Burning Mouth Syndrome and Salivary Brain Derived Neurotrophic Factor Level in Type 2 Diabetes Mellitus Patients on Metformin Monotherapy and on Combination of Metformin and Glibenclamide (A Comparative Study)

Suhair A. Hussain[1] , Enas F. Kadhim[2]

[1]M.Sc.student, [2]Assistant professor, Department of Oral Diagnosis, Collage of Dentistry, University of Baghdad

Abstract

Background: Diabetes mellitus is a chronic, progressive, incompletely understood metabolic condition chiefly characterized by hyperglycemia. Impaired insulin secretion, resistance to tissue actions of insulin, or a combination of both are thought to be the commonest reasons contributing to the pathophysiology of type 2 diabetes mellitus.

Objectives: The objectives of this study were to estimate the salivary Brain Derived Neurotrophic Factor level in type 2 diabetes mellitus patients on treatment of metformin as a monotherapy and those on combination treatment of metformin and glibenclamide. Also to evaluate the burning mouth syndrome in patients with diabetes mellitus under hypoglycemic agents.

Method: In this study 80 male subjects divided into 3 groups: Group 1: 30 subjects as healthy control group, Group 2: 25 subjects with type 2 diabetes under treatment of metformin as a monotherapy 500 mg twice/day and duration 12- 18 months, and Group 3: 25 subjects with type 2 diabetes treated with combination of metformin 500 mg twice/day and glibenclamide 5mg twice/day and duration 12- 18 months. Oral examination was done for each participant and the oral manifestations were recorded. Unstimulated whole saliva samples were collected to measure the brain derived neurotrophic factor level and comparing the results with each group. Body mass index for all participants was recorded. Salivary Brain derived neurotrophic factor concentrations were measured by enzyme linked immunosorbent assay ELIZA.

Results: The results showed that the burning mouth syndrome was seen in both patients groups. Statistical analysis showed a significant increase in burning mouth syndrome among the study groups. Regarding salivary brain derived neurotrophic factor, results revealed that there was a significant higher in combination group as compared to control and metformin monotherapy group.

Conclusion: Both monotherapy and combination therapy was affect salivary level of brain derived neurotrophic factor. Burning mouth syndrome was seen secondarily to diabetes and poor glycemic control and seen in both patients groups.

Keywords: *Burning mouth syndrome, metformin, glibenclamide, salivary brain derived neurotrophic factor, Diabetes Mellitus.*

Corresponding author:
Suhair Ali Hussain,
B.D.S., H.D.D. in Oral Diagnosis, Babylon Health Department, Ministry of Health, Hillah- Babil/Iraq,
E-mail: dr.suhairah@gmail.com

Introduction

Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Low levels of insulin to achieve adequate response and/ or insulin resistance of target tissues, mainly skeletal

muscles, adipose tissue, and to a lesser extent, liver, at the level of insulin receptors, signal transduction system, and/or effector enzymes or genes are responsible for these metabolic abnormalities[1]. Type 2 diabetes mellitus (T2DM) is a complex condition and serious health problem worldwide. In net terms, T2DM is a group of metabolic diseases characterized by chronic hyperglycemia followed by the abnormal secretion and actions of insulin. Genetic and environmental factors are thought to be responsible for the development of T2DM[2]. Besides these, it has been noticed that T2DM is associated with inflammation[3]. Brain-derived neurotrophic factor (BDNF) is a neurotrophin (NT) that plays an important role in maturation, synaptic connection, neuronal repair, and plasticity of the central nervous system (CNS); also it has an influence on the pathology and the treatment of neurological diseases[4, 5]. Besides the fundamental impact on the nervous system, several reports documented an association between plasma BDNF and systemic or peripheral inflammatory conditions, such as acute coronary syndrome and T2DM[6, 7]. One medication that has proved effective in treatment of type 2 diabetes and is considered the first choice for oral management, as recommended by the American Diabetes Association, is a drug in the biguanide class, metformin. Most side effects of metformin are mild and can include gastrointestinal distress, soft stools, and diarrhea[8]. It is well documented that these gastrointestinal side effects can lead to malabsorption of vitamin B12 in a dose- and time-dependent manner[9, 10]. Another medication used in the treatment of type 2 diabetes mellitus is sulfonylurea for example glibenclamide. Glibenclamide: Also known as glyburide is a medication used to treat diabetes mellitus type 2. Several soft tissue abnormalities have been reported to be associated with diabetes mellitus in the oral cavity[11]. Burning mouth syndrome (BMS) affects 1.3 million Americans. Burning painful sensation in the mouth is often linked with dysgeusia and xerostomia. Classically, its symptoms improve in the morning, worsen during the day, and diminish at night[12].

Aim and Objectives

The aim of this study was to investigate the oral changes in salivary brain-derived neurotrophic factor (BDNF) in male patients with type 2 diabetes taking anti-diabetic metformin as a monotherapy and comparing results with type 2 diabetic patients taking combination of metformin and glibenclamide. In addition to Clinical evaluation of the effect of the anti-diabetic medication in type 2 diabetes mellitus on burning mouth syndrome.

Materials and Method

This case control study was conducted in the period from february 2019 to May 2019 after approval from Ministry of Health and College of Dentistry University of Baghdad by the scientific committee. The samples collection was done in the Endocrine center/ Merjan Teaching Hospital in Al- Hillah city/ Iraq during the period from December 2018 to the end of March 2019. Laboratory work was done by Al- Nasih clinical laboratory/ Baghdad- Iraq. After explaining the aims and the objectives of the study, written informed consent was obtained from all participants. Unstimulated saliva collected by draining method. The brain derived neurotrophic factor was estimated in saliva samples by using Human Brain derived neurotrophic factor (BDNF) ELISA kit.

Exclusion criteria: Excluded from this study female patient, Patient with systemic disease other than type 2 diabetes mellitus, and Patients take any medications other than metformin or the combination of metformin and glibenclamide.

Inclusion criteria: 80 male subjects Age >\= 40 years old included in this sudy devided in to three groups: 30 control healthy male without any systemic disease, 25 male patients with type 2 diabetes mellitus under metformin monotherapy 500 mg twice/day for at least 6 months ago, and 25 male patients under the combination of metformin 500 mg twice/day and glibenclamide 5 mg twice/day for at least 6 months ago.

Statistical analysis: Data were written in computerized database using Microsoft Excel (2016). Statistical analysis was performed with SPSS (Statistical Package for Social Sciences; Version 24) and Sigma Stat 4.0 software. Descriptive statistical analysis including: mean, standard deviation and standard error was extracted for each group, Analysis of Variance One Way-ANOVA on rank was done to identify differences among the study groups, and Post- hoc tests Bonferroni (multiple comparisons).

Results Regarding patients on metformin monotherapy treatment, the burning mouth syndrome seen in 6 (24 %) while in combination group seen in 5 (20%). Statistical analysis using ANOVA test showed that there is highly significant difference among study groups in relation to BMS, table (1).

Table (1): The Mean standard deviation of the burning mouth syndrome

Groups	**N**	**Mean BMS**	**±SD**	**Percentage %**
Control	30	0.00	0.000	0
met	25	0.24	0.436	24%
met +g	25	0.20	0.408	20%
P value	0.01 HS			

Post hoc revealed that there was increase in BMS in those patient on metformin treatment compared to control group with significant difference (p=0.029) and there is non- significant differences between patients on metformin monotherapy treatment and those on combination of metformin and glibenclamide treatment (p=1.000), table (2).

Table (2): Post hoc test of BMS among study groups

Group	**Group**	**Std. Error**	**P value**	**95% Confidence Interval**	
				Lower Bound	**Upper Bound**
control	**met**	.090	**.029 S**	-.46	-.02
	met +g	.090	.089	-.42	.02
met	**control**	.090	**.029 S**	.02	.46
	met +g	.094	1.000	-.19	.27
met +g	**control**	.090	.089	-.02	.42
	met	.094	1.000	-.27	.19

Also there is an increase in the mean and the standard deviation of BMI in both metformin and combination groups as compared to control group; Statistical analysis using ANOVA test showed there is significant difference of BMI among the study groups, table (3).

Table (3): Mean standard deviation and standard error of BMI for the study groups.

	N	**Mean**	**Std. Deviation**	**Std. Error**
Control	30	23.143	1.0575	.1931
met	25	23.632	1.4056	.2811
met +g	25	24.460	1.3354	.2671
Total	80	23.708	1.3618	.1522
P value			**0.001 HS**	

Then by using post hoc Bonferroni test of BMI showed there is non-significant difference in those patient on metformin treatment compared to control group and those on combination of metformin and glibenclamide treatment show highly significant difference compared to control group in relation to BMI (p= 0.470, p=0.001) And there is non-significant difference between patients on metformin monotherapy treatment and those on combination of metformin and glibenclamide treatment in relation to BMI (p=0.069); table (4).

Table (4): post hoc test Bonferroni of BMI among study group

Group	**Group**	**Std. Error**	**P value**	**95% Confidence Interval**	
				Lower Bound	**Upper Bound**
control	met	.3418	.470 NS	-1.325	.348
	met +g	.3418	.001 S	-2.153	-.480
met	control	.3418	.470 NS	-.348	1.325
	met +g	.3570	.069 NS	-1.702	.046
met +g	control	.3418	.001 S	.480	2.153
	met	.3570	.069 NS	-.046	1.702

Results showed that there was increase in the mean and SD of BDNF in the two patients groups as compared to control group with overall mean= 0.576 SD ± 1.344. The mean of BDNF in control group 0.144 SD ± 0.069, in metformin group mean = 0.282 SD ± 0.125 and in combination group mean= 1.388 SD ± 2.217. ANOVA test showed that there was significant difference among the study groups in relation to salivary BDNF (p= 0.001), table (5).

Table (5): Mean of salivary BDNF among study groups

	N	**Mean ng/ml**	**Std. Deviation**	**Std. Error**
control	30	0.14460	.069345	.012661
met	25	0.28264	.125050	.025010
met +g	25	1.38864	2.217081	.443416
Total	80	0.57650	1.344138	.150279
P value	**0.001 HS**			

Post hoc test showed there is non-significant difference in those patients on metformin treatment compared to control group in relation to BDNF (p= 1.000) while there is significant salivary BDNF difference in combination group compared to control group (p= 0.001) and there is significant difference in combination group compared to metformin group (p= 0.007); table (6). Table (6): Post hoc Bonferroni test of salivary BDNF among the study group

Group	group	Std. Error	P value	95% Confidence Interval	
				Lower Bound	Upper Bound
control	met	.335922	1.000 NS	-.96018	.68410
	met +g	.335922	.001 HS	-2.06618	-.42190
met	control	.335922	1.000 NS	-.68410	.96018
	met +g	.350859	.007 HS	-1.96469	-.24731
met +g	control	.335922	.001 HS	.42190	2.06618
	met	.350859	.007 S	.24731	1.96469

Discussion

Diabetes mellitus is a wide spread complex disease with high morbidity and health care costs. Both diabetes mellitus and drugs used in the treatment of this disease can affect the mouth and salivary glands[13]. The current study is the first study carried regarding salivary BDNF and burning mouth syndrome in type 2 diabetes mellitus under metformin as a monotherapy and combination of metformin and glibenclamide (comparative study). Burning mouth syndrome was seen in 24% of those patients with monotherapy and 20% in combination group and there is significant increase in the BMS in metformin monotherapy group as compared to control group and no significant difference between the monotherapy and the combination group. This study agreed with previous study of [Moore et al., 2007] that showed BMS or related discomforts occurred slightly more frequently than in the control group. Burning mouth syndrome (BMS) has been attributed secondarily to diabetes, poor glycemic control, and diabetic neuropathy. The correlation between diabetes mellitus and BMS is still controversial. It has been suggested that type II diabetes mellitus plays a role in BMS development and a link between the type of insulin used for the diabetes treatment and BMS has also been proposed. In contrast, other studies report a lack of association between these two conditions[14]. A possible explanation for this controversy may be that these diabetic patients were erroneously classified as BMS. In fact, at the time of the above studies, a lack of strict criteria for BMS diagnosis could have affected the selection of the patients. For instance, burning oral complaints in diabetic subjects, who are more prone to oral infections, are probably caused by oral candidiasis. Although a large variety of drugs, medications, and miscellaneous treatments has been proposed in BMS, the treatment management of this syndrome is still not satisfactory, and there is no definitive cure. BMS patients have shown a good response to long-term therapy with systemic regimens of anti-depressants and anxiolytics[15]. In the current study, there was a significant difference among studied groups regarding BMI. A significant increase in the BMI in the combination group as compared to control group was seen and there is non-significant difference between the two patients groups. This study showed that there is significant difference among the studied groups. Also there is significant

difference in combination group compared to metformin group. A significant increase in the mean of salivary BDNF in combination group as compared to metformin group was seen and there is no previous study regarding salivary BDNF in type 2 diabetic patient under therapy. In order to understand the impact of metformin on BDNF levels, [Ma et al. 2015] investigated the effect of metformin on Schwann cells under hypoxia condition and they found that the mRNA levels of BDNF were significantly decreased. However, this detrimental effect of hypoxia on gene expression in Schwann cells was partially reversed by metformin. The mRNA level of BDNF in metformin-treated Schwann cells was higher than those without metformin under hypoxia condition. This beneficial effect of metformin on gene expression under hypoxia condition was significantly inhibited by compound C, which is an inhibitor of AMP-activated protein kinase (AMPK) and an important cellular regulator of lipid and glucose metabolism[16]. Taken all together, these findings suggest that the correlation between BDNF and metformin may be the reason of metformin-induced insulin action by insulin receptor binding, metformin-induced high BDNF levels due to increasing AMPK, and enhanced tyrosine kinase receptor activity which may amplify BDNF signaling. BDNF inhibited during hyperglycemic clamp conditions in humans. This may explain the concomitant finding of low circulating levels of BDNF in individuals with type 2diabetes[17]. Significant increase in the level of BDNF in combination group as compared to metformin group may explained by good glycemic control of combination treatment as compared to metformin mono-therapy treatment[18].

Conclusion

Both monotherapy and combination therapy was affect salivary level of brain derived neurotrophic factor. Burning mouth syndrome was seen secondarily to diabetes and poor glycemic control and seen in both patients groups.

Conflict of Interest: We declare that we have no conflicts of interest.

Human and Animal Rights All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional research committee.

Informed Consent Informed consent was obtained from all individual patients included in this study.

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