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Total and differential leukocytes count in type 2 diabetes mellitus patients in Iraq

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To find the correlation between total and differential leukocytes count and the presence of microalbuminuria in diabetic patients. The results show significant positive correlation between total leukocytes count and microalbuminuria in males and females also there is significant positive correlation between neutrophils count and microalbuminuria in males and females but there is no correlation with other types of leukocytes.

Key word : Diabetic Mellitus, Leukocytes, Microalbuminuria

Diabetes mellitus produces inadequate blood glucose control and leads to acute and chronic complications (Koda-Kimble & Carlisle, 1995). Acute complications include diabetic ketoacidosis and hyperosmolar non ketotic coma (Singh & Marshall, 1995). Chronic complications can involve the kidneys, eyes, nervous system, and cardiovascular system and can be classified as either macrovascular or microvascular (Koda-Kimble & Carlisle, 1995).

Peripheral white blood cell (WBC) count has been shown to be associated with insulin resistance, type 2 diabetes (Schmidt et al., 1999; Vozarova et al., 2002; Ford, 2002; Ohshita et al., 2004), coronary artery disease (CAD) (Kannel et al., 1992; Weijenberg et al., 1996; Danesh et al., 1998; Ohshita et al., 2004), stroke (Kannel et al., 1992), and diabetes micro- and macrovascular complications (Cavalot et al., 2002). An association between leukocytes counts and CAD has been observed in prospective and retrospective cohort studies as well as in case control studies; this association persists after adjusting for multiple coronary heart disease (CHD) risk factors, including smoking (Madjid et al., 2004).

Leukocytes can be activated by advanced glycation end products (Pertynska-Marczewska et al., 2004), oxidative stress (Shurtz-Swirski et al., 2004), angiotensin II (Lee et al., 2004), and cytokines (Scherberich, 2003) in a state of hyperglycemia. Experimental evidence links the progression of diabetic nephropathy to intrarenal inflammation and leukocytic cell infiltrates (Meyer, 2003; Schena & Gesualdo, 2005; Mora & Navarro, 2005; Tuttle, 2005; Galkina & Ley, 2006). Renal tissue macrophages, T cells, and neutrophils produce various reactive oxygen species (ROS), proinflammatory cytokines, metalloproteinases, and growth factors,

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which modulate the local response and increase inflammation within the diabetic kidney (Galkina & Ley, 2006).

Study in men have found that a higher number of eosinophils in the blood correlates with higher urine albumin—a critical early sign of diabetic kidney disease and the link between eosinophil count and albumin excretion rate was even stronger than for known risk factors like Hypertension and eosinophil count was unrelated to albumin excretion in diabetic women (Fukui et al., 2009).

Infiltrated macrophages are found within renal diabetic tissues, and studies demonstrated that macrophage-derived products can induce further inflammation in the diabetic kidney (Bohle et al., 1991; Furuta et al., 1993; Sassy-Prigent et al., 2000; Chow et al., 2004) One study revealed that peripheral lymphocyte count were negatively associated with systolic blood pressure, serum uric acid levels and this may provide some clues into the role of peripheral lymphocytes in protection from diabetic nephropathy (Chung et al., 2005). The aim of this study is to find the correlation between total and differential leukocytes count and the presence of microalbuminuria in diabetic patients.

MATERIALS AND METHODS

This study was done in Al-Hussein general hospital in Karbala province. The collection of samples was conducted during the period from June to November/ 2009. The study was conducted on 51 patients from the diabetic clinic in the mentioned hospital and 24 healthy subjects (10 males and 14 females) were taken as control (total persons 75). All patients were infected with type 2 diabetes from which 23 males and 28 females. The ages of patients and controls were ranges between 35—65 years old.

The medical history of each patient was taken which include age, gender, and duration of disease, type of treatment, family history, and history of any other illness. Measurements of height and weight were done to calculate body mass index and expressed as Kg/m2, measurement of blood pressure also done before takes samples of blood and urine, Blood pressure was measured after 30 min. rest, and the measurement was performed by using Mercury sphygmomanometer. The patient was seated with the back supported and the upper arm bore without constrictive clothing. The legs should not be crossed. The arm was supported at heart level and the bladder of the cuff was encircled at least 80% of the arm circumference with the stethoscope at the elbow crease over the brachial artery.

The mercury column was inflated at the above systolic pressure then deflated at 2 to 3 mm/s and the first and last audible sounds were taken as systolic and diastolic blood pressure. The column was read to the nearest 2 mmHg (Pickering et al., 2005). Samples were collected in fasting status. Blood samples were collected from healthy control and diabetic patients by vein puncture using 5 ml disposable syringes. Blood was divided into two parts. First part: 3 ml was put in the centrifuge tube and allowed to clot for 15 min then it was centrifuged for approximately 10 minutes at a relative centrifugal force (RCF) of 1000 xg to 2000 xg and separated the serum in plain tube for biochemical tests (Varley et al., 1991). Second part: 2 ml was put in EDTA tube; the blood was mixed gently and then used for hematological tests.

The first morning urine was collected in disposable containers from diabetic patients. Microalbuminuria was measured by using semi-quantitative dry immunochemical screening strips (MICRAL-TEST marker made in Germany). Serum glucose was estimated by enzymatic color test on basis of Trinder reaction and the glucose Kit was the Biocon marker made in Germany. HbA1c was measured by using quantitative colorimetric determination of glycohemoglobin in whole blood and the HbA1c Kit was the Stanbio

marker made in USA. Serum glucose and HbA1c were measured by photoelectric colorimeter from design APEL, AP 101/ Japan. Total leukocytes count was done manually by using 1 in 2 dilution of blood was made by adding 20 µl of well-mixed blood to 0.38 ml of lysing fluid (2% (20 ml/l) glacial acetic acid colored pale violet with gention violet) in glass tube. Mixed well and waited for 2 minutes then read by using counting chamber from type Improved Neubaer Slid (Lewis et al., 2001). Differential count of leukocytes was made by using Leishman's stain (The Leishman's stain Kit is the Crescent Diagnostics marker made in Saudi Arabia).

Statistical Analysis

The statistical analysis of this study was made by using SPSS program (Version 16.0) and the statistical processes used here were Means, Standard deviations, Independent sample T-Test, Correlation coefficient and Linear regression.

RESULTS

Table (1) shows the results of males group. The results show no significant increase in BMI and there is significant increase in systolic and diastolic blood pressure, fasting blood sugar, HbA_{1c} and microalbuminuria in patients group as compared with control group. Also the results show no significant increase in total and differential leukocytes count in patients group as compared with control group.

Table 1: The results of males group

Parameter	Control group n=10 (Mean ± SD)	Patients group n=23 (Mean ± SD)	P-value
Age (years)	**46.8±9.38**	**48.13±8.13**	**---**
BMI (Kg/m²)	**28.34±4.00**	**27.31±3.00**	**0.423**
Systolic BP (mmHg)	**112.0±9.19**	**126.96±13.03**	**0.003**
Diastolic BP (mmHg)	**72.6±9.19**	**82.17±8.77**	**0.005**
Fasting blood sugar(mmol/l)	**0.66 ±4.5**	**10.61±3.93**	**<0.001**
HbA1c (%)	**6.08±0.40**	**8.29±1.4**	**<0.001**
Total leukocytes count (X10⁹/L)	**1.11 ± 6.62**	**1.69 ± 7.16**	**0.362**
Neutrophils count(X10⁹/L)	**±0.99 4.01**	**4.47±1.48**	**0.370**
Basophils count (X10⁹/L)	**0.034 ± 0.037**	**0.014 ± 0.028**	**0.154**
Eosinophils count (X10⁹/L)	**0.17±0.11**	**0.11±0.05**	**0.168**
Lymphocytes count (X10⁹/L)	**2.21 ± 0.61**	**2.36 ± 0.72**	**0.544**
Monocytes count (X10⁹/L)	**0.18 ± 0.08**	**0.21 ± 0.12**	**0.563**
Microalbuminuria (mg/L)	**10.0 ± 1.76**	**33.91 ± 1.8**	**0.001**

The significant differences at P-value <0.05

Table (2) shows the results of females group. The results show no significant increase in BMI between control and patients group but there is significant increase in systolic and diastolic blood pressure, fasting blood sugar, HbA1c and microalbuminuria in patients group as compared with control group. The results of comparison in total and differential leukocytes count show significant increase in total leukocytes count in patients group as compared with control group also found significant increase in lymphocytes and monocytes count as compared with control group. The results of correlation between total and differential leukocytes count show significant positive correlation between total leukocytes count and micro-albuminuria in both sexes. Also found positive correlation between neutrophils count and microalbuminuria in both sexes but there is no significant correlation with other types of leukocytes as shown in table (3).

Table 2: The results of females group

Parameter	Control group n=14 (Mean ± SD)	Patients group n=28 (Mean ± SD)	P-value
Age (years)	**50.0 ± 10.87**	**52.07±8.46**	**---**
BMI (Kg/m²)	**27.71±4.56**	**29.07±5.04**	**0.402**
Systolic BP (mmHg)	**114.29±10.89**	**136.25±19.84**	**<0.001**
Diastolic BP (mmHg)	**72.86±9.94**	**85.71±11.60**	**<0.001**
Fasting blood sugar(mmol/l)	**790. ±534.**	**11.83±4.47**	**<0.001**
HbA1c (%)	**5.57±0.60**	**9.10±2.07**	**<0.001**
Total leukocytes count (X10⁹/L)	**6.84 ± 1.27**	**8.04 ± 2.17**	**0.030**
Neutrophils count(X10⁹/L)	**4.53±1.18**	**4.95±1.86**	**0.440**
Basophils count (X10⁹/L)	**0.011 ± 0.021**	**0.011 ± 0.026**	**0.916**
Lymphocytes count (X10⁹/L)	**2.02 ± 0.62**	**2.60 ± 0.92**	**0.039**
Monocytes count (X10⁹/L)	**0.16 ± 0.08**	**0.28 ± 0.18**	**0.002**
Microalbuminuria (mg/L)	**13.99 ± 2.60**	**33.42 ± 2.35**	**<0.001**

The significant differences at P-value <0.05

In males group the positive linear regression between total leukocytes count and microalbuminuria (Figure 1) was according to the linear equation (Y=-35.57+9.0X) at level P=0.001 and correlation coefficient r=0.63 and in females (figure 2) the positive linear regression was according to the linear equation (Y=-19.59+7.59X) at level P=<0.001 and correlation coefficient r=0.51. The positive linear regression between neutrophils count and microalbuminuria in males group (Figure 3) was according to the linear equation (Y=-21.88+11.36X) at level P<0.001 and correlation coefficient r=0.69 and in females group (Figure 4) was according to the linear equation (Y=0.23+8.32X) at P=0.01 and correlation coefficient r=0.48.

Table 3: The Pearson's Correlation between total and differential Leukocytes count and Microalbuminuria in males and females groups

Parameter	Microalbuminuria in males group (n=23)		Microalbuminuria in females group (n=28)	
	r	P	r	P
Total leukocytes count	0.63	0.001	0.51	0.006
Neutrophils count	0.69	<0.001	0.48	0.010
Basophils count	0.002	0.994	-0.233	0.233
Eosinophil count	0.049	0.824	0.272	0.162
Lymphocytes count	0.048	0.828	0.201	0.305
Monocytes count	-0.087	0.693	0.233	0.233

R = correlation coefficient, P= probability level (The correlation significant if P<0.05).

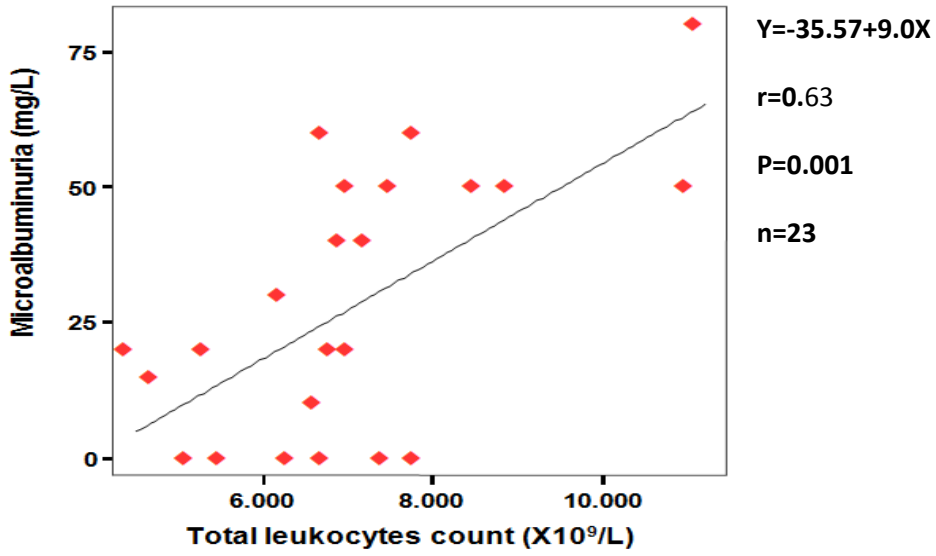

Figure 1: The relationship between microalbuminuria (mg/L) and total leukocytes count (X109/L) in males group.

DISCUSSION

The results show no significant differences in total leukocytes count in males group (Table 1) as compared with control group, despite this the mean of total leukocytes count was high and near the upper limit of normal leukocytes count, but there is significant increase in total leukocytes count in females group (Table 2) and we found elevation in Lymphocytes and monocytes count in females patients as compared with control group. This is may be because found inflammation response

companied with diabetes because leukocytes is one of the markers of inflammation. This finding is consistent with the hypothesis that a chronic activation of the immune system may play a role in the pathogenesis of type 2 diabetes (Vosarova et al., 2002) and other study revealed that low and high levels of leukocytes count were associated with an increased risk of diabetes in young workers (Du et al., 2009).

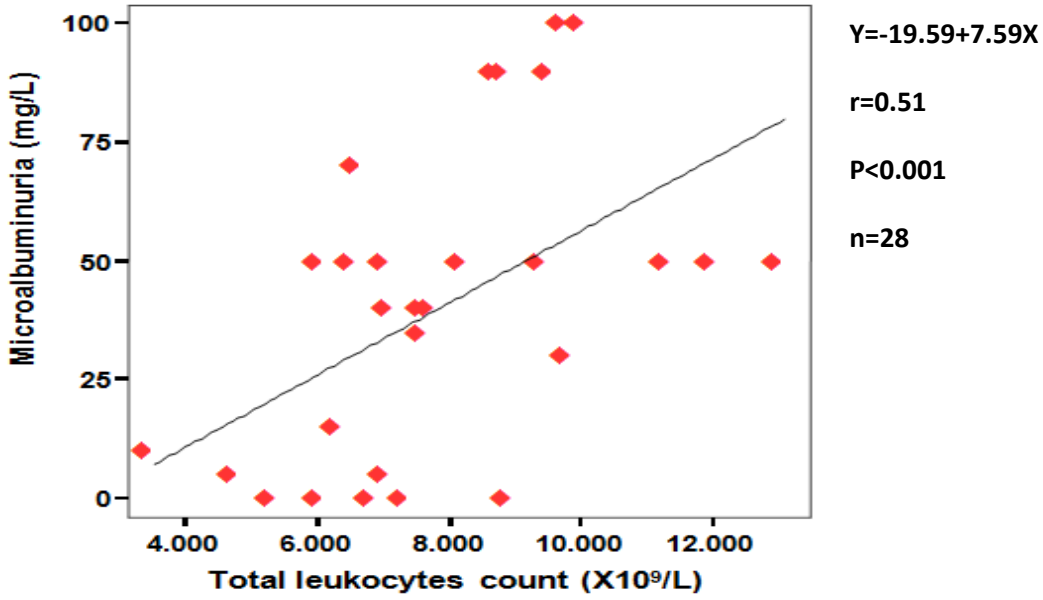

Figure 2: The relationship between micoalbuminuria (mg/L) and total leukocytes count (X109/L) in females group.

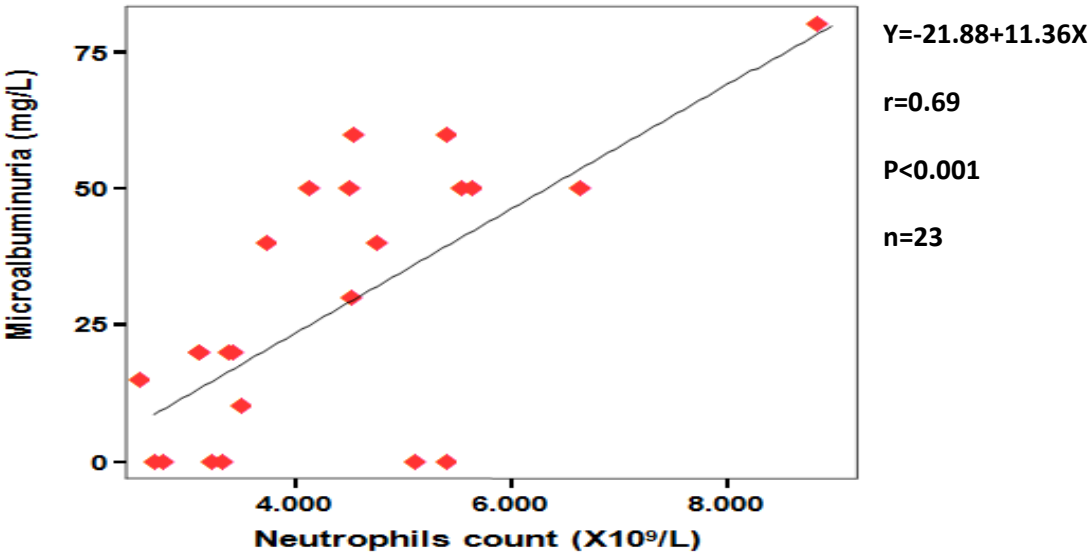

Figure 3: The relationship between neutrophils count (X109/L) and microalbuminuria (mg/L) in males group.

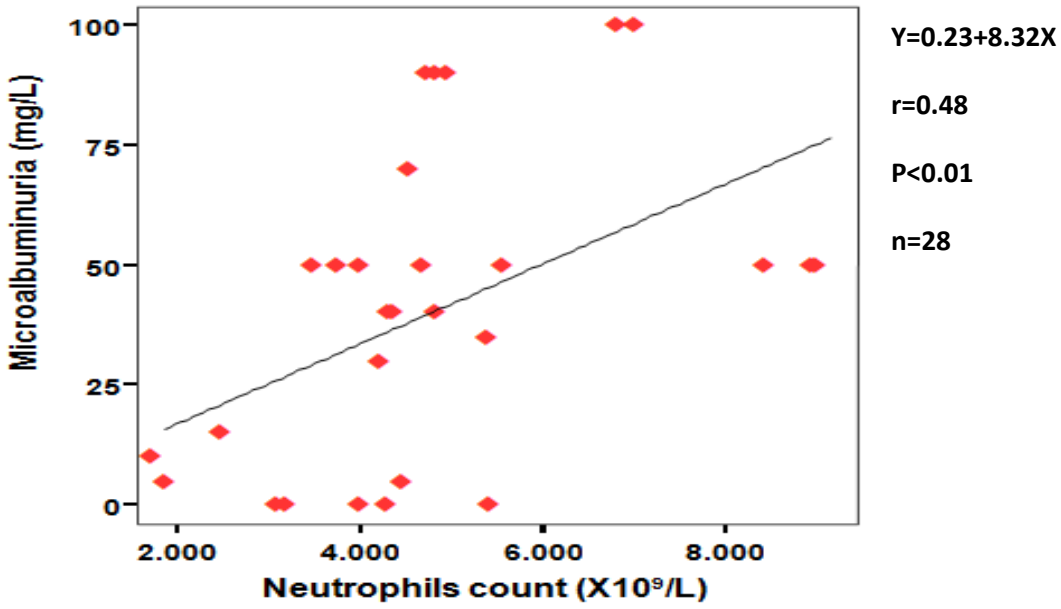

Figure 4: The relationship between neutrophils count (X109/L) and microalbuminuria (mg/L) in females group.

The study revealed significant positive correlation between micro-albuminuria and peripheral total leukocytes count in males (Figure 1) and females (Figure 2) and between microalbuminuria and neutrophils count in males (Figure 3) and females (Figure 4) and there is no significant correlation between microalbuminuria and other types of WBCs (Table 3), this study was supported by other study that found the peripheral WBCs, monocytes and neutrophils count increased in patients with the advancement of diabetic nephropathy (Chung et al., 2005) and the elevation in WBC count, even within the normal range, is associated with both macro and microvascular complications in type 2 diabetes by the relation between albuminuria and WBCs. Chronic inflammation, as indicated by a higher WBC count, may play a linkage role in the development of macro- and microvascular complications in diabetes (Tong et al., 2004).

The mechanisms responsible for the increased total and differential leukocytes in diabetic patients may be related to plasma cortisol and the changing insulin levels in renal disease. Both factors are known to increase WBCs count by increasing neutrophil influx from marrow storage and decreasing efflux from the blood stream (Bjornson et al., 1985; Collier et al., 1990). In addition, cortisol and insulin may increase the WBC count by stimulating leptin secretion from adipocytes (Wabitsch et al., 1996) and leptin might be involved in increased leukocyte counts (Gainsford et al., 1996; Laharrague et al., 2000). Leptin has been reported to stimulate myeloid differentiation from human bone marrow CD34_progenitors (Laharrague et al., 2000) and can induce proliferation, differentiation, and functional activation of hemopoietic cells (Gainsford et al., 1996).

The data in this study showed that leukocytes play an important role in the initiation and progression of renal disease, including inflammatory mechanisms independent of infection, causing proteolytic and oxidative damage to the mesangial cells

(Shanmugam et al., 2003). TNF-α (Tumor necrosis factor- α) is an inflammatory cytokine produced by neutrophils, macrophages and, importantly, adipocytes and studies have shown that TNF-α and inflammation in general can contribute to the pathogenesis of diabetic nephropathy (Schalkwijk & Stehouwer, 2005). Tumor necrosis factor has the widest variety of biological activities and effects that contribute to development of diabetic nephropathy. Importantly, though, it causes direct renal injury as a cytotoxin, as well as affecting apoptosis, glomerular hemodynamics, endothelial permeability, and cell-cell adhesion. It also seems to play an important part in the early hypertrophy and hyperfunction of diabetic nephropathy (DiPetrillo et al., 2003; DiPetrillo et al., 2004; Navarro-Gonzalez et al., 2008).

CONCLUSION

Elevation in leukocytes count despite in the normal range especially neutrophils may have effect on the development of microalbuminuria.

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