Estimation of Salivary Resistin, malondialdehyde and Lipid Profile levels in patients with Diabetes Mellitus

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Abstract
The present study is designed to verify the changes of resistin, malondialdehyde (MDA), glucose and lipid profile in type2 diabetes mellitus.
This study was carried out during the period from November 2013 till January 2014, on 96 subjects: 57 diabetic patients (11 males and 46 females, aged between 33 and 60 years, with a mean age of 40.32) and 39 healthy controls (24 males and 15 females, aged between 40 and 59 years, with a mean age of 47.43), who attended :outpatient, Health care center, and Emergency Department in Tikrit Teaching Hospital.
The results showed a significant increase in the salivary glucose, total cholesterol (TC), triglycerides (TG) resistin, and MDA level in diabetic patients compared to controls, while significant decrease in salivary HDL in diabetic patients compared to controls.

Keywords: Resistin, malondialdehyde, Diabetes Mellitus.

Introduction
Diabetes mellitus(DM) is a chronic metabolic disorder, characterized by Hyperglycaemia and hyperlipidaemia, due to insufficient insulin secretion, action or both. It has become a major cause of mortality and morbidity in the world [1].
Resistin, also known as 'found in inflammatory zone' (FIZZ), is a small inflammatory molecule with hyperglycaemic action . Although some authors indicate that it is secreted by adipocytes [2], it is still controversial since new data suggests that resistin is secreted by macrophages [3]. or other stromal cells present in the adipose tissue [4]. Resistin circulates in the plasma in several multimeric forms, but are those with small weight that seem to have an effect at the cellular level. Resistin reduces glucose uptake in muscles and is repressed by TZDs. This molecule can also modulate the secretion of other molecules, such as neuropeptide (NPY). This was observed when resistin was centrally administered to mice, resulting in increased NPY production in the arcuate nucleus. Resistin effects on glucose production were blocked in mice lacking NPY [5-6].Currently, it is reported that adipokines, such as adiponectin, leptin, resistin, and visfatin, can be detected in saliva of healthy subjects . To the best of our knowledge, no data on saliva resistin levels in T2DM patients are available at present [7].
Hyperglycaemia, generate reactive oxygen species (ROS) and increases the oxidative stress in the body. Oxidative stress plays important role in the development of many secondary complications in diabetes [8].Lipid peroxidation refers to the oxidative degradation of lipids. It is the process whereby free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage. This process proceeds by a free radical chain reaction mechanism resulting in formation of malondialdehyde.
MDA is a reactive aldehyde and is one of the many reactive electrophile species that cause toxic stress in cells and form advanced glycation end products. The production of this aldehyde is used as a biomarker to measure the level of oxidative stress in an organism. MDA is a stable end product of peroxidation of membrane lipids and is widely used as an indicator of increased lipid peroxidation [9].
In the recent years, efforts have been made to replace blood test with other biological material samples that could be collected by non invasive procedure. One of these samples can certainly be saliva. Saliva has many advantages over serum, such as inexpensive and non-invasive collection procedure, including ease of storage and delivery[10].Based on numerous studies, it has been proved that there is a modification of organic and inorganic constituents of saliva in diabetic patients [11-12].

Materials and Methods
Subjects:
This study was carried out at the Tikrit Teaching Hospital from November 2013 to January2014. In present study samples examined in 96 subjects. Group1: 57 diabetic patients (11 males and 46 females, aged between 33 and 60 years) .Group 2: 39 healthy controls persons (24 males and 15 females, aged between 40 and 59 years).
Saliva collection
Unstipulated whole saliva was collected from all patients and subjects in standard measures. Each individual was requested to abstain from eating, drinking, smoking, and brushing his/her teeth for at least 60 min prior to collection. Saliva samples were collected between 9 a.m. and 12 noon. Unstipulated whole saliva was collected using the drooling technique. Each subject rinsed their mouth with water before saliva collection, then the patient was asked to swallow to remove saliva from the mouth. The patient was seated upright, and leaned their head forward over a plastic test tube with a funnel, allowing their saliva to drain into the tube. Whole saliva (5 mL) was obtained from each individual. During saliva collection, the test tube was placed once immediately, we then centrifuged them at 3.000

rpm and the supernatant was removed and stored freeze until analysis[11].

Biochemical Test:

Resistin was determined by used an ELISA technique (RayBio-Human Resistin ELISA Kit Protocol. The intra- and interassay coefficients of variation in this assay kit ranged from 10 to 12%. saliva resistin levels were measured in ng/ml (Kit leaflet).

Malondialdehyde was estimated by the thiobarbituric acid assay method of Beuge and Aust [13]. The results were expressed as µmol MDA formed/l. Cholesterol was measured by by CHOD-POD method [14]., Triglycerides by GPO method [15]., HDL-C by Phosphotungstic acid method [16].

Statistical analysis: statistical comparison was performed by using T- test and X^2 statistics for nonparametric ones. P value of less than 0.05 was considered significant.

Result:

The results of the present study are described in Tables(1).The Glucose TC, and TG levels in saliva were substantially increased in the diabetic group as compared with the control group, while HDL level decreased in the diabetic group as compared with the control group. MDA increased significantly in diabetic patients when compared with control group. In addition, a significant rise of resistin values was observed in diabetic patients when compared with control group.

No significant difference was noticed in the Glucose, MDA, Resistin ,TC, and TG levels in the different weight groups between males and females, except there was significant difference in HDL levels between males and female .

Table(1): Salivary Glucose, MDA, and Resistin levels in diabetic patients compared with study groups

parameters	Male	Female	P Value	Total Patients	Control	P Value
Glucose (mmol/L)	5.8±1.4	6.4±1.9	NS	6.1±1.76	3.72±1.2	p≤ 0.01
MDA (µmol/l)	0.65±0.136	0.75±0.144	NS	0.7±0.14	0.25±0.08	≤0.001
Resistin (ng/ml)	4.18±1.240	3.82±1.3	NS	4±0.45	1.73±0.34	≤0.001
TC (mmol/L)	0.84±0.2	0.82±0.21	NS	0.83±0.23	0.41±0.07	≤0.001
TG (mmol/L)	0.62±0.145	0.58±0.159	NS	0.6±0.15	0.37±0.09	≤0.001
HDL (mmol/L)	0.145±0.05	0.175±0.07	p<0.05	0.16±0.04	0.2±0.05	≤0.001

Discussion

The present study was conducted to evaluate salivary glucose (SG) levels in diabetic group compared to control group. Similar findings have been reported by Forbat *et al* [18]on comparison of blood glucose with parotid saliva in diabetics.

On the contrary Darwazeh *et al* [19], Ben-aryeh *et al* [20], Prabal *et al* [21] and Shehla et al [22] have observed SG concentration to rise with rise in plasma glucose levels. Soares *et al* [23] found no difference in SG values between sexes and no correlation of SG to capillary glycaemia in healthy adults. The healthy control group of the current study was in accordance with this finding. Jurysta *et al* [24] studied the dependency of SG concentrations on glycaemia during oral glucose tolerance test in both diabetic and non-diabetic subjects. Furthermore, Prabal *et al* [21] also found SG levels to decrease in people with long standing diabetes. This latter finding is supportive of present observations. Carda *et al* [26] estimated various salivary biochemical parameters along with SG and correlated it with the morphological changes in parotid gland in type 2 diabetic patients. They observed that longer duration of the disease leads to fatty infiltration and micro angiopathies of salivary glands and Prabal *et al* [21] have attributed decreased SG levels in long standing diabetics to this aspect.

Source of resistin in saliva is not clear to date. Marchetti *et al*. [25]. found that diabetes increased salivary gland basement membrane permeability, allowing serum proteins to saliva by ultrafiltration. However, Carda *et al*. [26]. found that the parotid acinar and interstitial tissue of T2DM patients were rich in lipids, which suggested adipokines in saliva of these people may be secreted by fat cells in the salivary glands. Bostrom *et al*. [27]. found that the levels of resistin were upregulated locally in the salivary glands and corresponded to the intensity of lymphocytic inflammation in patients with Sjogren's syndrome, which suggested resistin ¨ is expressed in the salivary glands of those patients and may be a driving factor of local inflammation. Therefore, the source of saliva resistin in newly diagnostic T2DM is mainly derived from blood resistin by ultrafiltration.

Salivary MDA levels are directly affected by systemic oxidative stress[28], the salivary MDA level was significantly increased in the diabetic group of the present study which reflects a high oxidative stress status among diabetic patients. Salivary estimation of lipid peroxide along with other lipid profiles in diabetes mellitus is therefore considered very useful as it may serve as a useful monitor to judge the lipidemic status of the patients. However, other studies have demonstrated lower concentration of MDA in saliva of diabetic patients than in the control group [29].

Lipids may also be found in whole saliva as a result of gingival crevicular fluid outflow. Lipids may also originate from several membranes such as secretory vesicles, microsomes, lipid rafts and other plasma and intracellular membrane fragments of lysed cells and bacteria, although the lower percentage of

phospholipids indicates that the salivary lipids are not primarily of membrane origin. A large portion of salivary lipids is associated with proteins, especially to high molecular weight glycoproteins (i.e., mucins) and to proline rich proteins (PRPs) [30].

Al Rawi [31-32]. did two different studies and compared plasma and salivary lipid profile in individuals with ischemic heart stroke and the diabetes mellitus and suggested that lipid fractions particularly TGL can be assessed in saliva and may be used alone or in combination with other lipid parameters for monitoring disease activity and severity in such studies.

Conclusion:

There was a significant increase in the levels of Resistin, ,MDA, TC, TG, and glucose in saliva in diabetic patients. Resistin levels in saliva maybe used as a tool to evaluate diabetic patients.

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تقدير مستويات رزيستين ، المالون ثنائي الديهايد ومستوى الدهون اللعابية في المرضى الذين يعانون من داء السكري

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الملخص

تم تصميم هذه الدراسة للتحقق من التغييرات من رزيستين ، المالون ثنائي الديهايد ، الجلوكوز ومستوى الدهون في مرض السكري.

اجريت الدراسة خلال الفترة من نوفمبر 2013 حتى يناير عام 2014 على 96 شخص : 57 منهم مرضى السكري (11 من الذكور و46 من الاناث، تراوحت اعمارهم بين 33و 60 سنة مع متوسط عمر 40,32) و 39 من الاصحاء (24 منهم من الذكور و 15 من الاناث، تروحت اعمارهم بين 40 و59 عاما مع متوسط عمر 47,43) ، الذين راجعوا :االعيادات الخارجية، مركز الرعاية الصحية، وقسم الطوارئ في مستشفى تكريت التعليمية.

اظهرت النتائج زيادة ملحوظة في نسبة جلوكوز اللعاب ، TC, TG رزيستين ومستوى ، المالون ثنائي الديهاي في مرضى السكري مقارنة مع الاشخاص العاديين في حين اظهر انخفاض ملحوظ في HDL اللعاب في مرضى السكري مقارنة مع الاشخاص الطبيعيين.