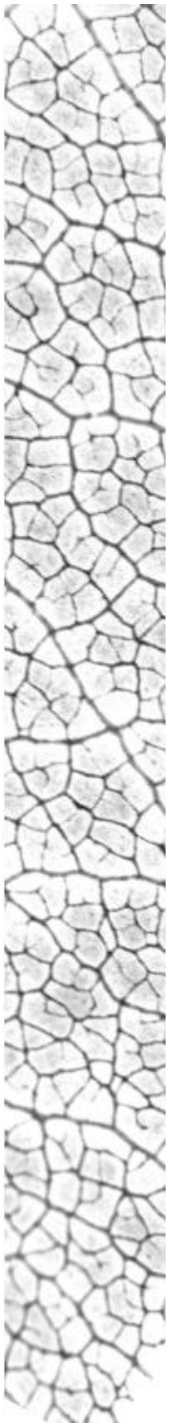

EurAsian Journal of BioSciences
Eurasia J Biosci 14, 5131-5139 (2020)

Correlation between DNA methylation with white blood cells count in Iraqi diabetic nephropathy patients

Anwar Abd-Al-Hameed Kamal [1*], Maysaa A. Hadi [2], Hussein Naji [1]

[1] College of Medicine, University of Babylon, IRAQ
[2] College of Science, University of Babylon, IRAQ
*Corresponding author: anwaralkhfagi77@gmail.com

Abstract

In the present study, there were significant increase in WBC count (P<0.001) and neutrophil% (P<0.001) in DN compared to DM and control groups, while there was significant decrease in eosinophil% (P<0.001), basophil% (P<0.001) and lymphocyte% (P<0.001) in DN compared to DM and control groups, so, there were no significant differences between study groups in their monocyte% (p 0.453). There were no significant differences of WBC count (p 0.323, p 0.733), neutrophil% (p 0.142, p 0.798), eosinophil% (p 0.338, p 0.402), basophil% (p 0.921, p 0.722), lymphocyte% (p 0.279, p 0.327) between duration of disease, while the mean differences of monocyte were significant decrease in diabetic nephropathy group (P 0.027) in all duration of disease. The mean differences of WBC count were significant increase (P 0.009) at duration of disease 6-10 years in diabetic nephropathy group, but no significant differences between WBC count in other durations 1-5 years and ≥ 11 years which was (p 0.094, p 0.37) respectively. Furthermore, neutrophil% had significant increase in diabetic nephropathy group in all duration of disease (p <0.001, P<0.001, P 0.002) respectively, and lymphocyte% were significant decrease in all duration of disease (P<0.001, P<0.001, P 0.001) respectively. The mean differences of monocyte was significant decrease (P 0.011) in diabetic without nephropathy at age group ≥ 60 years, while the mean differences of WBC was no significant differences between study groups in all age groups (p 0.439, p 0.506, p 0.41). Also, no significant differences between neutrophil% in all age groups (p 0.368, p 0.58, p 0.717), eosinophil% in all age groups (p 0.438, p 0.346, p 0.868), basophil% in all age groups (p 0.754, p 0.165, p 0.269), lymphocyte% in all age groups (p 0.682, p 0.312, p 0.653) and monocyte% in age groups of DN (p 0.671), and control group (p 0.257). However, the mean differences of neutrophil% was significant increase and lymphocyte % were significant decrease between study groups in all age groups (P 0.023, P <0.001, P <0.001) and (P 0.003, P <0.001, P<0.001) respectively in DN group compared to DM and control groups. In addition, the mean differences of eosinophil% was significant decrease in DN group in age groups between 46-60 years (P 0.001), and the mean differences of basophil% was significant increase in DN and DM groups 30-45 years (P<0.001) and age groups 46-60 years (P 0.005) compared to control group. There were no significant differences between WBC count in all age groups (p 0.497, p 0.085, p 0.087), monocyte% in all age groups (p 0.487, p 0.202, p 0.276), eosinophil% in age group 30-45 years (0.255) and ≥ 60 years (p 0.388) and basophil% in age group ≥ 60 years (p 0.191). There were significant increase of WBC count in female (P 0.001) of DN group compared to female of DM and control groups. In addition, there was significant increase of basophil % in DN and DM groups in both gender (male and female) (P 0.006, P 0.001) compared to control group, while the percentages of neutrophil% and lymphocyte% were significant increase and decrease respectively in DN group in both gender (male and female) (P<0.001, P 0.001), (P<0.001, P<0.001) compared to DM and control group. When compared between male and female, no significant difference of WBC count between study groups according to gender (p 0.227. p 0.609, p 0.241), eosinophil% (p 0.409, p 0.528, p 0.712), basophil% (p 0.934, p 0.593, p 0.326) and monocyte% (p 0.991, p 0.924, p 0.15). While, there were significant decrease (P 0.046) in neutrophil% and significant increase (P 0.015) in lymphocyte% of females compared to males in DN group. In addition, there was no significant between DNA methylation (p 0.174, p 0.111) in diabetic patients according to duration of disease. The mean differences of DNA methylation concentrations in study groups according to age were studied, it was significant increase in all age groups (P 0.005, P<0.001, P 0.026) compared to control group. There were no significant difference between DNA methylation concentration in study groups including (diabetic with nephropathy, diabetic without nephropathy and control group) according to age (p 0.323, p 0.5, p 0.736), there was significant difference between means of DNA methylation in this study for study groups with gender (male and female). DNA methylation (P<0.001, P<0.001).Furthermore, no significant difference between males and females in all study groups (diabetic with nephropathy, diabetic without nephropathy and control group) including DNA methylation concentration (p 0.771, p 0.179, p 0.5). The correlation between DNA methylation and study variables were significant found positive correlation between DNA methylation and basophil% (r=0.367, p=0.009) in patients with diabetic nephropathy.

Keywords: Diabetic Nephropathy, DNA methylation, white blood cells, diabetes mellitus

Kamal AA, Hadi MA, Naji H (2020) Correlation between DNA methylation with white blood cells count in Iraqi diabetic nephropathy patients. Eurasia J Biosci 14: 5131-5139.

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by high blood sugar (glucose) levels that result from defects in insulin secretion, or its action, or both (Paydar et al. 2019; Tazhbenova et al., 2019). Normally, blood glucose levels are tightly controlled by insulin, a hormone produced by the pancreas. In patients with diabetes, the absence of insufficient production of or lack of response to insulin causes hyperglycemia. Diabetes is a chronic medical condition, meaning that although it can be controlled, it lasts a

Received: May 2019
Accepted: April 2020
Printed: October 2020

Table 1. Mean difference of WBC parameters in study groups

Study variables	Study groups			P-value
	Diabetic nephropathy (N=50)	Diabetic without nephropathy (N=25)	Healthy control (N=25)	
WBC count 10e3/uL	9.52 ± 3.88	6.98 ± 2.06	6.95 ± 1.96	**<0.001***
Neutrophil %	74.31 ± 13.38	55.39 ± 6.33	62.45 ± 8.67	**<0.001***
Eosinophil %	2.20 ± 2.27	3.22 ± 1.82	4.41 ± 1.62	**<0.001***
Basophil %	0.61 ± 0.41	0.90 ± 0.38	0.37 ± 0.11	**<0.001***
Monocyte %	6.78 ± 3.25	6.53 ± 1.82	6.00 ± 1.05	0.453
Lymphocyte %	16.26 ± 10.39	32.11 ± 4.99	31.09 ± 9.78	**<0.001***

lifetime (Kalyani et al. 2014). The classic symptoms of untreated diabetes are unintended weight loss, polyuria (increased urination), polydipsia (increased thirst), and polyphagia (increased hunger) (Kumar et al. 2020). Symptoms may develop rapidly (weeks or months) in type 1 diabetes, while they usually develop much more slowly and may be subtle or absent in type 2 diabetes. Other symptoms of diabetes include weight loss and tiredness (Dhanavelu et al. 2019). Type 2 diabetes is characterized by insulin resistance, which may be combined with relatively reduced insulin secretion (Hesaka et al. 2019). The defective responsiveness of body tissues to insulin is believed to involve the insulin-t-receptor (Zang et al. 2017). It is the most common types of diabetes mellitus (Wu et al. 2018). The classic definition of epigenetics refers to the heritability of cell phenotypes, via either mitosis or meiosis that is not encoded by the genome (Zeni et al. 2017). Cell identities are defined by patterns of gene expression, and chromatin structure is the key mediator of epigenetic programming of gene function (Delaneau et al. 2019). More than 40 years ago, DNA methylation emerged as the first epigenetic modification. DNA methylation involves the addition of a methyl group to the cytosine ring of cytosines preceding a guanosine in the DNA sequence (cytosine-phosphate-guanine [CpG] dinucleotides) to form methyl cytosine (5-methylcytosine) (Zeni et al. 2017). It is a biological process in which a methyl group is added to the 5th carbon position of the cytosine (5MC) via methyltransferase enzymes (DNMTs) (Osman, 2020). The addition of methyl group changes the biophysical characteristics of DNA result in inhibiting the recognition of DNA by some proteins and permits the binding of others (Rea et al. 2017). Epigenetic programing has been linked to environmental factors (such as nutrition, smoking, chemical exposures, drugs, and other stresses), physical activity, and aging. Altered epigenetic processes are major contributors to disease (Tiffon, 2018). Epigenetic alterations in cancer have been known for >30 years because the observation that tumor progression is associated with the global loss of DNA methylation (Castilho et al., 2017).

MATERIALS AND METHODS

The present study is an observational case control design. The data of study were collected in the period from November 2019 to January 2020. The study was conducted in Marjan Teaching Hospital in Hilla City, Babylon province, Iraq. A total number of subjects involved in this study was 75 patients (50 patients suffering from diabetic nephropathy, 25 diabetic patients without nephropathy) and 25 as control healthy). All patients and control were from the same ethnic group (Arabic).

Research and sampling ethics

The project proposal and sampling method were approved by the Research Ethics Committee of Babylon Health Directorate according to the directorate administrative order No. 5393, date 12/11/2019. In addition, the project achieve the permission of research ethics in Marjan Medical City.

Hematological studies

All of hematological profile (CBC) have been done by use of an automated auto-analyzer. In this test, the blood is placed in the vibrator, after which the power switch is pressed. Blood 20 µl is blood is taken by probe, and taken out of the device, after a minute the result was appeared.

Estimation of 5-Methylcytosine DNA: by using ELIZA kit from Zymo- researchers / USA.

Statistical analysis

Statistical analysis was carried out using SPSS version 23. Continuous variables were presented as (Means ± SD). Student t-test was used to compare means between two groups

RESULTS AND DISCUSSION

Mean difference of WBC parameters between study groups

The mean differences between WBC parameters including (WBC count, neutrophil, eosinophil, basophil, monocyte, and lymphocyte) according to study groups was shown in **Table 1**. The results showed that, there were significant increase in WBC count (P<0.001) and neutrophil% (P<0.001) in DN compared to DM and control groups, while there was significant decrease in eosinophil% (P<0.001), basophil% (P<0.001) and lymphocyte% (P<0.001) in DN compared to DM and control groups, so, there were no significant differences between study groups in their monocyte% (p 0.453). These results were agreement with results of Khandare *et al*., (2017) who found that there were significant increase in WBC count according to diabetic patients and diabetic nephropathy. Leukocytes are activated and

Table 2. Mean differences of WBC parameters in diabetic patients according to duration of disease

Study variables	Study groups	Duration of disease						P-value
		N	1-5 years	N	6-10 years	N	≥ 11 years	
WBC count 10e3/uL	DN (N=50)	11	9.32 ± 3.88	12	10.99 ± 4.26	27	8.96 ± 3.68	0.323
	DM (N=25)	8	6.58 ± 2.28	11	6.98 ± 1.48	6	7.50 ± 2.86	0.733
P-value	Total	19	0.094	23	**0.009***	33	0.37	
Neutrophil %	DN (N=50)	11	81.32 ± 12.08	12	71.78 ± 9.33	27	72.57 ± 14.75	0.142
	DM (N=25)	8	54.75 ± 5.99	11	55.02 ± 6.36	6	56.95 ± 7.58	0.798
P-value	Total	19	**<0.001***	23	**<0.001***	33	**0.002***	
Eosinophil %	DN (N=50)	11	1.34 ± 1.48	12	2.18 ± 1.54	27	2.55 ± 2.73	0.338
	DM (N=25)	8	2.52 ± 1.35	11	3.41 ± 1.83	6	3.80 ± 2.32	0.402
P-alue	Total	19	0.095	23	0.096	33	0.31	
Basophil %	DN (N=50)	11	0.65 ± 0.52	12	0.61 ± 0.45	27	0.59 ± 0.35	0.921
	DM (N=25)	8	0.97 ± 0.39	11	0.90 ± 0.34	6	0.80 ± 0.45	0.722
P-value	Total	19	0.175	23	0.113	33	0.235	
Lymphocyte %	DN (N=50)	11	11.81 ± 9.56	12	17.41 ± 8.59	27	17.56 ± 11.23	0.279
	DM (N=25)	8	31.53 ± 4.29	11	33.70 ± 5.59	6	29.96 ± 4.45	0.327
P-value	Total	19	**<0.001***	23	**<0.001***	33	**<0.001***	
Monocyte %	DN (N=50)	11	5.21 ± 2.12	12	8.73 ± 3.83	27	6.56 ± 3.03	**0.027***
	DM (N=25)	8	6.59 ± 2.14	11	6.82 ± 1.84	6	5.92 ± 1.46	0.639
P-value	Total	19	0.18	23	0.144	33	0.622	

secrete cytokines in the diabetic state and stimulates leukocyte proliferation and differentiation, suggest that circulating leukocytes contribute to the development and progression of nephropathy, partially through the effects in patients with type 2 diabetes (Verdaguer *et al*., 2019). So, this results due to the fact that the WBCs play a role in the development and progression of diabetic complication (Rubattu *et al*., 2019).

Mean differences of WBC parameters in diabetic patients according to duration of disease

The mean differences of WBC parameters according to duration of disease between study groups including (diabetic nephropathy and diabetic without nephropathy) compared with control group respectively were shown in **Table 2**, the results showed that there were no significant differences of WBC count (p 0.323, p 0.733), neutrophil% (p 0.142, p 0.798), eosinophil% (p 0.338, p 0.402), basophil% (p 0.921, p 0.722), lymphocyte% (p 0.279, p 0.327) between duration of disease, while the mean differences of monocyte were significant decrease in diabetic nephropathy group (P 0.027) in all duration of disease. The mean differences of WBC count were significant increase (P 0.009) at duration of disease 6-10 years in diabetic nephropathy group, but no significant differences between WBC count in other durations 1-5 years and ≥ 11 years which was (p 0.094, p 0.37) respectively. Furthermore, neutrophil% had significant increase in diabetic nephropathy group in all duration of disease (p <0.001, P<0.001, P 0.002) respectively, and lymphocyte% were significant decrease in all duration of disease (P<0.001, P<0.001, P 0.001) respectively. These results were agreement with results of Abdelsalam *et al*., (2020) who found that there was significant increase of WBC at duration of disease in diabetic nephropathy patients. Different types of activated leukocytes play a crucial role in the pathogenesis of most kidney diseases from acute to chronic stages (Fani *et al*., 2018), however, diabetic nephropathy was not considered an inflammatory disease in the past. This view is changing now because there is a growing body of evidence implicating inflammatory cells at every stage of diabetic nephropathy (Feng *et al*., 2020). An elevated WBC count even within the normal range, is associated with both macro- and microvascular complications in type 2 diabetes (Pan *et al*., 2017). Higher WBC counts may be associated with the development of retinopathy, albuminuria, and peripheral arterial disease (Yan *et al*., 2019).

Mean differences of WBC parameters between study groups according to age groups

The mean differences of WBC parameters between study groups including (diabetic nephropathy and diabetic without nephropathy and control) according to age groups were shown in **Table 3**. The mean differences of monocyte was significant decrease (P 0.011) in diabetic without nephropathy at age group ≥ 60 years, while the mean differences of WBC was no significant differences between study groups in all age groups (p 0.439, p 0.506, p 0.41). Also, no significant differences between neutrophil% in all age groups (p 0.368, p 0.58, p 0.717), eosinophil% in all age groups (p 0.438, p 0.346, p 0.868), basophil% in all age groups (p 0.754, p 0.165, p 0.269), lymphocyte% in all age groups (p 0.682, p 0.312, p 0.653) and monocyte% in age groups of DN (p 0.671), and control group (p 0.257). However, the mean differences of neutrophil% was significant increase and lymphocyte % were significant decrease between study groups in all age groups (P 0.023, P <0.001, P <0.001) and (P 0.003, P <0.001, P<0.001) respectively in DN group compared to DM and control groups. In addition, the mean differences of eosinophil% was significant decrease in DN group in age groups between 46-60 years (P 0.001), and the mean differences of basophil% was significant increase in DN and DM groups 30-45 years (P<0.001) and age groups 46-60 years (P 0.005) compared to control group. There were no significant differences between WBC count in all age groups (p 0.497, p 0.085, p 0.087),

Table 3. Mean differences of WBC parameters between study groups according to age groups

Study variables	Study group	Age groups						P-value
		N	30-45 years	N	46-60 years	N	≥ 60 years	
WBC count10e3/uL	DN(N=50)	5	7.81 ± 1.89	17	9.14 ± 4.59	28	10.07 ± 3.65	0.439
	DM (N=25)	4	6.83 ± 1.37	13	6.58 ± 1.63	8	7.69 ± 2.88	0.506
	Control group	8	6.22 ± 2.78	13	7.43 ± 1.32	4	6.87 ± 1.87	0.41
P-value	Total	17	0.497	43	0.085	40	0.087	
Neutrophil %	DN(N=50)	5	73.52 ± 11.45	17	78.04± 16.72	28	72.18 ± 11.24	0.368
	DM (N=25)	4	56.65 ± 5.90	13	56.22 ± 7.25	8	53.41 ± 5.08	0.58
	Control group	8	60.66 ± 7.81	13	63.83 ± 9.96	4	61.55 ± 6.69	0.717
P-value	Total	17	**0.023***	43	**<0.001***	40	**<0.001***	
Eosinophil %	DN(N=50)	5	2.66 ± 1.88	17	1.62 ± 2.40	28	2.46 ± 2.26	0.438
	DM (N=25)	4	3.52 ± 2.07	13	3.61 ± 1.75	8	2.43 ± 1.80	0.346
	Control group	8	4.50 ± 1.78	13	4.48 ± 1.80	4	4.00 ± 0.61	0.868
P-value	Total	7	0.255	43	**0.001***	40	0.388	
Basophil %	DN(N=50)	5	0.64 ± 0.23	17	0.55 ± 0.46	28	0.64 ± 0.41	0.754
	DM (N=25)	4	1.22 ± 0.26	13	0.86 ± 0.42	8	0.80 ± 0.28	0.165
	Control group	8	0.42 ± 0.13	13	0.33 ± 0.10	4	0.37 ± 0.09	0.269
P-value	Total	17	**<0.001***	43	**0.005***	40	0.191	
Lymphocyte %	DN(N=50)	5	16.58 ± 9.76	17	14.46 ± 12.25	28	17.30 ± 9.46	0.682
	DM (N=25)	4	35.60 ± 1.66	13	31.18 ± 5.35	8	31.87 ± 5.17	0.312
	Control group	8	32.91 ± 7.60	13	31.17 ± 10.87	4	27.20 ± 11.34	0.653
P-value	Total	17	**0.003***	43	**<0.001***	40	**0.001***	
Monocyte %	DN(N=50)	5	7.75 ± 2.79	17	6.31 ± 3.54	28	6.89 ± 3.21	0.671
	DM (N=25)	4	6.34 ± 2.36	13	7.46 ± 1.41	8	5.13 ± 1.33	**0.011***
	Control group	8	6.51 ± 1.02	13	5.76 ± 0.94	4	5.75 ± 1.34	0.257
P-value	Total	17	0.487	43	0.202	40	0.276	

monocyte% in all age groups (p 0.487, p 0.202, p 0.276), eosinophil% in age group 30-45 years (0.255) and ≥ 60 years (p 0.388) and basophil% in age group ≥ 60 years (p 0.191). The results were agreement with results of Mosenzon *et al*., (2019) who found that there were significant decrease in diabetic patients at age ≥ 60 years. Leukocytes can be activated by glycation end products, oxidative stress, angiotensin II resulting from hyperglycemia, and can produce factors like tumor necrosis factor-α and interleukin β1 that involve chronic diabetes complication pathogenesis (Jha *et al*., 2018). The leukocyte count test can be added to the diabetes control protocol as an early predictor beside that of a routine physical examination. Leukocyte count can reflect the inflammatory situation of the whole system. This study was performed to discover whether leukocyte count is a suitable indicator for development of any type 2 diabetes complications (Abdel-Moneim *et al*., 2019). A study of de Marañón *et al*., (2020) found there was a relationship between age and diabetes duration to leukocyte count – it can be said that patients with higher leukocyte counts were older and had had diabetes for longer. But some other studies showed different results and demonstrated that age and diabetes duration did not have any significant difference in patients with high or low leukocyte counts (Miller *et al*., 2020; Zhu *et al*., 2020). Chronic inflammation induces development and progression of type 2 diabetes, implying that immunologic and inflammatory mechanisms can play a role in procession of the disease (Xia *et al*., 2018). Another theory is that a low insulin level in the blood stimulates neutrophil production in bone marrow (Miller *et al*., 2016). However, some receptors were found in diabetic patients' immune systems that induce inflammation in blood vessels. Chronic inflammation responses in addition to other risk factors can help to progress diabetes complications by inducing massive endothelium injury and an increase in some mediators and oxidative stress (Karan *et al*., 2020).

Mean differences of WBC parameters between study groups according to gender

The mean differences of WBC parameters between study groups including (diabetic nephropathy and diabetic without nephropathy and control) according to gender groups were shown in **Table 4**, the results showed that, there were significant increase of WBC count in female (P 0.001) of DN group compared to female of DM and control groups. In addition, there was significant increase of basophil % in DN and DM groups in both gender (male and female) (P 0.006, P 0.001) compared to control group, while the percentages of neutrophil% and lymphocyte% were significant increase and decrease respectively in DN group in both gender (male and female) (P<0.001, P 0.001), (P<0.001, P<0.001) compared to DM and control group. When compared between male and female, no significant difference of WBC count between study groups according to gender (p 0.227. p 0.609, p 0.241), eosinophil% (p 0.409, p 0.528, p 0.712), basophil% (p 0.934, p 0.593, p 0.326) and monocyte% (p 0.991, p 0.924, p 0.15). While, there were significant decrease (P 0.046) in neutrophil% and significant increase (P 0.015) in lymphocyte% of females compared to males in DN group. These results were agreement with results of Xiong *et al*., (2020) who found that there were significant increase of WBC count in female suffering from diabetic nephropathy, while a study of Kulathunga *et al*., (2020) found that the high percentage of Diabetic nephropathy in males could be related to male habits like alcohol and smoking.

Table 4. Mean differences of WBC parameters between study groups according to gender

Study variables	Study group	Gender				P-value
		N	Male	N	Female	
WBC count 10e3/uL	DN (N=50)	26	8.88 ± 3.99	24	10.22 ± 3.71	0.227
	DM (N=25)	13	7.19 ± 2.16	12	6.75 ± 2.01	0.609
	Control group	14	7.37 ± 2.13	11	6.42 ± 1.66	0.241
P-value	Total	53	0.20	47	**0.001***	
Neutrophil %	DN (N=50)	26	77.92 ± 13.10	24	70.39 ± 12.82	**0.046***
	DM (N=25)	13	55.03 ± 7.50	12	55.78 ± 5.08	0.771
	Control group	14	62.74 ± 7.93	11	62.09 ± 9.93	0.857
P-value	Total	53	**<0.001***	47	**0.001***	
Eosinophil %	DN (N=50)	26	2.45 ± 2.81	24	1.92 ± 1.49	0.409
	DM (N=25)	13	3.45 ± 1.90	12	2.97 ± 1.77	0.528
	Control group	14	4.52 ± 1.86	11	4.27 ± 1.32	0.712
P-value	Total	53	**0.04***	47	**0.001***	
Basophil %	DN (N=50)	26	0.61 ± 0.40	24	0.62 ± 0.42	0.934
	DM (N=25)	13	0.86 ± 0.41	12	0.94 ± 0.35	0.593
	Control group	14	0.39 ± 0.11	11	0.34 ± 0.12	0.326
P-value	Total	53	**0.006***	47	**0.001***	
Lymphocyte %	DN (N=50)	26	12.88 ± 9.07	24	19.92 ± 10.65	**0.015***
	DM (N=25)	13	31.98 ± 5.20	12	32.25 ± 4.99	0.898
	Control group	14	29.09 ± 8.79	11	33.64 ± 10.78	0.257
P-value	Total	53	**<0.001***	47	**<0.001***	
Monocyte %	DN (N=50)	26	6.78 ± 3.59	24	6.79 ± 2.93	0.991
	DM (N=25)	13	6.57 ± 1.44	12	6.50 ± 2.24	0.924
	Control group	14	6.27 ± 1.10	11	5.65 ± 0.91	0.15
P-value	Total	53	0.849	47	0.444	

Table 5. Mean differences of DNA methylation concentrations in study groups according to duration of disease

Study variables	Study groups	Duration of disease						P-value
		N	1-5 years	N	6-10 years	N	≥ 11 years	
DNA methylation (ng/ml)	DN (N=50)	11	4.69 ± 1.30	12	3.85 ± 1.24	27	4.00 ± 1.03	0.174
	DM (N=25)	8	3.88 ± 1.64	11	2.76 ± 0.77	6	3.12 ± 0.56	0.111
P-value	Total	9	0.251	23	0.02	33	0.158	

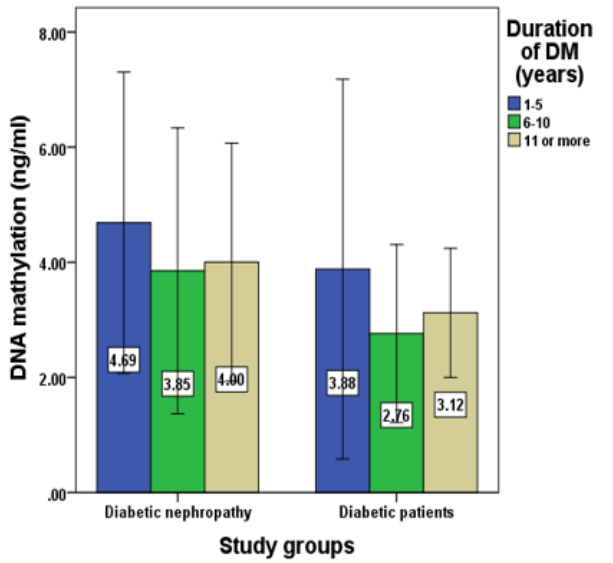

Fig. 1. The mean differences of DNA methylation according to duration of disease

Mean differences of DNA methylation concentrations in study groups according to duration of disease

In **Table 5**, there was no significant between DNA methylation (p 0.174, p 0.111) in diabetic patients according to duration of disease, Even though the importance of epigenetics was first recognized in light of its role in tissue development, an increasing amount of evidence has shown that it also plays an important role in the development and progression of many common diseases (Jin & Liu 2018), DNA methylation, in some common diseases, many new risk factors have been identified through the population-based epigenetic epidemiologic studies on the role of epigenetics in common diseases, this relatively new field still faces many unique challenges (Ma et al., 2020).

Mean differences of DNA methylation concentrations in study groups according to age

The mean differences of DNA methylation concentrations in study groups according to age were studied, it was significant increase in all age groups (P 0.005, P<0.001, P 0.026) compared to control group. There were no significant difference between DNA methylation concentration in study groups including (diabetic with nephropathy, diabetic without nephropathy and control group) according to age (p 0.323, p 0.5, p 0.736), as shown in **Table 6**. Aging is a process of becoming old with many physical and psychological consequences. Even though it is a natural process, it can be biologically classified as a disease (Cohen *et al*., 2020), many years ago, age-related DNA methylation changes were observed in diabetic nephropathy patients. Epigenetic mechanisms may play an important role in the aetiology of type 2 diabetes (Naidoo *et al*., 2018). Epigenome-wide association studies (EWASs) identified several DNA methylation markers associated with type 2 diabetes (Liu *et al*., 2019). Currently, a growing number of studies have shown that dynamic

Table 6. Mean differences of DNA methylation concentrations in study groups according to age

Study variables	Study group	Age group						P-value
		N	30-45 years	N	46-60 years	N	≥ 60 years	
DNA methylation (ng/ml)	DN (N=50)	5	3.79 ± 0.53	17	4.45 ± 1.23	28	3.96 ± 1.18	0.323
	DM (N=25)	4	2.62 ± 1.25	13	3.21 ± 1.10	8	3.48 ± 1.26	0.5
	Control group	8	2.20 ± 0.37	13	2.03 ± 0.71	4	2.24 ± 0.39	0.736
P-value	Total	17	**0.005***	43	**<0.001***	40	**0.026***	

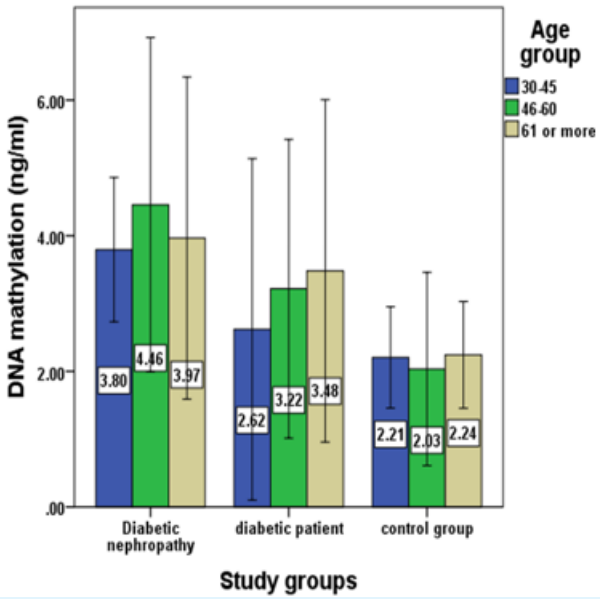

Fig. 2. The mean differences of DNA methylation according to age

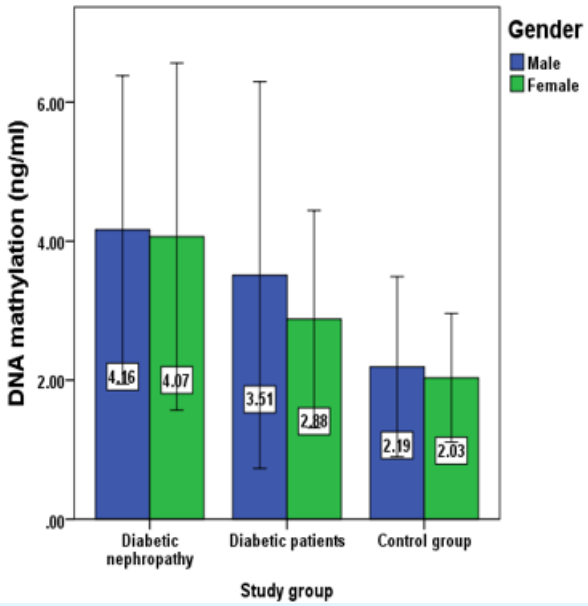

Fig. 3. The mean differences of DNA methylation according to gender

Table 7. Mean differences of DNA methylation concentrations in study groups according to gender

Study variables	Study group	Gender				P-value
		N	Male	N	Female	
DNA methylation (ng/ml)	DN (N=50)	26	4.16 ± 1.10	24	4.06 ± 1.24	0.771
	DM (N=25)	13	3.51 ± 1.39	12	2.87 ± 0.78	0.179
	Control group	14	2.19 ± 0.64	11	2.03 ± 0.46	0.5
P-value	Total	53	**<0.001***	47	**<0.001***	

DNA methylation throughout human lifetime exhibits strong correlation with age and age-related outcomes. Indeed, many researchers have built age prediction models with high accuracy based on age-dependent methylation changes in certain CpG loci (Xiao *et al*., 2019).

Mean differences of DNA methylation concentrations in study groups according to gender

The mean differences of DNA methylation according to gender were shown in **Table 7**, the results showed that, there was significant difference between means of DNA methylation in this study for study groups with gender (male and female). DNA methylation (P<0.001, P<0.001). Furthermore, no significant difference between males and females in all study groups (diabetic with nephropathy, diabetic without nephropathy and control group) including DNA methylation concentration (p 0.771, p 0.179, p 0.5). These results were agreement with results of Massart *et al*., (2019) who found that there was significant difference between means of DNA methylation in this study for study groups with gender. A study of Al-Rubeaan *et al*., (2017) found that DNA methylation levels were significantly higher in patients with diabetic nephropathy compared with those without nephropathy. No significance differences were observed in DNA methylation levels between men and women in three groups according to (Nowacka-Woszuk *et al*., 2019).

The correlation between DNA methylation and all study variables

The correlation between DNA methylation and study variables were shown in **Table 8**, it was significant found positive correlation between DNA methylation and basophil% (r=0.367, p=0.009) in patients with diabetic nephropathy. The results were agreement with results obtained by Santos *et al*., (2020) who found that was significant found positive correlation between DNA methylation and leukocyte specially basophil. Leukocyte differential counts and flow cytometry measurements (the gold standard for identifying subsets of cells within heterogeneous mononuclear cell samples) are often not

Table 8. The correlation between DNA methylation and study variables

Study variables	DNA methylation (ng/ml)					
	Diabetic nephropathy (N=50)		Diabetic without nephropathy (N=25)		Healthy control (N=25)	
	r	P-value	r	P-value	r	P-value
WBC count (10e3/uL)	-0.007	0.963	0.267	0.197	-0.107	0.609
Neutrophil (%)	0.035	0.81	-0.047	0.822	-0.107	0.609
Eosinophil (%)	-0.001	0.996	0.016	0.941	-0.022	0.917
Basophil (%)	0.367	**0.009***	0.029	0.892	0.195	0.351
Monocyte (%)	0.121	0.402	-0.160	0.446	0.269	0.194
Lymphocyte	-0.048	0.739	-0.121	0.564	0.015	0.945

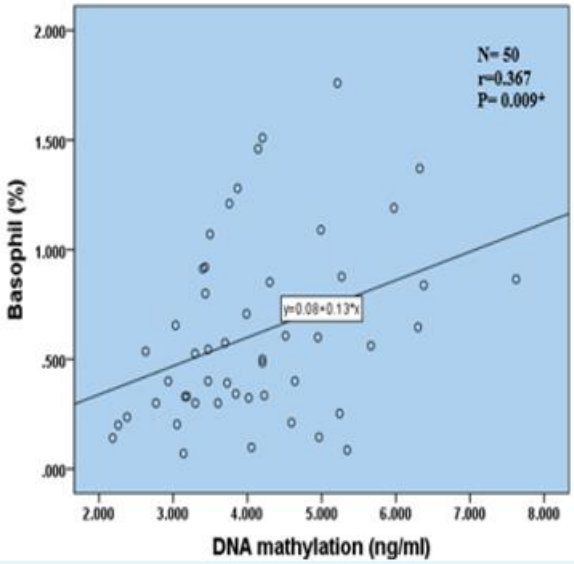

Fig. 4. Positive correlation between DNA methylation and basophil% (r=0.367,p=0.009)

possible because they require fresh samples with intact cells, or are too costly (Tay *et al*., 2018). Thus, as epigenome-wide DNA methylation can be measured using archival peripheral blood with relatively straightforward protocols and commercially available array technology or bisulfite sequencing, the capacity to accurately predict cell-type proportions using L-DMRs has important implications for any study of health, disease or pharmacologic intervention where measurement of leukocyte proportions is of interest (Walker *et al*., 2018).

CONCLUSION

There was no significant between DNA methylation (p 0.174, p 0.111) in diabetic patients according to duration of disease. The mean differences of DNA methylation concentrations in study groups according to age were studied, it was significant increase in all age groups (P 0.005, P<0.001, P 0.026) compared to control group. There were no significant difference between DNA methylation concentration in study groups including (diabetic with nephropathy, diabetic without nephropathy and control group) according to age (p 0.323, p 0.5, p 0.736), there was significant difference between means of DNA methylation in this study for study groups with gender (male and female). DNA methylation (P<0.001, P<0.001). Furthermore, no significant difference between males and females in all study groups (diabetic with nephropathy, diabetic without nephropathy and control group) including DNA methylation concentration (p 0.771, p 0.179, p 0.5). The correlation between DNA methylation and study variables were significant found positive correlation between DNA methylation and basophil% (r=0.367, p=0.009) in patients with diabetic nephropathy.

REFERENCES

Abdel-Moneim A, Abdel-Reheim E S, Semmler M, & Addaleel W (2019) The impact of glycemic status and metformin administration on red blood cell indices and oxidative stress in type 2 diabetic patients. The Malaysian Journal of Medical Sciences: MJMS, 26(4), 47.

Abdelsalam M, Wahab A M, El Sayed Zaki M, & Motawea M (2020) MicroRNA-451 as an Early Predictor of Chronic Kidney Disease in Diabetic Nephropathy. International Journal of Nephrology, 2020.

Al-Rubeaan K, Siddiqui K, Al-Ghonaim M A, Youssef A M, Al-Sharqawi A H & AlNaqeb D (2017) Assessment of the diagnostic value of different biomarkers in relation to various stages of diabetic nephropathy in type 2 diabetic patients. Scientific Reports, 7(1), 1-9.

Castilho R M, Squarize C H, & Almeida L O (2017) Epigenetic modifications and head and neck cancer: implications for tumor progression and resistance to therapy. International journal of molecular sciences, 18(7), 1506.

Cohen A A, Kennedy B K, Anglas U, Bronikowski A M, Deelen, J, Dufour, F & Friguet B (2020) Lack of consensus on an aging biology paradigm? A global survey reveals an agreement to disagree, and the need for an interdisciplinary framework. Mechanisms of ageing and development, 191, 111316.

de Marañón A M, Iannantuoni F, Abad-Jiménez Z, Canet F, Díaz-Pozo P, López-Domènech S & Víctor V M (2020) Association between proinflammatory markers, leukocyte–endothelium interactions, and carotid intima–media thickness in type 2 diabetes: Role of glycemic control. Journal of Clinical Medicine, 9(8), 2522.

Delaneau O, Zazhytska M, Borel C, Giannuzzi G, Rey G, Howald C & Ambrosini, G. (2019) Chromatin three-dimensional interactions mediate genetic effects on gene expression. Science, 364(6439).

Dhanavelu P, Senthilnathan R & Aravind R V (2019) Diabetes mellitus and extraction. Drug Invention Today, 11(9).

Fani F, Regolisti G, Delsante M, Cantaluppi V, Castellano G, Gesualdo L & Fiaccadori E (2018) Recent advances in the pathogenetic mechanisms of sepsis-associated acute kidney injury. Journal of nephrology, 31(3), 351-359.

Feng Q, Liu D, Lu Y, & Liu Z (2020) The Interplay of Renin-Angiotensin System and Toll-Like Receptor 4 in the Inflammation of Diabetic Nephropathy. Journal of Immunology Research, 2020.

Hesaka A, Sakai S, Hamase K, Ikeda T, Matsui R, Mita M & Kimura T (2019) D-Serine reflects kidney function and diseases. Scientific reports, 9(1), 1-8.

Jha J C, Ho F, Dan C, & Jandeleit-Dahm K (2018) A causal link between oxidative stress and inflammation in cardiovascular and renal complications of diabetes. Clinical Science, 132(16), 1811-1836.

Jin Z & Liu Y (2018) DNA methylation in human diseases. Genes & diseases, 5(1), 1-8.

Kalyani R R, Corriere M & Ferrucci L (2014) Age-related and disease-related muscle loss: the effect of diabetes, obesity, and other diseases. The lancet Diabetes & endocrinology, 2(10), 819-829.

Karan A, Bhakkiyalakshmi E, Jayasuriya R, Sarada D V L & Ramkumar K M (2020) The pivotal role of nuclear factor erythroid 2-related factor 2 in diabetes-induced endothelial dysfunction. Pharmacological Research, 153, 104601.

Khandare S A, Chittawar S, Nahar N, Dubey T N & Qureshi Z (2017) Study of neutrophil-lymphocyte ratio as novel marker for diabetic nephropathy in type 2 diabetes. Indian journal of endocrinology and metabolism, 21(3), 387.

Kulathunga M R D L, Wijayawardena M A A, Naidu R. Wimalawansa S J & Wijeratne A W (2020) Association between body mass index and estimated glomerular filtration rate in patients with chronic kidney disease of unknown aetiology in Sri Lanka. Environmental Geochemistry and Health, 1-9.

Kumar V, Kumar N & Prasad J (2020) Evaluation of changes in levels of glycated hemoglobin, total protein and albumin in patients diagnosed with type 2 diabetes mellitus. International Journal of Medical and Biomedical Studies, 4(3).

Liu D, Zhao L, Wang Z, Zhou X, Fan X, Li Y & Li Y (2019) EWASdb: epigenome-wide association study database. Nucleic acids research, 47(D1), D989-D993.

Ma J, Rebholz C M, Braun K V, Reynolds L M, Aslibekyan S, Xia R. & Joehanes, R (2020) Whole Blood DNA Methylation Signatures of Diet Are Associated with Cardiovascular Disease Risk Factors and All-cause Mortality. Circulation: Genomic and Precision Medicine.

Massart R., Nemoda Z, Suderman M J, Sutti S, Ruggiero A M, Dettmer A M & Szyf M (2016) Early life adversity alters normal sex-dependent developmental dynamics of DNA methylation. Development and psychopathology, 28(4 Pt 2), 1259.

Miller P H, Knapp D J, Beer P A, MacAldaz M, Rabu G, Cheung A. M & Eaves C J (2016) Early production of human neutrophils and platelets posttransplant is severely compromised by growth factor exposure. Experimental hematology, 44(7), 635-640.

Miller R G, Yu L, Becker D J, Orchard T J, & Costacou T (2020) Older age of childhood type 1 diabetes onset is associated with islet autoantibody positivity> 30 years later: the Pittsburgh Epidemiology of Diabetes Complications Study. Diabetic Medicine.

Mosenzon O, Wiviott S D, Cahn A, Rozenberg A, Yanuv I, Goodrich E L, & Bhatt D L (2019) Effects of dapagliflozin on development and progression of kidney disease in patients with type 2 diabetes: an analysis from the DECLARE–TIMI 58 randomised trial. The lancet Diabetes & endocrinology, 7(8), 606-617.

Naidoo V, Naidoo M, & Ghai M. (2018) Cell-and tissue-specific epigenetic changes associated with chronic inflammation in insulin resistance and type 2 diabetes mellitus. Scandinavian journal of immunology, 88(6), e12723.

Nowacka-Woszuk J, Grzemski A, Sliwinska M & Chmurzynska A (2019) Hepatic DNA methylation and expression profiles under prenatal restricted diet in three generations of female rat fetuses. PloS one, 14(4), e0215471.

Osman F (2020). DNA methylation mark in mitochondrial DNA.

Pan S, Liu Z W, Shi S, Ma X, Song W Q, Guan G C & Tang Z G (2017) Hamilton rating scale for depression-24 (HAM-D24) as a novel predictor for diabetic microvascular complications in type 2 diabetes mellitus patients. Psychiatry research, 258, 177-183.

Paydar S, Nezhad G S M, Karami M Y, Abdolrahimzadeh H, Samadi M, Makarem A and Noorafshan A (2019). Stereological comparison of imbibed fibrinogen gauze versus simple gauze in external packing of grade IV liver injury in rats. Bulletin of Emergency & Trauma, 7(1), 41.

Rea M, Eckstein M, Eleazer R, Smith C & Fondufe-Mittendorf Y N (2017) Genome-wide DNA methylation reprogramming in response to inorganic arsenic links inhibition of CTCF binding, DNMT expression and cellular transformation. Scientific reports, 7(1), 1-14.

Rubattu S, Forte M & Raffa, S (2019) Circulating leukocytes and oxidative stress in cardiovascular diseases: a state of the art. Oxidative Medicine and Cellular Longevity, 2019.

Santos J L Krause B J, Cataldo L R, Vega J, Salas-Pérez F, Mennickent P & Bravo C (2020) PPARGC1A Gene Promoter Methylation as a Biomarker of Insulin Secretion and Sensitivity in Response to Glucose Challenges. Nutrients, 12(9), 2790.

Tay H M, Yeap W H, Dalan R, Wong S C & Hou H W (2018) Multiplexed Label-Free Fractionation of Peripheral Blood Mononuclear Cells for Identification of Monocyte–Platelet Aggregates. Analytical chemistry, 90(24), 14535-14542.

Tazhbenova S T, Millere I, Yermukhanova L S, Sultanova G, Turebaev M, Sultanova B P (2019). Effectiveness of diabetes mellitus management program at primary health care level. Electronic Journal of General Medicine, 16(6), em172.

Tiffon C (2018) The impact of nutrition and environmental epigenetics on human health and disease. International journal of molecular sciences, 19(11), 3425.

Verdaguer Autonell J, Egia Mendikute L, Arpa Puigdemont B, Rosell Mases E, Corral Pujol M, Carrascal Sánchez J and Vives-Pi M (2019) B-lymphocyte phenotype determines T-lymphocyte subset differentiation in autoimmune diabetes. Frontiers in immunology, 10, 1732.

Walker R M, MacGillivray L, McCafferty S, Wrobel N, Murphy L, Kerr S M & Evans K L (2019) Assessment of dried blood spots for DNA methylation profiling. Wellcome Open Research, 4.

Wu H, Yang S, Huang Z, He J & Wang X (2018) Type 2 diabetes mellitus prediction model based on data mining. Informatics in Medicine Unlocked, 10, 100-107.

Xia C, Braunstein Z, Toomey A C, Zhong J & Rao X (2018) S100 proteins as an important regulator of macrophage inflammation. Frontiers in immunology, 8, 1908.

Xiao F H, Wang H T, & Kong Q P (2019) Dynamic DNA methylation during aging: a “prophet” of age-related outcomes. Frontiers in genetics, 10, 107.

Xiong C, Li L, Bo W, Chen H, XiaoWei L, Hongbao L, & Peng (2020) Evaluation of the efficacy and safety of TWHF in diabetic nephropathy patients with overt proteinuria and normal eGFR. Journal of the Formosan Medical Association, 119(3), 685-692.

Yan P, Zhang Z, Miao Y, Xu, Y, Zhu J, & Wan Q (2019) Physiological serum total bilirubin concentrations were inversely associated with diabetic peripheral neuropathy in Chinese patients with type 2 diabetes: a cross-sectional study. Diabetology & Metabolic Syndrome, 11(1), 100.

Zang L, Hao H, Liu, J, Li Y, Han W, & Mu Y (2017) Mesenchymal stem cell therapy in type 2 diabetes mellitus. Diabetology & Metabolic Syndrome, 9(1), 36.

Zeni L, Norden A G, Cancarini G, & Unwin R J (2017) A more tubulocentric view of diabetic kidney disease. Journal of nephrology 30(6) 701-717.

Zhu L, She Z G, Cheng X, Qin J J, Zhang X J, Cai J & Li H (2020) Association of blood glucose control and outcomes in patients with COVID-19 and pre-existing type 2 diabetes. Cell metabolism.