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Plasma Protein and Aggregation Changes in Diabetic Patients

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Abstract

Objective: Using a modified laser light scattering technique to study the red blood cells aggregation and sedimentation kinetics in normal and diabetic patients.
Method: Clinical records of (40) normal volunteers and diabetic patients from Educational Kadhimya Hospital were included in this study.
Results: In this method, the results of aggregation is presented in a time based graph, from which the slope (rate of aggregation can be calculated). Diabetic sample showed a shorter time of different aggregation and sedimentation stages. Samples from diabetic patients showed a lot of variability in regard with different stages of aggregation. These changes were correlated well with the abnormality in their proteins.
Conclusion: In diabetes mellitus a lot of rheological abnormalities could occur, like hyperglycemia, changes in lipid profiles and serum proteins and their sequel on the viscosity of blood on one hand and the structural and functional abnormalities of erythrocyte on the other hand. These modifications in the cellular properties and medium composition of blood in diabetic patients will affect the aggregation kinetics of erythrocytes, which may play a role in the pathogenesis of diabetic complication.

الخلاصة

قدمت هذه الدراسة على أساس أن المرضى المصابين بداء السكري يتعرضون الى العديد من المشاكل التي تصاحب دوران الدم مثل زيادة نسبة السكر في الدم (hyperglycemia) وكذلك التغيرات التي تطرا على تركيز الليبدات وبروتين المصل وبالتالي تأثير هذه التراكيز على لزوجة وتركيبه واداء كريات الدم الحمراء.كما أن هذه التغيرات بدورها سيكون لها تأثير كبير على خواص أغشية ومكونات خلايا الدم وبالتالي ميكانيكية تجمع وترسب كريات الدم الحمراء والتي بدورها تؤثر على انسيابية الدم ومضاعفات مرض السكري.

في هذه الدراسة اعتمدنا تقنية مطورة لدراسة استطارة ضوء الليزر من عينات تحتوي على 10%كريات دم حمراء موزعه في مصل مرضى مصابين بالسكري واخرين بصحة جيدة النتائج تضمنت دراسة ميكانيكية التجمع والترسب الكامل كريات الدم الحمراء مع معدل وسرعة حدوث كل مرحلة من مراحل العملية. ووجد إن النماذج المأخوذة من مرض السكر تختلف في قيم معلماتها عن تلك المحسوبة للنماذج المأخوذه من المتطوعين الطبيعيين ,وقد نوقشت النتائج على ضوء التغيرات الحاصلة في بروتين أمصال دم المرضى.

Introduction

Microangiopathy is a slowly progressive vascular change in diabetic patients, which may be pathological cause of many of the diabetic complications like neuropathy, retinopathy and nephropathy. Microangiopathy is related to the rheologic changes in diabetes, like increased red-cell aggregation and increased plasma viscosity.

The verification of the rheologic theory of diabetic microangiopathy is renal most difficult by the complex flow properties of human blood, and by the equally complicated, many factors that affect the microcirculation in normal and pathological conditions, like plasma viscosity and erythrocyte deformability (1).

Taking into consideration the plasma viscosity, blood is a suspension of erythrocyte in plasma, accordingly, the computed coefficient of apparent blood viscosity primarily depend on the true viscosity of the plasma and the effect of the dispersed erythrocytes on the flow of plasma i.e. hematocrite, red blood cell rigidity and viscosity. Moreover, blood is anomalous fluid, the viscosity of which (in vivo) can not be defined, as it varies with flow conditions. In vivo, when there is high shearing forces, erythrocytes adapt to the flow of plasma, but in the absence of such force, high molecular weight plasma aggregates the erythrocytes in rouleaux and three dimensional aggregate and sedimentation will occur. Accordingly, performing the test of aggregation in vitro will exclude the shearing force as an effective factor in the process (2).

There are evidences that there are plasma protein changes in diabetics, basically disturbed molecular shape of the protein rather than the pure effect of protein concentration (3). These protein changes will affect the process of aggregation and sedimentation and hence play a role in the progress of microangiopathy and pathogencity of diabetic complication.

In this study we try to correlate protein changes in diabetic patients with the changes that occur in the process of aggregation and sedimentation.

The physical point of view

When a photon beam (laser light) is passed through a material, the photon flux is reduced, because photons are removed, or deflected from the forward direction (4).

Figure (1) illustrates a flux incident on a sample with thickness (dx), and photons with flux (N-dN) emerges from the absorbing species of sample; where (dN) is the number of photons removed from source beam:

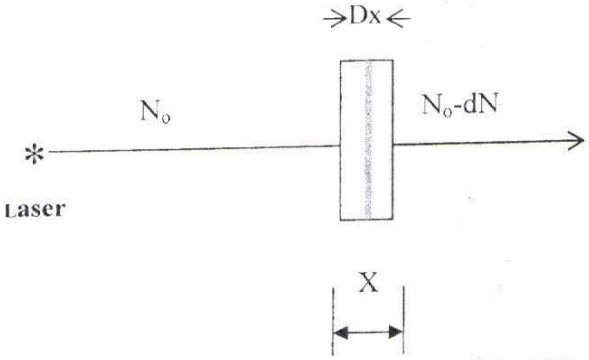


Figure.1: Monochromatic beam of light (N) incident on a sample cuvate with thickness (dx), photons with flux (N-dN) emerges from the absorbing species of sample.

$dN = -\mu N dx$

μ is the proportional constant (absorbing coefficient)

$$N\int_{0}^{N}(dN/N) = -\mu\int_{0}^{N}dx$$

$ln(N/N_o) = -\mu x$

or

$N = N_o e^{-\mu x}$ 1

Eq. (1) shows that the intensity of monochromatic electromagnetic radiation falls off exponentially.

In the term of monochromatic light (laser) [5].

$$\text{Intensity of a photon beam} = \frac{\text{energy}}{\text{photon}} + \frac{\text{no. of photons}}{\text{area} \times \text{time}}$$

The last term in the right is the photon flux N, the intensity of beam (I) is:

$I = (h\upsilon) N$ 2

Where (h) is Blank's constant and (υ) is the laser frequency. Using eq. (2), eq. (1) may be written as:

$I = I_o e^{-\mu x}$

Subjects and method

Subjects

Thirty-one normal volunteer and thirty-one diabetic patients were included in this study.

The volunteers were normal healthy subjects, their ages range between 20 and 45 years, with no past history of hematological or metabolic disorder.

Most of them were either medical staff or student of Saddam medical college of medicine. All were exposed for complete blood picture examination, total serum protein and serum albumin

estimation, fasting blood sugar test as well as ESR test. Any of them who showed abnormality in any of these tests was excluded from the study. The diabetic patients were with different duration of the disease ranging from few months to more than ten years. They were of varying metabolic status. Their ages range between 23 and 50 years.

Method

The method used is a modification of the method suggested by Muraldinharan et al., 1994. This method based on Laser light scattering technique (6, 7).

Procedure

Ten milliliter of venous blood was aspirated from each of the normal subject and diabetic patient, and was anticoagulated with .06 ml of heparin. The blood sample was divided into two parts. One part for measurement of fasting blood sugar, total protein and serum albumin estimation. The other part was centrifuged at 3000rpm for 5 minutes to separate the red blood cells from the plasma. Then the 10% PCV was prepared by adding 100 micro liter of packed cells to 900 micro liter of plasma. This 10% PCV suspension was then gently poured into the sample cell that is fixed in between the laser beam and the photocell that recorded the intensity of the transmitted light, as it is shown in figure (2):

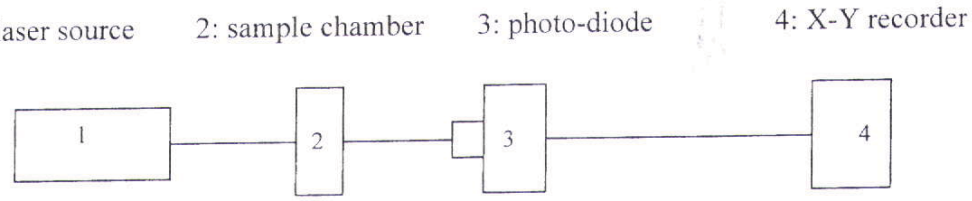


Figure (2) :The system layout

The transmitted light is changed with the progress in aggregation process. The test of recording the aggregate process was repeated again for normal and diabetic samples after removing the macromolecules (protein) by using high speed, cooling centrifuge at a rate of 15000 rpm, for 20 minutes.

Results

The pattern of aggregation of a sample derived from a normal subject and a sample derived from a diabetic subject (in the presence of macromolecule) were presented in figure (3 a. b.). It is clear that the diabetic sample showed a shorter time of different aggregation and sedimentation stage

Table (1, a.) presents the mean ± S.D. of the time obtained from the study of aggregation and sedimentation by Laser scattering technique at different stages during the process of sedimentation (in the presence of macromolecule). Generally diabetics showed a lower aggregation time. This decrease in aggregation time was statistically significant in rouleaux formation stage (AT), one dimensional aggregate formation time and sedimentation stage. While the decrease in the three-dimensional aggregation time was insignificant statistically.

The estimated total serum protein in the diabetic patients was higher than that of normal subjects (7.22 mg/100 cc ± 0.814, 6.59mg/100cc ± 0.94 respectively), this difference was statistically indifferent. While the serum albumin was slightly decreased in diabetics in comparison to that of the normal subjects (4.16 mg/100cc ± 0.792, 4.69 mg/100cc ± 0.472 respectively).

On removing the macromolecules from the plasma of normal subjects and that of diabetic patients, the latter showed similar or even statistically insignificant higher value of aggregation and sedimentation time in comparison to that of the normal subjects (table 1,b.), although their fasting blood sugar was not the same (189.45mg/100cc ± 84.33, 92.72mg/100cc ± 19.15) for diabetic and normal subjects respectively).

A study of the correlation between protein level and different stage of aggregation and sedimentation were performed table (2) and showed that the protein concentration is not the only parameter affecting the process, but the type of macromolecules also takes a significant role (9).

Table 1:
The mean±S.D.of aggregation time at different stages of aggregation,(AT= rouleaux formation, 1D=one dimension aggregate time, 3D=three dimensional aggregate and S.D.= sedimentation time)using : a. macromolecule rich plasma. b. macromolecule free plasma.

a.

Parameter	*Duration of phase*		*T test*
	Normal	*Diabetic*	
AT	14.13 ± 5.08	9.7 ± 2.08	0.011
1D	5.6 ± 2.64	2.98 ± 2	0.08
3D	8.61 ± 2.59	6.11 ± 4.52	0.44
ST	23.78 ± 7.37	23.78 ± 7.3	0.0001

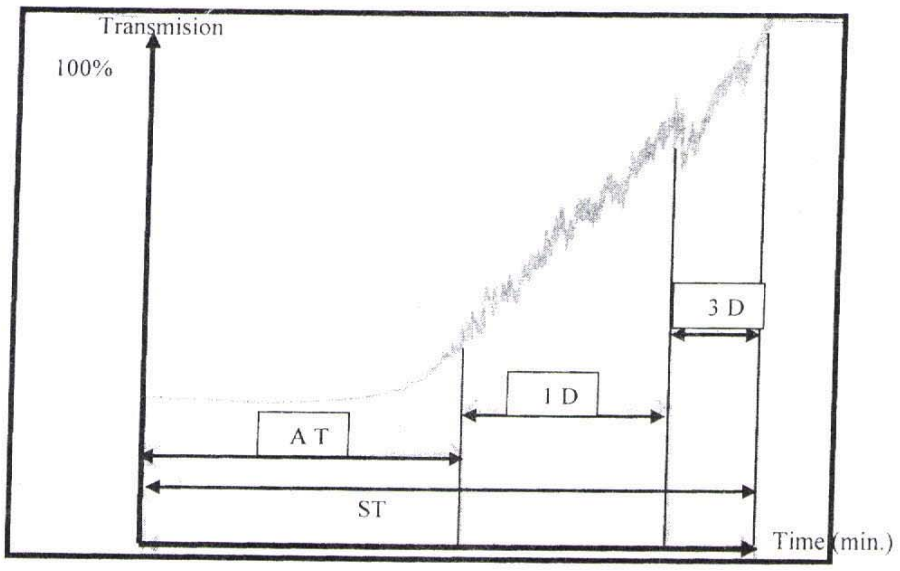
b.

Parameter	*Duration of phase*		*T test*
	Normal	*Diabetic*	
AT	12.6 ± 6.65	12.47 ± 4.86	0.131
1D	4.8 ± 2.12	5.83 ± 2.79	0.362
3D	9.2 ± 5.48	10.62 ± 8.5	0.195
ST	25 ± 8.6	30.06 ± 13.9	0.2

Table (2):
The relationship between phases of aggregation and the slopes (R) of the curves indicating total protein versus duration of samples.

Phase of aggregation	Slope of relation(R)	
	Normal	Diabetic
AT	0.563	0.217
1D	0.382	0.358
3D	0.063	0.421
ST	0.531	0.407

Figer 3a



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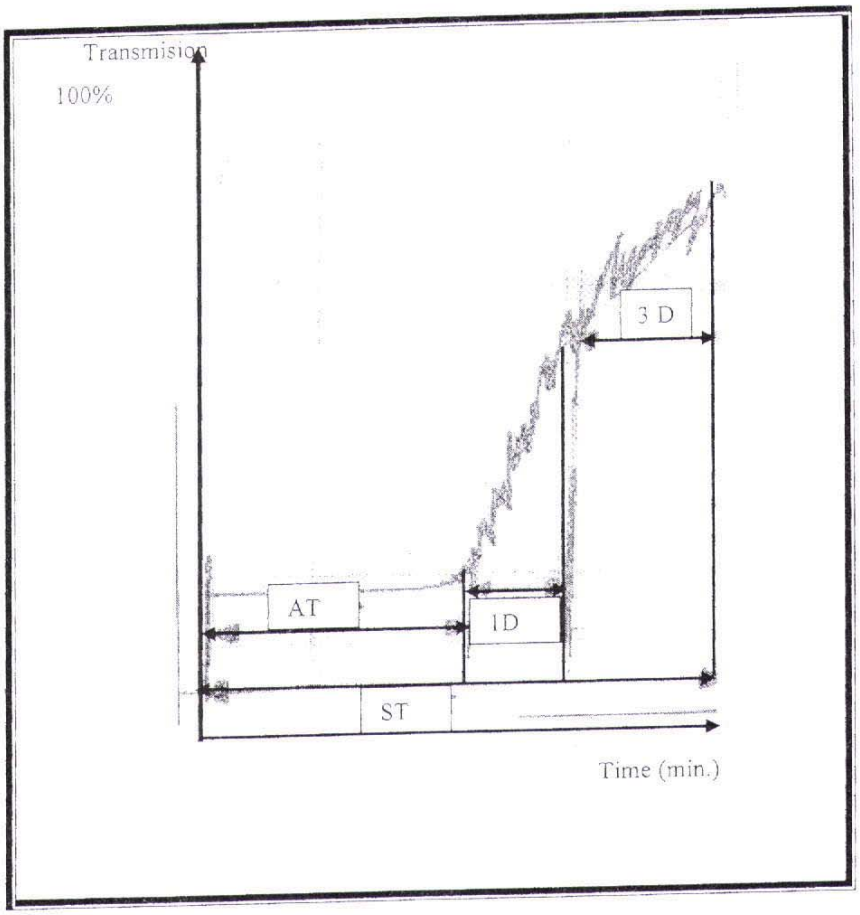
Figer 3b



Figer 3-a-b:Thy variation of transmitted laser light versus time (min)for(a)normal blood sample With 10%red blood cell concentration.(b)diabetic sample.Diabetic sample showed a shorter time of different aggregation and sedimentation stage.(AT=aggregation time,1D=one dimentional aggregation,3D=three dimensioal aggreation and ST=sedimentation time).

Discussion

Diabetic samples showed a shorter aggregation and sedimentation time, which indicate a higher tendency for aggregation. The physiological importance of erythrocyte aggregation in the circulation is its tendency to increase the blood viscosity and hence decrease speed of blood flow within the capillaries. This decrease in the speed of blood flow within the normal physiological limits will enhance oxygen transport between erythrocytes and tissues. On the other hand an increase in the erythrocyte aggregation beyond the normal limit will disturb the passage of blood within the capillaries and enhance the formation of sludge that may contribute to the pathogenesis of microangiopathies, which is one of the major complications in diabetics.

Many factors may contribute for the increased tendency of aggregation in diabetic patients, like increased blood viscosity by high blood glucose, decreased deformability of erythrocytes and increased concentration of macromolecules and their nature [10].

In our study, the level of macromolecules was higher in diabetics in comparison to that of the normal subjects. This may suggest that protein changes, and especially all types except Albumin are responsible for the increase in aggregation and sedimentation. On removing the macromolecules from the serum of the diabetic patients and that of the normal subject, there was no significant difference between their aggregation and sedimentation time although their fasting blood sugar was not the same,

indicating that these macromolecules were the major contributor for the increase in the aggregation tendency, and the blood sugar at such levels does not affect aggregation much.

To exclude the effect of the other variables that may affect aggregation in diabetics, like blood viscosity and erythrocyte deferability, the comparison was repeated between the diabetic erythrocyte suspended in the serum of the same diabetic patients before and after the removal of only the macromolecules. The results showed that the removal of macromolecules will decrease aggregation tendency and return the value of aggregation time to that of the normal subjects.

There are a lot of controversies whether the concentration or the type of macromolecule is more effective in the increase in aggregation tendency in diabetic patient. The correlation of protein before and after may confirm that the type rather than the concentration is more effective.

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