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Assessment of Alpha-Fetoprotein in Cerebrospinal Fluid, Plasma and Its Correlation to Some Biochemical Parameters in New Born with Congenital Hydrocephalus

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Abstract

Background: Congenital hydrocephalus is a type of hydrocephalus that a child is born with, and this hydrocephalus can develop with age in children and adults as well. Congenital hydrocephalus may occur due to: Complex interactions between genetic factors and environmental factors during fetal development during pregnancy. The signs and symptoms of hydrocephalus vary somewhat with age. In newborns (0 to 2 months) Abnormal head growth, thinning of the scalp and veins in the head becoming more prominent, vomiting, restlessness, eyes fixed downward (sign of sunset) or seizures, or inability to communicate. In children (2 months and over) Abnormal head growth, headache, nausea, vomiting, fever, double vision, insomnia, decline in the ability to speak or walk, communication disturbance, loss of sensory and motor functions, seizures, and vision disturbances may be encountered. Older children may have difficulty waking up or staying awake. Objective: Predicting the presence of alpha-fetoprotein in the cerebrospinal fluid of newborns and considering it as a marker for the detection of hydrocephalus. Method: This study was conducted at neuro surgical department in Ghazi Al-Hariri Hospital in the Medical City of Baghdad and Al-Mansour pediatric hospital in Baghdad, Iraq. from November 2022 to March 2023. It included 80 Iraqi patients with congenital hydrocephalus (40 female samples and the same number of males). Their age range from (0– 120) days. The part that deals with the practical aspect of the study were accomplished at the laboratory of the Ghazi AL-Hariri and Al-Mansour pediatric hospitals .Results: The results showed a highly significant increases ($p<0.001$) in AFP in CSF compared with the AFP in plasma specimen. While a significant decreases ($p<0.001$) are presented in T.protein, Glucose, and WBCs count in CSF in comparison with the plasma levels. Conclusion : The presence of alpha-fetoprotein in the cerebrospinal fluid of congenital hydrocephalus and its correlation with glucose , total protein and WBC

Keywords

Hydrocephalus is a neurological condition due to the aberrant circulation and/or obstruction of cerebrospinal fluid (CSF) flow with consequent enlargement of cerebral ventricular cavities (1). the enlargement of the brain ventricular system due to an excess in the volume of cerebrospinal fluid (CSF)(2). Recent data suggests that CSF plays an essential role in homeostasis and neuronal functions(3). The severity of hydrocephalus is assessed on the clinical symptoms and signs (4). The outcome, in terms of possible neurological deficits, may reflect the brain damage that can be inflicted by hydrocephalus(5). There are many vital molecules inside the body that are affected by brain damage resulting from hydrocephalus, such as some proteins, blood components, lipids and blood sugar levels (6). Biomarkers in the CSF are potentially more useful because they provide an insight into changes in the brain milieu associated with the condition, and consequently more research has been undertaken on the composition of the CSF (7). For example Changes in the protein composition of CSF may be indicative of altered CNS protein expression pattern, providing us with a link to the cause or diagnosis of a condition (8).Also, It was observed through similar studies that there was a change in the level of glucose in the serum of hydrocephalus children (9,10).Some of GLUTs spatially GLUT1 is located both at the luminal and abluminal sides of the brain capillaries, predominantly in intracellular membrane (11).other correlation of proteins and Hydrocephalus was found in Alpha-fetoprotein concentration, when fetal hydrocephalus is detected, elevated SAFP levels indicate that the fetus is at significant risk to have additional malformations and further investigations, including chromosome breakage studies, may be indicated (12).

Subjects and Method

The present study recruited 80 CSF and 80 plasma specimens from hydrocephalus children patients

Exclusion criteria included: Some causes that have symptoms similar to hydrocephalus have been excluded, especially the enlargement of the brain, for example :Some inflammatory diseases of the nervous system. Some storage and metabolism diseases that lead to an increase in the size of the brain itself, brain tumors, cerebral hemorrhage.

Study approval: The study was approved by Research Ethics Committee, Faculty of Medicine, Baghdad University, Iraq. The aim and methods of the study was explained to all participants, and an informed written consent was obtained from all participants.

Results

The results in Table 1 showed a highly significant increases ($p<0.001$) in AFP in CSF compared with the AFP in plasma specimen. While a significant decreases ($p<0.001$) are presented in T.protein, Glucose, and WBCs count in CSF in comparison with the plasma levels.

Table 1 Biochemical parameters in CSF and plasma specimens

Parameter	Plasma	CSF	P-value
AFP ng/ml	14.02±2.63	23.29±8.94	<0.001
T.Protein	60.23±14.20 g/l	82.52±35.58 mg/dl	<0.001
Glucose mg/dl	81.21±16.09	37.17±11.72	<0.001
W.B.C cell/mm3	6244.38±1488.84	61.85±38.27	<0.001

The correlations between CSF parameters and plasma parameters are presented in Table 2

Table 2 Correlations between CSF parameters and age and sex

		Afp	T.protein	Glucose	W.b.c	Ci	Na	K
CSF-AFP	r	0.078	-0.056	0.048	-0.151	0.203	0.134	0.008

	p	0.494	0.623	0.671	0.182	0.072	0.236	0.942
CSF-T.Protein	r	-0.040	-0.118	0.048	0.030	0.261*	0.153	0.035
	p	0.722	0.299	0.674	0.792	0.020	0.175	0.755
CSF-Glucose	r	0.284*	-0.118	0.119	0.254*	-0.081	0.105	-0.090
	p	0.011	0.297	0.294	0.023	0.473	0.356	0.425
CSF-W.B.C	r	-0.156	0.201	-0.165	-0.140	0.181	0.061	-0.164
	p	0.167	0.075	0.145	0.216	0.109	0.593	0.146

The results showed a significant correlation between CSF-glucose level and plasma AFpP (r=0.284, p=0.011) as presented in Figure 1

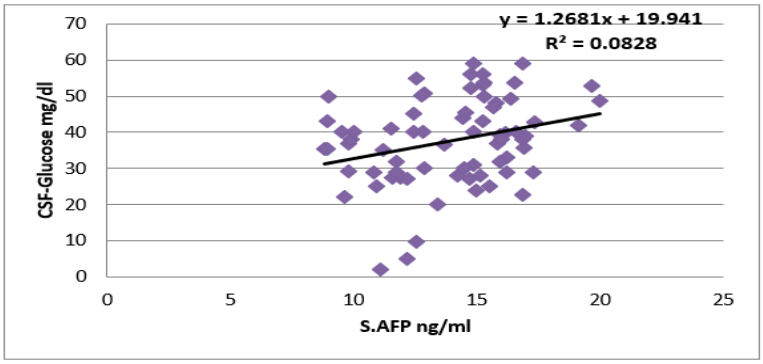

Figure 1. Correlation between CSF-glucose and plasma AFP.

The results also showed a significant correlation between CSF-glucose level and peripheral WBCs count (r=0.254, p=0.023) as presented in Figure 2

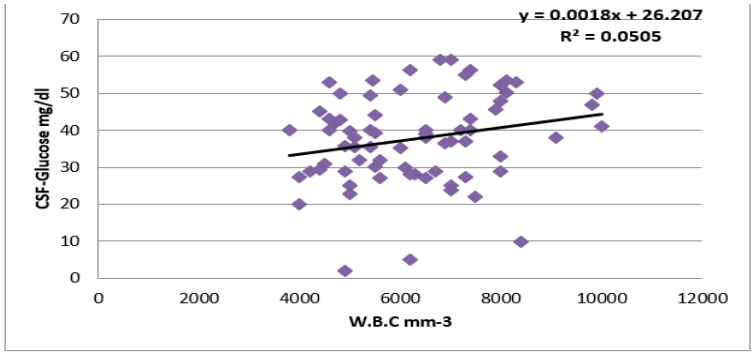

Figure 2 Correlation between CSF-protein and plasma chloride.

The results also showed a significant correlation between CSF-glucose level and peripheral WBCs count (r=0.261, p=0.020) as presented in Figure 3

The correlations among CSF parameters are presented in Table 4

Table 4 Correlations among CSF parameters

		CSF-AFP	CSF-T.Protein	CSF-Glucose	CSF-W.B.C
CSF-AFP	r	1	0.686**	-0.148	0.116
	p		0.000	0.189	0.308
CSF-T.Protein	r	0.686**	1	0.052	-0.048
	p	<0.001		0.646	0.674
CSF-Glucose	r	-0.148	0.052	1	-0.235*
	p	0.189	0.646		0.036
CSF-W.B.C	r	0.116	-0.048	-0.235*	1
	p	0.308	0.674	0.036	

The results showed a strong significant correlation between CSF-T.Protein and CSF-

AFP (r=0.686, p<0.001) as seen in Figure 3

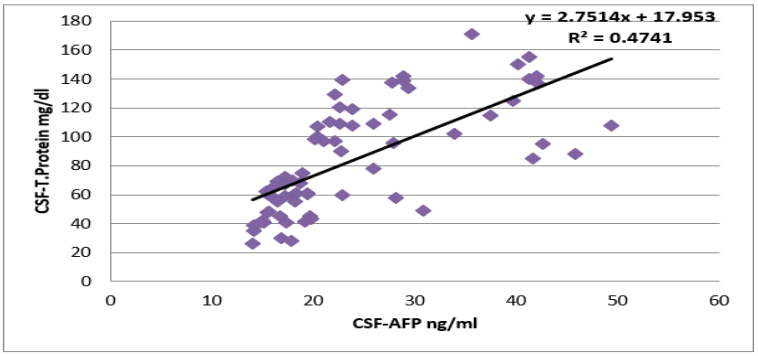

Figure 3 Correlation between CSF-T.Protein and CSF-AFP.

The results showed a negative significant correlation between CSF-glucose and CSF-WBCs count (r=-0.235, p=0.036) as seen in Figure 4

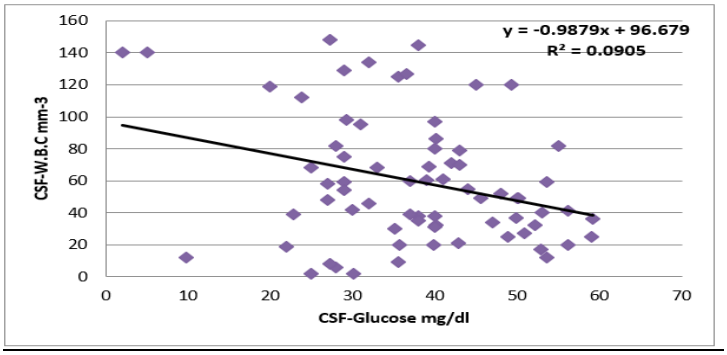

Figure 4 Correlation between CSF-WBCs and CSF-glucose.

While other parameters have no significant correlations between each other.

The samples were classified according to the AFP levels into two groups: more than 15 ng/ml and less or equal to 15 ng/ml groups. The levels of the measured parameters according to the sex are presented in Table 5

Table 5 The measured parameters according to the plasma AFP levels

Parameter/AFP category	S.AFP<15 N=47	S.AFP>15 N=33	p-value
Age days	85.79±87.97	96.82±122.42	0.640
T.Protein g/l	61.32±16.03	58.67±11.13	0.416
Glucose mg/dl	82.72±15.57	79.04±16.81	0.317
W.B.C mm-3	6172.34±1513.81	6346.97±1469.58	0.609
CI- mM	104.57±6.41	103.30±6.13	0.377
NA+ mM	138.34±4.53	138.30±3.33	0.968
K+ mM	4.95±0.77	4.82±0.72	0.464
CSF-AFP ng/ml	22.36±8.10	24.63±9.99	0.266
CSF-Protein mg/dl	80.15±36.96	85.90±33.79	0.481
CSF-Glucose mg/dl	34.59±12.25	40.84±9.98	0.018
CSF-WBCs mm-3	64.81±42.02	52.19±34.24	0.158

There is a significant increase in CSF-glucose in the high plasma AFP group (AFP>15ng/ml) in comparison with low AFP group (AFP≤15ng/ml) Comparison of CSF-Glucose according to the plasma AFP levels as seen in Figure 6

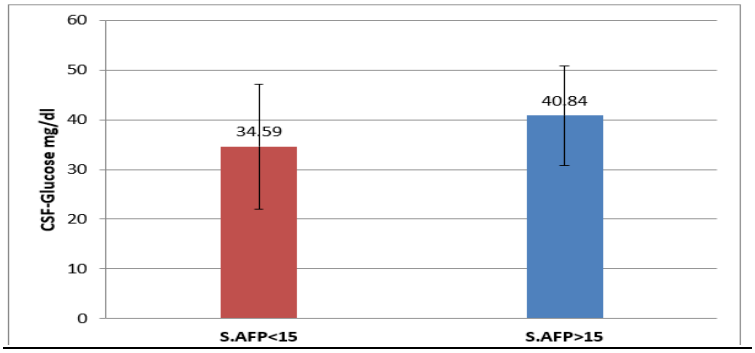

Figure 5 Comparison of CSF-Glucose according to the plasma AFP levels.

Prediction of AFP in CSF from blood components

In order to use the measured parameters in plasma to predict the AFP in CSF to dispense with the invasive lumber puncture, we recruit the linear regression analysis by using the most effective parameters by step by step method. We first used plasma AFP alone and then we add other parameters to the regression analysis to produce regression equations that can predict CSF-AFP efficiently. Table 6 summerizes the results of the regression analysis with the statistical data.

Table 6 The measured parameters according to the plasma AFP levels

# Equation	*Regression Equation*	*r*	R^2	*Mean*	*STD*	*SE**
1	*=1.05*AFP+8.571*	0.347	0.120	23.19	3.15	8.53
2	=1.139*AFP-0.001*WBCs+12.099	0.368	0.135	21.84	3.62	8.52
3	=0.967*AFP-0.014*Pr-0.001*WBCs+17.215	0.392	0.154	23.60	3.23	8.51
4	=0.997*AFP -0.007*Pr+0.082*Glu-0.001*WBCs+8.935	0.414	0.171	22.84	3.60	8.46
5	=0.995*AFP-0.023*Pr+0.067*Glu-0.001*WBCs +0.208*Cl+0.244*Na+0.076*K-45.032	0.454	0.106	22.42	4.11	8.43
Actual CSF-AFP				23.29	8.94	0.999**

*: Standard error of the y-predicted value for each x-value, **: Standard error of mean, STD: standard deviation, R2: Squared correlation coefficient, r: correlation coefficient.

Analysis number 1 showed that the equation for the prediction of CSF-AFP from plasma AFP (CSF-AFP=1.05*AFP+8.571) produce a predicted CSF-AFP with a significant correlation with the actual CSF-AFP ($r=0.347$, $p<0.05$). The predicted CSF-AFP level is 23.19±3.15 ng/ml with a standard error of 8.53. Figure 6 showed the correlation between the actual CSF-AFP and predicted CSF-AFP measured by the equation number 1.

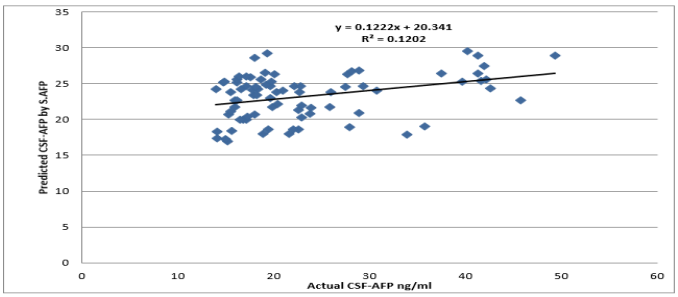

Figure 6 Prediction of AFP in CSF from serum AFP.

Figure 7 presented the correlation between the actual CSF-AFP and predicted CSF-AFP measured by the equation number 2.

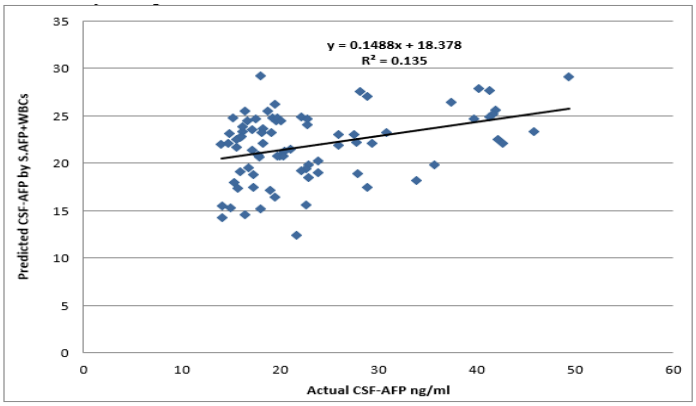

Figure 7 Prediction of AFP in CSF from serum AFP and WBC count.

Regression equation #2 constructed from plasma AFP and peripheral WBCs (CSF-AFP=1.139*AFP-0.001*WBCs+12.099). The equation produces a predicted CSF-AFP with a significant correlation with the actual CSF-AFP ($r=0.368$, $p<0.05$). The mean and standard deviation of the predicted CSF-AFP level is 23.84±3.62 ng/ml with a standard error of 8.52.

Analysis number 3 produce the equation for the prediction of CSF-AFP from plasma AFP, WBCs, and total protein (CSF-AFP=0.967*AFP-0.014*Pr-0.001*WBCs+17.215) produce a predicted CSF-AFP with a significant correlation with the actual CSF-AFP ($r=0.392$, $p<0.01$). The predicted CSF-AFP level is 23.60±3.23 ng/ml with a standard error of 8.51. Figure 8 showed the correlation between the actual CSF-AFP and predicted CSF-AFP measured by the equation number 3.

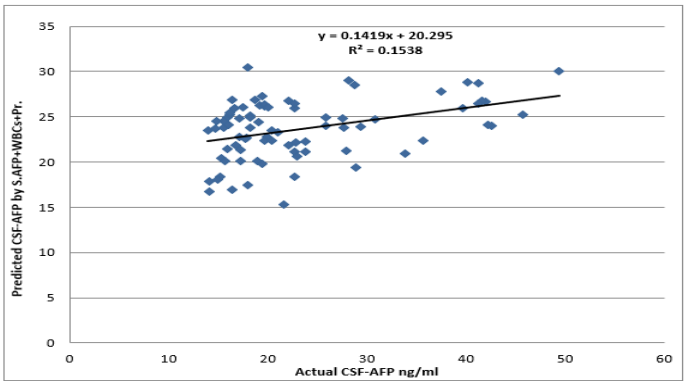

Figure 8 Prediction of AFP in CSF from serum AFP, total protein, and WBC count.

Regression equation #4 constructed from plasma AFP, peripheral WBCs, glucose, and total protein (CSF-AFP=0.997*AFP -0.007*Protein+0.082*Glucose-0.001*WBCs+8.935). The equation produces a predicted CSF-AFP with a significant correlation with the actual CSF-AFP ($r=0.414$, $p<0.01$). The mean and standard deviation of the predicted CSF-AFP level is 22.84±3.60 ng/ml with a standard error of 8.46. The correlation between the actual CSF-AFP and predicted CSF-AFP calculated by the equation number 4 is presented in Figure 9

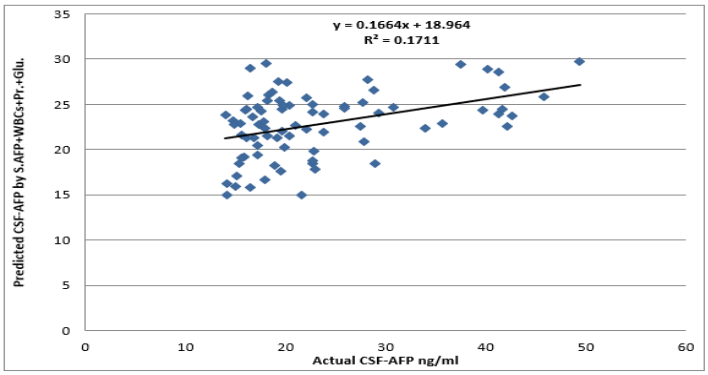

Figure 9 Prediction of AFP in CSF from serum AFP, total protein, glucose, and WBC count.

The last equation (#5) involves all the measured parameters in plasma with electrolytes (AFP, WBCs, glucose, protein, Cl, K, and Na). The regression equation is CSF-AFP=0.995*AFP-0.023*Pr+0.067*Glu-0.001*WBCs+0.208*Cl+0.244*Na+0.076*K-45.032 that, when applied, produces a predicted CSF-AFP level of 22.42±4.11 ng/ml with a standard error of 8.43. The correlation coefficient between the actual CSF-AFP and predicted CSF-AFP is 0.454 ($p<0.001$). Figure 10 plotted the actual CSF-AFP versus predicted CSF-AFP calculated by the equation number 5.

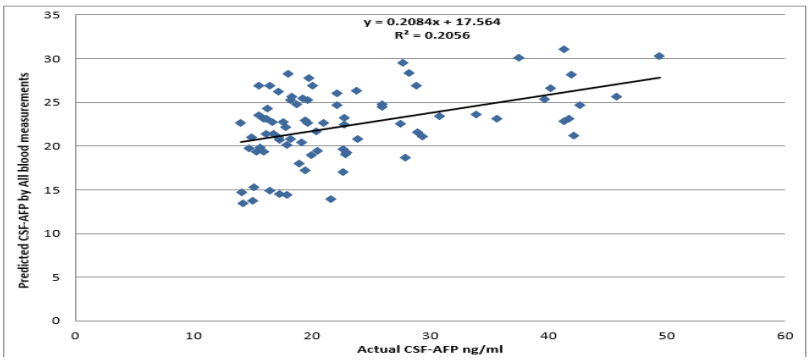

Figure 10 Prediction of AFP in CSF from serum AFP, total protein, glucose, WBC count, and electrolytes

Discussion

Data from the studies showed that CSF alpha-fetoprotein concentrations are significantly lower than those seen in blood plasma samples. The findings of the researchers cited in the source Qom are very comparable to these (14). Plasma or cerebrospinal fluid (CSF) AFP levels above or below normal range may be indicative of the presence of a germ cell tumor including yolk sac components (teratoma). In order to define normal ranges, we tested CSF AFP in children who did not have metastases. Other possibilities include genetic conditions like Down syndrome or Turner syndrome, or neural tube problems like spina bifida. An abnormal AFP level may not necessarily indicate a genetic disorder or neural tube abnormality in the developing embryo. It is possible that the child's AFP levels were abnormal because he was one of twins, that his birth was traumatic, or that he had a congenital abnormality of the brain as a result of the pressure he experienced during delivery. This is the consensus of numerous researchers, including (14 , 15,16) An aberrant tumor or a growth in the size of the parts of the yolk sac were blamed for the observed increase in concentration. The correlation between elevated blood sugar and congenital hydrocephalus stands out as the study's most significant finding. These results support our initial hypothesis, which predicted that hyperglycemia would lead to a

proportionate increase in polyol concentrations in the cerebrospinal fluid (CSF) of healthy individuals. Our proof-of-concept work is significant because we show that the polyol pathway is activated in a wide range of plasma and CSF glucose levels in people with and without diabetes. It is believed that AGE production, non-enzymatic glycation of myelin, and oxidative damage/stress, all of which are related with activation of the polyol pathway, contribute to brain abnormalities and cognitive impairment [17,18]. White matter hyperintensities, for instance, have been linked to enhanced glucose metabolism in the brain via the polyol route [19], and MRI has indicated that these hyperintensities are more common in individuals with affective disorders. In addition, structural neuronal alterations and decreased long-term spatial memory are linked to hyperglycemia [20]. A persistent, low-grade inflammatory condition results from impaired insulin action on the primary insulin-sensitive tissues (adipose tissue, muscle, and liver). Proinflammatory cytokines promote leukocyte differentiation and maturation, and they can be secreted in response to inflammation within an artery or to stimuli from outside the blood vessel. Every process associated with chronic inflammation will reduce insulin action, and insulin resistance will promote inflammation in a vicious loop [21,22], regardless of the triggering agents or starting events.

This may be due to the involvement of hypothalamus and brainstem autonomic regions by the growing ventricles during the development of hydrocephalus, as indicated by the aforementioned researchers in the cited source (17). A drop in CSF glucose levels is consistent with similar findings from earlier studies (23,24), and can be explained in this way. The low concentrations of glucose in cerebrospinal fluid (CSF) may be attributable to either central nervous system (CNS) inflammation or the excessive glucose consumption of white blood cells during the immune system's battle against infection and inflammation. Diseases of the central nervous system (CNS) have been linked to alterations in the glucose transporters that bring glucose into the brain. Due to the size of serum proteins, which prevents them from crossing the blood-brain barrier, the spinal fluid is often rich in protein. (25,26,27). Many conditions, including bacterial meningitis, brain tumor, aseptic meningitis, brain abscess, and cerebral hemorrhage, have been linked to elevated total protein levels in cerebrospinal fluid compared to their values in blood plasma, as shown by our study. Albumin makes up the vast majority of the normally occurring protein. There are two potential causes for an increase in cerebrospinal fluid (CSF) protein concentration: increased synthesis of proteins within the cerebrospinal canal by inflammatory or other invading cells, or increased permeability of the blood brain barrier, which allows more protein and higher molecular weight proteins to enter the CSF (28,29). Diseases like viral meningitis, neurosyphilis, subdural hemorrhage, cerebral thrombosis, brain tumor, and multiple sclerosis can all lead to a mild elevation of proteins in the blood. Acute bacterial meningitis, tuberculous meningitis, spinal cord tumor, cerebral haemorrhage, and Guillain-Barré syndrome(30,31) can all lead to a moderate or severe rise. Low amounts of CSF proteins may be an indicator of increased CSF production (32).

CSF-glucose level was found to have a statistically significant relationship with plasma AFpP ($r=0.284$, $p=0.011$). Comparing markers in plasma and cerebrospinal fluid of congenital hydrocephalus led us to the conclusions we did not find supported by other studies. We employ a data analysis technique called linear regression analysis, which seeks to estimate an unknown variable by analyzing its relationship to another variable for which an estimate is known, in order to use the parameters measured in plasma to predict the AFP in CSF and thus avoid the invasive lumber puncture. The most effective parameters are used

in a step-by-step process to create a linear equation that represents the relationship between the two variables (the unknown and the known). The statistical data regression analysis yielded are summarized in Table (6). Alpha-fetoprotein was originally employed as a marker for the monitoring and investigation of hydrocephalus patients based on these correlations and the results derived from the applied equations.

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