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Enhanced RBC Aggregation in Type 2 Diabetes Patients

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Background: This study aimed to investigate the relationship between HbA_{1c} and RBC aggregation in type 2 diabetes mellitus (T2DM) patients by analysis of data from routine clinical tests and from in vitro experiments. *Methods:* A total of 2,111 inpatients with type 2 diabetes were selected and among them, 364 patients (Group A) had limited influence of plasma proteins on erythrocyte sedimentation rate (ESR) and was compared with the rest of the 1,747 inpatients (Group B). ESR, HbA_{1c}, WBC, CRP, Fbg, and HCT were measured in all samples. Sixty samples were also collected from T2DM patients and used for in vitro ESR studies. Spearman's correlation coefficients were employed to reflect the correlation between ESR and other parameters. Mann–Whitney *U* test was used to compare the study parameters. *Results:* The test results for Group A were lower than Group B with respect to ESR, age, HCT, HbA_{1c}, CRP, WBC, and Fbg. Only the difference in HbA_{1c}, CRP, and Fbg values had statistical significance ($P < 0.05$). In addition, HbA_{1c} correlated better with ESR for Group A ($R = 0.622$) than Group B ($R = 0.563$), whereas CRP and Fbg were contrary to this. In the in vitro studies, the HbA_{1c} values were classified into the subgroups of 6.5–8.0%, 8.1–10%, and >10%. The corresponding ESR values were 28 ± 5.1 mm/h, 33 ± 2.7 mm/h, and 40 ± 4.1 mm/h, respectively. *Conclusion:* ESR results of T2DM patients were elevated that was mainly caused by Fbg levels, and in addition HbA_{1c} in part contributed to RBC aggregation. J. Clin. Lab. Anal. 29:387–389, 2015.

Key words: erythrocyte sedimentation rate; RBC aggregation; HbA_{1c}; T2DM

INTRODUCTION

The erythrocyte sedimentation rate (ESR) has been widely used as a nonspecific indicator of inflammation to help diagnose conditions and follow disease activity (1). The elevated ESR value is mainly due to the enhancement of specific plasma proteins and red blood cell (RBC) aggregation. Previous studies have investigated RBC aggregation in patients with diabetes (2–6). The presence of inflammatory conditions associated with diabetes is very likely to compromise the interpretation of ESR values. Le Devehat et al. report a positive correlation between hemoglobin A_{1c} (HbA_{1c}) and RBC aggregation in type 1 diabetes patients (T1DM) (7), but not in type 2 diabetes patients (T2DM).

In this study, we aimed to investigate the relationship between HbA_{1c} and RBC aggregation in T2DM patients by analysis of the data from routine clinical tests and from in vitro experiments.

MATERIALS AND METHODS

Patients

A total of 2,111 inpatients with T2DM were selected from the hospital database between October 2009 and October 2012. All included patients had samples that were tested simultaneously for the following study parameters; HbA_{1c} (≥6.5%), C reactive protein (CRP), ESR, Hematocrit (HCT), WBC count, and fibrinogen

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(Fbg). Of the 2,111 patients, 364 (Group A) had (i) no evidence of inflammatory or tumor diseases (normal WBC counts and no clinical diagnosis of tumor); (ii) normal hepatic and renal function (normal ALT, AST, Bilirubin, AFP and normal creatinine, urea and uric acid); (iii) no treatment with therapeutic agents known to specifically affect RBC aggregation (8). The remaining 1,747 subjects were classified into Group B. Another 20 samples were collected in each of the following HbA_{1c} ranges 6.5–8.0%, 8.1–10%, and >10%. These 60 samples were used for the ESR in vitro experiments.

Samples

Blood samples anticoagulated with K_3EDTA (Becton Dickinson, Franklin Lakes, NJ) were routinely obtained from inpatients. All samples were tested within 4 hours of venipuncture according to ICSH recommendations (1). All were tested for HbA_{1c} by Bio-Rad Variant (Bio-Rad Laboratories, Hercules, CA), ESR by Micro Test 1 (Ali Fax, Padova, Italy), WBC count by Sysmex XE-2100 (Sysmex corporation, Kobe, Japan), CRP by Beckman Immage 800 Immunochemistry System (Beckman Coulter, Brea, USA) and fibrinogen by Sysmex CA7000 (Sysmex corporation, Kobe, Japan).

In Vitro ESR Studies

To minimize the influence of components in each autologous plasma, RBC were separated from the plasmas by centrifugation at 2,000 *g* for 10 min, washed once in 0.9% NaCl solution, then washed and resuspended to a 40% HCT in a 3% solution of dextran 70 (9–11). DEX 70 is a water-soluble neutral polymer that causes RBC aggregation.

Statistical Analysis

Since the study parameters displayed skewed distributions, nonparametric statistical approaches were used. Spearman's correlation coefficients were employed to reflect the correlation between ESR and other parameters. Mann–Whitney *U* test was used for univariate comparison of the study parameters between Group A and Group B. Analysis of variance was used to check if the mean in vitro experiment had statistical difference. Results were considered statistically significant if *P* value was less than 0.05. The analysis was performed by SPSS 19.0 software (SPSS, Chicago, IL).

RESULTS

As shown in Table 1, the test results in Group A were lower than Group B with respect to ESR, age, HCT, HbA_{1c}, CRP, WBC, and Fbg. However, the Mann–Whitney *U* test showed that only HbA_{1c}, CRP, and Fbg had statistical significance ($P < 0.05$). In addition, by analysis of the Spearman correlation coefficients, it demonstrated that HbA_{1c} correlated better with ESR for Group A ($R = 0.622$) than Group B ($R = 0.563$), whereas CRP and Fbg were contrary to this.

TABLE 1. Test results and Correlation Coefficient of Group A and Group B

	Group A		Group B		
Parameters	Test results[1]	R^2	Test results[1]	R^2	*P* value
ESR (mm/h)	28(19, 45)	1	36(23, 56)	1	0.020
Age (years)	43(38, 64)	0.315	48(40, 59)	0.254	0.420
HCT (%)	41(38, 45)	–0.12	47(42, 51)	–0.09	0.157
HbA_{1c} (%)	7.4(6.8, 8.5)	0.750	7.9(6.7, 8.4)	0.563	0.037
CRP (mg/l)	7.4(6.4, 7.7)	0.471	13.5(9.4, 15.2)	0.598	0.031
WBC (10^9/l)	7.4(6.5, 8.1)	0.003	9.5(7.6, 10.3)	0.011	0.058
Fbg (g/l)	3.5(2.9, 3.9)	0.387	5.1(4.7, 6.3)	0.453	0.044

Note: 1. Test results are shown as median (25%, 75%); 2. *R* means the Spearman correlation coefficients that mean correlations with ESR.

In Vitro Observation of RBC Aggregation

Enhanced aggregation was observed for washed RBCs, free of CRP, Fbg, and other proteins. Samples with a range of HbA_{1c} values were classified into subgroups of 6.5–8.0%, 8.1–10%, and >10%. The corresponding ESR values were 28 ± 5.1 mmol/h, 33 ± 2.7 mmol/h, and 40 ± 4.1 mmol/h, respectively. It was observed that with the elevation of HbA_{1c}, ESR showed a statistically significant increase ($P < 0.001$).

DISCUSSION

ESR is a widely used indicator of the presence of inflammatory conditions and noninflammatory conditions such as stroke, coronary artery disease, and prostate cancer. Many ESR measurements are performed in patients with T2DM, a condition that may also directly contribute to the elevated ESR values and complicate the interpretation of test results.

Previous studies have found that RBC aggregation tended to be increased in diabetics (12), especially when the diabetes is characterized by poor metabolic control (13). Demiroglu et al. suggest that in patients with T2DM who developed clinically evident late complications also had enhanced RBC aggregation regardless of the degree of metabolic control (14). Our data add evidence to this finding as the ESR results had elevated median values of 28 mm/h for Group A and 36 mm/h for Group B. It should be noted that the WBC count (9.5×10^9/l) ap-

proached to the upper limits of normal reference value ($4–10 \times 10^9$/l) and together with the elevation of CRP (13.5 mg/l), may indicate the existence of concomitant infection (Group B, Table 1). In this case, the enhanced RBC aggregation in these T2DM patients is probably due to plasma protein changes caused by infection or inflammation, which was also observed in previous studies (15). In addition, it may be associated with the elevated plasma Fbg level 5.1 g/l (reference range: 2–4 g/l), which is known to be a primary determinant of RBC aggregation and was more highly correlated than other parameters with ESR for both groups.

An important feature of T2DM is the glycation of hemoglobin that affects both the charge and function of the RBC membrane. Whether the glycated hemoglobin directly contributes to the enhanced RBC aggregation is not fully clarified and there are findings both for and against this correlation (15). There are studies reporting a positive correlation between RBC aggregation and HbA_{1c} level in patients with type 1 DM (16), or in patients with coronary artery disease or acute coronary syndrome but no known diabetes (17, 18). However, since changes in plasma constituents in T2DM may have a strong relationship with the enhanced RBC aggregation, the RBC's effects are much likely to be covered.

Our retrospective analysis of routine test results (Table 1) revealed that compared to the 1,747 T2DM patients (Group B), the selected 364 one (Group A) had lower values of CRP and Fbg, suggesting these plasma constituents would have lower effects on the ESR. Meanwhile, the probable contribution of HbA_{1c} increased in Group A as its correlation coefficient rose to 0.622 compared to 0.563 in Group B. To further investigate the RBC aggregation without the influence of in vivo plasma proteins, in vitro experiments were carried out using RBCs washed free of CRP, Fbg, WBC, etc. The results showed that with increased HbA_{1c} values, the RBC aggregation reflected by ESR also increased.

The limitations of this study should be acknowledged. T1DM patients were not included who could serve as a comparison group, as several reports do not recommend the usage of HbA_{1c} in the diagnosis of T1DM. Further, there may be other factors that enhance the RBC aggregation in patients with T2DM, while this study did not investigate them comprehensively and chose only WBC, CRP, and Fbg as the indicator or direct reason to cause red cell aggregation. Finally, further studies are needed to sort out the independent effects of HbA_{1c} on RBC aggregation and to provide insights into the biological mechanisms involved. Thus the interpretation and extension of our results should proceed with caution.

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