Comparative Study on Saliva Proteins in Patients of Brain Tumors and Healthy Individuals

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Abstract: In the setting of neoplastic conditions, many biochemical substances deviate from their normal values; these may include proteins of body fluid. To verify this claim a prospective study was carried out to investigate the values of plasma proteins in salivary fluids in patients with primary brain tumors. This study had been conducted between September 2008 and July 2009 on 160 individuals. The qualified subjects for the study included, 80 patients (40 from each sex) with primary brain tumor and eighty age and sex normal subjects were used as control for salivary and serum proteins measurements. Concentrations of proteins were found higher in saliva and blood of male and female patients as compared with normal individuals. These results might suggest possible preliminary detection of primary brain tumor by observing the increase of protein concentration in the saliva of the patient which is easily carried out in clinical laboratory.

Key words: Primary brain tumors, saliva proteins, serum proteins, neoplastic disease

INTRODUCTION

Proteins play a central role in cell function and cell structure, in this respect serum contains a mixture of proteins differing in origin and function, the amount of proteins in the vascular compartment depends on the balance between the rate of synthesis and the rate of catabolism or loss. It is a well established and evidence-based fact that plasma proteins levels may suffer changes during a neoplastic disease process (Suzuki, 2006).

Saliva is a glandular secretion, which constantly bathes the teeth and oral mucosa to ensure the stability of the oral cavity environment. The volume of saliva is secreted by the major salivary glands; the rest is secreted by the minor salivary glands, which are scattered in the sub-mucosa in different parts of the oral cavity. Saliva is constituted by secretions of three paired major salivary glands: submandibular (70%), parotid (25%) and sublingual (5%), together with minor salivary glands (Rosen and Bailey, 2001). The daily secretion of saliva ranges normally between 800-1500 mL day⁻¹, about 0.5 mL is secreted each minute all the time except during sleep where it become very little (Guyton and Hall, 2000). Saliva is a readily available specimen, which can be collected by noninvasive procedures, the scientific and biomedical research have provided improved methods to measure and monitor the levels of ions, chemicals and molecules in saliva as well as all body fluids, in this respect the value of

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saliva as an indicator for systemic disease had been explored and found application in clinical
diagnosis (Hofman, 2001; Ginzkey et al., 2009). It is worth to mention that saliva had been
used to monitor levels of endogenous material as antibodies, e.g., IgA in hepatitis A virus
infection, immunoglobulins in lymphomas (Kazmi et al., 2006; Pink et al., 2009), also DNA
and nitrites levels had been studied in saliva of patients with cancer of gastrointestinal tract
(Tenovou, 1986); moreover hormones were also analyzed in saliva (Chang et al., 2005).

Primary Brain Tumors (PBT) originate in the brain and can be benign (slow growing) or
malignant (fast growing), these tumors can vary in malignancy but even so-called benign
tumors are commonly lethal because of their infiltrating properties and their tendency to
undergo malignant transformation over time (Behin et al., 2003; Doolittle, 2004). Brain tumors
are the second leading cause of cancer death in children under 15 years and young adults
up to the age of 34 years. These tumors are also the second fastest growing cause of cancer
death among humans older than 65 years (Xie and Zhu, 2009). Recent reports published by
Jordan’s Ministry of Health indicated that 196 new brain cancer cases were recorded during
2006, of these 138 cases were Jordanians and 58 cases for non Jordanians; during 2007 the
number of new cases of brain tumors increased to 237, of these 142 Jordanians and 95 non
Jordanians (Tarawneh and Nimri, 2006, 2007). Primary brain tumors have devastating
consequences and induced clinical signs and symptoms can result from the mechanical effect
of the tumor and/or chemical stimulation/signaling induced by the tumor. Thus, the aim of
the study is to investigate changes which might occur in the level of salivary proteins in the
setting of PBT.

MATERIALS AND METHODS

 Patients and Normal Individuals

This study had been carried out between September 2008 and July 2009 at both Public
and Private Hospitals in Amman, Jordan. Patients were evaluated by full medical history to
exclude any existing systemic disease that may affect the parameters to be diagnosed,
particularly diabetes, liver disease, renal disease and chronic drug intake, such a patient was
excluded from the study. One hundred sixty individuals qualified for the study, they include:
80 patients (40 for each sex) diagnosed by clinical and histological means as having a primary
brain tumor, their age ranged between 2-75 years; gender: 40 males and 40 females. Control
groups composed of age- and sex- matched normal subjects (40 for each sex).

 Serum and Saliva Collection and Preparation

Five to ten milliliters of venous blood were aspirated through venepuncture following
an overnight (about 10 h) of fasting. Unstimulated whole saliva was collected after the
patient and normal individuals have rinsed their mouths several times with deionized
water, then the accumulated saliva in the floor of the mouth under the tongue
(Nishanian et al., 1998) was drawn by a plastic disposable pipette, collection time was always
between 8.00-9.00 am (before surgery). The collected saliva was cold centrifuged at 2500 rpm
for 10 min at 5°C; this was done within one hour after collection to eliminate debris and
cellular matter. The centrifuged supernatants were divided into 5 equal parts. All sample were
stored frozen at (-20°C) in polyethylene tubes till assayed at the laboratories of Research
Centre at Zarka University College.

 Total Protein Determination

The total protein was measured by using kit which was obtained from TECO Diagnostics
Laboratory (Anaheim, CA 92807, USA). The determination of total protein in serum makes
use of the Biuret color reaction. The present method for quantitative determination of total protein in serum is based on the method proposed by the American Association for Clinical Chemistry (Doumas et al., 1981a, b) and National Committee for Clinical Laboratory Standards (1979). The principle of the method is depended on the enzymatic reaction sequence employed in the assay of total protein.

Statistical Analysis
Data were calculated and interred into a computerized data base structure. Statistical analyses were done by using SPSS (Statistical Package for Social Sciences). The mean and standard deviations (±SD) were calculated. The difference in mean of normally distributed variables between two groups was assessed by independent samples t-test.

RESULTS
In this study patients were subjected to clinical and histological diagnosis, the obtained results confirmed that 80 patients as having a PBT, their age ranged between 2-75 years; gender of patients were 40 males and 40 females. Protein concentrations were measured in each samples of saliva and serum obtained from patients and age-sex matched samples which were obtained from normal subjects as controls.

Protein Concentrations in Saliva of Male Patients
The measurements of saliva proteins (g dL−1) in both male patients and male normal individual showed consistent increase in protein concentrations in saliva of male patients in comparison with normal individuals. The range of protein concentrations in PBT patients were between 0.89 and 2.8 g dL−1, whereas in controls protein concentrations were in the range 0.24 to 0.57 g dL−1 (Fig. 1).

Protein Concentrations in Serum of Male Patients
The obtained results indicated that the values of protein concentrations in serum of male patients and control group (Fig. 2), were higher than the values of protein concentrations in saliva of male patients and control group (Fig. 1). But the results indicated that concentrations of proteins were still higher in patients as compared with control group in both saliva and serum. The range of protein concentrations in PBT were between 7.75 and 11.5 g dL−1, whereas in controls protein concentrations were in the range 5.88 to 7.15 g dL−1.

Fig. 1: Concentrations of saliva proteins in both groups of males, PBT patients and normal individuals
Fig. 2: Serum protein concentrations in both groups of males PBT patients and normal individuals

Fig. 3: Saliva protein concentrations in both groups of females PBT patients and normal individuals

**Protein Concentration in Saliva of Female Patients**

The values of protein concentrations in saliva obtained from female normal individuals and patients were comparable to that obtained in normal male individuals and PBT male patients. The range of protein concentrations in PBT patients were between 0.77 and 2.18 g dL$^{-1}$, whereas in controls were in the range 0.29 to 0.58 g dL$^{-1}$ (Fig. 3).

**Protein Concentration in Female Serum**

The results of the protein concentrations in the serum of female PBT patients and normal subjects indicated almost similar pattern of protein concentrations obtained in male PBT patients and controls. The range of protein concentrations in PBT patients were between 7.5 and 10.5 g dL$^{-1}$, whereas in controls protein concentrations were in the range 4.89 to 6.4 g dL$^{-1}$ (Fig. 4).

**Mean Protein Concentrations in Serum and Saliva**

The study revealed that the mean values of protein concentrations of saliva in the brain tumor group were higher than those in the healthy subjects with statistical significance (p<0.1). The mean values of protein concentrations in saliva of male and female patients were
DISCUSSION

Several types of cancers of the brain and Central Nervous System (CNS) have been reported among Jordanian patients. Brain tumors are the most frequent type of these tumors, there were 96.7 and 93.3% of all brain and nervous system tumors in males and females, respectively. The median age at diagnosis is 35 years. In 2007, there were 142 cases accounting 3.3% of all newly diagnosed malignant cancers among Jordanians. These cancers ranked the 8th in males and females and affected 82 males (4.0%) and 60 females (2.6%) with a male to female ratio of 1.4:1. Cancer incidence in Jordan is increasing at substantial rate, in this respect new recorded cases of brain tumors in 2007 increased by 17.3% as compared with new cases registered during 2006 (Tarawneh and Nimri, 2006, 2007).
Brain tumors are currently classified according to their grade (how quickly they may grow) and the type of cells they are composed of. This system, however, is not always accurate and sometimes two tumors that appear to be identical under the microscope will have very different growth patterns and responses to treatment. On the other hand the molecular classification system is based on molecular genomics and proteomics may be used to better predict a given tumor's behavior and response to therapy. Research in the field of molecular diagnosis is directed toward exploring the possibility of using genomic biomarkers (Hainfellner, 2009) and cytogenetic analysis which included interphase fluorescence in situ hybridization (Korshunov et al., 2008); others explored mass spectrometry and expression microarray profiling to identify candidate protein and mRNA biomarkers of various tumors (Hu et al., 2007). These methods are time consuming and expensive. Thus, it would useful to ascertain simple basic inexpensive test for diagnosis of PBT. In this study it was possible to show that estimation of protein concentrations in saliva can give preliminary indication of PBT. A highly significant increase in total protein levels in serum and saliva of primary brain tumor patients was noticed when compared to that of normal subjects. This increase could be explained on the basis that the whole body of cancer patients is engaged in protein synthesis of various forms like: C-reactive, proteins, tumor markers, enzymes and immunoglobulin and other proteins material. Present results are in agreement with the results of patients with oral cancers (Al-Rawi et al., 2005; Kashmoola et al., 2001), their results had shown that patients with oral squamous cell carcinoma had also markedly increased salivary total protein concentrations; furthermore they indicated that elevation in saliva total protein, may be due to increasing of salivary IgG and IgM. Other study grouped several plasma proteins as Acute Phase Reactant (APR), which significantly rises during inflammation and neoplasm (Schreiber et al., 1982).

It is worth mentioned that in comparison with the reported results in present work, molecular detection of cancer requires more sophisticated proteomic and genomic diagnostic assays as well as trained and skilled personnel, beside specialized equipments. In this respect DNA/RNA analysis is one possible way to develop effective molecular diagnostic assay (Hilton and Melling, 2004), on the other hand proteomics assays are considered promising to elucidate protein changes between healthy and diseased states (Colantonio and Chan, 2005).

CONCLUSION

The measurement of higher protein values in saliva of PBT patients as compared with healthy individuals might be considered a preliminary indication of diagnosis of PBT, which is easily carried out in clinic laboratory.

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REFERENCES


