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Low Hemoglobin Levels in Infected Diabetic Foot Ulcer

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Abstract: *Background: Studies have shown that anemia is twice as common in diabetics compared with non-diabetics. Objective: We aimed to evaluate the prevalence of anemia in diabetic foot ulceration (DFU) patients and it's correlation with infection. Methods: This study was conducted from October 2015 to the end of February 2016 and consisted of (55) Iraqi diabetic patients with foot ulcer that sub grouped to patients with non infected diabetic foot patients (n=25, group NIDFU), and patients with infected diabetic foot (n=30, group IDFU) according to erythrocyte sedimentation rate (ESR). Anthropometric indices and clinical examinations were done. Anemia is defined according to WHO as hemoglobin (Hb) levels <12 g/dL. Statistical analysis was done using t-test and Pearson correlation. Results: ESR of group NIDFU (25.2±14.26 mm/hr) was significantly lower (P = <0.001) in comparison to that of group IDFU (76.73±33.39 mm/hr).The mean values of Hb of NIDFU group (13.45±2.15 g/dl) was significantly higher (P = <0.001) than that of IDFU group (11.34±2.17 g/dl). In IDFU group; 63% were anemic while only 32% of NIDFU group have anemia. Pearson correlation of ESR in IDFU group revealed significant negative correlation with Hb (r= −0.386; P <0.05), while no significant correlation with Hb (r= 0.404; P >0.05) was found in NIDFU group. Conclusion: This prospective study showed a high incidence of anemia in patients with IDFU in contrast to NIDFU and a negative correlation was found between anemia and infection. Iron replacement and therapeutic strategies are recommended.*

Keywords: *diabetic foot ulcer, infection, hemoglobin, erythrocyte sedimentation rate.*

I. Introduction

T Diabetic Foot Ulcer (DFU) is one of many complications that accompanying fifteen percent of patients with diabetes mellitus. Amputation is recurrently the final event of serious diabetic foot ulcer [1, 2]. The prone of DFU to frequent chronic infection have an effect on the psychological health of the patient [3]. Disease of the peripheral nerves is known in diabetic patients and the management relies upon which nerve(s) is/are predisposed, this may include the peripheral nerves, the cranial and the autonomic nerves leading to numbness in the legs and may promote ulcer formation. When this problem is untreated, gangrene and amputation of the infected leg is provoked [4]. Diabetic nephropathy and retinopathy were associated with anemia for relatively some time [5, 6]. Wound healing is impaired by anemia. In the face of underlying arterial insufficiency, anemia may sudden rest pain. Few studies investigate the association between anemia and DFU that were limited to renal anemia in diabetes [6, 7]. Conway *et al.* (2009) [8] had proposed a mechanism is that hypoxia stimulate erythropoietin production that increase Hb level cause microvascular disease in both kidneys and retina of diabetic patients. According to some studies, hyperglycemia induces variation in hematological parameters [9, 10]. Erythrocyte shape is affected by glucose levels in blood that manipulate their flow properties and insulin level is frequently change blood viscosity [9]. Infection can indicate quick onset and advancement in diabetic foot ulcers. In day by day clinical practice, systemic markers of infection are the most settled method for supporting the clinical suspicion of infection in DFU. The traditional markers in this setting incorporate leukocyte count and C-receptive protein (CRP) and also the erythrocyte sedimentation rate (ESR). Existence of all markers of systemic inflammation (for example ESR) was found in T2DM patients [11]. In this study, we aimed to evaluate the prevalence of anemia in diabetic foot ulceration (DFU) patients and it's correlation with infection. The analytic precision of ESR, as inflammation marker, was evaluated to discriminate between infected and non-infected DFU. Hemoglobin levels were measured to diagnose the presence of anemia in patients with DFU.

II. Patients and Methods

2.1. Patients

This study took place between October 2015 and the end of February 2016 and involved (55) patients with DFU were chosen from National Diabetes Center/Al-Mustansiyria University, National Center of Hematology and Al-Yarmuk teaching hospital in Baghdad city/Iraq. T2DM is diagnosed and classified according to the American Diabetes Association criteria (2015) (FSG ≥126 mg/dl or 2 h postprandial glucose ≥200 mg/dl) [12]. Study groups consisted of patients with non-infected diabetic foot (group NIDFU; n=25), patients with infected diabetic foot (group IDFU; n=30). Diabetic foot ulcer was defined as redness, swelling of

wound, pus spots or exudates from the wound, fever, pain in the infected area, a full depth wound, and skin necrosis or gangrene below the ankle. All of the DFUs were graded using Wagner classification [13], as shown in table (1). The purpose of the study was informed to all participants and their oral consent was acquired. Demographic information (age, gender, body mass index, and duration of T2DM) with detailed history were recorded in well-structured questionnaire. Complete clinical examination for every patient was done by specialist physician in the center. Anemia is defined according to the World Health Organization (WHO) criteria [14], as hemoglobin (Hb) concentration of less than13 g/dL in men and less than 12 g/dL in women.

Table 1: Distribution of diabetic foot ulceration according to Wagner classification

Grade		NIDFU group		IDFU group	
		No.	%	No.	%
0		5	17	-	-
1		13	43	-	-
2		12	40	-	-
3		-	-	15	60
4		-	-	4	16
5		-	-	6	24
peripheral pulse	Positive	27	90	20	80
	Negative	3	10	5	20

2.2. Laboratory Analysis

About 5 milliliters of venous blood was taken from all participants after an overnight fasting 10-12 hours. For measuring ESR, HbA1c and RBC, blood sample was transferred into EDTA tube. ESR was measured using Westergren tube. CBC count was measured using autoanalyzer device (Abbott, U.S.A). HbA1c was measured using commercial kit, (SDA1cCareTM, SD Biosensor, Germany). Serum blood was processed for the measurement of fasting serum glucose (FSG) using autoanalyzer device (Biolabo, France). Lipid profile [Total Cholesterol (TC), Triglyceride (TG), High-Density Lipoprotein-Cholesterol (HDL-C)] were measured using commercial kits (Biolabo, France). Friedewald's equation [15] was used to calculate Low-Density Lipoprotein-Cholesterol (LDL-C) and Very Low-Density Lipoprotein-Cholesterol (VLDL-C).

2.3. Statistical Analysis

Analysis of the results was completed utilizing the statistical package of SPSS (Statistical Packages for Social Science –Version 22). Data was presented as mean and standard deviation (±SD). Independent student's t-test was used for comparison between two means. The correlation between two quantitative variables was presented in scatter diagram of the distribution, with the Bivariate Pearson's correlation coefficient (r) calculation and its significance. Significance difference was considered whenever the *P* value was less than 0.05. While high significant was considered whenever *P* value was less than 0.01.

III. Results

The clinical analysis of the two groups was presented in Table 2. ESR and PLT were significantly higher in IDFU group (P <0.01) than those of NIDFU. While, RBC, Hb and PCV in IDFU group were significantly lower (P <0.01) when compared with that of NIDFU. Anemia in IDFU group represent 63% while it was 32% in NIDF patients, in spite of nephropathy in NIDFU group was 20% while it was only 7% in IDFU group.

Table2: Demographic information and clinical characteristics of patients groups

Demographic		Patients (NIDFU)	Patients (IDFU)
Number		25	30
Gender (male/female)		15/10	22/ 8
Age (y)		36-65	40-65
Duration of disease (y)		10.6±5	12.24±5.67
Treatment (%)	Insulin	40	43
	OHD	40	27
	Insulin+OHD	20	30
Vasculopathy (%)		16	13
Neuropathy (%)		100	100
Retinopathy (%)		64	20
Nephropathy (%)		20	7
BMI (kg/m^2)		29.72±4.7	31.2±10.68
FSG (mg/dl)		252.64±98.73	258.5±104.03
HbA1c (%)		9.54±1.49	10.18±1.12

TC (mg/dl)	197.28±43.67	199.77±37.47
TG(mg/dl)	183.32±31.15	255.67±59.78 **
HDL-C (mg/dl)	38.11±8.67	33.29±6.97
LDL-C (mg/dl)	122.48±40.01	115.3±34.44
VLDL-C (mg/dl)	36.68±6.32	51.2±12**
ESR(mm/hr)	25.2±14.26	76.73±33.39 **
RBC	4.94±0.67	4.24±0.55**
Hb (g/dl)	13.45±2.15	10.34±2.17**
PCV (%)	40.46±5.65	35.03±5.52**
MCV (Fl)	81.96±3.26	83.57±6.09
MCH (Pg)	27.2±2	27.24±2.87
MCHC (g/dl)	33.09±1.58	32.55±1.91
RDW (%)	10.92±0.94	11.19±0.99
PLT	244.4±62.8	301.9±61.65**
MPV (Fl)	7.03±1.2	6.71±1.25

**: $P< 0.01$, *: $P <0.05$. Using student's t- test analysis

As shown in table (3), and Fig.1, ESR level shows a significant negative correlation with RBC, Hb, PCV and MPV in IDFU group. While, ESR level shows a significant positive correlation with PLT in IDFU group (Fig.2).

Table (3): Pearson correlation of ESR with hematological parameters in IDFU group

Parameter	ESR(mm/hr)	
	r	***P***
RBC	−0.386	0.035*
Hb (g/dl)	−0.386	0.035*
PCV (%)	−0.4	0.029 *
MCV (Fl)	−0.274	0.143
MCH (Pg)	−0.302	0.105
MCHC (g/dl)	−0.213	0.258
RDW (%)	0.097	0.609
PLT	0.404	0.027*
MPV (Fl)	−0.387	0.035*

*Correlation is significant at $P<0.05$

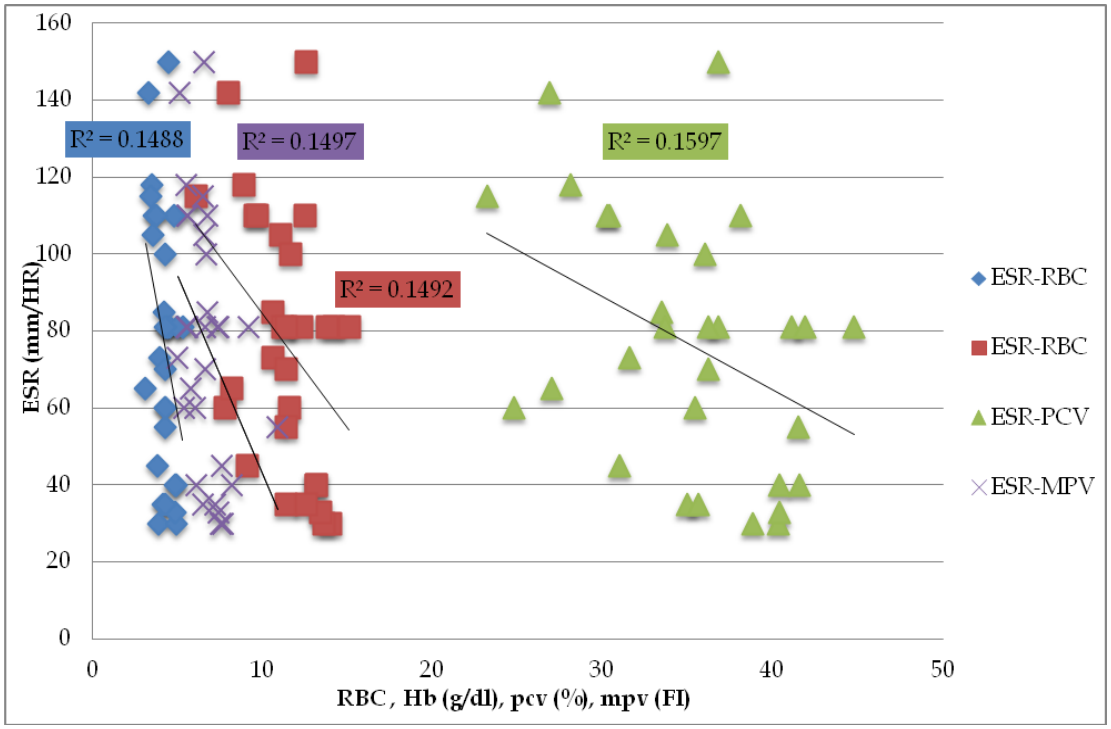

Figure (1): Pearson correlation of ESR with RBC, Hb, PCV and MPV in IDFU group.

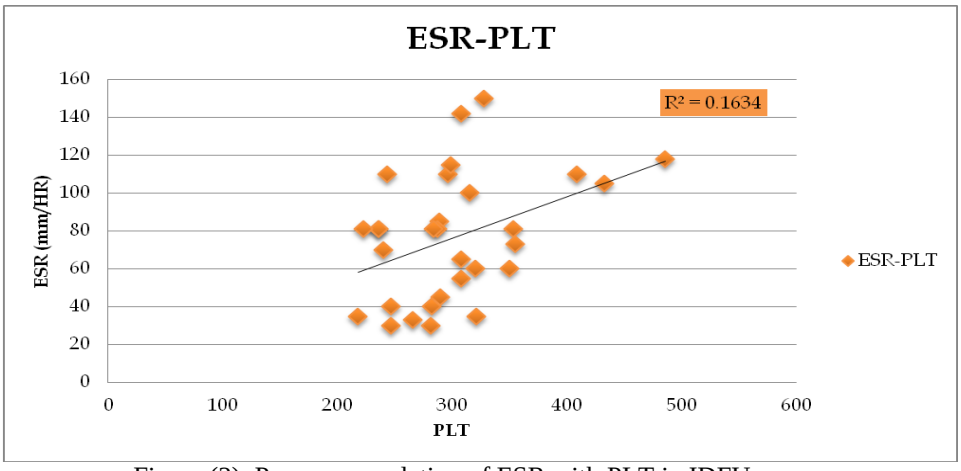

Figure (2): Pearson correlation of ESR with PLT in IDFU group.

IV. Discussion

Vascular complications of diabetes (nephropathy, retinopathy, and neuropathy) are associated with anemia that may lead to impaired wound healing, and macrovascular disease [16]. It was suggested that low blood oxygen level accompanying low hemoglobin levels may consequently worsen ischemia of the lower limb. Gene expression of endothelial adhesion molecules may happen due to induced hyperkinetic circulation by anemia which ultimately may generate thrombosis [17]. Anemia has been severally reported as a complication of diabetes mellitus [18]. According to previous studies, the proteins of RBC membrane undergo oxidation through non-enzymatic glycosylation due to increased oxidative stress in diabetes and reduce PCV, Hb, RBC levels that may lead to hemolysis and consequently to anemia [19,20,21]. It has been suggested that the rough utilization of angiotensin changing enzyme inhibitor (ACEI) inhibit the pro-erythropoeitic effects of angiotensin II on erythrocyte precursors, which may cause anemia in DM [22, 23]. The relationship between anemia and DFU are inadequately understood. A previous retrospective study suggested the existence of a relationship between anemia and clinical stages of DFU [18]. We found that 63% of IDFU patients have anemia which is in agreement with previous studies where 57% of 47 patients with DFU (2-3 Wagner grade) were anemic [24], while in a study enrolled all Wagner grades (1-5), the percent of anemia was 62% of 42 patients with DFU [25]. Fengning *et al.* (2016) [26] study the variables in the DFU patients with and without anemia. They noted that almost 50% of the diabetic foot patients have low hemoglobin levels, and concluded that anemia is common in patients with DFU and is associated with substantial morbidity and mortality. Thomas and Rampersad (2004) [27] had similar findings whereby the prevalence of anemia was higher in diabetic patients in spite of having safeguarded renal function. According to Avivit *et al*. 2015 [28] study, they measured deformable RBC in diabetic patients with and without DFU. They found significant increase in the of deformable RBCs percent in DFU patients in comparison with that in patients without DFU. Suggesting that this increase may slow blood flow in blood vesicle and delay wound healing. High levels of glycated hemoglobin have been appeared to impair endothelium mediated vaso- active responses, which can prompt hypertension and vascular diseases in diabetic patients [29]. Micro-angiopathy is a result of changes in the microcirculation which is caused by changes in blood viscosity and red blood cell deformability [30]. One of the variables deciding the cell deformability is the erythrocyte cytoplasmic viscosity that is basically determined by the properties and the concentration of hemoglobin in the erythrocyte [31]. Many factors were suggested as causing the frequent onset of anemia, including chronic inflammation, diabetic nephropathy, and malnutrition. Chronic inflammation is considered to be a common cause of anemia in diabetic patients, especially with DFU [32]. An explanation presented by Richards (2012) [33] which associate the inflammatory state with anemia. The transport of iron to the bone marrow is inhibited due to the scavenging of iron by macrophages and its storage in ferritin.

Wright *et al*. (2014) [31] found the prevalence of anemia in patients with severe DFU. Their explanation was due to systemic inflammation, repeated superficial, deep tissue infection, osteomyelitis, and antibiotic use which may delay healing of foot ulcer. Our finding of ESR correlated negatively with Hb is most likely related to the inflammatory and infective processes occurring in DFU which is in agreement with the result of khanbhai *et al.* (2012) [34]**.** They found an inverse correlation between hemoglobin and CRP, and they suggested that the progression of foot ulcer was related to decreased hemoglobin level and increased CRP level. The limitation of the study was small sample size which may be due to limitation of clinical centers available for diabetic foot ulcer care. Also most patients do not recruited to these centers, because they visit private clinics for wound treatment.

V. Conclusion

This prospective study showed a high incidence of anemia in patients with severe DFU. Negative correlation between ESR and Hb was found. Iron replacement and therapeutic strategies are recommended to improve their health and quality of life.

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