Sys Rev Pharm 2021;12(1):598-605
A multifaceted review journal in the field of pharmacy

Association between Glycated Hemoglobin A1c Levels with Genomic Instability in Type 2 Diabetes Mellitus Patients

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ABSTRACT

The incidence of type 2 diabetes mellitus globally increased. Genomic damage induced by oxidative stress play important role in the pathogenesis of diabetic complications. The objectives of this research are to determine whether increased blood glucose level in diabetes patients has been related with elevated levels of oxidation and DNA damage. **Case** –control study of (90) (Patients previously diagnosed with type 2 diabetes and 30 controls (non-diabetic apparently healthy volunteers) all participants were tested for plasma glycosylated hemoglobin (HbA1C), the DNA oxidation and genomic instability was performed by quantified of the level of 8-OHdG in urine samples and micronuclei and binucleated cells in exfoliated buccal cells respectively. The 8-OHdG contents in urine samples of diabetic patients were substantially high. However, there were non-significant differences within groups (P>0.05) The differences between8-OHdG contents in patients versus the control group were statistically significant (P<0.01) frequency of binucletied cells positive liner correlation with frequency of micronuclei strong positive correlation between frequency of micronuclei and HbA1C value. Our study points that micronuclei assay is a very valuable and noninvasive method predict genomic instability and its consequences mostly cancer in diabetes patients. Hence, clinical applications of MN assay may potentially improve the quality of administration of patients with diabetes and its complications.

Keywords: Diabetes, Micronuclei, (HbA1C), DNA oxidation, 8-hydroxy-2′-deoxyguanosine

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INTRODUCTION

Diabetes has become a commune and critical global health problem with predicated the global prevalence to rise from 8.3% in 2013 to 10.1% in 2035 (1). Diabetes is a chronic disease result from either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. Insulin is a hormone that controls blood sugar. Hyperglycaemia, or raised blood sugar, is a common effect of uncontrolled diabetes and over time cause serious damage to many of the body's systems, especially the nerves and blood vessels. (2)

In February 2020, the IDF reported that approximately 1,505,000 adult living with diabetes and the prevalence of diabetes in Iraq increased (7.6%) of adult Prevalence of diabetes in adults (3) Type 2 diabetes mellitus (T2DM), the most common subtype of diabetes, includes a complex heterogeneous group of metabolic diseases described by high levels of blood glucose (hyperglycaemia) and imperfect insulin action and/or insulin secretion (4).

DM2 patients recommended to controlling blood sugar levels through a healthy diet, exercise and medication to avoid the risk of long-term diabetes complications However, many diabetic patients with hyperglycemia (2).

The elevate in blood glucose levels even below the diagnostic threshold for diabetes result in increases formation of free radicals and lead to diabetes associated Complications (2,4)

overproduction of ROS and limited work of antioxidant defense system are important driving factors of oxidative stress. oxidative stress occurs when the generation of free radicals exceeds the system's ability to eliminate and neutralize them. (5,6)

Free radicals attack important macromolecules in the cells (DNA, proteins, carbohydrates, and lipids) (7) result in DNA mutations, protein modifications and lipid peroxidation Oxidative damages of nucleic acids are the most dangerous modifications observed among biomolecules. They can be grouped into oxidation of bases and/or sugar fragments, single/double strand breaks (SSB and DSB, respectively), basic/apurinic/apirimidinic (AP) sites, cross-linking, translocations and / or deletions of chromosome. The accumulation of such damages can result to alteration of genetic information, and subsequently to mutagenesis and programmed cell death (8,9). due to the fact that cancer initiation is promoted by genomic instability All previous mention alterations may be linked with risk for developing cancer in diabetes mellitus patients. (10,11,12) although some Epidemiological studies identify a positive correlation between diabetes mellitus and increased cancer risk, but the data is still insufficient regarding mechanism underlying this association. The objectives of this research are to determine whether increased blood glucose level in diabetes patients has been related with elevated levels of oxidation and DNA damage

Genomic damage was determined using the micronucleus test in exfoilted buccal mucosa and oxidative stress by measuring 8-hydroxy-2′-deoxyguanosine (8-OHdG).

MATERIALS AND METHODS

The samples collection and practical work of the present study extended through the period from December 2019 to April 2020 A total of (90) (Patients previously diagnosed with type 2 diabetes (39 male and 51 female) were enrolled from a local diabetes clinic (Baghdad). The control group included 30 non-diabetic apparently healthy volunteers (13 male, 17 females. All samples were obtained after learned consent of the participants earlier to their inclusion in the study and after the agreement of the Ethical Committee in College of Science /Department of Biology. Diabetes-specific things included number of years since diagnosis, And type of treatment (use of oral medications or insulin) moreover Age, marital status, smoking habit, alcohol consumption, diagnostic X rays, chemical exposure during occupation, family history of

cancer, medical and residential history. were collected by questionnaire.

BMI was calculated as weight (W) in kilograms divided by the square of height (H) in meters (13) with following formula (kg/m2) and

Collection Samples:

Four to five milliliters of venous blood were withdrawn from each participant (patients and control) via venipuncture under sterile conditions, Glycated hemoglobin were measure by use spineract kit (Spin). Urine samples were collected from each participant (patients and controls) using disposable sterile plastic tubes the samples centrifuged at 1000 rpm for 10 m. Supernatant taken and stored in sterile coded tubes at -20 until analysis. The 8-OHdG concentrations in urine were analyzed by use of 8-OHdG (8- Hydroxydeoxyguanosine) ELISA Kit (Catalogue: MBS764814; mybiosource.com). Exfoliated cells of the human buccal mucosa for the Buccal Micronucleus Cytome (BMCyt) assay were collected from all participant (patients and control) according to According to criteria described by Tolbert et al. (14), Sarto et al. (15) and Thomas et al. (16) Exfoliated buccal cells were screened under a total magnification of x1000 using a light microscope (Kruss, Germin).

Statistical analyses. Statistical analyses were done with SPSS (Version 20). The one-way analysis of variance (ANOVA) test was used for to compare the means of various groups with each other. Pearson's correlation was used for correlation analyses. Significance was accepted at $p<0.05$.

RESULTS AND DISCUSSION

To the best of our knowledge, the present study is the first study exploring association between genomic instability by micronuclei assay in diabetic patients in our country.

Table (1) below illustrates the some of the main characteristics of the study participants. According to HbA1c-levels the patients divided into three groups A (5-7%), B (7.1-9%) and C (≤9%).

There were no significant differences within all groups (A, B and C) regard age the mean age of patients was (46.41 ± 13.88,51.03±12.69 and 47.83±12.78) for all groups (A, B, and C) respectively. While they're highly significant difference between control and patients respect to age as shown in table (1). All diabetic patients were well matched for age the range of age of the sample was (18-79 years). According to previous reports middle-aged and older adults are still at the highest risk for developing type 2 diabetes. However, between 2011 -2012, about 23 % of new diabetes diagnoses in children were type 2 diabetes other study report that 5,300 subjects from ages 10 to 19 were diagnosed with type 2 diabetes. According to earlier reports, individuals under the age of 20 who had type 2 diabetes had an increasing number by up to 49 % by 2050 as a result of the spread of fast-food and more sedentary lifestyles (17,18). Previous studies report that early onset type 2 diabetes was more aggressive when compare with late-onset disease., Zou *et al*. Suggest that an age at type 2 diabetes onset less than 45 years was linked with a multiplied inherent susceptibility to diabetic retinopathy (19).

Table 1: Characteristics of all study samples stratified by level of HbA1c.

Type of character	Group A N=22 HbA1c% 5-7	Group B N=40 HbA1c% 7.1-9	Group C N=28 HbA1c% <9.1	Control N= 30	P value Between patient groups and controls
Age mean±SD	46.41±13.88	51.03±12.96	47.83±12.78	32.93±7.02	0.02
Gender: male (N.) female (N.)	 10 12	 14 26	 13 15	 13 17	 N.S 0.01
BMI	32.19	31.24	29.85	27.52	0.05
Smoker (N) Nonsmoker (N)	5 17	8 32	6 22	3 27	Ns 0.05
Diabetic duration (years)	7.18	7.25	5.55		
Drugs: Insulin Other Non	 3 16 3	 5 34 1	 6 22 0		 NS 0.001 NS
Occupation: Housewife Student Retired Employees other	 8 3 1 1 11	 24 3 1 1 11	 14 4 3 1 6	 6 12 0 9 3	 0.01 0.01 Ns 0.01 0.01

More females were represented in the current study, the difference within groups regarding the number of females in each group was statistically significant ($P< 0.001$).

The number of females in patient groups is (12, 26, 15) for (A, B and C) respectively. Since the number of the female more than the number of males in patients' groups as well as in control group, when divided the participants according the occupations most the participants list within housewife group there are significant difference within patients' groups based on the occupation as well as there are highly significant differences between the control and patients' groups. The female is easier engaging to participate in this study, hence the number of females in patient groups as well as in the control nearly more than male, another explanation of high percentage of females in the current study may be result from the nature of individuals, admitting to this clinic in that more of them seek medical attention than men under favour of owning more free time since most of them were housewives.

Out of the 90 patients, 19 patients were smoker, while 71 patients were nonsmoker. The difference within groups regarding the number of nonsmokers was statistically significant (P< 0.001) also the Chi-square test show a significant difference between control and patients regarding the number of nonsmoker individuals (P< 0.05). The mean duration of the diabetes for three groups were (7.18; 7.25; 5.55 years) for (A, B and C) groups, respectively, with a range (1–26 years) there is a significant difference within groups regarding duration of diabetes (P<0.05). The majority of patients (16, 34, 22 for groups A, B and C, respectively) were using oral antidiabetic agents for glycemic control, while 2and 1 patients within groups A and B don't use diabetes medications.

The results show that all patient groups have higher BMI, the Chi-square test show no significant difference within groups of patients, according to the BMI, (the mean of BMI of patient groups (A, B and C) were 32.19± 1.68; 31. 24±0. 39; 29. 85±1. 1. 08; respectively. While there was a significant difference (P< 0.05) between control and patient groups based on mean on BMI.

The current result is conceded with previous studies reveled that high BMI associated with hyperglycemia in DM2 patients (20,21,22). A body mass index more than 30 kg/m 2 is reflected to be the central formal criterion for the description of obesity. World Health Organization subdivided obesity into three classes based on the severity of excessive body fat. The BMI range of (25 – 29.9 kg/m ^{2}) is the category of overweight or pre – obesity. In Western countries, 30 – 50% of the population fall into the category of overweight (23). Obesity is the most potent risk factor for type 2 diabetes, according to the prospective Nurses' Health Study, the risk of diabetes in woman with a BMI of (23.0–24.9 kg/m$^{2)}$ was four to five -fold higher compared with woman have normal BMI range. Nearly most of newly diagnosed women with T2DM were obese at the time of diagnosis (24,25). Similar results were reported for males in the Health Professionals' Study (26). Furthermore, changes in body weight also anticipated the risk of diabetes. Recent analysis of the EPIC Potsdam cohort demonstrates that a weight gain of one BMI unit between the age of 25 and 40 years amplified the relative risk of T2DM by 25% also had a higher effect than the identical weight gain between 40 and 55 years of age (27). It is also important to note that the duration of obesity has a strong impact on the risk of developing T2DM (28).

HbA1c proportion providing average blood glucose levels over (8-12 weeks) and consider useful long-term gauge of blood glucose control. In fact, the blood glucose level in diabetic patient's dependent on the severity and duration of disease and successful treatment (2,4). For that reason, the study sample classifies to three groups according to level of HbA1C to examine the significance of hyperclasemia in the rise of genomic damage in diabetic patients with type 2.

- **Genotoxic and cytotoxic biomarkers in diabetes patients' groups and control**

All individuals in the study sample (patients and healthy control) were with a positive expression of the MN as well as BN in exfoliated buccal cells in different numbers.

Micronucleus frequencies in the epithelial cells of the patients were significantly higher than in the control group (P<0.001). The mean value of the MN ± SD of patient groups were (19.05 ± 8.39; 17.98±11.37,27.90±11.78) for groups A, B and C respectively while the MN ± SD of control group was (10.33 ± 4.49). Also, there were a significant difference within groups (A, B and C) regarding the frequencies of MN (P< 0.01) (figure 1). With respect of frequencies of binucleated cells the mean value of BN ± SD for patient groups were (3.14± 0.83; 3.18 ± 1.57; 3.90 ±1.23) for groups A, B and C, respectively, however there were no significant differences within groups, this result is significant at the P = 0.05 level when compare with frequency of BN cells in control group (4.67±4.23) (figure 2).

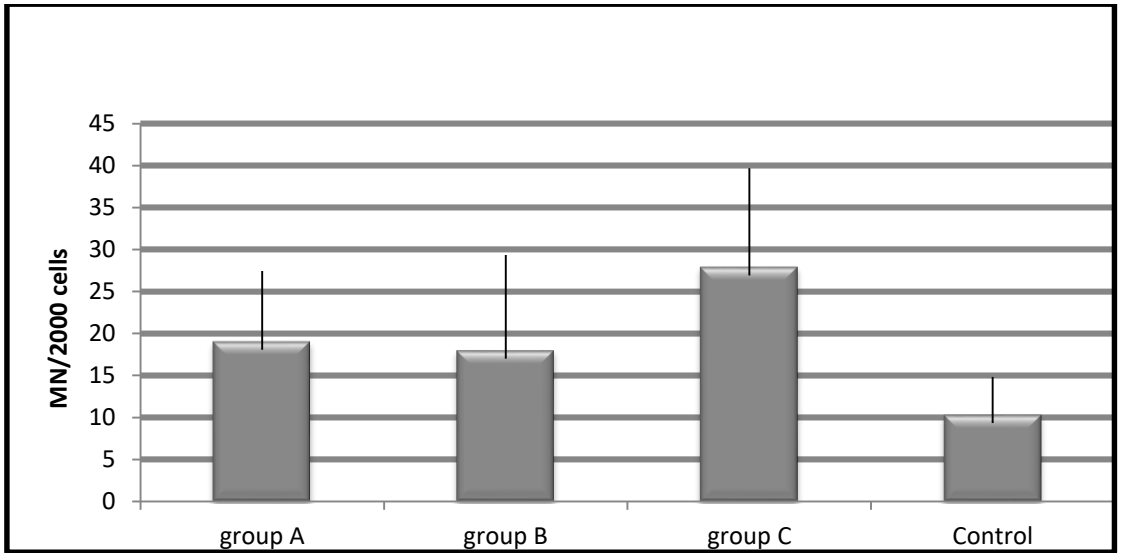

Figure 1: The frequencies of micronuclei in exfoliated buccal cells of all studied subjects. All value is given mean ±S. D per group.

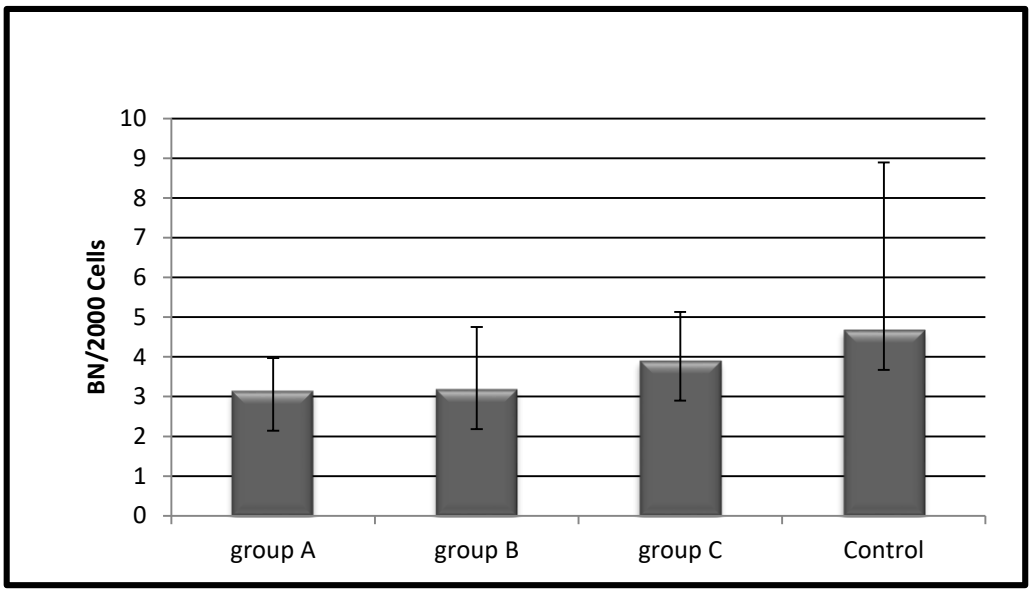

Figure 2: The frequencies of binucleated cells in exfoliated buccal cells of studied subjects. All value is given mean ±S. D per group.

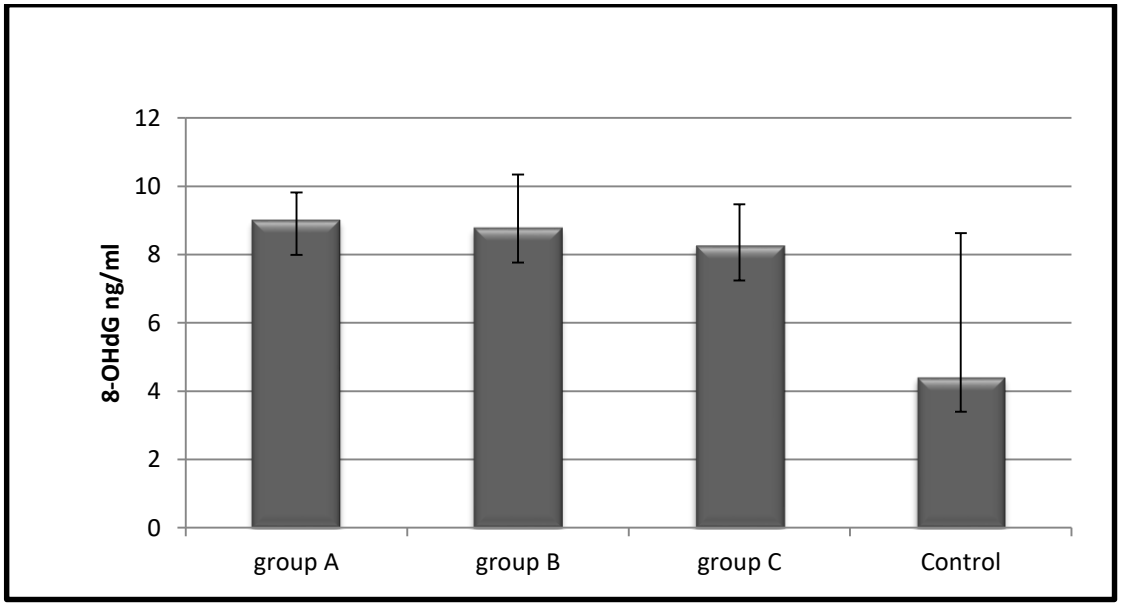

Figure 3: The mean and S.D. of 8-OHdG among all study samples stratified by level of HbA1c.

The 8-OHdG contents in urine samples of diabetic patients were substantially higher. However, there were non-significant differences within groups (P>0.05). The differences between 8-OHdG contents in patients versus the control group were statistically significant (P<0.01) the mean value of the 8-OHdG ± SD of patient groups were (8.99±1.56.58; 8.78± 1.38; 8.24±1.00) for A, B, and C groups respectively (figure 3).

The results of a current study reflect that there is strong correlation between genomic damage and glucose control in DM2 patients. One of the more significant findings to emerge from the present study is that higher frequencies of MN in buccal cells as well as an increased proportion of 8-OHdG of nearly all DM2 patients as matched to controls. Hyperglycaemia in DM2 has been linked with a rise risk of DNA damage result from the reduce of the DNA repair system and the increase of oxidative stress parameters (22,29). The current result is supported by previous studies demonstrate that the urinary 8-OH-dG levels in T2DM patients or model animals were significantly greater than those in a non-diabetic group (30,31).

Previous studies have identified induced oxidative stress in T2DM patients, via decrease antioxidant biomarkers and increase oxidative steers biomarker furthermore, strong linear association where detect between the level of HbA1c and oxidative markers (32,33,34,35,36). When analyses the correlation between studied parameters (age, BMI, HbA1c, 8-OHdG, MN, BN, diabetic duration) in all samples (N=90), positive correlation between BMI and diabetic duration (r=0.211* P=0.046 (Figure 4). Moreover, positive correlation between frequency of MN and level of HbA1c (r= 0.317, P= 0.002) (Figure 5). The positive liner correlation was found between frequency of MN and BN (r= 0.248*, P=0.018) (Figure 6)

Hence, it could conceivably be hypothesized that longer diabetes duration; treatments and severity of

hyperglycaemia, result in elevation of genomic instability which expressed in the current study as increases in concentration of urine (8-OHdG) and score of MN in buccal cells. This finding, more conform when analyze correlation in all diabetic patients (n=90) the frequency of binucletied cells positive strong positive correlation between frequency of MN and HbA1c all these results provide answer for the main research question addressed in this paper (whether or not hyperclasemia rise genomic damage in T2DM patients. This finding supports previous research into this brain area which links genomic instabilities and high level of HbA1c. Previous studies showed that T2DM patients have lower DNA repair capacity compared with healthy controls (37,38). Moreover, another researcher reported that enhanced expression of DNA repair gene in T2DM with higher HbA1c compared to T2DM with lower HbA1c. The researcher suggests that induce expression of DNA repair genes is a compensatory mechanism towards higher genomic damage in their high-HbA1c group (39,40).

Grinder *et al*. (41) reported that the total of MN was significantly amplified in DM2 patients compared to healthy controls. Additionally, it was doubled in DM2 patients with HbA1c more than 7.5% compared to those with lower HbA1c value (less than 7.5%). Singh *et al*. (42) and Müllner *et al*. (43) stated that the levels of buccal MN in diabetic people are approximately two -fold higher than in nondiabetic participants. One possible expected mechanism of formation of binucleated (BN) cells is due to a failure in cytokinesis during cell division (44).

Significant positive correlations between binucleted and MN in all participants confirm that higher cytotoxic and genomic damage in diabetic patients. Although the number of bincleated cells in control group more than patient groups. This finding was unexpected and suggests that control individuals have an efficient genomic repair system that led to suppress division and multiply of cells that contain abnormal chromosomal aberration. Cytokinesis is the last stage of cell division at this stage the two daughter cells completely. Separately, the malfunction of this process has been proposed to promote carcinogenesis by driving to tetrapliody, aneuploid cells can result from unbalanced tetraploid intermediates. Cells have many protective mechanisms that prevent multiplying and survival of cells contain many chromosomal rearrangements for example the AuRORA B-controlled checkpoint that prevents cytokinesis via prevents furrow regression in cells with irregular chromosomal segregation (45,46). Buccal cells have been shown to have imperfect DNA repair capacity result in increased rates of genomic instability. Increased frequency of MN in exfoliated buccal cells of cancer patients may reveal an amplified susceptibility of those to genomic instability. This hypothesis was strongly confirmed through a case-control study nested in a large European cohort, which followed up healthy individuals surveyed with cytogenetic tests for cancer incidence (47). Findings from this survey study indicated that the higher incidence of chromosomal damage detected in subjects who develop cancer later was largely owing to a higher subject's susceptibility rather than to the exposure to carcinogen (48). A meta-analysis review done by Khlifi *et al*. (49) stated the clinical applications of the MN assay in exfoliated buccal mucosa cells in patients with, head-and-neck, oral, breast, bladder, and other cancers.

CONCLUSION

The present study pointed that micronuclei assay is a very valuable and noninvasive method predict genomic instability and its consequences mostly cancer in diabetes patients. Hence, clinical applications of the MN assay may potentially improve the quality of administration of patients with diabetes and its complications.

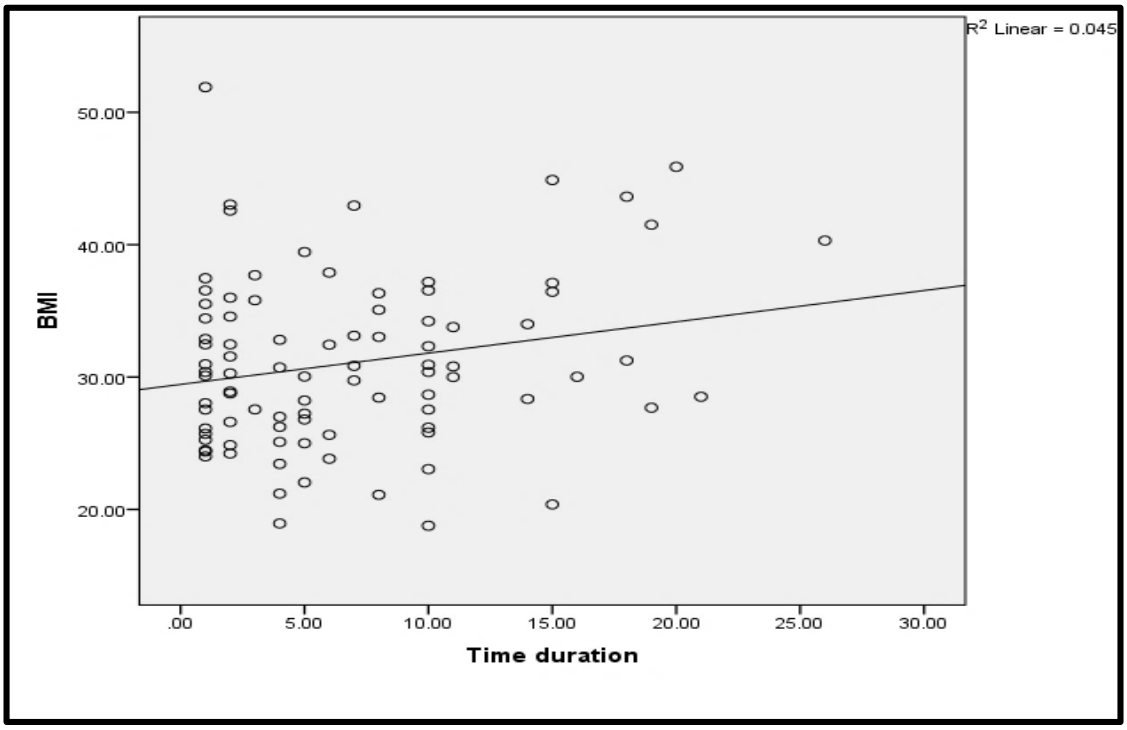

Figure 4: Correlation between BMI and diabetic duration in all diabetes patients (N=90).

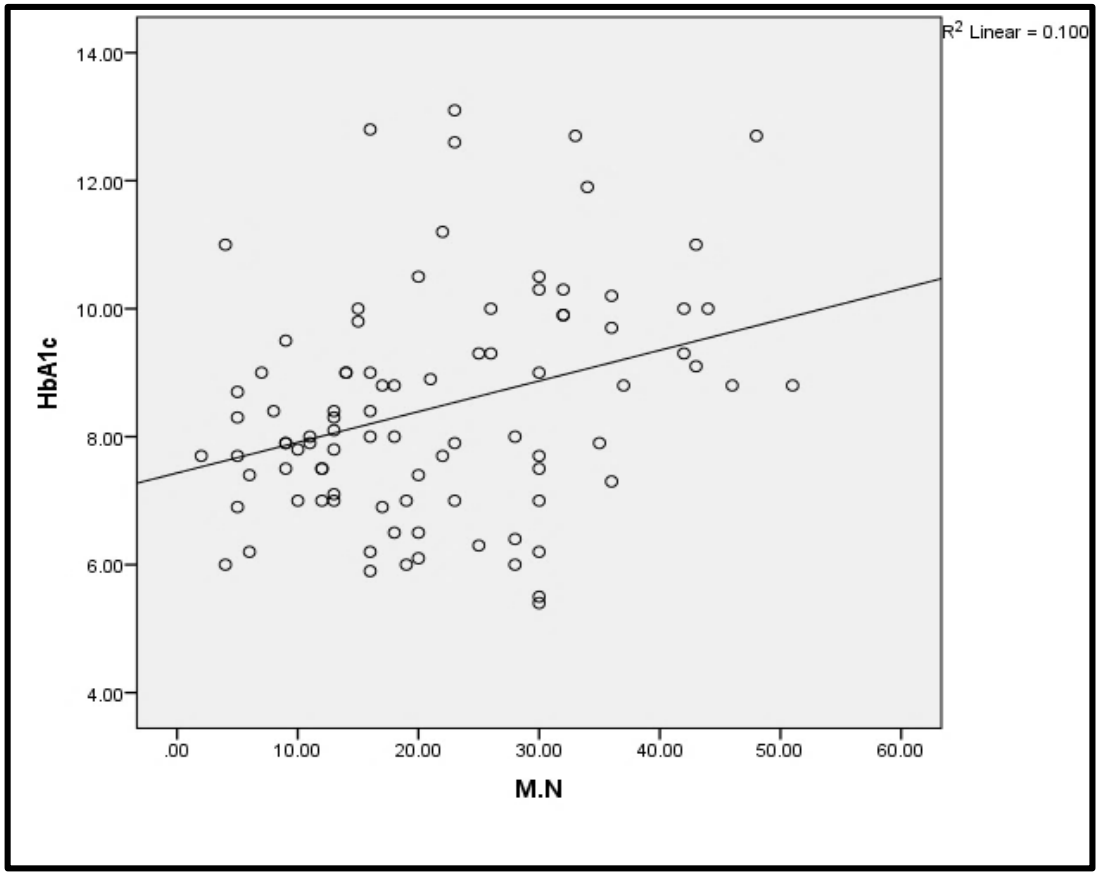

Figure 5: Correlation between MN and HbA1c in all diabetes patients (N=90).

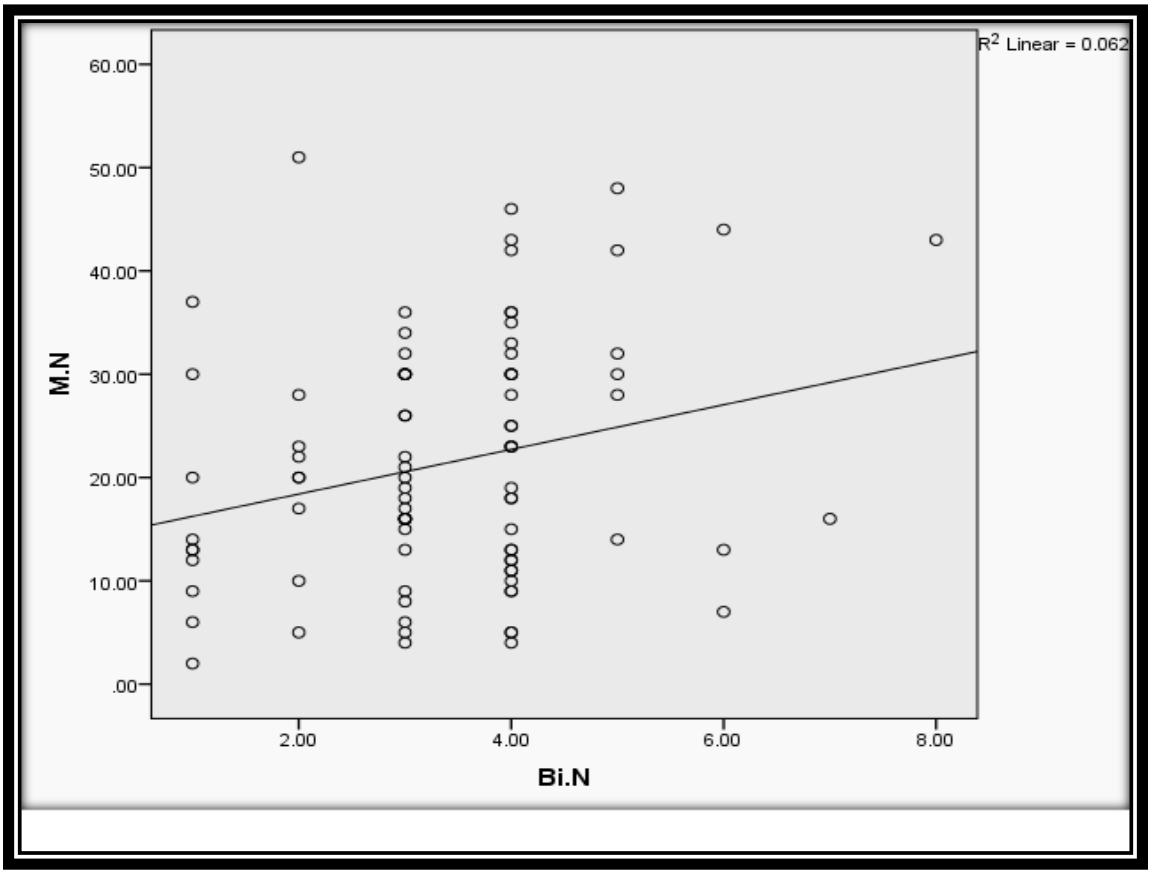

Figure 6: Correlation between BN and MN in all diabetes patients (N=90).

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