The use of saliva samples for diagnosis and monitoring of systemic health conditions

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الخلاصة:-

كان الهدف من هذه الدراسة هو تقييم نتائج التحاليل المعتمدة على اللعاب في تشخيص الحالات المرضية ومقارنتها بنتائج التحاليل المعتمدة على الدم .....

أشترك في هذا البحث خمسة و سبعين فرد من الذكور مقسمين الى أربع مجاميع المجموعة الاولى وتشمل خمسة عشر فرد من الاصحاء وتعرف بالمجموعة A والثانية وهي المجموعة B وتشتمل على ثلاثين فرد يعانون من أنسداد رئوي مزمن وقد قسمت هذه الفئة الى مجموعتين فرعييتين الاولى تشمل خمسة عشر مريض لايعانون من فقدان العضلات والمجموعة الثانية وتشمل خمسة عشر مريض يعانون من فقدان للعضلات ، المجموعة الثالثة وهي المجموعة C وتشمل خمسة عشر فرد من مرضى السكر بينما تشمل المجموعة الاخيرة وهي المجموعة D على خمسة عشر مريض بالقلب.....

تم أخذ عينات من اللعاب والدم لكل المشتركين وبعد اجراء التحاليل المختبرية الخاصة بكل مرض وجدت حالة من التقارب في النتائج بين عينيتي الدم واللعاب.

Abstract:-

The aim of the present study was to evaluate the ability of using saliva samples for investigation and monitoring of some of the systemic disease condition.

Seventy- five male were involved in this study, their age range from (43-56)years old, their body mass index (BMI) is less than 27%. They were arranged in four groups, group A are control group consist of fifteen individual who are clinically healthy, group B are patients with chronic obstructive pulmonary disease(COPD) consist of thirty individual , group C are patients with recently diagnose diabetes mellitus (DM) consist of fifteen individual and the last group is group D patients with coronary heart disease (CHD) consist of fifteen individual .

A salivary and serum sample were taken to assess total protein (TP), total cholesterol(TC), triglyceride (TGL) and glucose concentration. Results shows a significantly higher ($P<0.05$) concentration of TC, TGL and glucose in patients (serum and saliva) more than control group (serum and saliva), while TP was significantly higher in control group.

Introduction :-

Saliva is the watery and frothy substance produced in the mouths of humans and most other animals[1]. Human saliva contains informative components that can be used as diagnostic markers for human disease[2]. Saliva is already used to rapidly diagnose HIV(human immune deficiency virus) and soon will be widely available for detecting oral cancer[3].

Research is ongoing in the determination of how the composition of human saliva reflects tissue fluid levels of hormonal, immunological and toxicological molecules as well as specific protein biomarkers for infectious, neoplastic and neurodegenerative diseases [4].

Saliva is increasingly used and well validated in diagnosing, monitoring systemic disease status predicting disease progression. Biomarkers detected in saliva can be valuable in a wide range of clinical pathology, forensic medicine and sport medicine[5]. Salivary assays present a lot of advantages when compared to blood assay: the sampling is very easy to do especially in non medical environment, multiple samples could be collected providing more information than that of single blood sample[6].

Dr.Philip C.Fox, who testified on behalf of the American Association for dental Research and the American Dental Education Association said (Imagine a future in which a saliva sample is used for quick, painless and less expensive diagnostic tests, monitoring for many systemic health condition and exposure to chemical and biological agent) [7].

This study tests the above hypothesis for some of systemic disease it measure the concentration of TP, TC, TGL and glucose in both serum and saliva of the same individual depending on the type of the disease and the first biochemical marker to be affected to determine the difference between control group and patients groups like COPD which is characterized by a limitation of the airflow in the lung which increases overtime and is not totally reversible. Proteins is the most biochemical marker affected by COPD and since muscles is the major protein store in body it will be affected by the metabolic changes caused by this disease lead to muscular wasting[8].

Diabetes mellitus (DM) is the second systemic disease it is characterized by arise in blood glucose concentration(there are two types of DM type I insulin dependent, type II non insulin dependent) so glucose is the first biochemical marker to be affected[9].

Many epidemiological studies indicate that the risk of coronary heart disease (CHD) increases significantly with increase in cholesterol and triglyceride levels[10].

Subjects and Methods :-

The study population was composed of seventy-five male their age range (43-56) their BMI is between(23-27%). They were arranged in four groups :-

Group (A)control:- consist of fifteen individual who are clinically healthy their clinical characteristic matched with patients groups.

Group(B)COPD:- consist of thirty patients(based on recall of physician diagnosis all patients had chronic air way limitation) but they were in clinically stable condition and not suffering from respiratory tract infection or exacerbation of their disease, the patients divided in to two subgroups the first without muscular wasting (+) and the second with muscular wasting (-), the body mass index of this subgroup is less than other groups, each subgroup consist of fifteen patients.

Group(c)DM:- consist of fifteen patients with non insulin dependent diabetes recently diagnose (based on recall of physician diagnosis and fasting blood glucose, the samples (serum and saliva) were collected two times from this group. The first sample was taken in the first week after diagnosis and beginning of treatment and the second sample was taken after two weeks from beginning of treatment.

Group(D)CHD:- consist of fifteen patients with coronary heart disease (angina and myocardial infarction based on physician diagnosis) they were in clinically stable condition.

Participants were asked to refrain from eating, drinking, smoking or oral hygiene procedures prior to sample collection, each participant underwent a physical

examination by a dentist, to ensure that no suspicious mucosal lesion or inflammation was present in the oral cavity, the oral mucosa appeared healthy, without erythema and epithelial desquamations. The whole saliva was collected for five minutes by the subject leaning forward and spitting saliva into test tubes that were kept in crushed ice and immediately after collection the samples were cold centrifuged for five minutes. The supernatant was aspirated and stored at -8ºc until analyzed.

Ten ml of venous blood was drawn with minimal trauma. The serum was separated by centrifugation supernatant were aspirated and stored at -8ºc until analyzed. The serum and saliva total protein was measured by colorimetric biuret method (Spinreact Girona Spain Kit). Total cholesterol concentration and triglyceride in serum and saliva were measured by enzyme colorimetric testing (Biomerieux Kit, France) and glucose concentration was measured by by glucose oxidase method using Randox kit..

All data were analyzed with SPSS program, comparison between groups were made by analysis of variance (ANOVA) test. The significance of difference in mean between each pair of group was performed by Benferonni test. A P value <0.05 was considered statistically significant.

Results:-

The measurement of the mean concentration of total protein in the serum of control group was not significantly differ from (non muscular wasting) COPD patients, while it was significantly higher in control group($P<0.05$) than (muscular wasting) COPD patients as shown in table (1).

The mean concentration of total cholesterol and TGL in the serum of patients with CHD were significantly higher ($P<0.05$) than control group as shown in table (1). The measurement of the mean concentration of serum glucose in patients with DM was significantly higher ($P<0.05$) than control group in the first and second weeks after treatment as shown in table (1).

Table (1) The concentration of T-protein, T-cholesterol, TGL and B-glucose in the serum of the four groups (M±SD)

Serum sample	Total protein g/dl	Total cholesterol mg/dl	TGL mg/dl	Blood glucose mg/dl	P<0.05
Control (A)group	6.6 ± o.2	181.8 ± 5.7	91.9 ± 5.95	91.9 ± 4.4	
COPD (B)group(+)	5.6 ± 0.23				**NS**
(-)	3.92 ± 0.21				**S**
DM (C) group 1st W				165.5 ± 3.2	**S**
2nd W				121.1 ± 5.7	**S**
CHD (D) group		225.8 ± 6.1	167.5 ± 3.4		**S**

TGL : Triglyceride
(+) : without muscular wasting
(-) : with muscular wasting
1st : first week
2nd : second week
(M±SD): mean ±standard deviation

The measurement of the mean concentration of total protein in the saliva of control group was significantly higher (P<0.05) than COPD patients in both subgroups as shown in table (2). The measurement of the mean concentration of total cholesterol and TGL in the saliva of CHD were significantly higher (P<0.05) than control group as shown in table (2). The mean concentration of glucose in saliva of DM group was significantly higher (P<0.05) than control group as shown in table (2).

Table (2) The concentration of T-protein, T-cholesterol, TGL and B-glucose in the saliva of the four groups(M±SD)

Saliva sample	Total protein g/dl	Total cholesterol mg/dl	TGL mg/dl	Blood glucose mg/dl	P<0.05
Control (A)group	1.2 ± 0.15	14.8 ± 2.9	20.6 ± 2.3	21.9 ± 2	
COPD (B)group(+)	0.72 ± 0.03				S
(-)	0.61 ± 0.03				
DM (C) group 1st W				40.3 ± 2.5	S
2nd W				32.4 ± 1.9	
CHD (D) group		30.9 ± 4.3	27.7 ± 4.9		S

TGL : Triglyceride
(+) : without muscular wasting
(-) : with muscular wasting
1st W: first week
2nd W: second week
(M±SD) : mean ± standard deviation

Discussion:-

Diagnosis by using saliva sample is it convenient ??

Can we depends on saliva only to diagnose a systemic disease condition ??

If this is possible can we apply it for all or only specific systemic disorder ??

Many recent research try to answer those question, only a comparative analysis between saliva and blood in the same individual can help to discover the useful of saliva as a diagnostic test [(11)].

Researcher have identified all 1-116 unique proteins found in human saliva, as many as 20% of the proteins found in saliva are also found in blood [(12)].

The researcher hope saliva based tests could be used to diagnose cancer, heart disease, diabetes and a number of other conditions, researcher from five universities (university of Rochester, the Scripps research Institute, the university of southern

California, the university of California San Francisco and the university of California Los- Angeles sought to determine the complete set of proteins secreted by the major salivary glands [13].

Early analysis has already turned up a number of proteins with known roles in Alzheimer's, Huntington's and Parkinson's disease, breast colorectal and pancreatic cancer and diabetes [14].

Most of the proteins were part of signaling pathway, which are key to the body's response to system wide disease they said the work should accelerate the development of new tools for tracking disease throughout the body[15].

In this study the measurement of total protein in the saliva of patient with COPD disease were significantly lower than control healthy group.

The increase in cholesterol and TGL represent a major cause of heart disease and most of the circulatory disorder, some studies found increased risk of ischemic stroke associated with increased total cholesterol levels, while other found no clear association[16]. A study which concentrated on risk group found that all lipid parameters measured in serum and saliva of stroke patients and risk group patients showed significant difference in comparison with healthy control[17].

In this study the measurement of total cholesterol and TGL in the saliva of patients with coronary heart disease were significantly higher than control group.

Many studies confirm the association of increase glucose concentration in saliva and diabetes mellitus disease[18]. In this study the measurement of glucose concentration in the saliva of patients with DM were significantly higher than control group.

Further research are needed in different systemic disease condition in order to decide the usefulness of saliva sample in the diagnosis of the disease.

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