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HBA1C% INDUCED CATARACT IN DIABETIC PATIENTS

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Summary

Cataract formation in the diabetic patients was studied on the basis of the determination of (Hb A1c%) using variant HPLC instrument and blood glucose enzymatic method for (42) blood specimens. These patients are; neglected blood glucose levels (patients are not using any treatment), on oral antidiabetic medications and insulin dependent. The effect of (Hb A1c %) when raise above the normal value (5.8%) in diabetic patients, is leading to disturb the transparency of the eye lens. The aim of this study is to show the relation between high values of (Hb A1c%) and cataract lens changes. (HbA1c%) of ten normal blood specimens were measured as control group.

Introduction

Blood glucose determinations in the diabetic patients at the time of blood collection explain the current metabolism of the carbohydrate. Glycohemoglobin (or glycated hemoglobin) in contrast, allow retrospective estimation of glycemia, largely independent of circadian patients dietry, and other transient fluctuations in the blood glucose concentration by integrating the latter over the course of days or weeks[1].

Hemoglobin is nonenzymatically glycated when blood glucose enters the erythrocytes and it's anomeric hydroxyl derivatives amino groups present on n-terminal value [2]. Total glycohemoglobin is subdivided into subtractions depending on each of the glycation sites and reaction partners[1]. The sum sub of reactions is called (HbA_1), in diabetic patients (HbA1c%) is the most important glycated parameter. Besides plasma proteins which differ in their half lives and concentrations and sometimes subject to relatively large antra individual fluctuations, glycated hemoglobins are particularly suited as long – term parameter for assessing glycemic control.

The half life of hemoglobin is defined by the red cell several time which is relatively constant at (100-120) days. The degree of the glycation, a part from the half- life of hemoglobin , depends essentially on the concentration as well as the duration of the blood glucose

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elevation. Glycation is irreversible and enzymatic system for the degradation of glycation sites of hemoglobin's are not known[1].

The terminology of glycation produced glucose and other carbohydrate with hemoglobin has changed several times since the first description in (1968) of an abnormal hemoglobin found in diabetic patients[3] since (1968), IUPAC-IUB (international union of pure & applied chemistry) has recommended the use of terms glycohemoglobin for the spontaneously, i.e. non – enzymatically occurring glycating of hemoglobin[4] compared to most other tissue, the lens has particularly high protein content & a low water content. The high protein concentration is necessary for the lens to maintain a high refractive index [5]. The transparency of the lens is largely the result of the high ordered arrangements of the macromolecular components of the lens cell & the small differences in refractive index between light scattering components[6]. Sine transparency of the lens is so highly dependent on protein order and structural integrity, it is not surprising that relatively small changes in any of these parameters might lead to the development opacification of the lens leading to cataract[5]

Cataract is a complication of diabetes and galactosemia. Human diabetic cataract can develop rapidly in uncontrolled diabetes mellitus[5.] There is also evidence that sugars might directly interact with lens proteins by a process called glycation, leading protein aggregation and ultimately cataract formation[7.]

It has been also suggested that glycation of protein might cause conformational changes, increasing the chance of oxidation and formation of disulfide bonds and subsequent aggregation[8]. Aspirin has been suggested as an anti-cataract agent on the basis of its ability to block protein glycation[9].

More recently have been demonstrated specific blockade of protein glycation by aminoguanidine[10.] The relative role of protein glycation in sugar cataract development remains an open question[11.]

Several techniques are available for the analysis of glycohemoglobins[12,13,14] most of them by HPLC (High performance liquid chromatography) using ion exchange principle. The rest of techniques used for glycohemoglobins measurements are: calorimetrically (using thiobarbituric acid), electrophoresis, isoelectric focusing using PH gradient principle, affinity chromatography using pheny1 baronet column and immunochemical methods.

In our study, the "Variant" instrument by (BIO-RAD), (ion-exchange) high performance liquid chromatography (HPLC) was used for the automatic & accurate separation of glycated hemoglobin (HbA1c%). The aim of this study is to evaluate the effect of glycated hemoglobin (HbA1c%) on eye lens & the formation of cataract in the diabetic patients. Blood glucose fasting was measured using enzymatic method (oxidase – peroxidase).

Materials and Methods

Blood specimens for (42) diabetic patients age (36-52) years, (28) males and (14) females were collected in EDTA tubes. These patients are;

i- Insuline dependant. (16- patients)
ii- On oral ant diabetic medication. (17- patients)
iii- Neglected blood glucose levels. (9 – patients)with out using medication

All of them were having cataract. Hemoglobin (HbA1c%) were measured for every specimen using "variant" instrument, the "variant" total GHB program utilizes the principles of

baronet affinity (Ion exchange) high performance liquid chromatography (HPLC) for automatic and accurate separation of glycated hemoglobin from non-glycated hemoglobin. The separation is performed rapidly and precisely, without interference from labile glycated hemoglobin's, lipemia, bilirubin, temperature fluctuations or age related degradation. "Variant" instrument was used according to the set–up- procedure explained in the instruction manual[15]. The non-glycated hemoglobin's first alluted from the column with buffer one, and the glycated hemoglobin's (HbA1c %) are displaced using buffer two equilibration is re-established by washing the column with buffer one. The separated hemoglobin's pass through the flow cell of the filter photometer, where changes in the absorbency are measured at (415)nm . calibrator is analyzed with each run . The calibrator adjusts the calculation parameter used in the determination of the relative percent of glycated hemoglobin (HbA 1c %).

Results

Blood results (HbA lc % and blood glucose) of 42 specimens for diabetic patients with cataract changes were shown in tables (I), (II) and (III).

Table I. Results of (HbA1c %) and fasting blood glucose for diabetic patients (Neglected blood glucose levels) with cataract changes. Blood glucose (280 –480 mg %)

Patient No.	*(HbA1c %)*	*Glucose mg %*
1	14.9	439
2	9.4	302
3	13.9	480
4	15.7	464
5	11.3	290
6	9.1	280
7	12.0	392
8	13.8	317
9	13.4	308

X =12.6 X =364
s.d = +2.2 sd= + 79

Table II. Results of (HbA1c%) and fasting blood glucose for diabetic patients (on oral anti diabetic medication), with cataract changes. Blood glucose (176-231 mg %)

Patient No.	*(HbA1c %)*	*Glucose mg %*
10	11.0	226
11	7.7	179
12	9.3	176
13	8.1	192
14	11.2	186
15	14.6	212
16	11.4	220
17	7.9	180
18	8.9	210
19	14.4	189
20	12.2	185
21	12.0	215
22	12.4	222
23	9.8	192
24	12.8	231
25	12.2	177
26	13.3	182

X=11.1 X= 198.0
s.d= +2-1 sd= + 19.2

Table III. Results of HbA1c% and fasting blood glucose for diabetic patients (insulin dependant), with cataract changes. Blood glucose (128- 170 mg %).

Patient No.	*HbA1c%*	*Glucose mg %*
27	8.4	162
28	12.1	163
29	12.1	168
30	10.9	169
31	7.9	170
32	9.2	158
33	8.9	168
34	12.4	170
35	8.0	160
36	7.2	148
37	8.7	132
38	8.5	165
39	11.2	170
40	10.5	165
41	11.2	163
42	10.0	128

X= 9.8 X= 159-0
s.d =+1.6 sd = +13.0

Measurements were performed for (HbA1c %) and fasting blood sugar using "variant" and the enzymatic,

oxidase- peroxidase method respectively in the same day of specimens collection.

Normal value of (HbA1c %) is not exceeding (5.8 %) [1]. Results for (Hb A1c %) ranging (7.2 –15.7 %) and blood glucose (128-480 mg %) are shown in table (1, 2,3). (9) High values of (HbA1c %), (9.1- 15.7 %) with high values of blood glucose (more than 280 mg %) are shown in table (I), (neglected blood glucose level). In table (II), (17) high values of (HbA1c %) (7.7-14.6 %) with results of blood glucose (176 – 231 mg%) are tabulated. (On oral antdiabetic medication).

In table (III), (16) high values of (HbA1c %) (7.2-12.9 %) and moderate values of blood glucose (128-170 mg%) are shown (insulin dependent patients) .

Evaluation of "variant" technique for (HbA1c %) measurements was also performed using the same quality control specimen [15] by two different methods." Variant and "diamat": (HbA1c %), and the results obtained were treated statically using linear regression program and the values of (r = 0.973, slop = 0.94 and intercept = 0.53) were calculated. diamat (HbA1c %) is another program for (HbA1c %) measurements which has been considered as a reference technique for (HbA1c %) [15] in table (IV) results of (HbA1c %) of ten normal values are shown as control group.

Table IV. Control Group

Patient No.	*HbA1c%*	*Glucose mg %*
43	4.3	118
44	4.2	121
45	5.2	120
46	4.9	117
47	4.7	117
48	5.4	119
49	4.6	109
50	3.9	98
51	5.0	118
52	4.4	113

X = 4.7 X= 115
sd = + 0.47 s.d = +7.4

Discussion

From a clinical stand point, cataract is defined as visual impairment as a result of disturbance of eye lens transparency [5]. Accumulation of polyols would seem to be a major factor in the cataractogenic processes, called Glycation, leading to protein aggregation and ultimately cataract formation. According to the results of (HbA1c %) shown in tables (I), (II) and (III), elevated values of (HbA1c %) in blood is more influential cause for cataract formation in diabetic patients as (HbA1c %). In the blood much longer than glucose and its bulky molecular having great negative effect on lens transparency. Such changes in the lens might include aggregation, change in tissue hydration phase separation of molecular components, breakdown of cell membranes and changes in the structure of the cytoskleton [5]. Most, if not all, of these changes can do take place during aging and cataract development. There is no simple treatment [1] like blood glucose level) to suppress it is high level in the blood and then to eliminate any serious effect on lens.

In table (I), 9 high result of (HbA1c %) with high values of blood glucose (more than 280% fasting), are shown. As explained above, high glucose level can be reduced to normal or a bit above normal within hours, thus might eliminate it's effect on lens, while high level (HbA1c %) can not.

Therefor the change in lens transparency could be due to (HbA1c %) high levels. In table (II), although blood glucose levels of these patients are treated (highest values (231) mg %, fasting), but (HbA1c %) is high enough to cause cataract formation. In table (III), blood glucose levels are less than (170mg %) and (HbA1c %) is much higher than normal values. As blood glucose level less than (170mg %) is not a cause of cataract formation in diabetic

patients (insulin dependant and controlled blood glucose levels), according to a number of research works[5]. As mentioned earlier, patients ages (36-52) years, this means that, the age of the patients is not the cause of cataract formation because they are not old patients (age factor can be neglected), clinically, all these patients are fit and having no problem of their diseases. As shown in the tables (I), (II), (III), values of (HbA1c %) are not increasing or decreasing linearly with blood glucose mg % this is due to the high level of glucose lasting time in the blood before reduce it (diet control or treatments) to a lower or normal levels of glucose existing in the blood stream will give a great chance for (HbA1c %) to form freely. According to the values of (SD and x), all the values (HbA1c%) and blood glucose are acceptable.

Conclusion

Measurements of (HbA1c %) in the blood of the diabetic patients are of vital importance for all diabetic patients (neglected, on oral antidabetic medication and insulin dependant).

Blood glucose measurements are not sufficient for diabetic patients to prevent any complication as blood glucose levels increasing or decreasing every single hour depends on; diet, anxiety type of work and medications. While (HbA1c %) does not. (HbA1c %) is non-enzymatic reaction, therefore it may form smoothly and simply in the blood stream of the diabetic patient as long as blood glucose level is higher than normal for a particular time. Glucose can be metabolized in the blood according to the certain metabolic rules to produce energy leading to reduce its high levels, while (HbA1c %) does not[1]. Therefore (HbA1c %) can be reflected a major cause for many implications in blood compositions of the diabetic patients and may lead to the change of lens transparency and cataract formation.

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