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RED BLOOD CELLS AGGREGATION IN THE PATIENTS WITH TYPE 2 DIABETES AND T2D ASSOCIATED ANEMIA, A CASE CONTROL STUDY IN BANGLADESH

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Abstract

Background and Aims**:* Type 2 Diabetes (T2D) is a chronic metabolic disorder. Nowadays the number of people with diabetes mellitus is quadrupling in the world as well as in Bangladesh. It is related to different types of disease morbidity and socio-demographic parameters. The present study aimed to investigate the association of RBC aggregation (measured by ESR level) with T2D and T2D associated anemia. ***Material and method: A total of 234 subjects were included in the study. Sociodemographic and anthropometric information about study subjects were collected using a standard questionnaire. Blood samples were tested for random blood glucose levels, Hb percentage, and ESR. Association of these data with metabolic disorders was analyzed by statistical analysis software, SPSS. ***Results:*** *This study provides a piece of evidence that anemia is highly prevalent in T2D patients than the healthy control subjects in Bangladesh (59.1% vs 22.5%; P value <0.001). The ESR values were significantly elevated in T2D patients as well as anemic and aged subjects (P value <0.001).* ***Conclusion:*** *The study thus suggests that anemia was prevalent in T2D patients in Bangladesh. The RBC aggregation was significantly elevated in patients with T2D as well as anemia, indicating increased inflammatory response in these diseases.*

key words: *Type 2 Diabetes (T2D); Hb percentage; Anemia; RBC aggregation; ESR; BMI; Blood pressure.*

Background and aims

Diabetes mellitus is one of the most widespread and serious non-communicable diseases (NCDs). A plethora of evidence showed that type 2 diabetes (T2D) is a major threat to global development that can lead to a multitude of complications, such as cardiovascular disease and cancer [1]. The global prevalence of diabetes and impaired glucose tolerance in adults has been increasing over recent decades. The rate of change in prevalence in many countries and regions has been boosted by rapid urbanization and dramatic changes towards a sedentary lifestyle [2].

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Globally, the prevalence of diabetes is 8%. Almost 80% of them live in low- and middle-income countries [3]. A recent report showed that the prevalence of diabetes was 9.7%, and prediabetes was 22.4% in Bangladesh [4]. It was projected that the prevalence will increase in such a rate by 2030 that place Bangladesh as the 8th highest diabetic populous country in the world [5]. The prevalence of T2D was expected to increase to 693 million by 2045. It was estimated that almost half of them were undiagnosed, and approximately 5 million deaths worldwide were attributable to diabetes in 2017 [6]. Multiple genetic, environmental, and behavioral risk factors contribute to developing T2D [7].

Anemia was shown to be a risk factor for microvascular complications, including retinopathy, nephropathy, and neuropathy in diabetic patients [8]. The management of Diabetes in Bangladesh is inadequate, [9] moreover the causes have not been investigated. It is possible that hidden anemia can be a leading reason behind the complication of T2D in Bangladesh [10]. So, the present study aims to evaluate the prevalence of anemia in diabetic patients in the Bangladeshi population.

The RBC aggregation and the enhancement of specific plasma proteins result in an elevated level of erythrocyte sedimentation rate (ESR). The ESR has been widely used as a nonspecific marker of inflammation to diagnose the disease conditions and consequent follow-ups. Most of the inflammatory markers are associated with T2D [11]. The inflammatory conditions in T2D patients shown by ESR levels [12] suggested that RBC aggregation occurs in diabetes. Another aim of our study was the measurement of the ESR level to delineate the inflammatory condition in T2D and T2D associated anemia.

Materials and method

Study subjects and Collection of information

Total 234 study subjects are included in this study, 132 of them were T2D patients and 102 of them were control subjects. Blood samples were collected from the study subjects during the period of January 2017 to December 2018 from different area of Bangladesh including i. SSS hospital, Tangail (n = 43), ii. Diabetes association, Tangail (n = 50), iii. Mirzapur general hospital (Pvt.) (n = 28), iv. Bashabo diabetes association, Dhaka (n = 31), v. BIRDEM, Dhaka (n = 10), vi. Mawlana Bhashani Science and Technology University, Tangail (n = 45), vii. Jamalpur Diabetic Association, Jamalpur (n=12) and viii. Kurigram Diabetic Association, Kurigram (n=15). Healthy individuals were selected as control including students, university employees, and relatives of the students who did not have clinical features of diabetes or other complications, such as acute or chronic infection or kidney and liver diseases. Pregnant women and the patient routinely take insulin were excluded from this study. During the sample collection the sociodemographic and anthropometric data were collected by using standard questionnaire. The information about age, gender, smoking habit, red meat consumption, family history of diabetes, diabetic drug taken were collected. The following risk factors were measured at baseline using standardized methods based on World Health Organization recommendations [10]. Height and weight of the subjects were measured to calculate the BMI and the blood pressure was average of two separate readings taken at least 5 min apart and height, weight were measured using meter scale and weight machine respectively. BMI was calculated as weight in kilograms divided by squared height in meters (kg/m^2). The study was conducted following the guidelines approved by the ethical review

committee of the Bangladesh medical research Council (BMRC).

Clinical tests

Expert phlebotomist collected 4-5 microliter of blood samples from each study subjects. Blood samples anticoagulated with K3EDTA (Becton Dickinson, Franklin Lakes, NJ). All samples were tested within 4 hours of venipuncture according to ICSH recommendations [11]. The blood samples were tested for ESR by Micro Test 1 (Ali Fax, Padova, Italy), Hb percentage by ERMA PCE-210 Hemoglobinometer and blood plasma glucose level by humaLyzer 3000.

Statistical Analysis

Descriptive statistics were reported using relative frequencies (percentages). Chi-square test was performed to assess any association of different parameters with ESR, anemia and diabetes. Unconditional logistic regression analyses was performed to calculate odds ratio and 95% confidence intervals for these parameters. We have also calculated the odds ratio of ESR for the patients affected by both T2D and anemia to understand the combined effect of these disease on RBC aggregation and/or Inflammation. The odds ratios were further adjusted with confounding factors, Age and Gender. Data analyses was performed using SPSS for windows (version 17.0 SPSS Inc, Chicago, IL, USA) all hypothesis testing was 2-tailed with P value, <0.05 set as statistically significant.

Results

Two hundred and thirty four (234) subjects were included in the present study, of them 102 (43.6%) were healthy control and 132 (56.4%) were T2D patients. We collected 43 (18.4%) samples from SSS hospital, Tangail, 50 (21.4%) samples from Tangail diabetes association, Tangail, 28 (12.0%) samples from Mirzapur general hospital (Pvt.), 31 (13.2%) samples from Bashabo diabetes association, Dhaka, 10 (4.3%) samples from Bangladesh Institute of Research and Rehabilitation for Diabetes, Endocrine and Metabolic Disorders (BIRDEM), Dhaka, and 45 (19.2%) samples from Mawlana Bhashani Science and Technology University (MBSTU), Tangail, 12 (5.1%) samples from Jamalpur Diabetic Association, Jamalpur, and viii. 15 (6.4%) samples from Kurigram Diabetic Association, Kurigram. From these, the students of MBSTU, patients from Bashabo diabetes association and BIRDEM (36.7%) were majorly heterogeneous population from various districts of Bangladesh. The socio-demographic and anthropometric data of the study subjects were described in Table 1. 141 (60.3%) of the study subjects were male and 93 (39.7%) were female. 43.2% (101/234) of the study subjects were 20-30 years old, whereas 15.4% (36/234) were 31-40 years old, 24.4% (57/234) were 41-50 years old and 17.1% (40/234) were more than 50 years old.

Table 1. The characteristics of 234 subjects enrolled and analyzed in this study.

Characteristics	Number (Percentage)
Age of the Respondent	
20-30	101 (43.2)
31-40	36 (15.4)
41-50	57 (24.4)
>50	40 (17.1)
Gender	
Male	141 (60.3)
Female	93 (39.7)
Smoking Habit	
Yes	32 (13.7)
No	202 (86.3)
BMI Category	
Normal	149 (63.7)
Overweight	61 (26.1)
Obese	13 (5.6)
Underweight	11 (4.7)
Red meat consumption	
No	46 (19.7)
Rare	77 (32.9)
One or more	111 (47.4)

Blood Pressure	
Normal	149 (63.7)
Pre-High	57 (24.4)
High	28 (12.0)
Disease Type	
Normal	102 (43.6)
Type 2 Diabetes	132 (56.4)
ESR	
Normal	99 (42.3)
Elevated	135 (57.7)
Hb percentage	
Normal	133 (56.8)
Decreased	101 (43.2)
Total	234 (100)

Hb percentage was measured for all the study subjects to assess their anemic condition and it was observed that 133/234 (56.8%) subjects had normal hemoglobin level and 101/234 (43.2%) were anemic (Table 1). Anemia is significantly associated with age (*P* value, <0.001) and females were more prone to anemia than male (52.7% vs 36.9%; *P* value 0.022) (Table 2). T2D patients were significantly affected by anemia (59.1% vs 22.5%; *P* value < 0.001). The result was found to be significant when analyzed by binary logistic regression model, moreover, after adjusted with age and gender it was also showing statistically significant result (OR: 4.19; 95% CI: 2.19-8.03; *P* value <0.001) (Table 3). The mean hemoglobin level of the diabetic patients was 11.76±0.14 g/dl and healthy control subjects was 13.15±0.16 g/dl.

Table 2. The association of Hb% level with different anthropometric parameters and disease status.

Characteristics	Hb percentage		Chi-square	*P*-value
	Normal N (%)	Decreased N (%)		
Age of the respondent				
20-30	78 (77.2)	23 (22.8)	30.99	<0.001
31-40	14 (38.9)	22 (61.1)		
41-50	22 (38.6)	35 (61.4)		
>50	19 (47.5)	21 (52.5)		
Gender				
Male	89 (63.1)	52 (36.9)	5.70	0.022
Female	44 (47.3)	49 (52.7)		
Smoking Habit				
Yes	23 (71.9)	9 (28.1)	3.71	0.065
No	110 (54.5)	92 (45.5)		
BMI				
Normal	88 (59.1)	61 (40.9)	2.67	0.444
Overweight	35 (57.4)	26 (42.6)		
Obese	5 (38.5)	8 (61.5)		
Underweight	5 (45.5)	6 (54.5)		
Red meat Consumption				
No	20 (43.5)	26 (56.5)	5.80	0.055
Rare	42 (54.5)	35 (45.5)		
One or more	71 (64.0)	40 (36.0)		
Blood Pressure				
Normal	87 (58.4)	62 (41.6)	0.69	0.708
Pre-High	32 (56.1)	25 (43.9)		
High	14 (50.0)	14 (50.0)		
Disease Status				
Normal	79 (77.5)	23 (22.5)	31.31	<0.001
Type 2 Diabetes	54 (40.9)	78 (59.1)		
ESR				
Normal	73 (73.7)	26 (26.3)	19.97	<0.001
Elevated	60 (44.4)	75 (55.6)		
Total	133 (56.8)	101 (43.2)		

Table 3. Logistic Regression Coefficient of Hb percentage values in the subjects by some selected factors.

Genotype	Haemoglobin percentage*			
	Crude		Adjusted	
	Odd-ratios (95% CI)	*P*-value	Odd-ratios[a] (95% CI)	*P*-value
BMI				
Normal®	1		1	
Overweight	1.07 (0.58, 1.95)	0.822	0.86 (0.44, 1.70)	0.681
Obese	2.31 (0.72, 7.39)	0.159	2.70 (0.73, 9.99)	0.137
Underweight	1.73 (0.51, 5.92)	0.382	2.53 (0.68, 9.35)	0.164
Blood Pressure				
Normal®	1		1	
Pre-High	1.09 (0.59, 2.03)	0.770	1.00 (0.50, 1.99)	0.99
High	1.40 (0.62, 3.15)	0.412	0.78 (0.32, 1.91)	0.591
Disease Status				
Normal®	1		1	
Type 2 Diabetes	4.96 (2.77, 8.85)	<0.001	4.19 (2.19, 8.03)	<0.001
ESR				
Normal®	1		1	
Elevated	3.51 (2.01, 6.15)	<0.001	2.30 (1.23, 4.31)	0.009

*Subjects with normal Hb percentage value were used as reference.
® Reference Category
[a.] Odd ratios are adjusted by Age and Gender.

From 234 study subject ESR has been measured, among them 99 (42.3%) have normal level of ESR and 135 (57.7%) have elevated level of ESR. We found that age, T2D, and Hb percentage are significantly associated with ESR. Elevated level of ESR mostly occur in older subjects than 20-30 years young adults (*P*-value, <0.001) (Table 4). Moreover, the anemic patients have significantly elevated level of ESR (74.3% VS 45.1%; *P* value, <0.001). However, there was no association found between ESR level and the gender, smoking habit, BMI, red meat consumption, and blood pressure (Table 4). The logistic regression analysis also showed the significant relationship between ESR and T2D (OR; 4.81; 95% CI, 2.54-9.09) (Tables 5 and 6). The mean ESR value of Diabetic patients was 31.73±1.68 mm/hr and healthy control subjects was 13.94±0.96 mm/hr.

Table 4. The association of ESR level with different anthropometric parameters and disease status.

Characteristics	ESR Category		Chi-square	*P*-value
	Normal N (%)	Elevated N (%)		
Age of the respondent				
20-30	62 (61.4)	39 (38.6)	26.74	<0.001
31-40	11 (30.6)	25 (69.4)		
41-50	16 (28.1)	41 (71.9)		
>50	10 (25.0)	30 (75.0)		
Gender				
Male	60 (42.6)	81 (57.4)	0.09	0.517
Female	39 (41.9)	54 (58.1)		
Smoking Habit				
Yes	13 (40.6)	19 (59.4)	0.04	0.497
No	86 (42.6)	116 (57.4)		
BMI				
Normal	60 (40.3)	89 (59.7)	0.70	0.873
Overweight	28 (45.9)	33 (54.1)		

Obese	6 (46.2)	7 (53.8)		
Underweight	5 (45.5)	6 (57.7)		
Red meat Consumption				
No	15 (32.6)	31 (67.4)	2.72	0.256
Rare	32 (41.6)	45 (58.4)		
One or more	52 (46.8)	59 (53.2)		
Blood Pressure				
Normal	67 (45.0)	82 (55.0)	2.59	0.273
Pre-High	24 (42.1)	33 (57.9)		
High	8 (28.6)	20 (71.4)		
Disease Status				
Normal	66 (64.7)	36 (35.3)	37.16	<0.001
Type 2 Diabetes	33 (25.0)	99 (75.0)		
Hb Percentage				
Normal	73 (54.9)	60 (45.1)	19.97	<0.001
Decreased	26 (25.7)	75 (74.3)		
Total	99 (42.3)	135 (57.7)		

Table 5. Logistic Regression Coefficient of Disease status of the patients by some selected factors.

		Type 2 Diabetes*				
Genotype		Crude			Adjusted	
		Odd-ratios (95% CI)	P-value		Odd-ratios[a] (95% CI)	P-value
BMI						
Normal®		1			1	
Overweight		2.23 (1.18, 4.22)	0.013		3.18 (1.47, 6.86)	0.003
Obese		1.49 (0.46, 4.78)	0.497		0.88 (0.22, 3.51)	0.865
Underweight		0.53 (0.15, 1.90)	0.333		0.39 (0.08, 1.79)	0.231
Blood Pressure						
Normal®		1			1	
Pre-High		1.42 (0.76, 2.63)	0.267		1.24 (0.60, 2.58)	0.553
High		3.52 (1.35, 9.18)	0.010		3.13 (1.03, 9.47)	0.043
ESR						
Normal®		1			1	
Elevated		5.50 (3.12, 9.86)	<0.001		4.81 (2.54, 9.09)	<0.001
Hb percentage						
Normal®		1			1	
Decreased		4.96 (2.77, 8.85)	<0.001		4.22 (2.19, 8.12)	<0.001

*Healthy control subjects were used as reference.
® Reference Category
[a.] Odd ratios are adjusted by Age and Gender.

Table 6. Logistic Regression Coefficient of ESR values in the subjects by some selected factors.

	Erythrocyte Sedimentation rate*			
Genotype	Crude		Adjusted	
	Odd-ratios (95% CI)	*P*-value	Odd-ratios[a] (95% CI)	*P*-value
BMI				
Normal®	1		1	
Overweight	0.79 (0.43, 1.44)	0.453	0.51 (0.25, 1.04)	0.06
Obese	0.78 (0.25, 2.45)	0.679	0.46 (0.12, 1.73)	0.252
Underweight	0.81 (0.23, 2.77)	0.736	0.88 (0.22, 3.48)	0.859
Blood Pressure				
Normal®	1		1	
Pre-High	1.12 (0.61, 2.08)	0.711	1.02 (0.51, 2.06)	0.941
High	2.04 (0.84, 4.93)	0.112	1.51 (0.56, 4.03)	0.405
Disease Status				

Normal®	1		1	
Type 2 Diabetes	5.50 (3.12, 9.68)	<0.001	4.73 (2.51, 8.94)	<0.001
Hb percentage				
Normal®	1		1	
Decreased	3.51 (2.01, 6.16)	<0.001	2.27 (1.21, 4.24)	0.010

*Subjects with normal ESR value were used as reference.
® Reference Category
[a.] Odd ratios are adjusted by Age and Gender.

Anemic patients were also shown to have significantly elevated level of ESR (74.3% vs 45.1%; OR: 2.27; 95% CI: 1.21- 4.24; *P* value 0.01) (Table 2 and S2). However insignificant relation was found between anemia and other factors e.g. smoking habit, BMI, red meat consumption, and blood pressure (Table 2). The mean ESR value of the anemic patients was 31.45±1.93 mm/hr and control subjects was 18.30±1.29 mm/hr.

Type 2 Diabetes is associated significantly with most of the variables used in this study except smoking habit (Table 7). However, age and gender adjusted results of the logistic regression analysis showed that overweight (OR: 3.18; 95% CI: 1.47-6.86; *P* value 0.003) and high blood pressure (OR: 3.13; 95% CI: 1.03-9.47; *P* value 0.043) was associated with T2D. The result also indicated that Elevated level of ESR (OR: 4.81; 95% CI: 2.54-9.09; *P* value <0.001) and anemia (OR: 4.22; 95% CI: 2.19-8.12; *P* value <0.001) were significantly related to T2D (Table 5).

Table 7. The relationship of different parameters with type 2 diabetes.

Characteristics	Disease Status		Chi-square	*P*-value
	Normal N (%)	Type 2 Diabetes N (%)		
Age of the respondent				
20-30	90 (89.1)	11 (10.9)	152.65	<0.001
31-40	12 (33.3)	24 (66.7)		
41-50	13 (22.8)	44 (77.2)		
>50	7 (17.5)	33 (82.5)		
Gender				
Male	73 (51.8)	68 (48.2)	9.66	0.002
Female	29 (31.2)	64 (68.8)		
Smoking Habit				
Yes	17 (53.1)	15 (46.9)	1.37	0.242
No	85 (42.1)	117 (57.9)		
BMI				
Underweight	72 (48.3)	77 (51.7)	8.21	0.042
Normal	18 (29.5)	43 (70.5)		
Overweight	5 (38.5)	8 (61.5)		
Obese	7 (63.6)	4 (36.4)		
Red meat Consumption				
No	10 (21.7)	36 (78.3)	30.37	<0.001
Rare	23 (29.9)	54 (70.1)		
One or more	69 (62.2)	42 (37.8)		
Blood Pressure				
Normal	73 (49.0)	76 (51.0)	7.61	0.022
Pre-High	23 (40.4)	34 (59.6)		
High	6 (21.4)	22 (78.6)		
ESR				
Normal	66 (66.7)	33 (33.3)	37.16	<0.001
Elevated	36 (26.7)	99 (73.3)		

HB percentage				
Normal	79 (59.4)	54 (40.6)	31.31	<0.001
Decreased	23 (22.8)	78 (77.2)		
Total	102 (43.6)	132 (56.4)		

It has been found that T2D, anemia and combination of these have significant effect on the ESR level (*P* value <0.05). T2D has greater effect on ESR (OR, 11.23; 95% CI, 3.86-32.63) than anemia (OR, 3.53; 95% CI, 1.42-8.78). Moreover, the patients who were affected by both T2D and anemia have even more effect on ESR level (OR, 14.02; 95% CI, 4.95-39.68) (Table 8).

Table 8. The combined effect of type 2 diabetes and Anemia on the ESR level of subjects.

Characteristics	ESR Category		Odd-ratios* (95% CI)	*P*-value	Odd-ratios*[a] (95% CI)	*P*-value
	Normal N (%)	Elevated N (%)				
Normal®	63 (70.8)	26 (29.2)	1		1	
T2D only	10 (22.2)	35 (77.8)	8.48 (3.66-19.61)	<0.001	11.23 (3.86-32.63)	<0.001
Anemia only	13 (44.8)	16 (55.2)	2.98 (1.26-7.07)	0.013	3.53 (1.42-8.78)	0.007
Both T2D and Anemia	13 (18.3)	58 (81.7)	10.81 (5.08,23.00)	<0.001	14.02 (4.95-39.68)	<0.001
Total	99 (42.3)	135 (57.7)				

*Subjects with normal ESR value were used as reference.
® Reference Category
[a.] Odd ratios are adjusted by Age and Gender.

Discussion

Type 2 Diabetes is a metabolic disorder linked to overweight and obesity [13]. This multifactorial disease is also linked to hypertension due to unhealthy eating, sedentary behavior, sodium retention, abdominal obesity, autonomic derangements, premature arterial stiffening, and endothelial dysfunction. A combination of these disorders has increased the risk of cardiovascular disease [14,15]. Knowing the status of BMI and hypertension is important for diabetic patients as these can be manageable by proper measures and can reprieve cardiovascular complications. The present analyses suggest that overweight is a contributing factor for T2D, whether obesity is not significantly associated (Table 5). The number of obese people in our dataset was low, so more samples of obese people can give a clear picture of the relationship. On the other hand, age and gender-adjusted results point out that hypertension has been associated with T2D.

Our analysis showed that females were significantly affected by anemia than the males (*P* value, 0.022). This result supports the previous study where it has been shown that the incidence of anemia in female subjects was significantly higher than in male subjects in their adulthood [16]. We have performed unconditional logistic regression, and the odds ratios (OR) were adjusted for the confounding factors Age and Gender. The adjusted result showed that there is a significant relationship existed between T2D and anemia (*P* value, <0.001) (Table 3). It was found that T2D patients have 4.19 times risk of having anemia than healthy control subjects. However, obesity and hypertension do not affect anemia (Table 3). The prevalence of anemia increased in T2D patients might be resulted from a high incidence of renal insufficiency [17] however, it gives rise in the persons with normal kidney function [18].

It harms the quality of life of diabetic patients, [19] even a causal factor for cardiovascular disease and affects the mortality of diabetic patients [20]. It was shown in diabetic nephropathy patients that reduced production of erythropoietin by kidney resulted in an increased risk of anemia [21]. The decreased renal function and proinflammatory cytokines are also contributing factors in the reduction of hemoglobin levels in diabetic patients. So, anemia in diabetic patients is a crucial indicator of kidney disease [22]. Patients with diabetes have been reported to have a deformation in their erythrocyte properties leading to impaired red cell survival causing anemia. However, anemia in diabetic patients is mostly ignored due to overlapping symptoms in both of these diseases. Anemia in diabetic patients can cause a more severe condition leading to hospitalization and premature death [23]. In our study subjects, 59.1% of the diabetic patients were affected by anemia, indicating a hidden complication existed in diabetic patients of Bangladesh.

While analyzing the red meat consumption status, a vicious cycle was identified that might be contributing to an anemic condition in Diabetic patients. It has been shown in our analysis that red meat consumption moderately decreases the risk of anemic condition (*P* value, 0.055) (Table 2). However, T2D patients consume a significantly reduced level of red meat (P value <0.001) (Table 7). The reduced consumption of red meat in Diabetic patients can be happened due to their consciousness about the risk of cardiovascular diseases. So, alternative preventive measures should be taken to reduce anemic conditions in T2D patients after diagnostic confirmation.

One of the non-specific inflammatory markers, ESR is associated with T2D, and hence Diabetes was associated with enhanced aggregation of RBCs [12]. ESR values alongwith other inflammatory markers are increased in diabetic patients with cardiovascular complications, and an indicator of diabetic foot ulcer [24-26]. In our study, we compared the ESR level of T2D patients with that of the healthy controls. We found that T2D patients had a significantly elevated level of ESR level (Table 5). Moreover, the subjects with anemia also showed a significant association with ESR level (*P* value, 0.012) (Table 6). However, there was no relation found between the ESR level with BMI and hypertension. So, our study indicates that the ESR level is increased, hence RBC aggregation/Inflammation occurred mainly due to T2D and anemia among the metabolic disorder included in this study. T2D mainly involves insulin resistance, and progressive b-cell dysfunction resulted in decreased insulin-mediated glucose uptake. Research suggests that oxidative stress and inflammation can play an important role in the pathogenesis of T2D, and reversibly T2D can explicit into a chronic low-grade inflammation [27,28]. The previous report also suggests that there is an association between T2D and pro-inflammatory mediators, and these mediators have diagnostic values [29].

The present analyses showed that ESR value was significantly elevated in both the case of T2D and anemia. We have also analyzed the ESR level with combined data of T2D and anemia to underpin the comparative effect of these two diseases on the elevated level of ESR (Table 8). The anemic persons have 3.53 times risk to have an elevated level of ESR than the normal subjects, while the T2D patients have 11.23 times risk. The patients who have both T2D and anemia have even more (14.02 times) risk, showing a combined effect on ESR level. So, T2D has a prominent effect on ESR level than the anemia and have a combinatorial effect on the inflammatory response. To the best of our knowledge, this is the first report that showed

the combinatorial effect of diabetes and anemia on the ESR level.

Though an increased level of the inflammatory mediators is found in diabetic patients, one report showed that common inflammatory diseases, such as chronic gastritis or ulcer, chronic bronchitis, chronic gastroenteritis, asthma, and chronic hepatitis were at lower incidence in diabetic patients [30]. So, diabetes individually and along with anemia might act as the key elicitor of inflammation. This study points out the requirement of a large scale study of anemia and related complications in the diabetic population in Bangladesh. The major limitation of the present study was the lower sample size of the study population. For that reason, a lower number of subjects were obtained in the subclasses not sufficient to be compared.

In conclusion, our study finds that anemia is significantly higher in T2D patients in Bangladesh, which should be widely investigated in the south Asian population to reduce the disease burden in this region. The inflammatory response of the diabetic patients is mainly associated with T2D and anemia, as well as a combination of both these diseases. However, ESR was not associated with other metabolic disorders, like obesity and hypertension.

Conflict of interests. The authors declare that they have no conflict of interests.

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Authors' contributions. KMKK conceived, designed and guided the study. AK collected the samples, performed biochemical tests and drafted the manuscript. KMKK and GKP performed the statistical analysis. KMKK helped to draft the manuscript and make critical revision. All authors read and approved the final manuscript.

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