation unrepresentative[4,34,35].

In type 2 diabetic, a higher correlation (r = 0.868, P <0.001, Fig 2) between fructosamine and glucose was noted, a finding that is comparable with other reports[36,17, 31]. The closer correlation between these indices may be attributed to the relatively more stable glycaemic state. An agreement between the two glycaemic parameters was found in the majority of studied patients (90.5%) while the discordance was noted in a minority of cases (9.5%) because they evaluate different aspects of glycaemic state and reflect the degree of control over different intervals[6,10,37, 40] .

The highly significant correlation between measured and corrected fructosamine in the control and diabetics, besides the non-significant correlation between albumin and fructosamine, suggests the independence of fructosamine from albumin, as long as protein level is within normal, as supported by other studies[26,37,41]. Since the majority of tested patients had albumin >35 g/l and no one had albumin <30 g/l, correction does not seem to be necessary and the uncorrected values are valid indicators provided that there is no coexisting hypoalbuminemia or disturbed protein metabolism[40-42].

CONCLUSION

Serum fructosamine correlates significantly with plasma glucose and so presents a suitable index for the evaluation and monitoring of glycaemic control particularly in those with unstable diabetes. The highly significant relationship between measured and corrected fructosamine renders correcting fructosamine according to albumin concentration unnecessary particularly in those with normal protein status. It is also a practicable assay and hence its application as a complementary glycaemic index merits consideration in diabetic care assessment.

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