

Role of S-100B as a Serum Biochemical Marker for Brain Injury in Egyptian Patients with Phenylketonuria

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Abstract :Background: Phenylketonuria (PKU) is a metabolic disorder characterized by high phenylalanine (Phe) levels in blood. Tissue accumulation of L-phenylalanine (Phe) is the biochemical hallmark of human phenylketonuria (PKU), an inherited metabolic disorder clinically characterized by mental retardation and other neurological features. The mechanisms of brain damage observed in this disorder are poorly understood. S-100B protein is highly specific for nervous tissue where its role is not yet fully understood. **Objective:** The aim of our study was to determine the diagnostic value of measuring S-100b in the serum of PKU patients as a marker for brain lesion. Additional validity should be acquired by a comparison with plasma levels of phenylalanine. **Methods:** Nineteen PKU patients from 15 families were selected from the clinic for special needs at the National Research Centre. Their age ranged between 2 and 20 years in addition to 15 healthy controls with same age. Blood samples were drawn to investigate circulating serum levels of S-100b using ELISA technique for all the studied cases. **Results:** Statistical significant increase of serum S-100B concentrations was present in PKU patients compared to controls. Regarding sensitivity and specificity, PKU patients, serum neural protein S-100b showed high sensitivity and specificity values. In addition, there was non significant negative correlation between S100B and Phe **Conclusion:** we concluded that serum S-100B blood could be a useful peripheral marker of nervous system damage in patients with phenylketonuria.

[Mohga S Abdallah, Hayat M Sharada, Esmat Ashour, Lamiaa Mageed, Nagwa Meguid. **Role of S-100B as a Serum Biochemical Marker for Brain Injury in Egyptian Patients with Phenylketonuria.** Journal of American Science 2010;6(12):971-978]. (ISSN: 1545-1003). <http://www.americanscience.org>.

Key Words: Phenylketonuria, Serum neural protein S-100B

1. Introduction

Phenylketonuria is a rare metabolic disorder with an estimated prevalence of 1:10,000, i.e. an orphan disease. Inheritance is autosomal recessive caused by mutations in the phenylalanine hydroxylase gene. This enzyme converts phenylalanine to tyrosine which is also a precursor for dopamine. Partial or complete enzyme inactivity results in the accumulation of phenylalanine in tissues, blood (hyper-phenylalaninemia) and other body fluids (Albrecht *et al.*, 2009).

Untreated phenylketonuria causes severe neurological impairment, mental retardation and behavioral difficulties. Although there is no doubt that blood phenylalanine has a detrimental effect in the brain (Scriver, 2007), the ultimate causes for this effect remain hypothetical (Hoeksma *et al.*, 2009). If left untreated from birth, this results in rapid accumulation of toxic concentrations of Phe in the blood, leading to severe brain damage and

microcephaly (van Spronsen *et al.*, 2009). The vast majority of cases are now identified via neonatal screening programmes, allowing timely intervention to avoid severe consequences. However, insufficiently controlled Phe concentrations may still result in neuro-cognitive symptoms, such as a decrease in intelligence quotient (IQ), attention, executive function (such as planning, working memory, inhibition, flexibility and behavioral issues), and psychosocial effects in later childhood, continuing into adulthood (DeRoche *et al.*, 2008; Waisbren *et al.*, 2007).

A low-Phe diet currently forms the principal strategy to limit Phe accumulation in the blood and, therefore, in tissues such as the brain (MacDonald and Asplin, 2006). The low-Phe diet restricts the intake of high-protein foods, and the remaining nutritional requirements must be obtained from Phe-free amino acid supplements (protein substitute) and special or natural foods that are low in Phe, the nature

and availability of which can vary geographically (Weetch and MacDonald, 2006).

The term S-100B refers to members of a multigenic family of calcium-modulated proteins (S100 proteins), mostly of low molecular mass ($\approx 10\,000$ Da), that were first identified (on the basis of methods available at the time) as a protein fraction detectable in brain but not in non-neural extracts and called S100 because of their solubility in a 100%-saturated solution with ammonium sulfate (Moore, 1965). At present, at least 20 proteins have been identified as belonging to the S100 protein family, the members of which are characterized by the presence of a pair of so-called EF-hand (i.e., helix-loop-helix) calcium-binding motifs (Kawasaki *et al.*, 1998), first discovered in the crystal structure of parvalbumin (Kretsinger and Nockolds, 1973), that induce conformational changes of the protein after binding to calcium (Donato, 2001; Heizmann, 1999). This conformational change may facilitate the interaction of S100 proteins with secondary effectors: S100 proteins are generally thought to be calcium sensor proteins that modulate biological activity via calcium binding (Ikura, 1996). In addition, some S100 members (Schafer *et al.*, 2000; Nishikawa *et al.*, 1997) have been shown to bind Zn^{2+} and Cu^{2+} , suggesting the possibility that their biological activity in some cases might be regulated by Zn^{2+} and/or Cu^{2+} , rather than by Ca^{2+} (Heizmann and Cox, 1998). Aim of this study: Since S-100B protein is a biomarker of neural tissue damage and PKU is a neurodegenerative disease, we aimed to investigate whether S-100B is related to CNS damage of PKU patients and to their blood Phe levels.

2. Material and methods

Patients screening

The present study was carried out on 19 PKU patients (12 males and 7 females) from 15 families selected from the children with Special Needs Clinic and Clinics Department, National Research Centre. Their age ranged between 2 and 20 years. In addition, 15 healthy matched subjects of age and sex were ascertained as control group.

All selected cases were selected at the time of the diagnosis of the index cases by elevation of serum levels of Phenylalanine (Phe) which was done by an enzymatic colorimetric assay (Scriver *et al.*, 1989) for all studied cases.

Participants were classified into three age groups: children with mean age below 13 years, adolescents with mean age between 13 years and 18 years, and adults older more than 18 years.

All PKU patients were managed with low phe diet the time they diagnosed

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Clinical Investigation: All PKU patients were on low phe diet.

- 3 patients had abnormal EEG and received Carbamazepine in addition to low phe diet
- Also 6 patients received Sodium valproate.
- On the other hand 5 patients had autistic features receiving Risperidone.

Intelligence quotient (IQ):

All the patients were tested using Stanford-Binet Intelligence scale: Fourth Edition (Thorndike *et al.*, 1986) according to their age. The degree of mental retardation (MR) was evaluated according (Menon *et al.*, 2003): normal ~ 80 , borderline = 70-90, mild = 51-70, moderate = 36-50, severe = 21-35, profound = 0-20.

Clinical Investigation

Collection of Blood Samples

Blood samples were collected from (controls and PKU patients) by venous arm puncture into tubes, The tubes containing blood without adding anticoagulant were centrifuged after 30 minute at (3000 r.p.m for 10 minutes at 4° C) to separate the serum. The serum samples were divided into small aliquots and kept frozen until analysis.

Laboratory Investigations

- Serum concentrations were performed by using a commercially available the RD 192090100 Human S100B ELISA Kit.
- Total serum protein was assessed by colorimetric method using commercially Stanbio Total Protein Loiqui-Color Kit. This method is based on the reports of Weischeslbaum (1946) and Gornal *et al.*, (1949).
- Determination of Total Calcium in serum by colorimetric method using commercially available Kit from STANBIO Company. The procedure presented is based essentially on the micro-method of Saker and Chauhan (1967).
- Ionized calcium was calculated according to Zeisler Formula (1954)

Statistical analysis of data, Package for

Social Science (SPSS for Windows Release 11; SPSS Inc., Chicago, IL, USA) was used. Data were analyzed by two-way ANOVA. As no variable was simultaneously Gaussian in the groups of study, differences were tested with the Mann-Whitney U test. Correlation coefficients were estimated with the Spearman test. For the evaluation of categorical variables, Chi square test was used. All tests were

two sided and P values < 0.05 were considered significant.

3. Results

Among the 19 patients with PKU studied, PKU was proved to have high level of Pha concentration. The diagnosis of Mental retardation was based on IQ Score (Table 1)

Table 1. Demographic and clinical data of the studied group

		#N
No. of Family groups		15
Gender	Females	7
	Males	12
	Total	19
Age (years)	0-12 yrs	12
	13-18 yrs	6
	Over 18yrs	1
Severity of PKU	Classic(severe)	10
	Moderate	7
	MHP	2
Degree of Mental Retardation	Borderline	2
	Mild	1
	Moderate	7
	Severe	8
	Not available	1

#N.: no of cases

The results of the S100B in PKU showed that S100B level was significantly increased in PKU

cases when compared to healthy control group (F=43.771, P<0.0001). In addition, Total calcium (Ca) and ionized Calcium (Ca⁺²) were significantly decreased in PKU when compared to healthy controls (F=35.121, P<0.0001 and F=27.155, P<0.0001 respectively). In addition the results of the Total Protein (TP) in PKU Showed that there was no statistical variation between the mean values of TP in PKU (7.71±0.22gm/dL) when compared to healthy controls(7.54+ 0.19gm/dL) (F=0.333, P=0.568) (Table 2)

The best Cut off value of S100B was 1866 pg/ml (the sensitivity and PPV values were 100 % and 86.4% respectively but the specificity and NPV were 80% and 100 % respectively)(Table 3) The area under the curve of S100B was 0.942 (Table 4, Fig.1). The predictive value of S100B for discriminating between individuals with and without PKU was assessed by calculating the area under the ROC curve.

Correlation between S100B and Phe, IQ, TP, Ca and ionized Ca⁺² in PKU Cases:

There was no significant positive correlation between S100B and both of IQ, Ca and ionized Ca⁺² (r=0.368, P=0.133, r=0.256, P=0.290 and r=0.272, P=0.260 respectively) in PKU Cases. In addition, there was non significant negative correlation between S100B and both of Phe and TP (r=0.173, P=0.479 & r=-0.157, P= 0.521 respectively) in PKU Cases (Table 5).

Table 2. Summarized for data Age, Pha Conc., IQ Score, Total Protein (TP)(g/dL), Total Calcium (Ca), Ionized Calcium (Ionized Ca⁺²) mg/dL) and S100B (Pg/ml) in Control and PKU Groups.

Group		Age (years)	Phe (mg/dl)	IQ	TP (gm/dl)	Total Ca (mg/dl)	Ionized Ca ⁺² (mg/dl)	S100 B (pg/ml)
Controls (C)	Range	5.00 19.00	2.5 5.5	83 98	6.50 8.50	8.50 11.90	3.00 5.40	398.00 3981.00
	Mean	9.87	4.03	102.5	7.54	10.66	4.47	1541
	+SE	1.33	0.25	1.23	0.19	0.25	0.17	256.381
	N	15	15	15	15	15	15	15
PKU	Range	2.00 21.0	7.00 37.0	21. 80.	6.20 8.90	5.10 13.00	2.00 5.90	1995.00 5248.00
	Mean	9.89	20.67	41.50	7.71	7.50	3.07	3767.1
	+SE	1.19	1.71	3.83	0.22	0.43	0.20	220.07
	N	19	19	18	19	19	19	19
ANOVA test	F				0.333	35.121	27.155	43.771
	P				0.568	0.000	0.000	0.000

Table (3): Cut off, Sensitivity, Specificity, PPV, NPV, and Accuracy for S100B

Parameter	Cut off (pg/ml.)	Sensitivity	Specificity	PPV*	+NPV	Accuracy
S100B	1866	100	80	86.4	100	91.17(31/34)

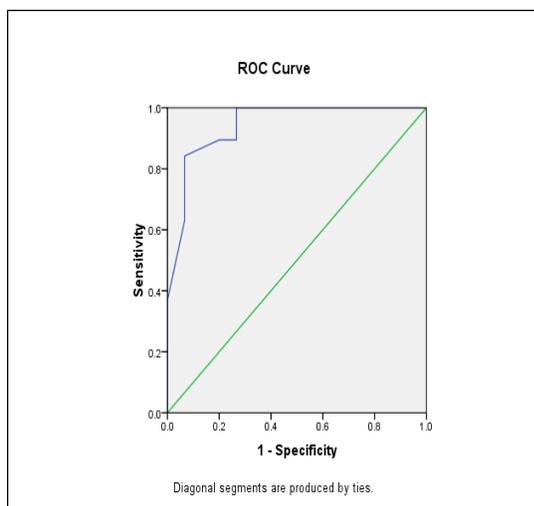


Fig (1): ROC curve of S100B

Table (4): Area under Curve of S100B

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.942	0.040	0.000	0.864	1.020

Table (5): Correlation between S-100B and Other Biochemical Parameters in PKU Cases

Diagnosis		100B	Ionized Ca ⁺²	Ca	TP	
KU	age	Pearson Correlation	0.285	-0.056-	-0.036-	0.217
		Sig. (2-tailed)	0.238	0.818	0.885	0.373
		N	19	19	19	19
	Phe	Pearson Correlation	-.173-	-.361-	-.320-	.405
		Sig. (2-tailed)	.479	.129	.181	.085
		N	19	19	19	19
	IQ	Pearson Correlation	.368	.262	.410	.475*
		Sig. (2-tailed)	.133	.294	.091	.046
		N	18	18	18	18
	Ca	Pearson Correlation	.256	.965**	----	-.016-
		Sig. (2-tailed)	.290	.000	-----	.947
		N	19	19	-----	19
Ionized Ca ⁺²	Pearson Correlation	.272	----	.965**	-.242-	
	Sig. (2-tailed)	.260	----	.000	.318	
	N	19	----	19	19	
TP	Pearson Correlation	-.157-	-.242-	-.016-	---	
	Sig. (2-tailed)	.521	.318	.947	---	
	N	19	19	19	---	

* P values < 0.05 were considered significant.

** P values < 0.0001 were considered very high significant.

According to the different ages group

There were no statistical variations in S-100B between different age groups [$F=0.050$, $P>0.05$ ($P=0.826$)] (Fig.2). In the group of age (0-12yrs), there was no significant negative correlation between S100B and both of Phe, Ca and ionized Ca^{2+} ($r=-0.0222$, $P=0.0607$, $r=-0.237$, $P=0.651$ and $r=-0.0590$, $P=0.217$ respectively) However, there was no significant positive correlation between S100B and IQ and TP ($r=0.575$, $P=0.232$ and $r=-0.411$, $P=0.419$ respectively).

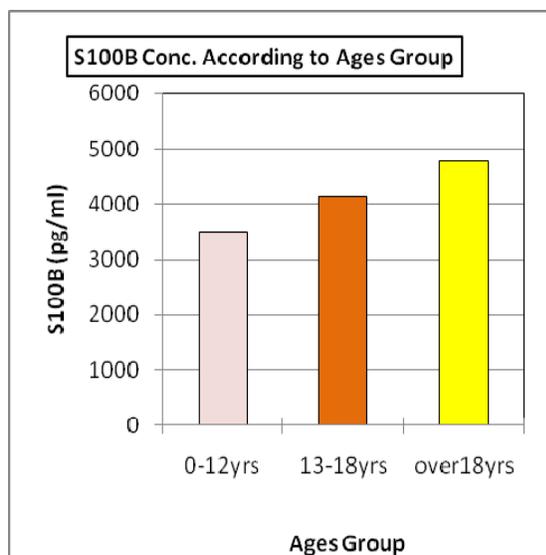


Fig. (4): Mean Values for S100B Conc. in Different Age groups of PKU

According to Severity of PKU:

The results revealed that there was no variation between different severity of PKU groups ($F=2.998$, $P=0.104$).

In Classic Phe severity. There was no significant positive correlation between S100B and both of Phe, IQ, Ca and ionized Ca^{2+} ($r=0.251$, $P=0.0484$, $r=0.317$, $P=0.373$, $r=0.330$, $P=0.352$ and $r=0.463$, $P=0.0178$ respectively). As well as in Moderate Phe severity, there was non significant positive correlation between S100B and both of Phe and IQ ($r=0.490$, $P=0.262$ and $r=-0.0233$, $P=0.615$ respectively), but there was non significant negative correlation between S100B and both of Ca and ionized Ca^{2+} ($r=-0.083$, $P=0.859$ and $r=-0.146$, $P=0.754$ respectively)

According to Degree of Mental Retardation (Depended on IQ Score) in PKU:

The results indicated that the mean values \pm SE of S100B in Borderline (B), Moderately (Mod) retarded and Severely (S) retarded of Mental retardation in PKU were 4898.5 ± 112.5 , 3941.9 ± 252.71 and 3380.1 ± 411.26 pg/ml respectively with ranges of 4786-5011, 3162-5128 and 1995-5248 pg/ml respectively, Statistically, there was no significant changes between these groups ($F=0.05$, $P=0.826$).

As well as the result showed one case have Mild Mental retardation and another one haven't IQ score.

4. Discussion

Phenylketonuria (PKU) is the most common inherited disorder of amino acid metabolism that arises from a single enzyme deficiency of phenylalanine hydroxylase, which converts the essential amino acid phenylalanine to tyrosine. Failure of this conversion results in the accumulation and elevation of phenylalanine in blood and tissues. Elevated concentrations of phenylalanine and its metabolites interfere with normal development of the central nervous system, leading to severe mental retardation. In PKU, plasma Phe concentrations may reach 400–1800 $\mu\text{mol/L}$ and are harmful especially during the first year of life (Leader *et al.*, 2008).

Our study showed a significant negative correlation between concurrent Phe level (ranged from 7 to 37 mg/dl) and IQ score (ranged from 21 to 80) for the affected individuals'. Our results agreed with previous investigators (Antshel *et al.*, 2003; White *et al.*, 2002).

It is generally accepted that early-treated children with classic PKU suffer loss of IQ scores if the diet is discontinued, and children who are maintained on phe- restricted diet are more likely to achieve higher IQ scores and greater school achievement than those who discontinue the dietary management (Sharman *et al.*, 2009; Susan *et al.*, 2007).

On the other hand, another longitudinal study of intelligence among early-treated patients with PKU concluded that for each 300 $\mu\text{mol/l}$ (5mg/dl) increase in phe level, IQ measured during pre-school years decreased by 0.5 SD (approximately 7 points) up to 10 years of age, after which time IQ remained reasonably stable (Burgard, 2007).

The measurement of biomarker of neural tissue damage such as S-100B protein may offer an alternative and direct indicator of cell damage in the nervous system when clinical and radiologic signs are not yet fully manifest and it is recommended to additional advantage of providing a quantitative

indicator of the extent of brain lesion (Steiner *et al.*, 2010).

Several reports have been published regarding the expression of S100B in patients with Phenylketonuria, Down's syndrome, in children with cerebral palsy or delayed development (Steiner *et al.*, 2010).

Previous authors reported that higher concentrations of serum S-100B protein in normal neonates and children compared to adults could reflect the ongoing central neuro-developmental processes occurring during these different stages of life (Mori *et al.*, 2009) the proliferation and maturation of glial cells, growth of neuritis and formation of synapses have been documented to be the most important functions of S-100B in morphogenesis (Tateihi *et al.*, 2006).

Our results showed a significant increase in S-100B levels in the affected cases and a significant correlation between S-100B and phe and total protein (TP). Our data are in agreement with corresponding data reported by other investigators (Boneh *et al.*, 2006; Alarcon *et al.*, 2005; Foerch *et al.*, 2005; Marchi *et al.*, 2004; Park *et al.*, 2004; Schulpis *et al.*, 2004).

Marchi *et al.*, (2004) reported that serum S100B is an ideal marker of blood-brain barrier integrity, because with a molecular weight of 21 kDa (S100B dimer) it may not penetrate through an intact blood-brain barrier. Furthermore, its concentration is high in central nervous system fluids and normally low in blood. Indeed, serum levels of S100B were directly correlated with an experimental damage of the blood-brain barrier.

One hypothesis is that S-100B accumulates in the extracellular space after astrocyte death or due to increased release by activated astrocytes, or after cellular disintegration of the damaged parenchyma. Under these conditions, the S-100B concentration may be in the micromolar range and the protein may become toxic due to its stimulatory effects on nitric oxide (NO) production by astrocytes and microglia. It interacts with receptor for advanced glycation end product (RAGE) on neurons and RAGE mediated neuronal apoptosis, or stimulates of interleukin-6 (IL-6) secretion by neurons (Alarcon *et al.*, 2005). In this regard, it is noteworthy that RAGE is up regulated in many tissues including brain, during development and in the course of pathologic states i.e., under conditions in which many cells are destined to die by apoptosis. RAGE therefore may be an important progression factor in several disease states, and S-100B might be one crucial RAGE ligand in the brain (Alarcon *et al.*, 2005).

Similarly, it was suggested that elevated S-100B levels are involved in the development of Down's syndrome; its expression was also correlated with the density of dystrophic neuritis with over-expressed β -amyloid precursor proteins (A β PP) (Medana *et al.*, 2007). Recent evidence indicates that high levels of S-100B in cerebrospinal fluid CSF increase the risk of repeated seizures in children with severe infection due to axonal injury (Medana *et al.*, 2007).

Our results showed a significant negative association between S-100B and both Total Calcium (Ca) and Ionized Calcium (Ca²⁺) in the affected cases although S-100B plays an important role in Calcium homeostasis. Our data agree with previous studies that reported similar findings (Wojda *et al.*, 2008; Mattson *et al.*, 2004; Petzold *et al.*, 2003).

S-100B is involved in calcium homeostasis. For example, glial cells from mouse S100B null cerebellar cultures have been reported to display enhanced calcium transients in response to potassium chloride (KCl) or caffeine suggesting a role of S-100B in cytosolic calcium buffering. This observation is difficult to reconcile with the relatively low affinity of S-100B for calcium in vitro but one cannot exclude the possibility that normally S-100B modulates the activity of one or more effect or proteins (calcium channels/endoplasmic reticulum ATPase) that regulate the levels of cytosolic calcium with ensuing abnormality in calcium handling in the absence of S100B (Petzold *et al.*, 2003).

In conclusion, our findings support the hypothesis of a positive relationship between serum S-100B level and Phe blood level in PKU patients, and estimation of S-100B blood could be a useful informative peripheral marker monitoring the toxic effect of phe on central nervous system and direct indicator of cell damage in nervous system. Moreover, the combination of this marker with other diagnostic techniques may the way forward to improve the clinical assessment of patients with PKU.

Our study emphasized the correlation between early-dietary intervention and IQ score improvement in children with classic PKU.

Further studies with increase number of cases are highly recommended.

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References

- Alarcon R, Fuenzalida C, Santibanez M and von Bernhardt R 2005. Expression of scavenger receptors in glial cells. Comparing the adhesion of astrocytes and microglia from neonatal rats to surface bound beta amyloid. *J Biol Chem* 280:30406–30415.
- Albrecht J., Garbade S. F., Burgard P. 2009. Neuropsychological speed tests and blood phenylalanine levels in patients with phenylketonuria: A meta-analysis. *Neuroscience and Biobehavioral Reviews* 33 : 414–421
- Anderson P., Wood S., Francis D., Coleman L., Warwick L., Casanelia S., Anderson V., Boneh A. 2004. Neuropsychological functioning in children with early-treated phenylketonuria: impact of white matter abnormalities. *Dev Med Child Neurol* 46:230-238.
- Antshel K, Waisbren S 2003. Timing is everything: executive functions in children exposed to elevated levels of phenylalanine. *Neuropsychology* 17: 458–468.
- Boneh A, Dorothy EM, Humphrey M, Upton J and Prters L. 2006. Three- year audit of the hyperphenylalaninemia/phenylketonuria spectrum in Victoria. *Journal of pediatrics and Child Health* 42: 496-498.
- Burgard P. 2007. Development of intelligence in early treated phenylketonuria. *Eur. J. Pediatr.* 159 :74–79.
- DeRoche K, Welsh M. 2008. Twenty-five years of research on neurocognitive outcomes in early-treated phenylketonuria: intelligence and executive function. *Dev Neuropsychol*; 33:474–504.
- Donato R. 2001. S100B: a multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles. *Int J Biochem Cell Biol*; 33:637–68.
- Foerch C, Singer OC, Neumann-Haefelin T, du Mesnil de Rochement R, Steinmetz H and Sitzer M. 2005. Evaluation of serum S100B as a surrogate marker for long-term outcome and infarct volume in acute middle cerebral artery infarction. *Arch eurol* 62:1130–1134.
- Gornal A.G, Bardawil C.J, David MM. 1949. Determination of serum protein by means of the biuret reaction. *J. Biol. Chem.* 177: 751-766.
- Guthrie R. and Susi A.. 1963. A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. *Pediatrics* 32 (1963), pp. 318–34313.
- Heizmann CW, Cox JA. 1998 New perspectives on S100 proteins: a multifunctional Ca²⁺, Zn²⁺, and Cu²⁺-binding protein family. *Biometals*; 11:383–97.
- Heizmann CW. 1999. Calcium-binding proteins in the central nervous system. *Neurochem Res*; 24:1097–100.
- Hoeksma M, Reijngoud D.J, Pruijm, H.W., Paans A.M, van Spronsen F.J., 2009 Phenylketonuria: high plasma phenylalanine decreases cerebral protein synthesis, *Mol. Genet. Metab.*; 96 177–182.
- Ikura M. 1996. Calcium binding and conformational response in EF hand proteins. *Trends Biochem Sci*; 21:14–7.
- Kawasaki H, Nakayama S, Kretsinger RH. 1998. Classification and evolution of EF-hand proteins. *Biometals*; 11:277–95.
- Kretsinger RH, Nockolds CE. 1973 Carp muscle calcium-binding protein: II Structure determination and general description. *J Biol.Chem*; 248:3313–26.
- Leader b, Baca QJ, Golan DE. 2008. Protein therapeutics: a summary and pharmacological classification, *Nature Reviews* 7:21-39.
- MacDonald A, Asplin D. 2006. Phenylketonuria: practical dietary management. *J Fam Health Care*; 16:83–5.
- Marchi N., Cavaglia V., Fazio S., Bhudia K., Janigro D. 2004. Peripheral markers of blood-brain barrier damage. *Clinica Chimica Acta*, 342:1–12.
- Mattson MP. 2007. Calcium and neurodegeneration. *Aging Cell.* 6:337-350.
- Medana IM, Idro R, Newton CR. 2007. Axonal and astrocyte injury markers in the cerebrospinal fluid of Kenyan children with evere malaria. *J Neurol Sci*, 258:93-98.
- Menon PSN, Nair MKC, Mathur YC, Mukherjee D, Bhave S, Hathi GS, Sachdev HPS and Vijayakumar M. 2003. *IAP Textbook of Pediatrics*, 2nd edition.
- Moore BW. 1965. A soluble protein characteristic of the nervous system. *Biochem Biophys Res Commun*; 19:739–44.
- Mori T, Tan J, Gary W, Koyama N, Nojima Y and Town T. 2009. Overexpression of Human S100B Exacerbates Brain Damage and Periinfarct Gliosis After Permanent Focal Ischemia. *Strok* (39): 2114-2121.
- Nishikawa T, Lee ISM, Shiraishi T, Ishikawa Y, Ohta M, Nishikimi I. 1997 Identification of S100b protein as copper-binding protein and its suppression of copper-induced cell damage. *J Biol Chem.*; 272:23037–41.

- Park IH, Yeon SI, Youn JH, Choi JE, Sasaki N, Choi IH, Shin JS. 2004. Expression of a novel secreted splice variant of the receptor for advanced glycation end products (RAGE) in human brain astrocytes and peripheral blood mononuclear cells. *Mol Immunol* 40:1203–1211.
- Petzold A, Keir G, Lim D, Smith M, Thompson EJ. 2003. Cerebrospinal fluid (CSF) and serum S100B: release and wash-out pattern. *Brain Res Bull* 61:281–285.
- Rohr F.J., Allred E.N., Turner M., Simmons J. and Levy H.L. 1996 Use of the Guthrie bacterial inhibition assay to monitor blood phenylalanine for dietary treatment of phenylketonuria. *Screening* 4 : 205–211.
- Sarkar RC, Chauhan SP. A new method for determining micro quantities of calcium in biological materials. *Anal Biochem* 1967;20:155-156
- Schafer BW, Fritschy JM, Murmann P, Troxler H, Durussel I, Heizmann CW, and Cox JA. 2000. Brain S100A5 is a novel calcium-zinc-, and copper ion-binding protein of the EF-hand superfamily. *J Biol. Chem*; 275:30623–30.
- Schulpis KH, Kariyannis C, Papassotiropoulos I. 2004. Serum levels of neural protein S-100B in phenylketonuria. *Clin Biochem* ;37(1):76-79.
- Scriver CR. 1998. Commentary on: A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants, by Robert Guthrie and Ada Susi, 20: 120-125.
- Scriver, C.R., 2007. The PAH gene, phenylketonuria, and a paradigm shift. *Hum. Mutat.* 28 (9), 831–845.
- Sharman R, Sullivan K, Young R, McGill J. 2009. Biochemical markers associated with executive function in adolescents with early and continuously treated Phenylketonuria. *Clin Genet*, 75: 169-174.
- Sitta A., Aletheia G., Barschak D., Amanda T., Barden A., Giovana B., Biancini A., Paula R., Carolina F. de Souza A., Netto C., Wajner M., Carmen R. 2009. Effect of short- and long-term exposition to high phenylalanine blood levels on oxidative damage in phenylketonuric patients. *Int. J. Devl Neuroscience* 27 : 243–247.
- Steiner J., Myint M., Schiltz K., Westphal S., Bernstein H., Walter M., Matthias L., Markus J., Bogerts B. 2010. S100B Serum Levels in Schizophrenia Are Presumably Related to Visceral Obesity and Insulin Resistance. *Cardiovascular Psychiatry and Neurology* 11:1-11.
- Tateishi N, Shimoda T, Yada N, Shinagawa R and Kagamiishi Y. 2006. S100B: astrocyte specific protein, 26(1):11-6.
- Thorndike, R. L., Hagen, E. P., & Sattler, J. M. 1986. *Stanford-Binet Intelligence Scale: Fourth Edition*. Itasca, IL: Riverside Publishing.
- Tsoporis N., Izhar S., Leong-Poi H., Desjardins F., Huttunen J., Parker G. 2009. S100B Interaction With the Receptor for Advanced Glycation End Products (RAGE). A Novel Receptor-Mediated Mechanism for Myocyte Apoptosis Postinfarction. *American Heart Association*;10: 35-42.
- van Spronsen F.J., Hoeksma M., Reijngoud D.J., 2009. Brain dysfunction in phenylketonuria: is phenylalanine toxicity the only possible cause? *J. Inherit. Metab. Dis.* 32 :46–51.
- Waisbren SE, Noel K, Fahrbach K, Cella C, Frame D, Dorenbaum A, Levy H.. 2007 Phenylalanine blood levels and clinical outcomes in phenylketonuria: a systematic literature review and meta-analysis. *Mol Genet Metab*; 92:63–70.
- Weetch E, MacDonald A. 2006 The determination of phenylalanine content of foods suitable for phenylketonuria. *J Hum Nutr Diet*; 19:229–36.
- Weichselbaum TE. 1946. Quantitative colorimetric determination of total protein in serum. *Am J Clin Pathol* 7:40-42.
- White D, Nortz M, Mandernach T, Huntington K, Steiner R. 2002. Age-related working memory impairments in children with prefrontal dysfunction associated with phenylketonuria. *J. Int. Neuropsychol. Soc.* 8:1–11.
- Wojda U, Salinska E, Kuznicki J. 2008. Calcium ions in neuronal degeneration. *IUBMB Life*, 60:575-590.
- Zeisler, E. B., Determination of diffusible serum calcium. 1954. *Am. J. Clin. Pathol.* 24(5), 588-593.

11/6/2010