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Anisocytosis is Associated with High Body Mass Index and Poor Adaptation to Oxidative Stress in Obese Patients with Type 2 Diabetes Mellitus

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KEYWORDS Complete Blood Count. Ferric Reducing Ability of Plasma. Malondialdehyde. Red Cell Distribution Width. Superoxide Dismutase

ABSTRACT This paper aims to evaluate interrelationships among components of complete blood count (CBC), especially red cell distribution width (RDW) and oxidative stress markers, malondialdehyde (MDA) and superoxide dismutase (SOD) in erythrocytes and ferric reducing ability of plasma (FRAP) in obese diabetics (DM-OB). A cross section of 55 DM-OB were compared with 55 healthy non-obese non-diabetic controls (C). Of CBC indices, RDW was maximally impacted indicating anisocytosis in DM-OB accompanied by increased MDA and lowered SOD and FRAP. RDW correlated significantly with MDA in DM-OB but not in the C. Odds ratio (OR) indicated a 15 times higher risk of having a high RDW among the DM-OB. RDW directly correlated with BMI but not with antioxidant enzyme SOD or total non-enzymatic antioxidant activity indexed by FRAP. The overall result indicated that adaptation achieved through increased SOD observed in the C broke down in the DM-OB, resulting in anisocytosis in obese diabetics.

INTRODUCTION

Obesity and diabetes are 21st century pandemics which are interlinked. The risk of diabetes increases linearly with BMI; the prevalence of diabetes was reported to increase from two percent in those with a BMI of 25 to 29.9 kg/m^2, to eight percent in those with a BMI of 30 to 34.9 kg/m^2, and finally to thirteen percent in those with a BMI greater than 35 kg/m^2. Obesity in early middle age, between 38 to 55 years, is related to a higher risk and earlier onset of Type 2 diabetes (Colditz et al. 1990). Obesity is a major cause of enhancing diabetes which is a rapidly growing problem worldwide. The number of diabetics with obesity have doubled from 1980 to 2015 (WHO 2015).

It is well accepted that both diabetes and obesity are associated with a variety of clinical and biochemical derangements, elevated fasting blood glucose and disturbed oxidative stress and antioxidant properties (Ceriello 2006). However, obesity and metabolic syndrome are not generally expected to modulate parameters evaluated in a complete blood count (Vuong et al. 2014).

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One of the most common and inexpensive clinical investigations, ordered by physicians as a first line diagnostic tool, is the Complete Blood Count (CBC), because it summarizes basic information about an individual's health. It assesses the overall blood composition, the plasma volume and gives detailed qualitative as well as quantitative information about the three types of blood cells: the white blood cells (WBC), the red blood cells (RBC) and the platelets. This minimally invasive conglomerate of haematological parameters has elicited renewed interest, largely due to recent findings related to one of its components, the red cell distribution width (RDW).

RDW is the ratio of the under root of variance in RBC volume and the mean volume of circulating RBC. Hence, an increase in RDW, known as anisocytosis, is caused by an increase in RBC volume variance or a decrease in mean cell volume (MCV), or both. The causes of increased volume variance can be categorized based on when they act during the RBC lifecycle. It could be during their release from the bone marrow as immature reticulocytes due to increased reticulocyte volume variance. It could also be during their maturation while circulating in the blood due to increased heterogeneity in the rate of RBC volume reduction occurring in the peripheral circulation, or it could also be at

the end of their lifespan, due to delay in clearance of the senescent RBC. Recent research suggests that it is this delayed RBC clearance that is the major cause of elevations in RDW in pathological conditions (Patel et al. 2010).

More recently it has been reported to be associated with increased risk for a remarkably wide range of morbidity and mortality: all-cause mortality, mortality from heart disease, pulmonary disease, sepsis, advanced stage cancer, complications in heart failure, coronary artery disease, diabetes, chronic obstructive pulmonary disease, stroke, anemia, poor outcomes in autoimmune disease, and many more dyscrasias (Felker et al. 2007; Horne 2012; Patel et al. 2015). The pathophysiological mechanism explaining the relationship between RDW and mortality from a wide range of diseases is largely unknown, but the role of inflammatory and oxidative stress markers has been implicated (Scharte and Fink 2003; Ghaffari 2008).

Oxidative stress is one of the major causes of inflammation in living systems which disrupt the homeostasis of free radical and antioxidant levels. Oxidative stress plays a major role in the pathogenesis of obesity and diabetes by disrupting the release of adipokines such as TNF-α and IL-6 by adipose tissue. Thus elevation in levels of Reactive Oxygen Species (ROS) in diabetes has been attributed to disturbances in antioxidant enzymes such as catalase, superoxide dismutase, glutathione peroxidase, dietary antioxidants and a variety of other factors, which cause widespread impact such as DNA damage, protein oxidation and lipid peroxidation (Baynes et al. 1999).

Since RDW is an important indicator of proinflammatory and oxidative stress states, it is not surprising that recent reports have indicated its relationship with type 2 diabetes mellitus and the metabolic syndrome (Mansourian et al. 2014; Nada 2015; Kachekouche et al. 2018; Yin et al. 2018; Jaman et al. 2018) and with body weight (Nada 2015; Kim et al. 2018).

It has been further suggested that an increased RDW mirrors a profound deregulation of erythrocyte homeostasis involving both impaired erythropoiesis and abnormal red blood cell survival, which may be attributed to a variety of underlying metabolic abnormalities such as shortening of telomere length, oxidative stress, inflammation, poor nutritional status, dyslipidemia, hypertension, erythrocyte fragmentation and alteration of erythropoietin function (Salvagno et al. 2014).

It has been pointed out that more studies are required which explore whether there are any interrelationships among components of CBC, especially RDW and markers of oxidative stress in obese respondents suffering from diabetes. This is an important question because it can enhance the predictive value of the inexpensive and minimally invasive CBC, especially due to the recent interest in RDW as a morbidity and mortality indicator.

Objectives

The present case-control study was undertaken to assess relationships among components of complete blood count, including haemoglobin, haematocrit, quantitative parameters of white blood cells, red blood cells, and platelets, the RBC distribution width, and markers of oxidative stress such as malondialdehyde, superoxide dismutase and ferric reducing ability of plasma in obese (body mass index > 30) diabetic respondents.

METHODOLOGY

Selection of Respondents

In this case-control study, a cross section of 55 obese volunteers (Body mass index (BMI) > 30) who had been diagnosed by a physician with type 2 diabetes mellitus (DM-OB) were compared with 55 healthy non-obese non-diabetic controls (C). Data collection was done between January 2016 to March 2016. All volunteers were in the age range of 50-65 years (59.4±6.59). Height and weight were measured following standard procedures, as recommended by WHO (WHO 1995), using a metal tape and a setsquare for height and digital bathroom scales with a least count of 100 g for weight. Body mass index (BMI) was computed from these data, using the formula:

$BMI = \text{weight (kg)} / (\text{height (m)})^2$.

Diabetics who had a Body Mass Index (BMI) > 30 (Mean ± SD: 33.3±1.34) were enrolled if they satisfied the exclusion criteria, namely, that they did not suffer from any cardiac, metabolic or other abnormalities. The controls had a BMI between 18 and 25 kg/m^2 (Mean±SD: 22.5±1.27). The study protocol was approved by the Institutional Ethics Committee of Population Re-

source and Research Centre, Allahabad. Respondents were requested to volunteer for collection of about 5-6 ml of blood, and informed consent was obtained.

Collection and Processing of Blood Samples

Blood was obtained by a trained medical technician after overnight fasting, in sodium citrate coated vials and processed in cold conditions within an hour to obtain plasma and packed red blood cells (RBC)/erythrocytes. Hemolysates were prepared by using packed RBCs and stabilizing solution (comprising of 0.05 ml of beta mercapto ethanol and 10% ethylene diaminetetraacetic acid) and stored at -80^{0}C until analysis, as described earlier (Tripathi et al. 2011).

Assessment of Hematological and Biochemical Parameters

Complete Blood Count

Complete Blood Count (CBC) was assessed using *Mythic-18 Hematology* Cell Counter (human) (Orphee). CBC comprised of total white blood cell count (WBCs), differential white blood cell count including average number per 10^3µl and percent of total WBC of lymphocytes, monocytes and granulocytes, total red blood cells (RBCs), haemoglobin (g/dL), haematocrit (%), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin content (MCHC), red cell distribution width (RDW), mean platelet volume (MPV), platelet count (PCT) which is a measure of total platelet mass, and Platelet distribution width (PDW).

RDW was defined by dividing the standard deviation of the mean cell size by the mean corpuscular volume (MCV) of the red cells, defined by the following formula:

$$RDW = \frac{\sqrt{\text{Variance (MCV)}}}{\text{Mean (MCV)}}$$ where MCV: Volume of RBC

Malondialdehyde (MDA), was estimated in hemolysate by the method of Niehaus and Samuelsson (1968), modified as described earlier (Tripathi et al. 2011). Activity of the antioxidant enzyme, SOD was also estimated in hemolysate by the modified method of Marklund and Marklund (1974), which utilizes the inhibition of auto-oxidation of pyrogallol by superoxide dismutase. FRAP was estimated in plasma by the method of Benzie and Strain (1996).

Statistical Analysis of Data

Data analysis was performed using Prism Graphpad version 5 software. All data was expressed as Mean ± Standard deviation (SD). Difference between C and DM-OB was assessed using unpaired student's t-test. Odds ratios (OR) and ninety-five percent confidence intervals (CI) were obtained for bivariate analysis to assess DM and obesity as risk factors for alterations in various indices of CBC and oxidative stress. Pearson's correlation coefficients 'r' were also calculated to study the relationships between various factors.

RESULTS

The respondents enrolled for the study were matched for age, with a Mean±SD of 59.4±6.6 years. The Body Mass Index (BMI) of the obese group suffering from diabetes was 33.3±1.34 kg/m^2 while that of the control group of healthy respondents was 22.5±1.27 kg/m^2.

The constituents of complete blood count (CBC) of obese diabetic (DM-OB) volunteers were compared with those of non-obese, non-diabetic controls and findings are presented in Table 1.

It can be observed from Table 1 that some components of CBC showed significant difference between DM-OB and control groups. All values for leucocytes were within the respective reference range, but total WBCs were lower in DM-OB than C ($p<0.001$), (9.71±0.95 and 5.70±0.85), but they were within the recommended range of 4.0-12 for both groups. This was mainly due to the reduction in granulocytes (C: 5.97±0.99; DM-OB: 3.44±1.09). The division of these WBC into percent lymphocytes (C: 32.86±1.05; DM: 33.96±1.38), monocytes (C: 2.74±0.57; DM-OB: 4.65±0.56) and granulocytes (C: 62.01± 3.78; DM-OB: 61.56± 1.60), however, did not indicate any statistically significant difference between C and DM-OB, and all values were within the normal range.

No statistically significant difference was observed between number of RBC, haemoglobin, mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC), indicating lack of significant effect on iron status. Haematocrit was marginally higher in the DM-OB (C: 37.62±2.50, DM-OB: 42.58±2.58) but all values were within the accepted range of 35-55.

Table 1: Complete Blood Count (CBC) of non-diabetic, non-obese Controls and Obese Diabetics (DM-OB)

N=55	*Units*	*Range*	*Control (N=55)*	*Diabetes –OB (N=55)*	*P-value*
WBCs	10^3µl	4.0 -12.0	9.71 ±0.95	5.70 ±0.85	0.001**
Lymphocytes	10^3µl	1.0 -5.0	2.81 ±0.31	3.92 ±0.27	0.8
Monocytes	10^3µl	0.1 -1.0	0.81 ±0.13	1.03 ±0.11	0.08
Granulocytes	10^3µl	2.0 -8.0	5.97 ±0.99	3.44 ±1.09	0.15
Lymphocytes	%	25.0 -50.0	32.86 ±1.05	33.96 ±1.38	0.5
Monocytes	%	2.0 -10.0	2.74 ±0.57	4.65 ±0.56	0.02*
Granulocytes	%	50.0 -80.0	62.01 ±3.78	61.56 ±1.60	0.9
RBCs	10^6µl	4.0 -6.20	4.59 ±0.38	6.02 ±0.34	0.2
Haemoglobin	g/dl	11.0 -17.0	13.46 ±0.66	12.44 ±0.91	0.3
Haematocrit	%	35 -55	37.62 ±2.50	42.58 ±2.58	0.02*
MCH	Pg	26.0 -34.0	27.52 ±0.85	29.07 ±0.76	0.3
MCHC	g/dl	31.0 -35.5	30.5 ±0.35	35.01 ±0.73	0.6
RDW	%	10.0 -16.0	14.75 +0.13	18.84 ±1.08	0.0001***
Platelets	10^3µl	150-400	172.4 ±19.01	104.45 ±14.02	0.005
MPV	µm^3	7.0 -11.0	11.4 5±0.34	8.99 ±0.24	0.6
PCT	%	0.20 -0.50	0.19 ±0.02	0.16 ±0.02	0.08
PDW	%	10.0 -18.0%	15.74 ±0.49	13.44 ±0.51	0.6

All values are expressed as mean ± SD
WBCs - White Blood Cells, RBCs - Red blood Cells, MCH - Mean corpuscular haemoglobin, MCHC – Mean corpuscular haemoglobin concentration, RDW – Red cell distribution width, MPV – Mean Platelet Volume, PCT –Platelet count, PDW – Platelet distribution width.

The most significant difference ($p<0.0001$) was observed with regard to percent of red cell distribution width (RDW) (14.75±0.13 and 18.84±1.08 for C and DM-OB respectively). The mean RDW of DM-OB was more than the upper limit of the normal range of 10.0-16.0 percent.

The number of platelets was statistically higher ($p<0.005$) in the DM-OB (172.4±19.01) than in the C (104.45±14.02) but again, both remained within the normal range of 150-400 10^3µl. Since both diabetes and obesity are associated with an increase in oxidative stress, selected parameters to assess OS, such as malondialdehyde (MDA), superoxide dismutase (SOD) and ferric reducing ability of plasma (FRAP) were measured, and results are presented in Table 2.

Table 2: Oxidative stress indices in blood of non-diabetic, non-obese Controls (c) and Obese Diabetics (DM-OB)

Biochemical stress markers	*Control (n=55)*	*DM-OB*	*p value*
MDA (Nanomoles / ml)	0.36± 0.08	0.73 ± 0.14	0.0001***
SOD (unit/mg of Hb /min)	0.38 ± 0.16	0.20 ± 0.13	0.001**
FRAP (µmol/ml of plasma)	0.69±0.39	0.41±0.22	0.0001***

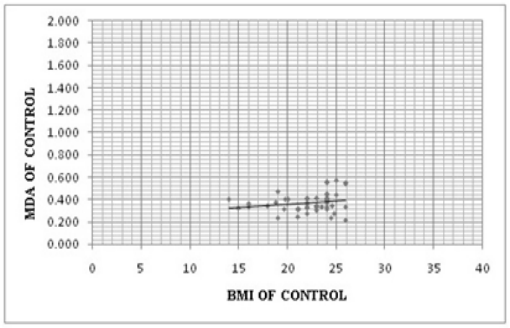
All values are expressed as mean ± SD

MDA – Malondialdehyde, SOD – Superoxide dismutase, FRAP- Ferric reducing ability of plasma.

As expected, it can be seen from Table 2 that MDA, measured in the hemolysate to assess lipid peroxidation in the erythrocyte membrane was twice as high in the DM-OB group (0.73±0.14) as compared to the controls (0.36±0.08), while the antioxidant enzyme, SOD, was significantly lower, almost half, in the DM-OB group (0.20±0.13) as compared to the controls (0.38±0.16), indicating inability of the DM-OB to adapt to the increase in free radicals. This was reflected in the lower total antioxidant capacity of plasma, as indexed by FRAP, which was significantly lower in the DM-OB (0.41±0.22) as compared to the controls (0.69±0.39), corroborating their poor adaptation to OS.

The question arises whether there is any direct or inverse relationship of BMI with those major components of CBC which are statistically significantly different between C and DM-OB, namely TLC and RDW, between BMI and OS markers; and between these CBC components and markers of OS, namely MDA, SOD and FRAP. The question also arises whether these relationships are the same for controls and DM-OB. Table 3 and Figure 1(a-c) and Figure 2 (a-b) present the correlation coefficients between pairs of these parameters.

It can be seen from Table 3 and Figure 1(a) that MDA increased significantly as BMI increased in DM-OB ($r=0.56$, $p<0.005$), which is the group with all respondents suffering from



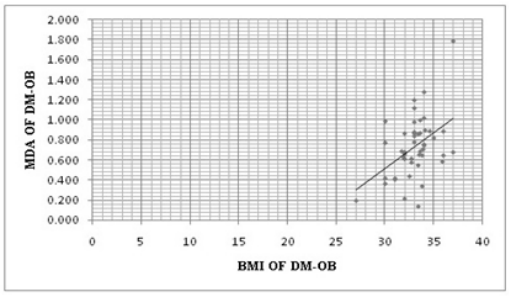
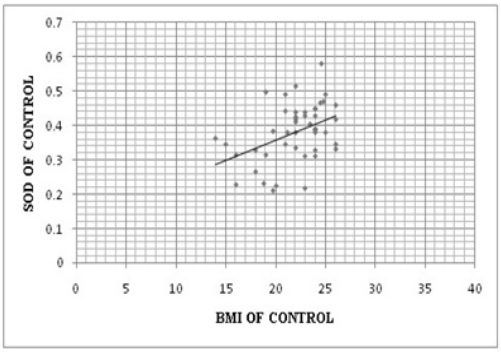


Fig. 1a.



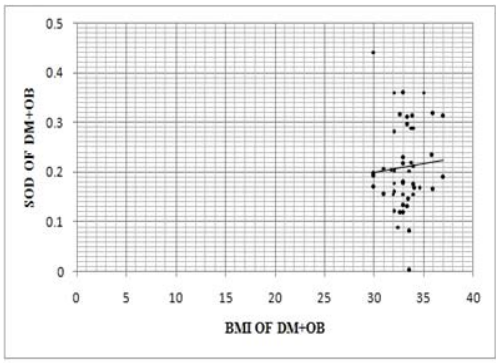
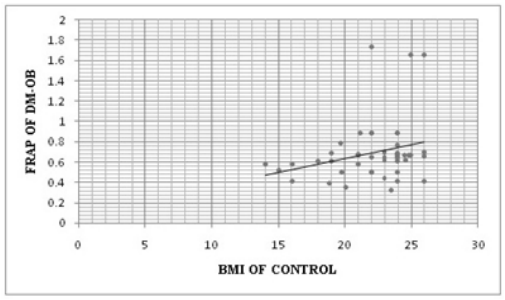


Fig. 1b.

Table 3: Comparison of correlation coefficients among Body mass index (BMI), and Red cell distribution width (RDW) with indices of oxidative stress (OS) in respondents suffering from diabetes mellitus - obesity (DM-OB) and Controls (C).

		BMI	*MDA*	*SOD*	*FRAP*
BMI	C	1	0.28*	0.41**	0.39**
	DM-OB	1	0.56**	-0.03	0.02
RDW	C	0.28*	0.22	0.20	0.12
	DM-OB	0.34**	0.49**	0.05	0.19

*Indicates significance at $p<0.05$; ** at $p<0.005$.
BMI – Body Mass Index, RDW – Red cell distribution



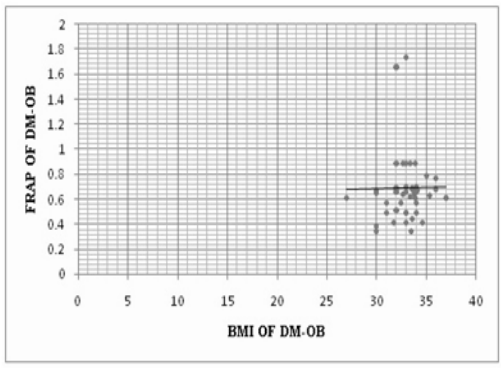


Fig. 1c.

Fig. 1. Comparison between respondents suffering from diabetes mellitus - obesity (DM-OB) and controls (C) of correlation coefficients between body mass index (BMI) and (a) Malondialdehyde (MDA); (b) Superoxide dismutase (SOD); (c) Ferric reducing ability of plasma (FRAP)

obesity. However, it was interesting that MDA increased with BMI even in the controls (C) ($r=0.28$, $p<0.05$) where BMI of all respondents was in the normal range, indicating that MDA is very sensitive to BMI. The spread of MDA values was much more in the DM-OB group, while

it was more clustered in the controls, indicating greater variability in the diseased. Antioxidant enzyme, SOD (Fig. 1b), and total non-enzymatic antioxidant activity, FRAP (Fig. 1c) increased with increasing BMI in C (r=0.41 and 0.39 respectively for SOD and FRAP) but not in DM-OB (r= -0.03 and 0.02 respectively for SOD and FRAP), The higher correlation between BMI and SOD in the controls, evident in Fig. 1b could be the reason for the low MDA in controls. FRAP correlated with BMI as long as BMI was in the normal range but not in the diseased,indicating that as long as BMI remained within the normal range, adaptation was observed due to increase in SOD and FRAP, hence MDA did not increase, but the adaptive response was not sustained in the DM-OB.

RDW was positively correlated to BMI (Fig. 2a) in both C (r=0.28) and DM-OB (r=0.34). Increase in RDW correlated with increase in MDA (Fig. 2b) in DM-OB (r=0.49) but not in the controls (r=0.22), indicating a relationship between OS and red cell maturity in the DM-OB. No significant relationship was found between RDW and SOD as well as FRAP in either groups.

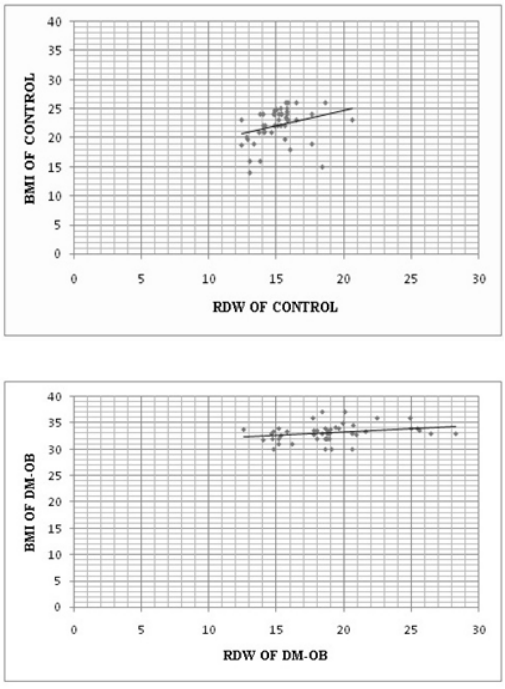


Fig. 2a.

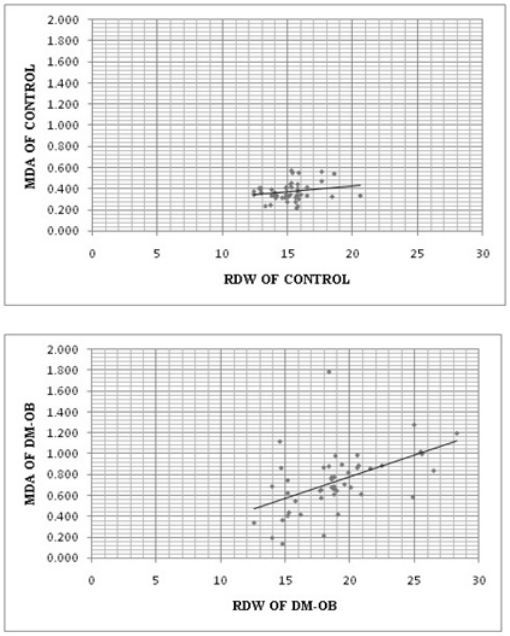


Fig. 2(B)

Fig. 2. Comparison between respondents suffering from diabetes mellitus - obesity (DM-OB) and Controls (C) of correlation coefficients between Red cell distribution width (RDW) and (A) Body Mass Index (BMI);(B) Malondialdehyde

It can be seen from Figure 2a that although the correlation coefficients between BMI and RDW were similar in controls and in DM- OB, the spread of values of RDW was much more in the DM-OB, indicating a greater variability in RDW in the diseased group.

To further evaluate the extent of risk of having diabetes with obesity on OS and RDW, as compared to the non-obese non diabetic controls, odds ratios were calculated and are presented in Table 4.

Table 4 presents the odds ratios for assessment of Diabetes - Obesity as risk factors for changes in red cell distribution width (RDW), total leucocytes count (TLC), malondialdehyde (MDA), superoxide dismutase (SOD), and ferric reducing ability of plasma (FRAP). It can be seen that the odds of having a high RDW was 15 times more in those who were suffering from diabetes - obesity (DM-OB) as compared to C. The odds of having a high MDA was 6.5 times more, and of lower SOD, eighty-eight percent in DM-OB as compared to C. The total antioxidant

Table 4: Odds ratio (OR) for assessment of Diabetes + Obesity as risk factors for changes in RBC distribution width (RDW), Total leucocyte count (TLC), malondialdehyde (MDA), Superoxide dismutase (SOD), and ferric reducing ability of plasma (FRAP).

DM-OB as Risk factor for	*OR*	*95 % CI*
High RDW	15	5.17, 43.55
High MDA	6.5	2.83, 14.92
Low SOD	0.12	0.05, 0.29
Low FRAP	0.13	0.05, 0.3

Cut offs for OR:
RDW- >13.5 %, WBC- > 7500 10^3µl, SOD - >0.3U/gm of Hb, MDA- > 2.3µmol MDA/gm of Hb, FRAP- > 0.69 U/ml of plasma.

capacity as indexed by FRAP was also eighty-seven percent more likely to be lower in diseased respondents than controls.

DISCUSSION

The most striking observation from the present case-control study was the significantly high red cell distribution width in the obese diabetics, which correlated with body mass index and with the oxidative stress marker, malondialdehyde. The odds of having a high RDW and a high MDA were respectively 15 and 6.5 times more in the obese diabetics than in the non-obese non-diabetic controls. This could be attributed to their lower antioxidant enzyme, SOD, and lower total antioxidant capacity as indexed by FRAP.

It is not surprising that obese respondents suffering from diabetes mellitus have a high RDW because it has been reported to be an index of inflammation and has been found to increase with increasing body weight (Nada 2015).

It is known that inflammation is accompanied by higher oxidative stress. The higher oxidative stress status of the obese diabetics in the present study is evident from the higher malondialdehyde and lower levels of antioxidant enzyme superoxide dismutase as well as lower total antioxidant activity as measured by FRAP. The higher SOD in the controls, where the BMI is within the normal range, as compared with the DM-OB indicates a breakdown of the normal homeostatic pattern, leading to loss of adaptation by SOD and FRAP in DM-OB. This is in contrast to studies by Mizobuchi et al. (1993) who had reported significantly higher SOD activity in diabetes patients as compared to healthy controls. In controls, adaptation due to higher SOD is observed hence MDA does not increase, but the adaptive response is not sustained in the obese diabetics. It is well documented that MDA is a stable end product of free radical damage induced by lipid peroxidation, and serves as a reliable marker for the assessment of free radical induced damage to tissues (Rashida et al. 2010). In diabetic patients a major factor that is responsible for enhanced free radical generation is hyperglycemia through auto-oxidation of glucose and may be an important risk factor for cardiovascular disease. Red blood cell homeostasis is an excellent example of redox balance: erythroid progenitors accumulate hemoglobin during development, and erythrocytes continuously transport large amounts of oxygen over the course of their approximately 120-day lifespan. This results in a high level of oxidative stress (Fruehauf and Meyskens 2007). Erythrocytes, more than other cells, are exposed to mechanical damage, biochemical disturbance and damage associated with oxidative stress (Petibois and Deleris 2005). The increased lipid peroxidation in diseased conditions such as diabetes and obesity, observed in the erythrocyte membrane in the present study, is well known.

Recently this has been attributed to destabilization of the cell membrane skeleton, which causes changes in transmembrane transport and increase in erythrocyte membrane rigidity as a result of lipid oxidation and indirectly affects the cell lifespan (Gwozdzinski et al. 2017). Since cell lifespan directly affects RDW, it is surmised that increase in MDA may be responsible for accelerated aging of the erythrocyte, accompanied by a decrease in cell volume, and an increase in haemoglobin content and cell density, leading to the increase in RDW observed in present study. This is expected because redox balance in ageing cells is disturbed in favour of the oxidation processes, as explained by Mohanty et al. (2014). Macrophages identify and phagocytize RBCs that have attained a critical age (120 days in humans and 60 days in mice) in a process known as erythrophagocytosis (Piomelli and Seaman 1993).

Since reduced RBC clearance has been suggested to be a major cause of elevations in RDW (Patel et al. 2010), and as obesity is related to increased inflammatory pattern, anisocytosis is related with erythrocyte deformability and delayed RBC clearance (Donma et al. 2015) in the

obese respondents in the researhers' study. Both RDW and BMI are directly and significantly associated, strengthening the notion that RDW is an inflammatory marker, as obesity is considered a state of subclinical inflammation (Nada 2015; Kim et al. 2018).

Al-Kindi et al. (2017) have analysed large datasets from the NHANES study and concluded that RDW is a powerful and an independent marker for prediction of all-cause mortality and cardiovascular mortality in patients with diabetes. Agarwal (2012) has reported that in a large representative database of general US population, he observed a significant association between elevated RDW and elevated CRP with low cardiorespiratory fitness.

In another study (Vayá et al. 2014), undertaken to assess the relationship between RDW and inflammatory parameters in morbidly obese patients, RDW and CRP were not found to be correlated even though RDW was higher in the morbidly obese as compared to controls. This led the researchers to conclude that RDW should not be considered to be an inflammatory marker in morbid obesity. It is being increasingly recognized that RDW has for long been neglected as an aid for preliminary risk stratification of patients, despite being an inexpensive valuable parameter. The researchers' study strengthens this viewpoint. Oxidative stress is responsible for shortening the lifespan of red blood cells, which intensifies both the production and the release of young cellular forms into the circulation, which is reflected by increased RDW levels (Friedman et al. 2004). The relationship of RDW with type 2 diabetes mellitus and the metabolic syndrome, reported by the researchers' is corroborated by recent studies by several other researchers (Kachekouche et al. 2018; Yin et al. 2018; Jaman et al. 2018). These groups have also reported similar relationships as researchers' between pro-inflammatory and oxidative stress states.

CONCLUSION

RDW is a minimally invasive, inexpensive haematological marker, and anisocytosis has recently found application in study of inflammatory and oxidative stress conditions. In the present study, obese patients with type 2 diabetes mellitus had a 15 times higher risk of anisocytosis than non-obese non-diabetic healthy controls. Anisocytosis correlated with body mass index and high lipid peroxidation in the erythrocytic membrane. The association pattern between antioxidant enzyme and non-enzymatic antioxidant capacity were different in the control group and the obese diabetic group, and indicated presence of adaptation in the healthy group which broke down in the diseased group.

RECOMMENDATIONS

It is recommended that more work is undertaken to explore the utility of RDW as a marker of inflammation and oxidative stress, because limited data is available which explores these interrelationships among components of CBC, especially RDW and markers of oxidative stress. This is important because it can enhance the predictive value of this inexpensive and minimally invasive test.

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