

## Evaluation of Proteins in Serum and Cerebrospinal Fluid in Primary Brain Tumors

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Proteins play a central role in cell function and cell structure, serum contains a mixture of proteins differing in origin and function, the amount of protein 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 serum (plasma proteins) levels may suffer changes during a neoplastic disease process. A prospective study to explore the serum and cerebrospinal fluid (CSF) proteins levels in the setting of primary brain tumours (PBT) among Iraqi persons suffering the latter condition and to have it compared with hydrocephalic and healthy individuals. This study had been conducted at both University and Neurosurgical Dept. between November 2018 and October 2019. Out of the 107 patients suffering from primary brain tumors with an age range 2-75 years (mean 35, the standard SD  $\pm$  19), 56 were males (52.33%), and 51 were females (47.66%). Although 89% of patients were under the age of 60 years, however, the most affected age group was 31-40 years (17.75%). Fifty age- and sex-matched patients with hydrocephalus (non-neoplastic disease) were used as control group in CSF measurements. Forty age- and sex-matched normal subjects were used as controls (in serum). A highly significant increase in total protein levels in serum of primary brain tumor patients was noticed when compared to that normal subject. Elevation in total protein content in malignant tissue was also observed in comparison to benign tissue. Serum proteins levels changed in PBT.

**Keywords:** Cerebrospinal Fluid (CSF); CSF Proteins; PBT; Plasma Protein(s).

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Proteins play a central role in cell function and cell structure,<sup>1-2</sup> serum contains a mixture of proteins differing in origin and function, the amount of protein in the vascular compartment depends on the balance between the rate of synthesis and the rate of catabolism or loss. Hepatocytes synthesize many plasma proteins<sup>3-4</sup>, those of the complement system are synthesized both in these cell, and by macrophages.

Control of growth and differentiation: controlled sequential expression of genetic

information is essential for the orderly growth and differentiation of cells. In higher organisms, growth and differentiation are controlled by growth factor proteins. Hormones coordinate the activities of different cells in multicellular organisms, many of them, such as insulin<sup>5</sup> and thyroid stimulating hormone<sup>1,4</sup> are proteins.

All proteins normally present in cerebrospinal fluid (CSF) are derived from serum. Only a few proteins, such as beta-trace protein and gamma-trace protein (Cystatin C), are synthesized intrathecally<sup>6</sup>.

Protein entry into the CSF is thought to be mainly dependent on pinocytosis by the capillary endothelial cells of the brain and spinal cord that constitute the blood-brain barrier<sup>7</sup>. The normal protein content of CSF is several fold lower compared to serum and depends upon the relative exclusion of macromolecules by the blood-brain barrier<sup>8</sup>.

The identification of proteins in the CSF that are secreted by the tumor or its environment may reveal cellular mechanisms relevant to cancer biology. Also, it may result in the development of new tumor markers and may ultimately target new therapies. Protein expression profiling has been suitable a valuable tool in obtaining information about the state of protein circuits inside tumor

The spread of cancer into the brain and central nervous system is a serious problem leading to neurological symptoms and rapid mortality. Malignant cells in the brain and CSF derived from primary neural cancers can be able to detect and characterize these cells, this allows us to answer important questions about metastatic through identification and characterization of the cancer cell populations capable of infiltrating the CSF. Cancer may reach the CSF through hematogenous spread, direct extension from the tumor itself, or by migration along perineural or perivascular spaces<sup>10, 11</sup>

Controlled sequential expression of genetic information is essential for the orderly growth and differentiation of cells. In higher organisms, growth and differentiation are controlled by growth factor proteins. Hormones coordinate the activities of different cells in multicellular organisms, many of them, such as insulin<sup>18-19</sup> and thyroid stimulating hormone<sup>1,4</sup> are proteins.

The aim of the study is to investigate plasma proteins in the context of PBT among a group of patients in their sera and CSF and to compare the CSF figures with that of cohort of hydrocephalic patients and sera of another cohort of healthy volunteers.

## MATERIALS AND METHODS

This study had been conducted between November 2018 and October 2019 at both The University and Neurosurgical Dept. 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, otherwise the patient was excluded from the study. Ages and sex-matched normal subjects were used as controls (in serum).

Out of the 107 patients suffering from primary brain tumors with an age range 2-75 years, (mean 35, the standard SD  $\pm$  19), 56 were males and 51 were females. Although 89% of the patients were under the age of 60 years, however, the most affected age group was 31-40 years (Table 1).

Fifty age- and sex-matched patients with hydrocephalus (non-neoplastic disease) were used as control group in CSF measurements.

### Duration of the Disease

The duration of the disease range from  $<1 - >9$  years. The majority of the patients were presented within less than 1-year from the onset of symptoms.

### Histological Findings and Grading

The age and sex distribution in 110 primary brain tumors patients is shown in Table 1. Histological grading of the malignant varieties has shown that the highest percentage was that of grade IV (34%) followed by grade III (27%) giving a total percentage of 61%.

### Chemical and Reagents

All Chemical and standard solutions used in this work, were the highest analytical grade obtained from commercial source, and used without further purification. All volumetric glassware were cleaned in a solution of 5 N HCL for at least 24 hrs, then washed repeatedly in deionized water prior to use.

Total protein kit, and Albumin kit were obtained from Randox Laboratories Ltd. United Kingdom.

### Sample Collection and Preparation

The 107 patient included in this study had fasted for 8 to 12 hrs. before surgery. The operation is usually performed under a general anesthetic and endotracheal intubation.

### Tumour Tissue

At craniotomy operation, while tumour removal is being done, a sufficient amount of the abnormal growth is kept for processing and tissue protein estimation.

**CSF**

A CSF specimen (3 to 4 ml) was collected in a plastic specimen container catheter following opening the dura and arachnoid matters or via a ventricular puncture.

Control group: fifty patients with hydrocephalus were included as a control group; CSF samples were collected via a ventricular catheter that was used in treatment. The CSF specimens were collected in plastic containers, promptly frozen, and stored at (-20 °C) until analysis.

Assays were done within one week to one month of collection at the laboratories of The Neurosurgery Hospital, and The research center.

**Serum**

About 5 ml venous blood was drawn aseptically into sterile test tube with silicon coated, by utilizing disposable needle and plastic syringes. The blood was allowed to clot (10 minutes), centrifuged at 4000 rpm for 15 min. Serum sample were immediately transferred into four tube and frozen at (-20°C) for subsequent analysis, haemolyzed samples were discarded.

One milliliter of venous blood, after clothing, was centrifuged at 600 rpm for 10 min. serum was diluted with 0.3 ml (0.2 M)

sodium potassium phosphate buffer (pH8.4) and centrifuged for 20 min. at 1000 rpm thoroughly to remove protein. The filtrate was kept frozen at (-20°C) until analyzed. (20-100 ml) aliquot of the filtrate was used for HPLC analysis.

**RESULTS**

Data of proteins in Malignant, Benign, CSF and in serum albumin concentration are shown in tables 2, 3, and 4.

**Table 1.** Distribution of PBT patients according to age and sex

| Age (years) | Male        | Female      | Total       |
|-------------|-------------|-------------|-------------|
| 1-10        | 5 (41.66%)  | 7 (58.33%)  | 12 (11.21%) |
| 11-20       | 10 (58.82%) | 7 (41.17%)  | 17 (15.88%) |
| 21-30       | 9 (60%)     | 6 (40%)     | 15 (14.01%) |
| 31-40       | 10 (52.63%) | 9 (47.36%)  | 19 (17.75%) |
| 41-50       | 8 (44.44%)  | 10 (55.55%) | 18 (16.82%) |
| 51-60       | 8 (57.14%)  | 6 (42.85%)  | 14 (13.08%) |
| 61-70       | 4 (44.44%)  | 5 (55.55%)  | 9 (8.41%)   |
| >70         | 2 (66.66%)  | 1 (33.33%)  | 3 (2.80%)   |
| Total       | 56 (52.33%) | 51 (47.66%) | 107 (100%)  |

**Table 2.** Mean protein concentration in malignant and benign Tissue of PBT patients

| Malignant tumour tissue<br>Mean ±SD(g/dl) | Benign tumour tissue<br>Mean ±SD(g/dl) | P value |
|---|--|---------|
| 4.5251±0.9216                             | 1.5472±0.9680                          | <0.01   |

**Table 3.** Mean CSF protein concentration in malignant and benign PBT patients

| CSF of malignant tumours<br>Mean ±SD(mg/dl) | CSF of benign tumours<br>Mean ±SD(mg/dl) | P value |
|---|--|---------|
| 156.7741±46.4748                            | 77.9791±14.0174                          | <0.01   |

**Table 4.** Mean serum albumin concentration in PBT patients and normal subjects

| Patient serum<br>Mean ±SD(g/dl) | Normal serum<br>Mean ±SD(g/dl) | P value |
|---------------------------------|--------------------------------|---------|
| 2.2186±0.533 2                  | 3.908±0.724                    | <0.01   |

**DISCUSSION****Total Serum Protein**

A highly significant increase in total protein levels in serum of primary brain tumor patients was noticed when compared to that normal subject (Table 4). Elevation in total protein

content in malignant tissue was also observed in comparison to benign tissue<sup>12</sup>.

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 immunoglobulins and other proteins material. Our results are in agreement with Fiandra *et al* 1993 and Shrotriya *et al.* 2015<sup>13,14</sup> who found that patients with neoplasm had higher values of total protein ( $P < 0.01$ ).

In addition, patients with oral squamous cell carcinoma had also markedly increased total protein concentrations<sup>15</sup>. While Nagashima and Schreiber (1984) grouped several plasma proteins as acute phase reactant (APR), which significantly rise, rise during inflammation and neoplasms.

On the other hand, it was found that the increase in synthesis of acute phase reactant (APR) is accompanied by a decrease in the synthesis of prealbumin, albumin and transferrin, which are so called negative APR. inflammatory tissue lesions generally induce changes in the concentrations of various serum proteins. The acute phase reactants may be increased, albumin may be decreased and the immunoglobulin production may be enhanced<sup>17, 18, 19</sup>.

The increase in total protein concentrations, in this study, indicated that synthesis of APR proteins had exceeded the synthesis of negative APR, this imbalance leads to a marked increase in total protein levels.

#### **Total Protein in CSF**

In the current study, it was found a significant increase in total protein concentration in CSF of malignant PBT ( $156.77 \pm 46.47$  mg/dl) compared to that of benign nature ( $77.97 \pm 14.01$ ) as shown in table 3, with statistical significance ( $p < 0.01$ ).

The CSF protein concentration depends of the serum protein concentration, permeability of the blood-CSF barrier, and immunoglobulin synthesis<sup>20</sup> Abnormal concentrations of the serum proteins influence the corresponding protein levels in CSF, this is due to increased capillary permeability, with a similar increase in the permeability of the blood- brain barrier, which may be demonstrated by finding relatively high molecular weight proteins not normally present in CSF

The non –specific pattern is associated with a large number of inflammatory conditions, but may sometimes aid diagnosis such a pattern may be due to: cerebral tumors, multiple sclerosis and increased intrathecal immunoglobulin synthesis (acute phase reactants)<sup>21, 22</sup>.

#### **Albumin**

Reduced serum albumin concentration is a common finding in patients with neoplastic diseases. Hypo-albuminemia can appear early in the course of the disease, and indeed it may occur despite normal nutrition and even without effusion, protein loss or evident clinical signs of liver damage.<sup>23, 24</sup> The presence hypoalbuminaemia has been widely confirmed in the malignant lymphomas<sup>25</sup>, prostatic cancer<sup>26</sup>, melanoma<sup>27</sup>, colorectal cancer<sup>28</sup> and leukemia<sup>29</sup>

It is well established that the acute phase reaction, usually involved interleukins, tumor necrosis factor (TNF), and C-reactive proteins causing a reduction in the concentration of albumin is associated with the risk and development of cancer<sup>30</sup>.

However, it has been shown that TNF may increase the permeability of the microvasculature, thus allowing an increased trans-capillary passage of albumin<sup>31</sup> and hence a lowering of the serum albumin concentrations

The present study demonstrated a significant decrease in albumin concentration in serum of patients with primary brain tumors ( $2.21 \pm 0.53$  g/dl) in comparison to normal group ( $3.90 \pm 0.72$  g/dl) with statistical significance ( $p < 0.01$ ) as seen in table (4).

## **CONCLUSIONS**

Both the serum and CSF have shown a significant increase in the total proteins while, on the contrary to that, the albumin value has been less than normal.

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