AIM OF THE WORK

The aim of this work was to study the clinical significance of S100 B protein and neopterin in patients with encephalitis.

PATIENTS AND METHODS

The study was conducted on 40 subjects who were divided into two groups.

- **Group I:** consisted of twenty patients with clinical manifestations and positive CSF criteria of suspected viral encephalitis.
- **Group II:** consisted of twenty apparently healthy volunteers of matching age and sex.

Patients categorization were based on clinical manifestations, laboratory features and positive CSF criteria of suspected viral encephalitis.

Patients were selected from those attending in Alexandria Fever hospital.

Patients with the following associated conditions were excluded from the study:

1. Epilepsy
2. Brain abcess
3. Previous neurological surgery
4. Past history of stroke
5. Other neuropychiatric disorders

Informed consent for participation in the study was obtained from all patients and controls.

All patients were subjected to the following:

1. Detailed history taking including risks for acquiring infection, any treatment received before, investigations done before, recent travel and any associated medical diseases.
2. Thorough clinical examination focusing on :
   a. The presenting symptoms including fever, cough, vomiting, diarrhea, skin rash.
   b. Signs including seizures, consciousness level, meningeal signs, focal neurological signs (neck rigidity, babinski's sign and limb weakness), ataxia, personality change, visual and hearing hallucination, myoclonic jerk, and blurred vision.
3. Routine laboratory investigations:
   a. Complete blood picture.
   b. Random blood sugar.
   c. Lumbar puncture for CSF analysis. (184)
   d. ESR 1st, 2nd hour.
   e. C-reactive protein (CRP). (185)
4. Routine radiological investigations:
   a. CT brain.
   b. MRI brain.
5. CSF culture for bacteria infection.
6. Estimation of serum S100 B protein by ELISA. (186)
7. Estimation of serum neopterin by ELISA. (187)

All controls were subjected to the following:

1. Detailed history taking and thorough clinical examination to confirm that they were completely healthy.
2. Complete blood count (CBC).
3. Random blood sugar.
4. CRP. [185]
5. ESR 1st, 2nd hour.
6. Estimation of serum S100 B protein byThe enzyme-linked immunosorbent assay (ELISA). [186]
7. Estimation of serum neopterin by ELISA. [187]

S100B Protein

1. Principle

- The S100B ELISA TEST is based on binding of S100B by two antibodies, one immobilized on microwell plates, and the other one conjugates with horseradish peroxidase (HRP).
- The assay was on two steps binding procedure and after every incubation step, the bound/free separation was performed by a simple solid-phase washing, then the substrate solution 3,3',5,5' - tetramethylbenzidine (TMB) is added.
- After an appropriate time had elapsed for maximum colordevelopment, the enzyme reaction was stopped and the absorbance were determinated.
- The S100B concentration in the sample was calculated based on a series of standard.The color intensity was roportional to the S100B concentration in the sample.

2. Reagents and materials supplied in the kit

1. S100B Standards (6 vials, lyophilized)
2. Controls (2 vials, lyophilized)
3. Conjugate buffer (1 vial, 20 mL)
4. Conjugate (1 vial, 1 mL) Anti S100B-HRP conjugate
5. Assay buffer (1 vial, 12 mL)
6. Coated microplate (1 breakable microplate) Anti S100B adsorbed on microplate
7. TMB Substrate (1 vial, 15 mL) Hydrogen peroxide H_2O_2-TMB 0.25g/L (any skin contact was avoided)
8. Stop solution (1 vial, 15 mL) Sulphuric acid 0.15 mol/L (any skin contact was avoided)
9. 50X Conc.Wash Solution (1 vial, 20 mL)

3. Procedure

A. Preparation of the sample:-
   - The S100B determination carried out in human serum. We didn't use hemolyzed samples.
   - We stored samples at -20°C.

B. Preparation of the Standards and Controls:-
- Standards and controls were reconstituted with 1 mL of distilled water before use; and stored at -20°C.

C. Preparation of the Conjugate:-
- Preparation started 2 hours before used.
- We added 50 µL conjugate (reagent 4) to 1.0 mL of Conjugate Buffer (reagent 3).
- We mixed gently for 5 minutes, with a rotating mixer.

D. Preparation of the wash solution:-
- We diluted 10 mL of Wash Solution Concentrate (50X) with 490 mL of distilled or deionized.

E.Procedure:-
- As it was necessary to perform the determination in duplicate, we prepared two wells for each of the seven points of the standard curve (S0-S5), two for each Control, two for each sample, one for Blank.

Reagent	Standard	Sample/ Controls	Blank
Standard S0-S5	50 µL		
Sample/Controls		50 µL	
Assay Buffer	50 µL	50 µL	
• It was incubated 2 *h* at room temperature (22-28°C). • We removed the content from each well, washed the wells six times with 300 µL of diluted wash solution			
Diluted Conjugate	100 µL	100 µL	
• It was incubated 1 *h* at room temperature (22-28°C). • We removed the content from each well, washed the wells six times with 300 µL of diluted wash solution			
TMB-Substrate	100 µL	100 µL	100 µL
• It was incubated*30 minutes* at room temperature (22-28°C), in the dark.			
Stop solution	100 µL	100 µL	100 µL
• We shaked gently the microplate. • We readed the absorbance (E) at 450 nm against Blank			

4. Results

A. Mean Absorbance:-

- We calculated the mean of the absorbencies (Em) for each point of the standard curve and of each sample.

B. Standard Curve:-

- We ploted the values of absorbance of the standards (S0 – S5) against concentration. We drawed the best-fit curve through the plotted points.

C. Calculation of Results:-

- We interpolated the values of the samples on the standard curve to obtain the corresponding values of the concentrations in pg/mL.

Neopterin

1. Principle of the test

- The Neopterin assay is a competitive enzyme immunoassay for the quantitative determination of neopterin in serum, plasma and urine using high affinity monoclonal antibody specific for neopterin. An unknown amount of antigen (neopterin) in the sample and a fixed amount of enzyme labelled antigen (neopterin bound horse radish peroxidase) compete for the antibody binding sites (mouse monoclonal antibody against neopterin).
- Both antigen-monoclonal antibody complexes binded to the wells of the microtiter strips coated with a goat-anti-mouse antibody. Unbounded antigen and conjugate (neopterin bound horse radish peroxidase) were removed by washing.
- The intensity of the color developed after the substrate incubation was directly proportional to the amount of bound conjugate and inversely proportional to the amount of neopterin in the standard or sample.
- Therefore, as the concentration of neopterin in the sample or standard increased, the intensity of the blue color was decreased. An acidic stop solution was added which changed the chromogen color from blue to yellow. The microwells were measured optically by a microplate reader with an absorbance filter of 450nm (OD450).
- The optical densities of the samples were compared to the OD's of the kit standards and an interpretative result was determined. The results were calculated by plotting a Standard curve (optical density versus concentrations of neopterin standard), from which the neopterin concentrations in the patient samples could be readed off directly.

2. Specimen collection, handling andstorage

- The usual precautions for venipuncture was observed. The chemical integrity of a blood specimen from the moment it was collected until it was assayed .
- Samples were stored at -20 °C.

3. Test procedure

A. general remarks

1. Before performing the assay, samples and assay kit were brought to room temperature (about 30 minutes beforehand) and we ensured the homogeneity of the solution.
2. All reagents were mixed without foaming.
3. All Standards were run with each series of unknown samples.
4. It was recomended that each standard and sample should be assayed in duplicate each time the test was performed.
5. Standards were subjected to the same manipulations and incubation times as the samples being tested.
6. Once the test had been started, all steps were completed without interruption.
7. We used new disposable plastic pipette tips for each reagent, standard or specimen in order to avoid cross contamination.
8. We avoided direct sunlight during the assay.

B. Assay procedure

1.	We used a pipette 25 µl of each Standard, controls, serum/plasma sample into the appropriate wells.
2.	We used a pipette 50 µl of Ready-to-Use Enzyme Conjugate into each well.
3.	We used a pipette 100 µl of Ready-to-Use Neopterin monoclonal antibody into each well.
4.	We covered the plate with the adhesive foil and incubated it in the dark for 90 minutes at room temperature (20 – 25 °C).
5.	We removed adhesive foil. We aspirated the incubation solution. We washed the plate 3 times with 300 µL of diluted Wash Buffer. We removed excess solution by tapping the inverted plate on a paper towel.
6.	We used a pipette 200 µL of Ready-to-Use TMB Substrate Solution into each well.
7.	It was incubated 15 min at 18-25°C in the dark. (Without adhesive foil.)
8.	We stopped the substrate reaction by adding 100 µL of Stop Solution into each well. We briefly mixed contents by gently shaking the plate. Color was changed from blue to yellow.
9.	We measured optical density with a photometer at 450 nm within 15 min after pipetting of the Stop Solution.

C. Calculation of results

1. We calculated the average absorbance values (OD450) for each set of duplicate Standards, samples and controls.
2. If individual absorbance values differed by more than 15% from the corresponding mean value, the result was considered suspect and the sample was re-assayed.
3. Either logit-log or semi-log graph paper was used for manual construction. We constructed a standard curve by plotting the OD450 nm for each of 6 standards (disregarding thezero standard) on the vertical linear y-axis versus the corresponding neopterin concentration on the horizontal logarithmic x-axis, thus we created a smooth standard curve with maximum 1 tuning point.
4. The concentration of the samples were readed directly from this standard curve. We used the mean absorbance value for each sample, we determined the corresponding concentration of neopterin from the standard curve.
5. Any sample reading was greater than the highest standard was diluted appropriately with zero standard. In this case the concentration determined from the standard-curve was multiplied by the dilution factor.

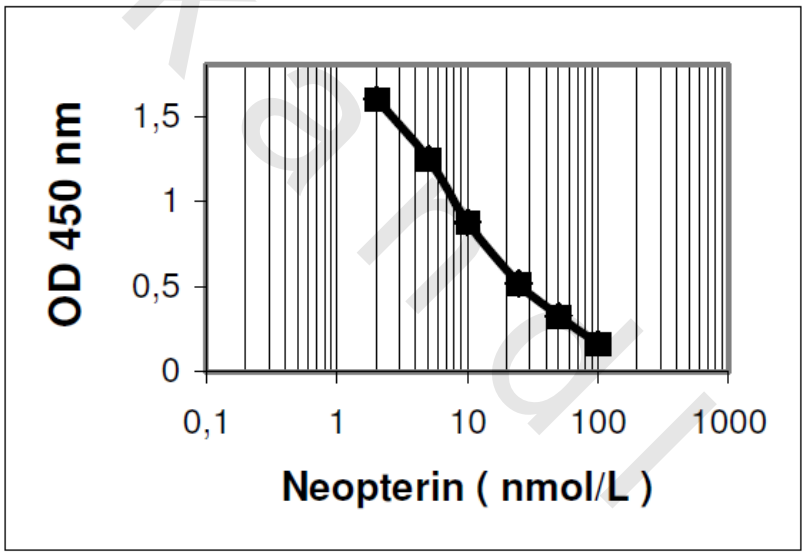

Figure (4): Typical Calibration Curve

4. Assay characteristics

A. Expected physiological ranges:

- Normal: < 10 nmol/L (0.3-3.0 ng/mL).
- Conversion: Neopterin (nmol/L) x 0.253 = ng/mL.

RESULTS

The present study was conducted on 40 subjects classified into 2 groups:

- Group I: consisted of twenty patients with clinical manifestations and positive CSF criteria of suspected viral encephalitis.
- Group II: comprised of twenty apparently healthy volunteers of matching age and sex.

Demographic data distribution: Table (5)

Age and gender

In group (I), the mean age was 22.45 ± 13.83 with range of 5 to 45 years. While in group (II) the mean age was 22.45 ± 13.83 with range of 5 to 45 years, with no statistical significant difference between the two studied groups regarding to age.

As regards gender, in group (I) the males were 40% and females were 60%, and in group (II) the males were 40% and females were 60%, with no statistical significant difference between the two studied groups regarding to gender.

Table (5): Comparison between the two studied groups according to demographic data

	Group I (n = 20)		Group II (n = 20)		Test of sig.	p
	No	%	No	%		
Gender						
Male	8	40.0	8	40.0	$\chi^2 = 0.0$	1.000
Female	12	60.0	12	60.0		
Age						
Min. - Max.	5.0 – 45.0		5.0 – 45.0		t = 0.0	1.000
Mean ± SD	22.45 ± 13.83		22.45 ± 13.83			

χ^2: Chi square test
t: Student t-test

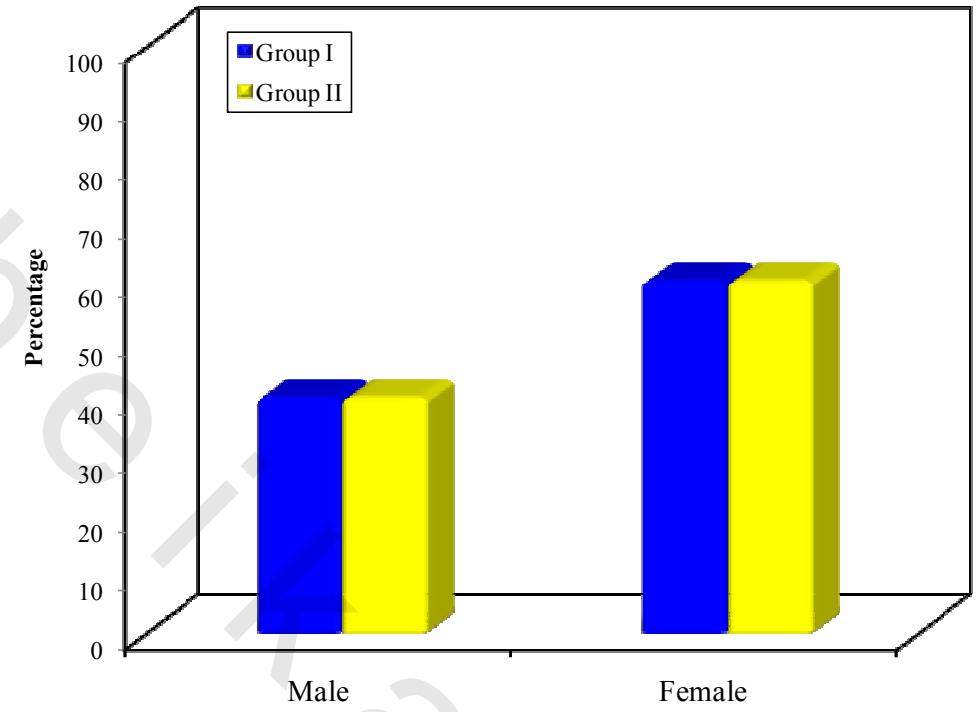

Figure (5): Comparison between the two studied groups according to gender

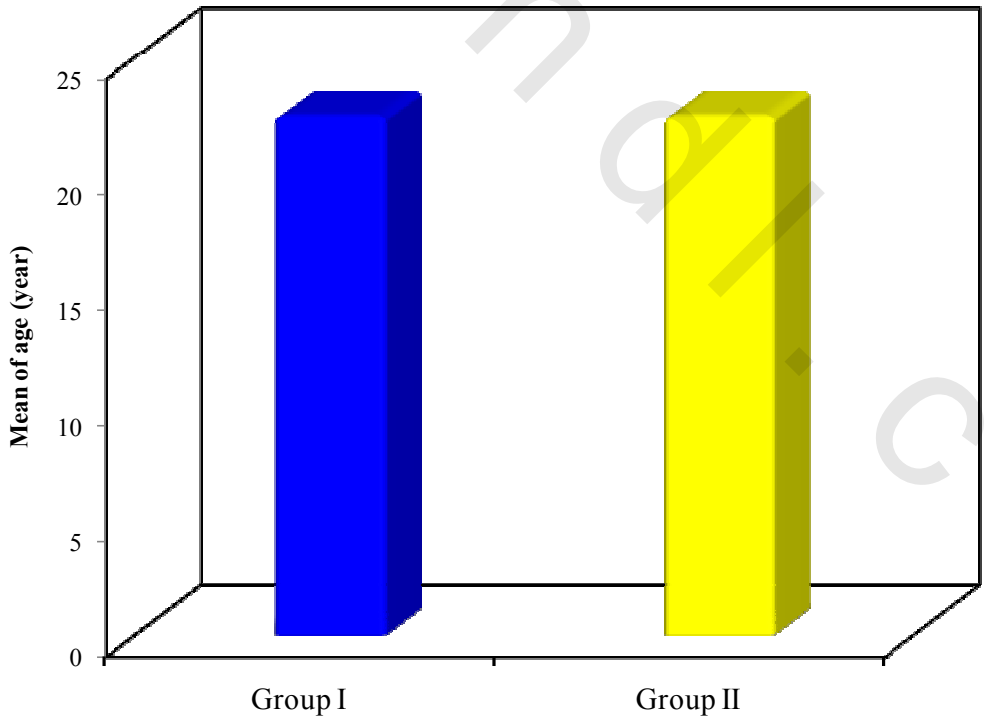

Figure (6): Comparison between the two studied groups according to age

The clinical manifestations: Table (6)

Regarding to repiratory manifestations there was 6 of the patients had acute bronchitis represents 15%.

Regarding to acute gastero-entestinal manifestations there was 13 of the patients had acute gastero-enteritis represents 32.5%.

Regarding to skin rash there was 1 of the patients had vesicular rash represents 5 %.

Regarding to days fever ranged from 2.0 -15.0, with a mean of 5.95 ± 3.66.

Table (6): Distribution of the studied cases according to prodromal symptoms (n = 20)

	No	%
Acute bronchitis	6	30
Acute gastero-enteritis	13	65
Vesicular rash	1	5.0
Days of fever Min. - Max. Mean ± SD	 2.0 - 15.0 5.95 ± 3.66	

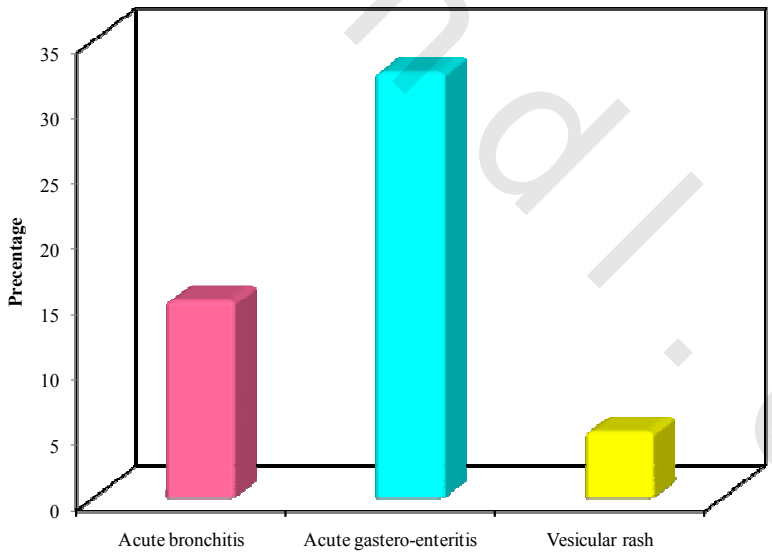

Figure (7): Distribution of the studied cases according to prodromal symptoms

The CNS manifestations: Table (7)

Regarding to conscious level there was 10 of the patients were drowsy representing 50%, 2 of the patients were comatosed representing 10% and 8 of the patients were semi-consious representing 40%.

Regarding to ataxia in there was 5 of the patients had ataxia representing 25%. Regarding to convulsion there was 14 of the patients had convulsion representing 70%. Regarding to abnormal gait there was 3 of the patients had abnormal gait representing 15%. Regarding to delayed speech there was 3 of the patients had delayed speech representing 15%. Regarding to eye symptoms there was 5 of the patients had eye symptoms representing 15%.

Table (7): Distribution of the studied cases according to central nervous system manifestations (n = 20)

	No	%
Conscious level		
Drowsy	10	50.0
Comatosed	2	10.0
Semi-conscious	8	40.0
Conscious	0	0.0
Ataxia	5	25.0
Convulsion	14	70.0
Abnormal gait	3	15.0
Delayed speech	3	15.0
Eye symptoms	5	25.0

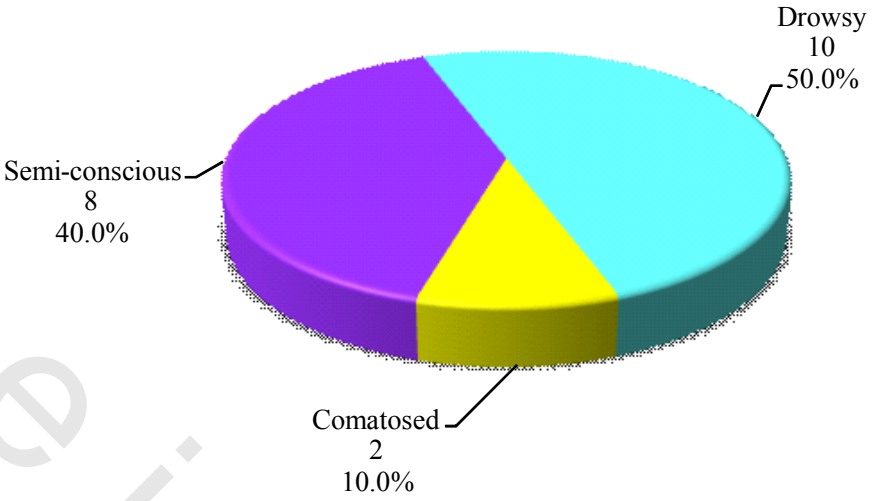

Figure (8): Distribution of the studied cases according to conscious level

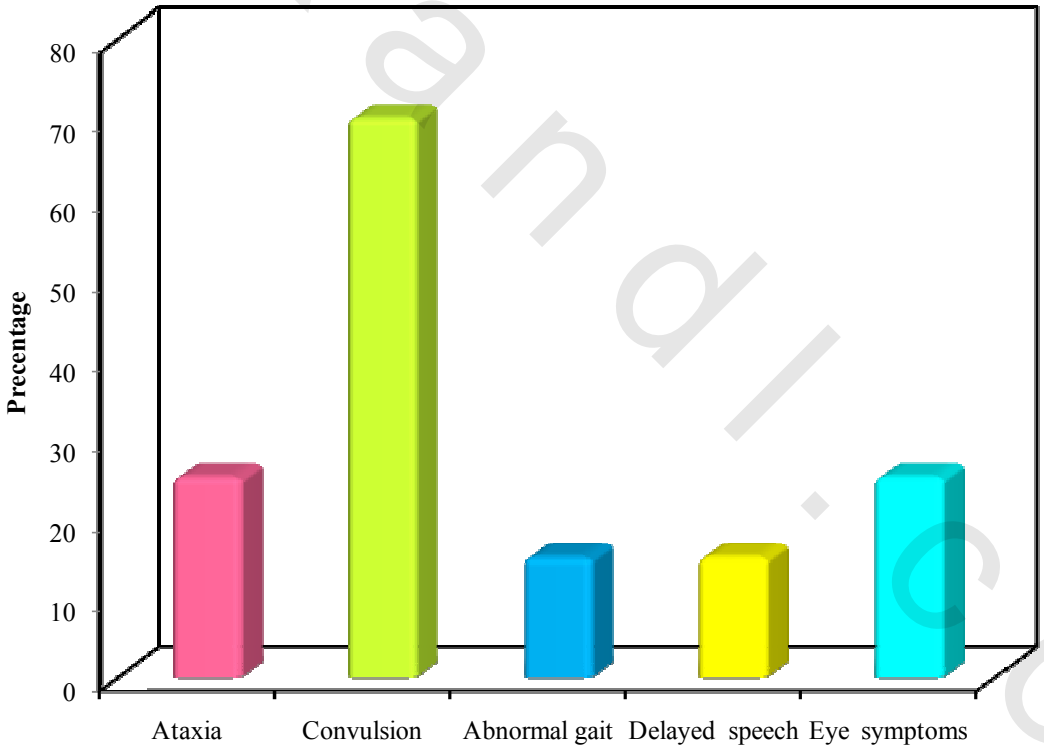

Figure (9): Distribution of the studied cases according to central nervous system manifestations

Laboratory investigations: (Table 8)

A- complete blood picture (Table 8)

Table (8) shows comparison between the two studied groups regarding to routine investigations.

Regarding to haemoglobin concentration (Hb) (gm/dl), Group (I) Hb ranged from 9.50 - 15.70 gm/dl with a mean value of 12.15 ±1.68 gm/dl. Group (II) Hb ranged from 9.50 -14.10 gm/dl with a mean value of 12.60 ±1.22 gm/dl. There was no statistically significant difference between two studied groups regarding to Hb.

Regarding to red blood cells count (RBCs) (million/ul), Group (I) RBCs ranged from 3.06 – 5.70 with a mean value of 4.34 ± 0.78. Group (II) RBCs ranged from 3.17 – 5.88 with a mean value of 4.79 ± 0.80. There was no statistically significant difference between the two studied groups regarding to RBCs.

Regarding to white blood cells count (WBCs) (thousands/ul), Group (I) WBCs ranged from 5.15 – 21.13 with a mean value of 10.04 ± 4.41. Group (II) WBCs ranged from 5.50 – 10.10 with a mean value of 7.58 ±1.48. There was no statistically significant difference between the two studied groups regarding to WBCs.

Regarding to platelet count, Group (I) platelets ranged from 162.0 – 390.0 with a mean value of 298.85 ± 61.13. Group (II) platelets ranged from 160.0 – 400.0 with a mean value of 256.05 ± 70.18. There was no statistically significant difference between the two studied groups regarding to platelets.

B- Random blood sugar (Table 8)

Regarding to Random blood sugar Group (I) Random blood sugar ranged from 109.0 – 460.0 with a mean value of 152.35 ± 74.82. Group (II) Random blood sugar ranged from 120.0 – 140.0 with a mean value of 129.25 ± 6.20. There was no statistically significant difference between the two studied groups regarding to Random blood sugar.

Table (8): Comparison between the two studied groups according to laboratory investigations

	Group I (n = 20)	Group II (n = 20)	Test of sig.	p
HB (gm/dL)				
Min. - Max.	9.50 - 15.70	9.50 - 14.10	t = 0.969	0.339
Mean ± SD	12.15 ± 1.68	12.60 ± 1.22		
RBCs *1000000/uL				
Min. - Max.	3.06 – 5.70	3.17 – 5.88	t = 1.811	0.078
Mean ± SD	4.34 ± 0.78	4.79 ± 0.80		
WBCs *1000/uL				
Min. - Max.	5.15 - 21.13	5.50 - 10.10	Z = 1.651	0.099
Mean ± SD	10.04 ± 4.41	7.58 ± 1.48		
Platelets *1000/uL				
Min. - Max.	162.0 - 390.0	160.0 - 400.0	t = 1.624	0.113
Mean ± SD	298.85 ± 61.13	265.05 ± 70.18		
Random blood sugar (mg/dl)				
Min. - Max.	109.0 - 460.0	120.0 - 140.0	t = 0.677	0.498
Mean ± SD	152.35 ± 74.82	129.25 ± 6.20		

p: p value for comparing between the two studied groups
t: Student t-test
Z: Z for Mann Whitney test
*: Statistically significant at $p \leq 0.05$

C-The acute phase reactant tests:

Table (9) show comparison between the two studied groupsregarding to C-reactive protein (CRP) and erythrocytesedimentation rate ESR 1st and 2nd.

Regarding to serum CRP, Group (I) serum CRP ranged from 10.0-30.0 mg/dl with a mean value of 14.90± 5.06 mg/dl .Group (II) serum CRP ranged from 1.0- 3.0 mg/dl with a mean value of 1.90 ± 0.85 mg/dl. The mean CRP was significantly higher in groups (I) than in group (II).

Regarding to serum ESR 1st, Group (I) serum ESR 1st ranged from 15.0-40.0 with a mean value of 23.35± 8.24. Group (II) serum ESR 1st ranged from 5.0- 14.0 with a mean value of 8.40 ± 2.78. The mean ESR 1st was significantly higher in groups (I) than in group (II).

Regarding to serum ESR 2nd, Group (I) serum ESR 2nd ranged from 25.0-80.0 with a mean value of 47.50 ± 14.82. Group (II) serum ESR 2nd ranged from 10.0- 24.0 with a mean value of 17.70 ± 3.54. The mean ESR 2nd was significantly higher in groups (I) than in group (II).

Table (9): Comparison between the two studied groups according to CRP and ESR 1st and 2nd

	Group I (n = 20)	**Group II (n = 20)**	**Test of sig.**	**p**
CRP (mg/dl)				
Min. - Max.	10.0 - 30.0	1.0 - 3.0	Z = 5.454*	<0.001*
Mean ± SD	14.90 ± 5.06	1.90 ± 0.85		
ESR 1st hrs				
Min. - Max.	15.0 - 40.0	5.0 - 14.0	t = 7.692*	<0.001*
Mean ± SD	23.35 ± 8.24	8.40 ± 2.78		
ESR 2nd hrs				
Min. - Max.	25.0 - 80.0	10.0 - 24.0	t = 8.744*	<0.001*
Mean ± SD	47.50 ± 14.82	17.70 ± 3.54		
t_1 (p)	14.679* (<0.001*)	25.587* (<0.001*)		

p: p value for comparing between the two studied groups
Z: Z for Mann Whitney test
t: Student t-test
t_1: Paired t-test for comparing between 1st and 2nd ESR
*: Statistically significant at $p \leq 0.05$

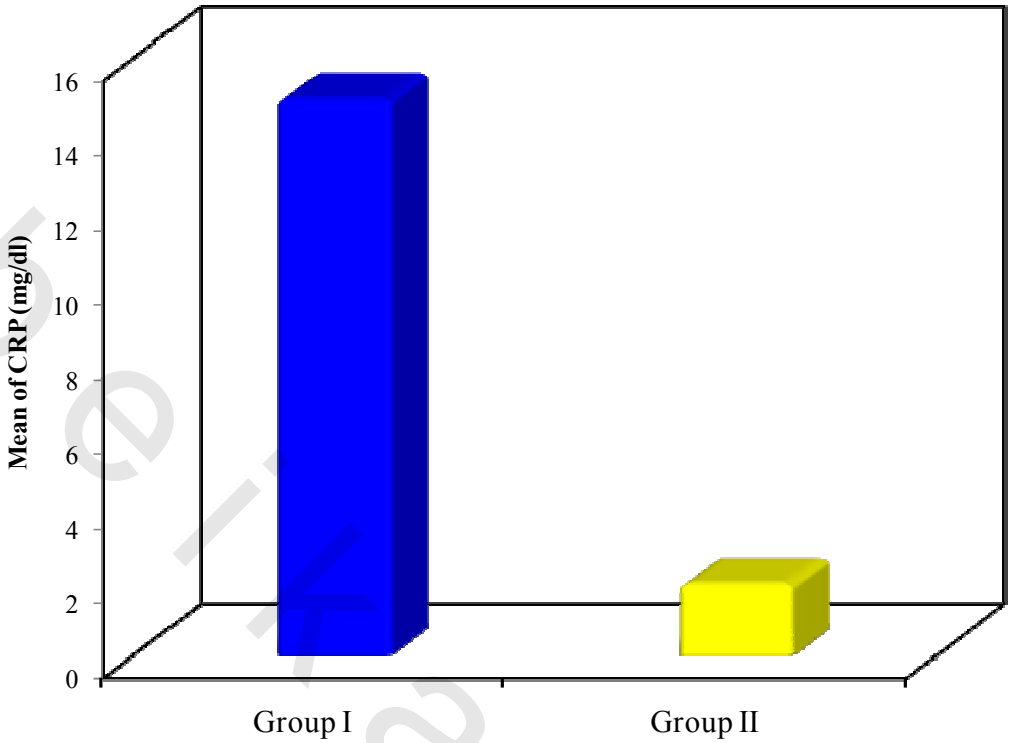

Figure (10): Comparison between the two studied groups according to CRP

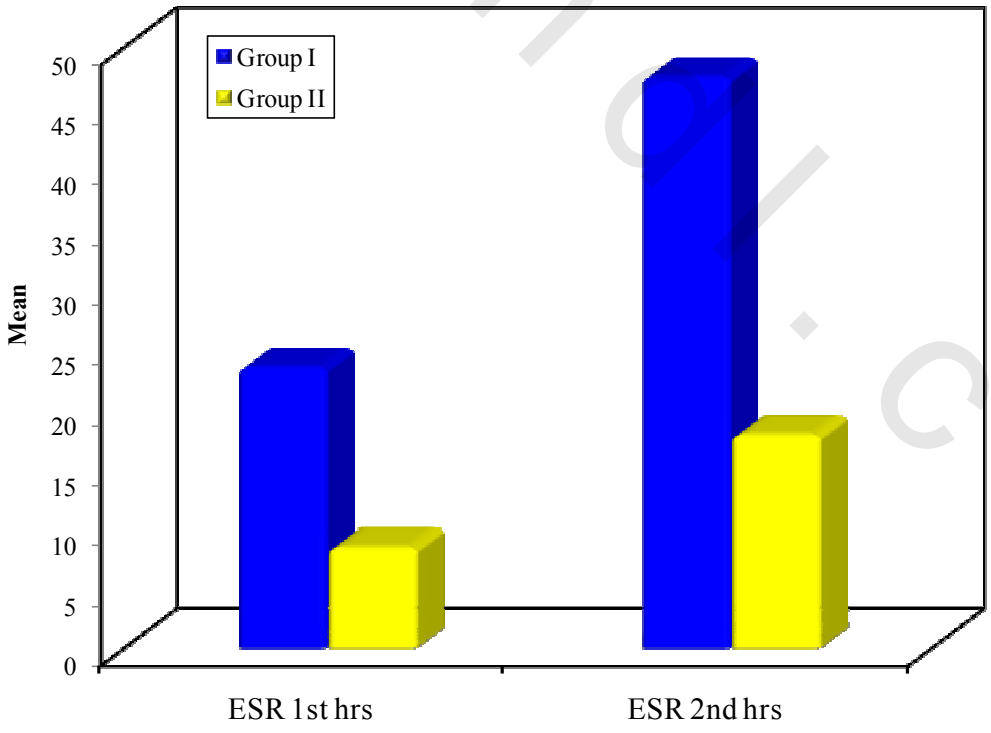

Figure (11): Comparison between the two studied groups according to ESR 1st and 2nd

CSF crieteria (Table 10)

Table (10) show distribution of the studied cases according to CSF crieteria.

Regarding to CSF appearance, there was 18 patients had clear appearance representing 90% and 2 patients had opalescent appearance representing 10%. Regarding to CSF culture for bacterial infection, there was 20 patients had negative culture for bacteria representing 100% and zero patients had positive culture for bacterial infection. Regarding to CSF glucose ranged from 70.0-171.0 with a mean value of 85.25± 23.57. Regarding to CSF protein ranged from 11.0-30.0 with a mean value of 22.20 ± 5.43. Regarding to CSF WBCs ranged from 30.0-1700.0 with a mean value of 287.25 ± 457.52. Regarding to CSF lymphocyte it was 100.0 with a mean value of 100.0± 0.0. Regarding to CSF polymorph nuclear leukocytes there was no cells. Regarding to CSF lactate ranged from 9.0-65.0 with a mean value of 19.15 ± 12.63.

Table (10): Distribution of the studied cases according to CSF (n = 20)

	No	%
CSF Appearance		
Clear	18	90.0
Opalescent	2	10.0
CSF Culture for bacteria		
Positive	0	0%
Negative	20	20%
Glucose (mg/dl)		
Min. - Max.	70.0 - 171.0	
Mean ± SD	85.25 ± 23.57	
Protein (mg/dl)		
Min. - Max.	11.0 - 30.0	
Mean ± SD	22.20 ± 5.43	
WBCs /mm		
Min. - Max.	30.0 - 1700.0	
Mean ± SD	287.25 ± 457.52	
Lymphocytes		
Min. - Max.	100.0 - 100.0	
Mean ± SD	100.0 ± 0.0	
Polymorph nuclear leukocytes		
Min. - Max.	0.0 - 0.0	
Mean ± SD	0.0 ± 0.0	
Lactate (mg/dl)		
Min. - Max.	9.0 - 65.0	
Mean ± SD	19.15 ± 12.63	

S 100 B Protein (Table 11) (Figure 12)

Regarding to S100 B protein in group (I) it ranged between 0.22-10.0 with a mean value of 1.72 ± 2.29.Group (II) it ranged between 0.01-0.04 with a mean value of 0.02 ± 0.01. The mean S100 B protein was significantly higher in groups (I) than in group (II).

Serum neopetrin (Table 11), (Figure 13)

Regarding to Neopetrin in group (I) it ranged between 162.06 – 434.78 with a mean value of 320.24 ± 93.92. Group (II) it ranged between 5.14 – 18.90 with a mean value of 12.48 ± 3.64. The mean Neopetrin was significantly higher in groups (I) than in group (II).

Table (11): Comparison between the two studied groups according to S 100 B Protein and Neopetrin

	Group I (n = 20)	**Group II (n = 20)**	**Test of sig.**	**p**
S 100 B Protein (µg/l)				
Min. - Max.	0.22 - 10.0	0.01 - 0.04	Z = 5.413*	<0.001*
Mean ± SD	1.72 ± 2.29	0.02 ± 0.01		
Neopetrin (nmol/l)				
Min. - Max.	162.06 - 434.78	5.14 - 18.90	t = 14.643*	<0.001*
Mean ± SD	320.24 ± 93.92	12.48 ± 3.64		

p: p value for comparing between the two studied groups
t: Student t-test
Z: Z for Mann Whitney test
*: Statistically significant at $p \leq 0.05$

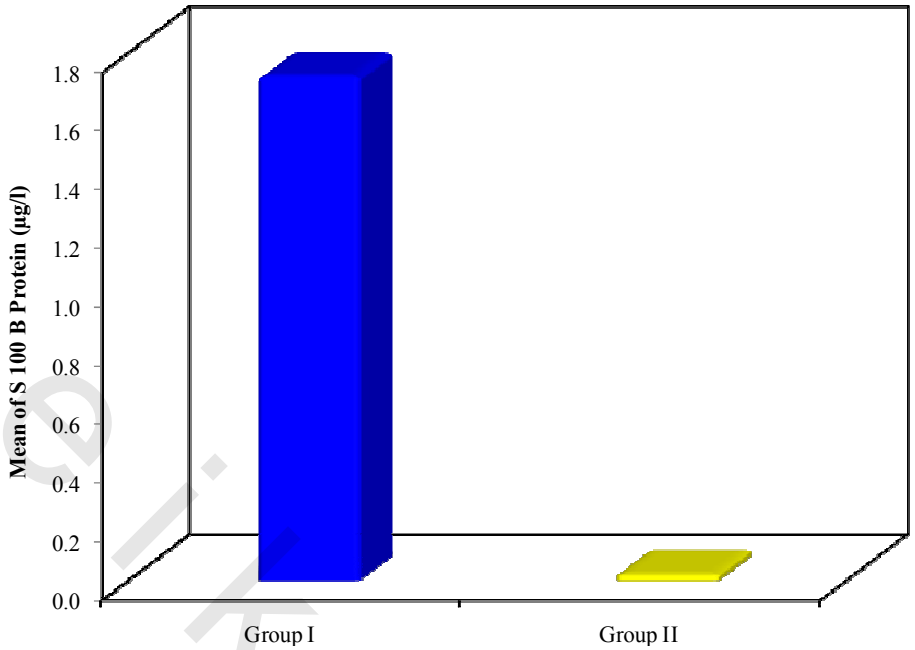

Figure (12): Comparison between the two studied groups according to S 100 B Protein.

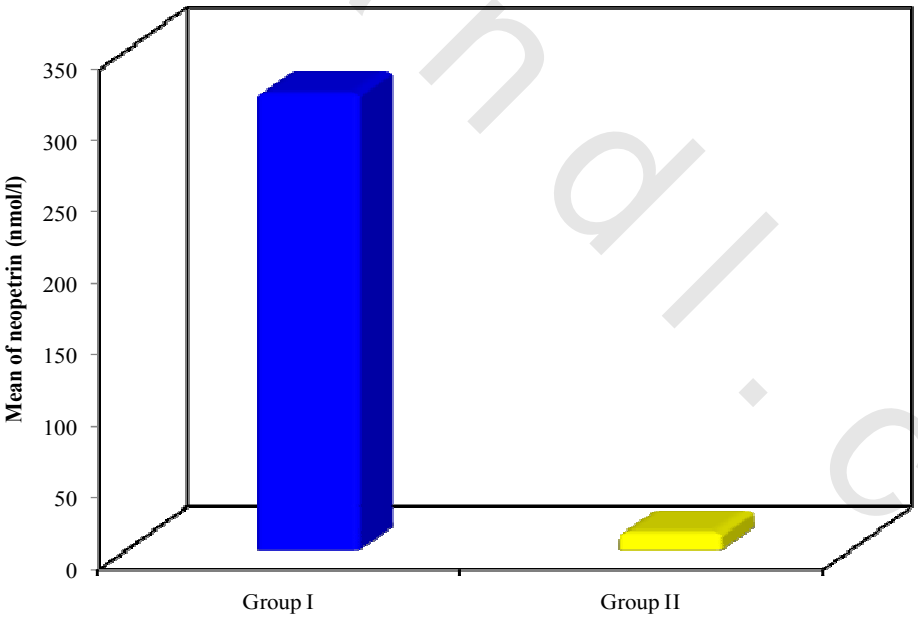

Figure (13): Comparison between the two studied groups according to neopetrin

Imaging studies (Table 12)

Regarding to MRI criteria there was 13 patients had unremarkable image (65%), 4 patients had brain oedema (20%), 2 patient had frontal lobe and subcortical white matter (distribution of enterovirus) (10%) and one patient had demylinating disorder of paieto-occipital region (5%).

Regarding to CT crietaria there was 18 patients had unremarkable image (90%) and 2 patients had remarkable image (10%).

Table (12): Distribution of the studied cases according to MRI criteria and CT brain (n = 20)

	No	%
MRI criteria		
Unremarkable	13	65.0
Remarkable	7	35.0
Criteria of MRI		
Nil	13	65.0
Brain oedema	4	20.0
Frontal lobe and subcortical white matter (enterovirus)	2	10.0
Demylinating disorder of paieto-occipital region	1	5.0
CT brain		
Unremarkable	18	90.0
Remarkable	2	10.0

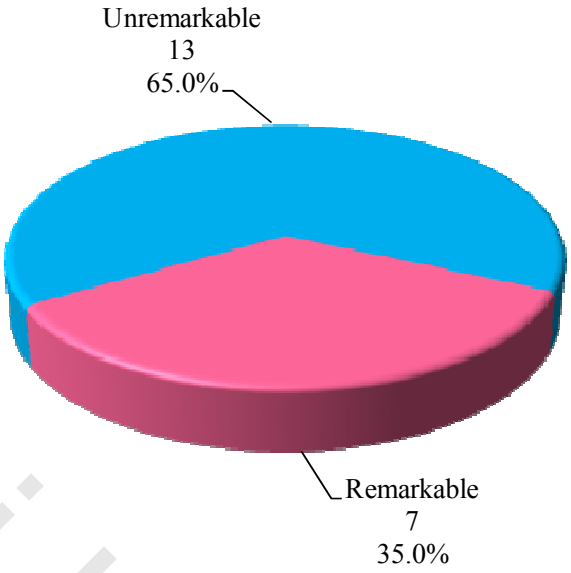

Figure (14): Distribution of the studied cases according to MRI criteria

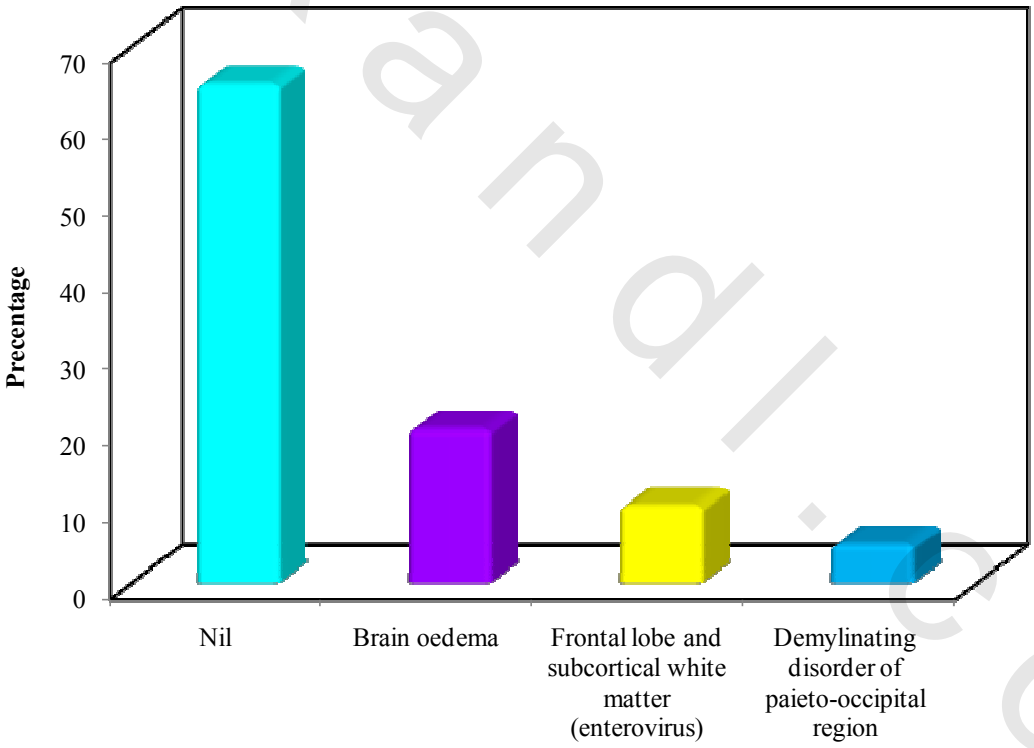

Figure (15): Distribution of the studied cases according to criteria of MRI

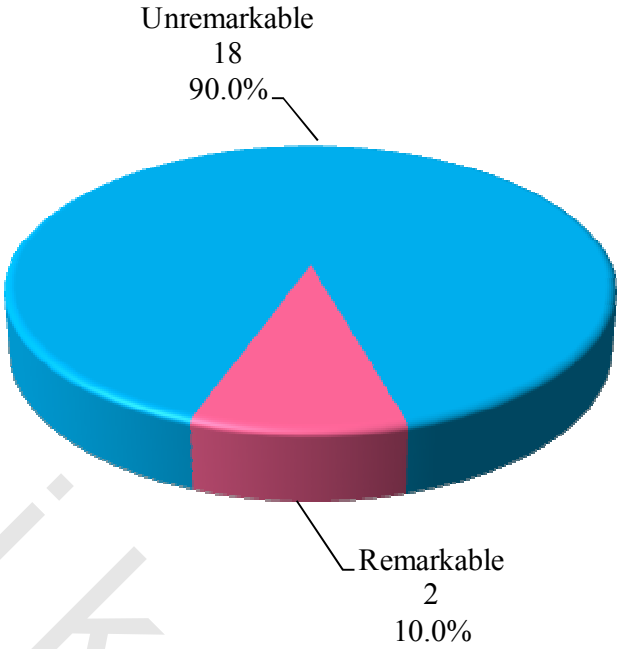

Figure (16): Distribution of the studied cases according to CT brain

The course of the disease

Table (13) show distribution ofthe studied cases according to progress of disease.

Regarding to patients were ventilated or not there was 16 patients had not ventilated (80%), 4 patients had ventilated (20%). Considering days in intensive care unit (ICU) ranged from 1.0 to 18.0, with a mean of 4.35 ± 5.14.

As regards to days in word ranged from 0.0 to 26.0, with a mean of 12.45 ± 5.86.

Regarding to complications there was 10 patients had complications (50%), 10 patients had not complications (50%).

According to death there was 16 patients lived (80%), 4 patients died (20%).

Table (13): Distribution of the studied cases according to progress of disease (n = 20)

	No	%
Ventilated or not		
No	16	80.0
Yes	4	20.0
Days in ICU		
Min. - Max.	1.0 - 18.0	
Mean ± SD	4.35 ± 5.14	
Days in word		
Min. - Max.	0.0 - 26.0	
Mean ± SD	12.45 ± 5.86	
Complications		
No	10	50.0
Yes	10	50.0
Death		
No	16	80.0
Yes	4	20.0

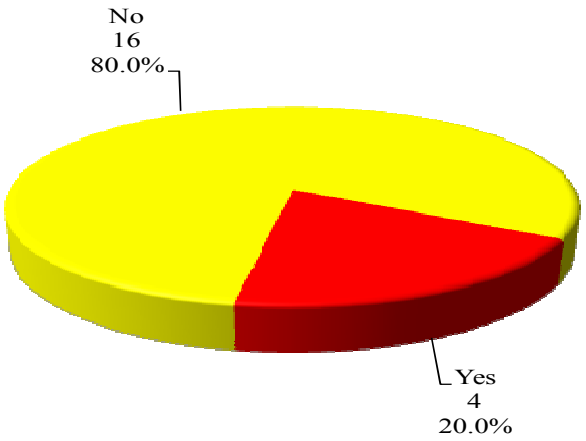

Figure (17): Distribution of the studied cases according to patients who ventilated or not

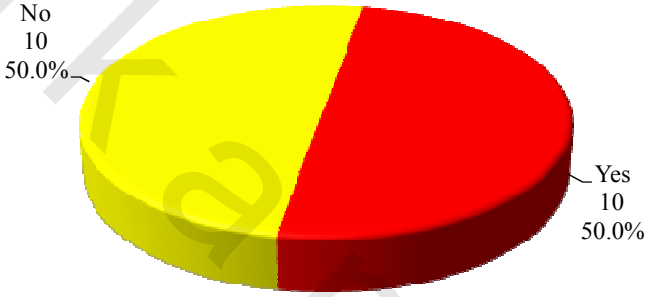

Figure (18): Distribution of the studied cases according to complications

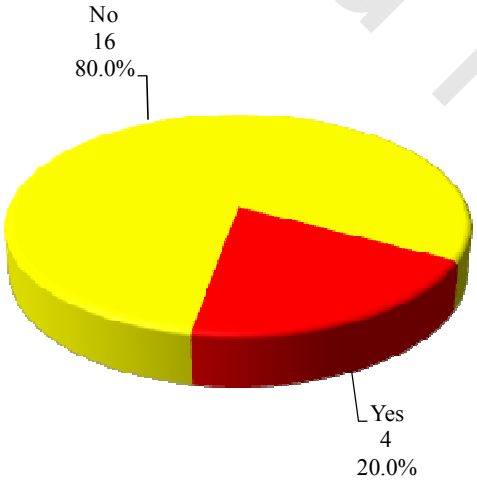

Figure (19): Distribution of the studied cases according to death

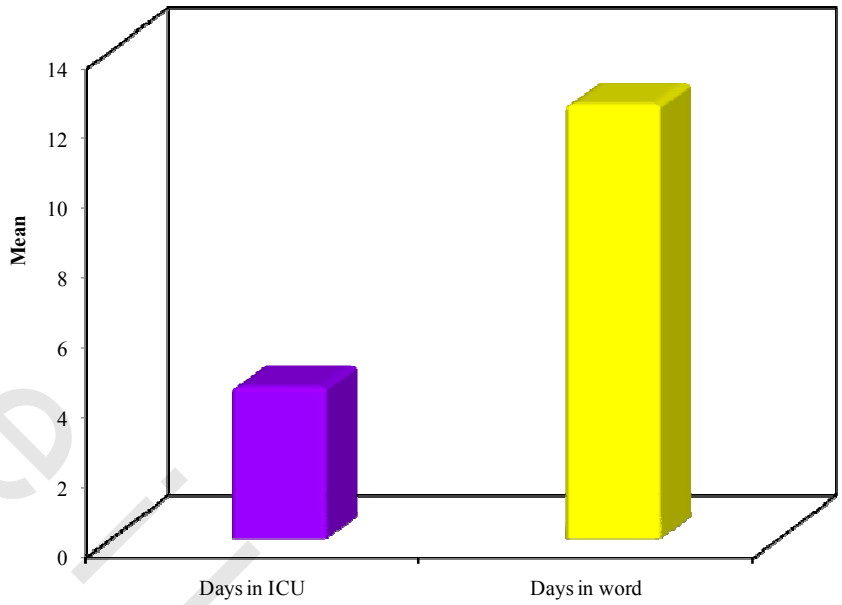

Figure (20): Distribution of the studied cases according to progress of disease

Table (14) show distribution of the studied cases according to Complications.

Regarding to complications there was 5 patients had ataxia (25%), 3 patients had abnormal gait (15%), 3 patients had delayed speech (15%), 5 patients had eye symptoms (25%).

Table (14): Distribution of the studied cases according to Complications (n = 20)

	No	%
Complications		
No	10	50.0
Yes	10	50.0
Ataxia	5	25.0
Abnormal gait	3	15.0
Delayed speech	3	15.0
Eye symptoms	5	25.0

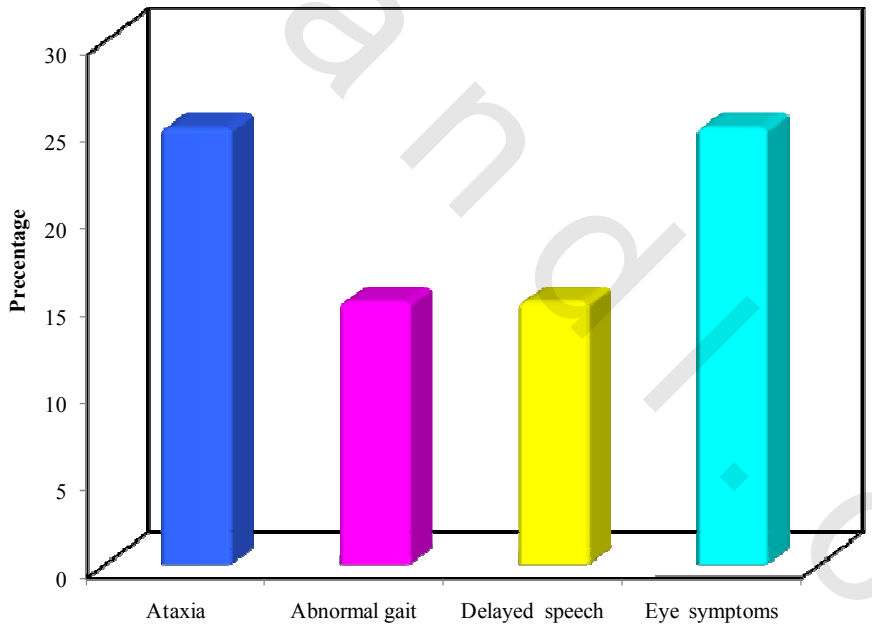

Figure (21): Distribution of the studied cases according to Complications

Correlations between gender with S 100 B protein andNeopetrin in patients

Table (15) show relation between gender with S 100 B proteinand neopetrin in patients.

The mean of S100 B protein in male patients was 1.58± 0.82 while in female patients was 1.82± 2.94. There were no statistical significant difference between males and females regarding to S 100 B protein (P= 0.059).

As regards neopetrin, the mean in male patients was 309.78± 88.08 while in female patients was 327.21± 100.83. There were no statistical significant difference between males and females regarding to neopetrin (P= 0.696).

Table (15): Relation between gender with S 100 B protein and neopetrin in patients.

	gender		Test of sig.	p
	Male (n = 8)	**Female (n = 12)**		
S 100 B Protein (µg/l)				
Min. - Max.	0.84 - 3.11	0.22 - 10.0		
Mean ± SD	1.58 ± 0.82	1.82 ± 2.94	Z = 1.891	0.059
Median	1.13	0.81		
Neopetrin (nmol/l)				
Min. - Max.	221.34 – 434.78	162.06 – 422.92		
Mean ± SD	309.78 ± 88.08	327.21 ± 100.83	t = 0.398	0.696
Median	276.67	387.36		

p: p value for comparing between male and female
Z: Z for Mann Whitney test
t: Student t-test

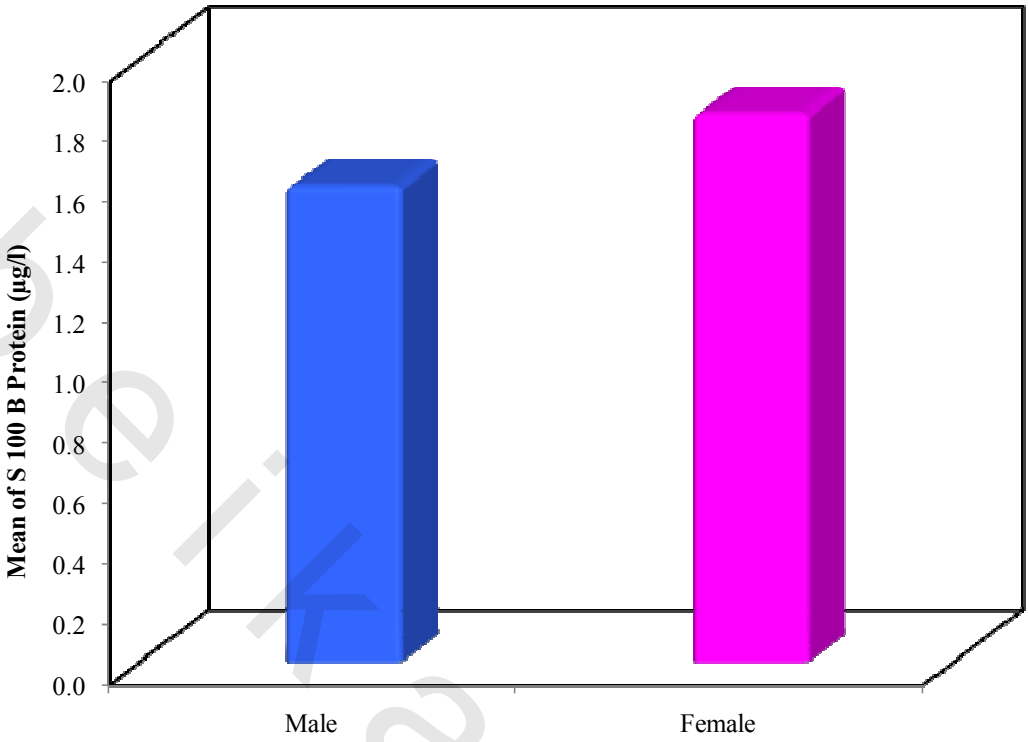

Figure (22): Relation between gender with S 100 B protein in patients.

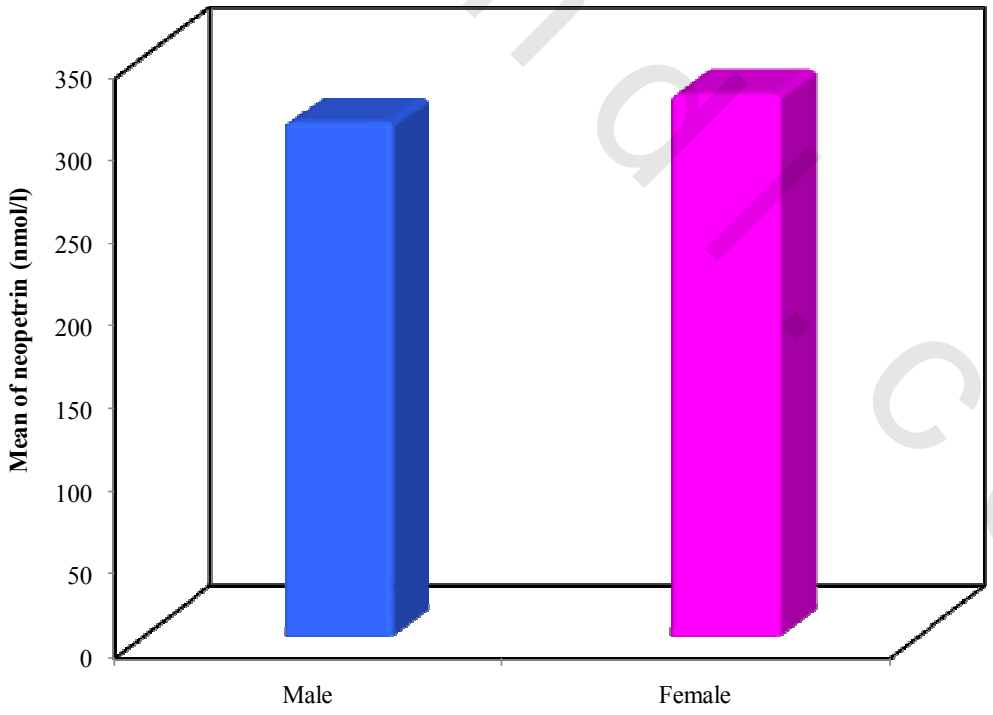

Figure (23): Relation between gender with neopetrin in patients.

Table (16) show relation between convulsion with S 100 Bprotein and neopetrin in patients.

Regarding to convulsion, 6 patients had no convulsion with a mean of S100 B protein 1.75 ± 1.95 while 14 patients had convulsion with a mean of S100 B protein 1.71 ± 2.94. There were no statistical significant difference between patients had no convulsion and patient had convulsion regarding to S 100 B protein (P= 0.902).

The mean of neopetrin in those patients without convulsion was 303.69 ± 95.34 while in those with convulsion was 327.33 ± 96.0. There were no statistical significant difference between patients had no convulsion and patient had convulsion regarding to neopetrin (P= 0.619).

Table (16): Relation between convulsion with S 100 B protein and neopetrin in patients.

	Convulsion		**Test of sig.**	**p**
	No (n = 6)	**Yes (n = 14)**		
S 100 B Protein (µg/l)				
Min. - Max.	0.22 – 5.40	0.31 – 10.0		
Mean ± SD	1.75 ± 1.95	1.71 ± 2.49	Z= 0.124	0.902
Median	1.13	0.96		
Neopetrin (nmol/l)				
Min. - Max.	197.63 – 395.26	162.06 – 434.78		
Mean ± SD	303.69 ± 95.34	327.33 ± 96.0	t = 0.506	0.619
Median	304.35	381.42		

p: p value for comparing between no convulsion and convulsion
Z: Z for Mann Whitney test
t: Student t-test

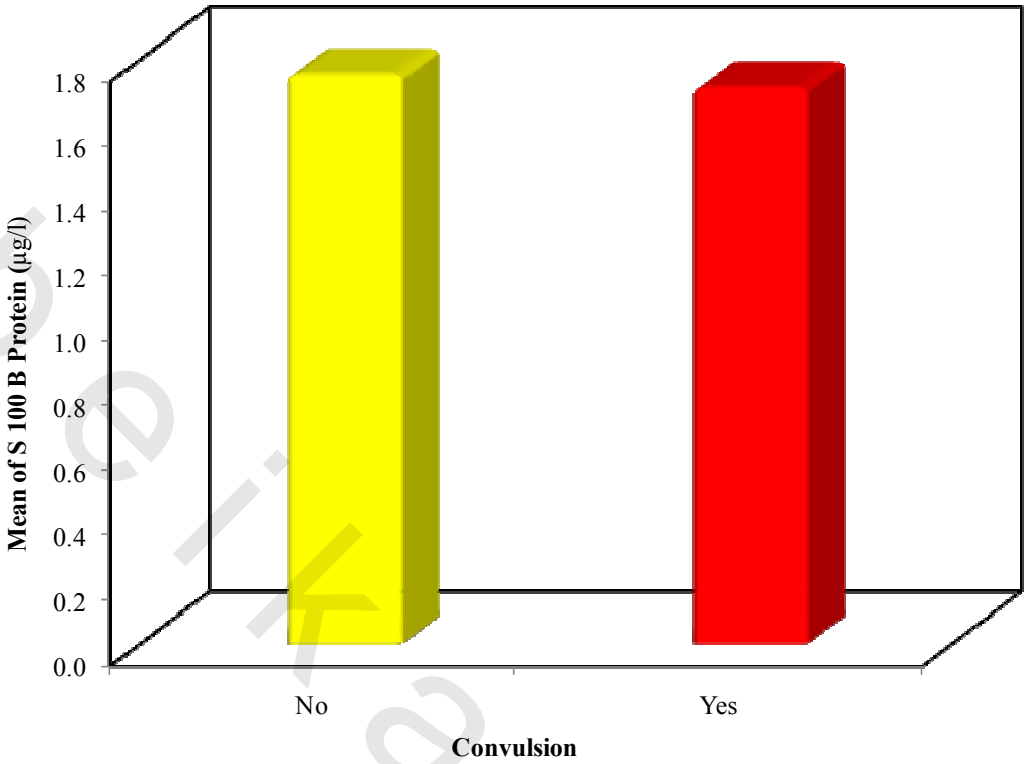

Figure (24): Relation between convulsion with S 100 B protein in patients.

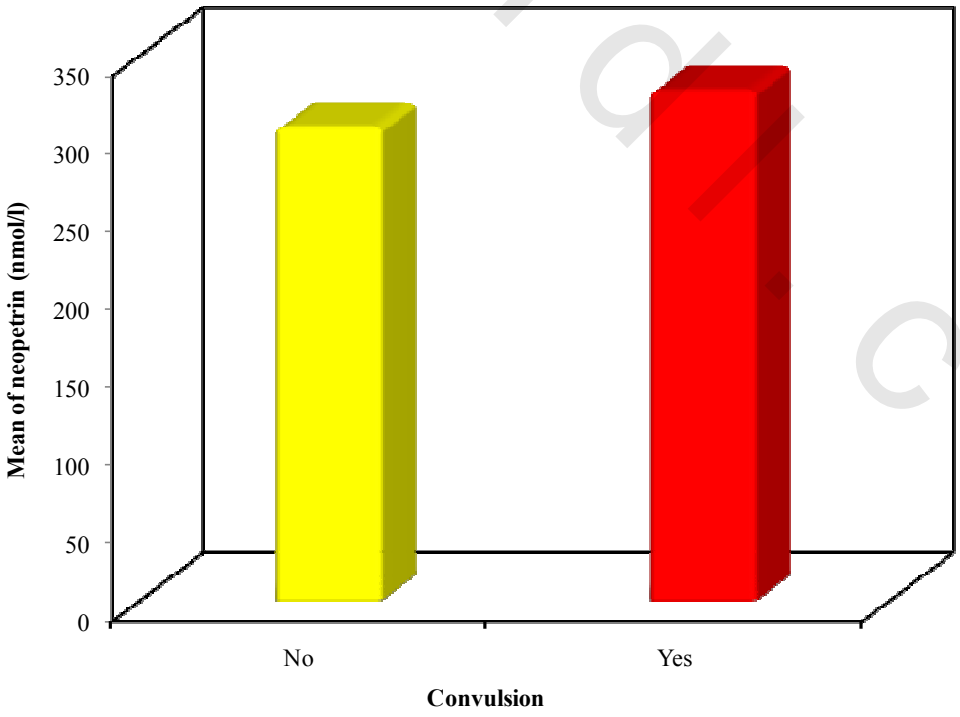

Figure (25): Relation between convulsion with neopetrin in patients

The relation between complications with S 100 B protein and neopetrin in patients

Table (17) show relation between abnormal gait with S 100 Bprotein and neopetrin in patients.

Regarding to abnormal gait, 17 patients had no abnormal gait with a mean of S100 B protein 1.79 ± 2.48 while 3 patients had a mean of S100 B protein 1.33 ± 0.67. There were no statistical significant difference between patients who had no abnormal gait and patient with abnormal gait regarding to S 100 B protein (P= 0.672).

Regarding to abnormal gait, 17 patients had no abnormal gait with a mean of neopetrin 303.98 ± 92.47 while 3 patients had abnormal gait with a mean of neopetrin 412.38 ± 20.28. The mean value of serum neopetrin was significantly higher in those patients with abnormal gait than in patients without abnormal gait.

Table (17): Relation between abnormal gait with S 100 B protein and neopetrin in patients.

	Abnormal gait		**Test of sig.**	**p**
	No (n = 17)	**Yes (n =3)**		
S 100 B Protein (µg/l)				
Min. - Max.	0.22 – 10.0	0.84 – 2.10		
Mean ± SD	1.79 ± 2.48	1.33 ± 0.67	Z= 0.424	0.672
Median	0.91	1.06		
Neopetrin (nmol/l)				
Min. - Max.	162.06 – 422.92	395.26 – 434.78		
Mean ± SD	303.98 ± 92.47	412.38 ± 20.28	t = 4.285*	0.001*
Median	284.58	407.11		

p: p value for comparing between no abnormal gait and abnormal gait
Z: Z for Mann Whitney test
t: Student t-test
*: Statistically significant at $p \leq 0.05$

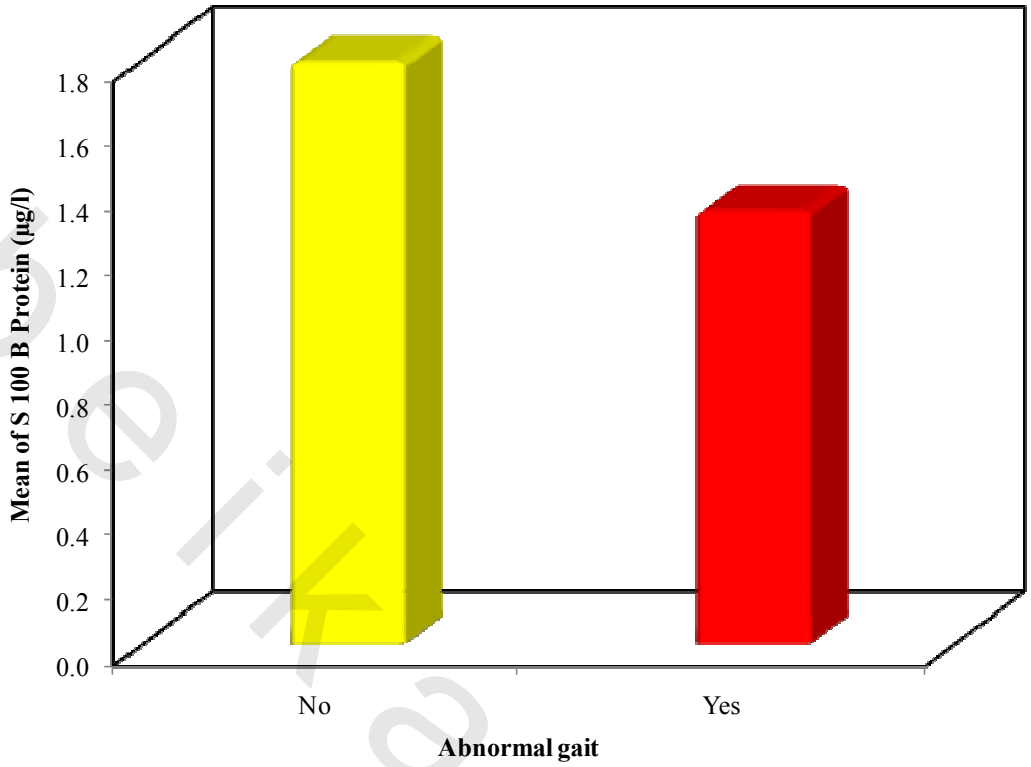

Figure (26): Relation between abnormal gait with S 100 B protein in patients.

Figure (27): Relation between abnormal gait neopetrin in patients.

Table (18) show relation between Delayed speech with S 100 B protein and neopetrin in patients.

Regarding to delayed speech , 17 patients had no delayed speech with the mean of S100 B protein 1.79 ± 2.48 while 3 patients had delayed speech with the mean of S100 B protein 1.33 ± 0.67. There were no statistical significant difference between patients had no delayed speech and patient had delayed speech regarding to S 100 B protein (P= 0.672).

Regarding to delayed speech, 17 patients had no delayed speech with the mean of neopetrin 303.98 ± 92.47 while 3 patients had delayed speech with the mean of neopetrin 412.38 ± 20.28. The mean value of serum neopetrin was significantly higher in those patients with delayed speech than in patients without delayed speech.

Table (18): Relation between Delayed speech with S 100 B protein and neopetrin in patients.

	Delayed speech		**Test of sig.**	**p**
	No (n = 17)	**Yes (n =3)**		
S 100 B Protein (µg/l)				
Min. - Max.	0.22 – 10.0	0.84 – 2.10		
Mean ± SD	1.79 ± 2.48	1.33 ± 0.67	Z= 0.424	0.672
Median	0.91	1.06		
Neopetrin (nmol/l)				
Min. - Max.	162.06 – 422.92	395.26 – 434.78		
Mean ± SD	303.98 ± 92.47	412.38 ± 20.28	t = 4.285*	0.001*
Median	284.58	407.11		

p: p value for comparing between no delayed speech and delayed speech
Z: Z for Mann Whitney test
t: Student t-test
*: Statistically significant at p ≤ 0.05

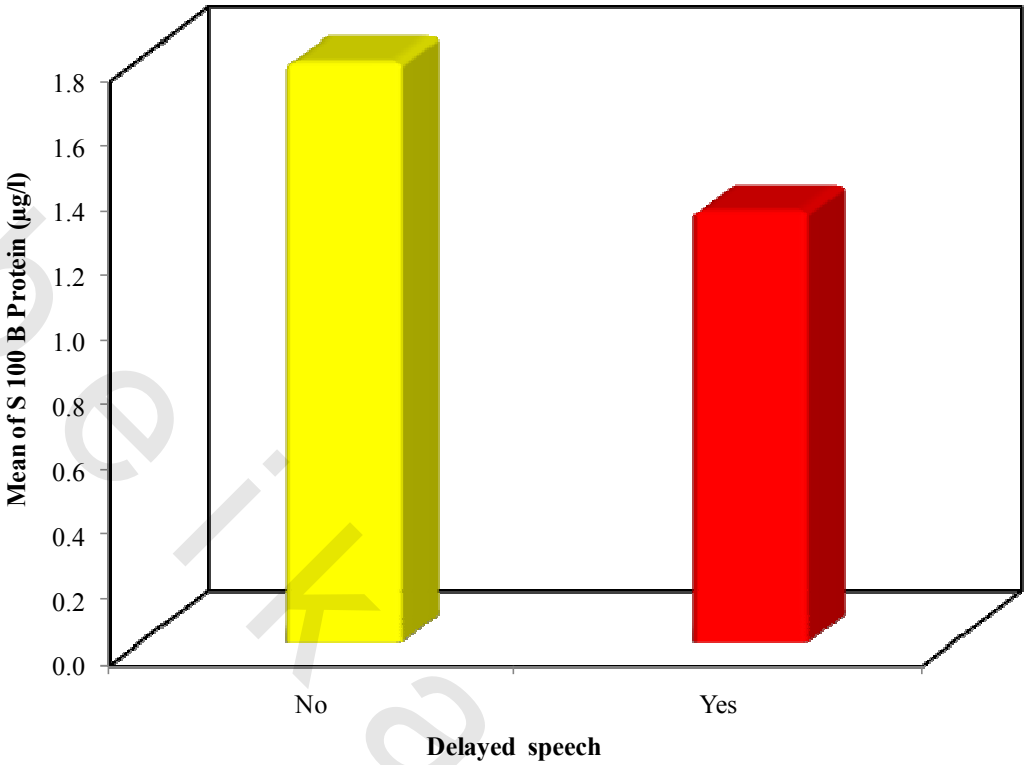

Figure (28): Relation between Delayed speech with S 100 B protein in patients.

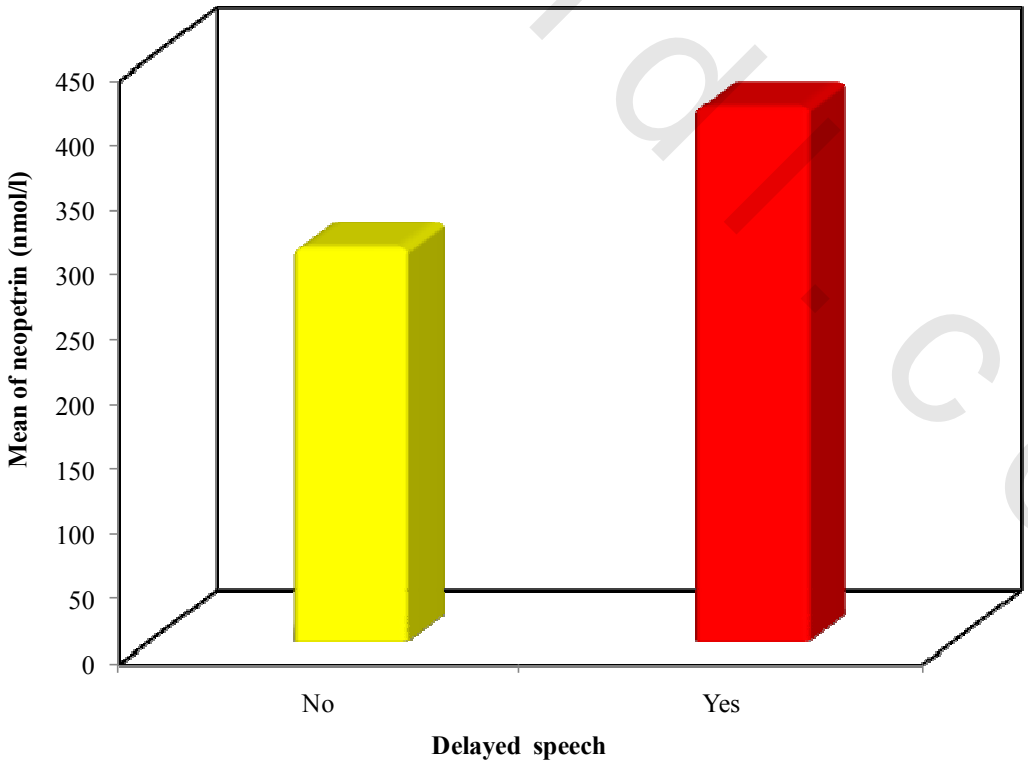

Figure (29): Relation between Delayed speech with neopetrin in patients.

Table (19) show relation between Eye symptoms with S 100 B protein and neopetrin in patients.

Regarding to eye symptoms, 15 patients had no eye symptoms with the mean of S100 B protein 1.77 ± 2.61 while 5 patients had eye symptoms with the mean of S100 B protein 1.60 ± 1.06. There were no statistical significant difference between patients who had no eye symptoms and patient with eye symptoms regarding to S 100 B protein (P= 0.315).

Regarding to eye symptoms, 15 patients had no eye symptoms with mean of neopetrin 299.98 ± 96.27 while 5 patients had eye symptoms with mean of neopetrin 381.03 ± 57.46. The mean value of serum neopetrin was significantly higher in those patients with eye symptoms than in patients without eye symptoms.

Table (19): Relation between Eye symptoms with S 100 B protein and neopetrin in patients.

	Eye symptoms		**Test of sig.**	**p**
	No (n = 15)	**Yes (n =5)**		
S 100 B Protein (µg/l)				
Min. - Max.	0.22 – 10.0	0.31 – 3.11		
Mean ± SD	1.77 ± 2.61	1.60 ± 1.06	Z = 1.004	0.315
Median	0.85	1.42		
Neopetrin (nmol/l)				
Min. - Max.	162.06 – 422.92	284.58 – 434.78		
Mean ± SD	299.98 ± 96.27	381.03 ± 57.46	t = 2.267*	0.043*
Median	268.77	399.21		

p: p value for comparing between no eye symptoms and eye symptoms
Z: Z for Mann Whitney test
t: Student t-test
*: Statistically significant at $p \leq 0.05$

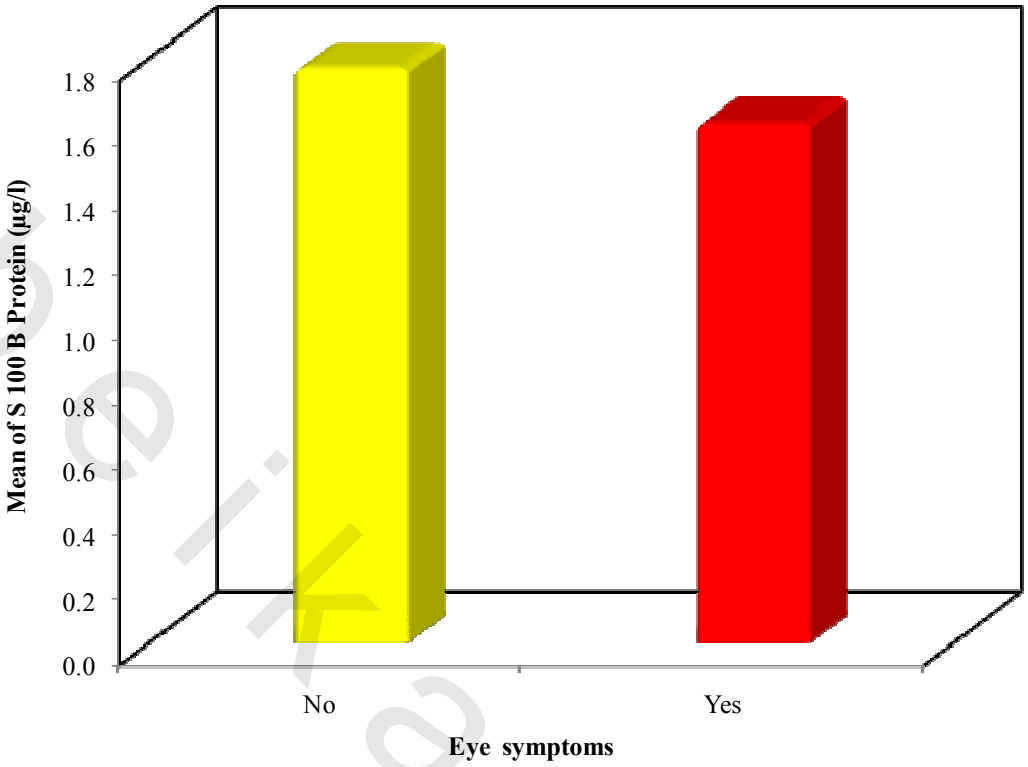

Figure (30): Relation between Eye symptoms with S 100 B protein in patients.

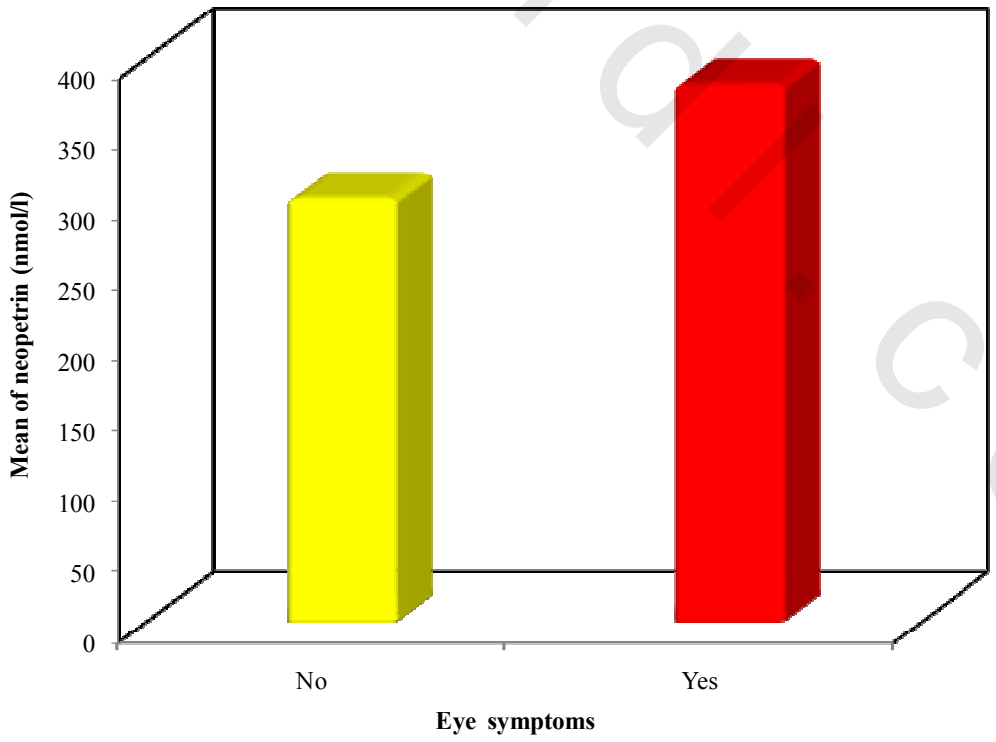

Figure (31): Relation between Eye symptoms with neopetrin in patients.

Table (20) show relation between conscious level with S 100 Bprotein and neopetrin in patients.

Regarding to conscious level, 10 patients were drowsy with mean of S100 B protein 1.28 ± 1.58 while 2 patients were comatosed with mean of S100 B protein 5.58 ± 6.26 and 8 patients were semi-consious with mean of S100 Bprotein 1.31± 0.87. There was no statistical significant difference between patients who were drowsy, comatosed or semi-consious regarding to S 100 B protein (P=0.148).

Regarding to conscious level, 10 patients were drowsy with mean ofneopetrin 315.02 ± 98.07 while 2 patients were comatosed with mean of neopetrin 316.21 ± 111.80 and 8 patients were semiconsious with mean of neopetrin 327.77 ± 98.41. There were no statistical significant differencebetween patients were drowsy,comatosed or semi-conscioueregarding to neopetrin (P=0.962).

Table (20): Relation between conscious level with S 100 B protein and neopetrin in patients.

	Conscious level			Test of sig.	p
	Drowsy (n = 10)	**Comatosed (n = 2)**	**Semi-conscious (n= 8)**		
S 100 B Protein (µg/l)					
Min. - Max.	0.22 – 5.40	1.15 – 10.0	0.37 – 3.11		
Mean ± SD	1.28 ± 1.58	5.58 ± 6.26	1.31 ± 0.87	$^{KW}\chi^2$=3.819	0.148
Median	0.81	5.58	1.03		
Neopetrin (nmol/l)					
Min. - Max.	162.06 – 399.21	237.15 – 395.26	187.26 – 434.78		
Mean ± SD	315.02 ± 98.07	316.21 ± 111.80	327.77 ± 98.41	F= 0.039	0.962
Median	373.52	316.21	339.99		

p: p value for comparing between conscious level categories
$^{KW}\chi^2$: Chi square for Kruskal Wallis test
F: F test (ANOVA)

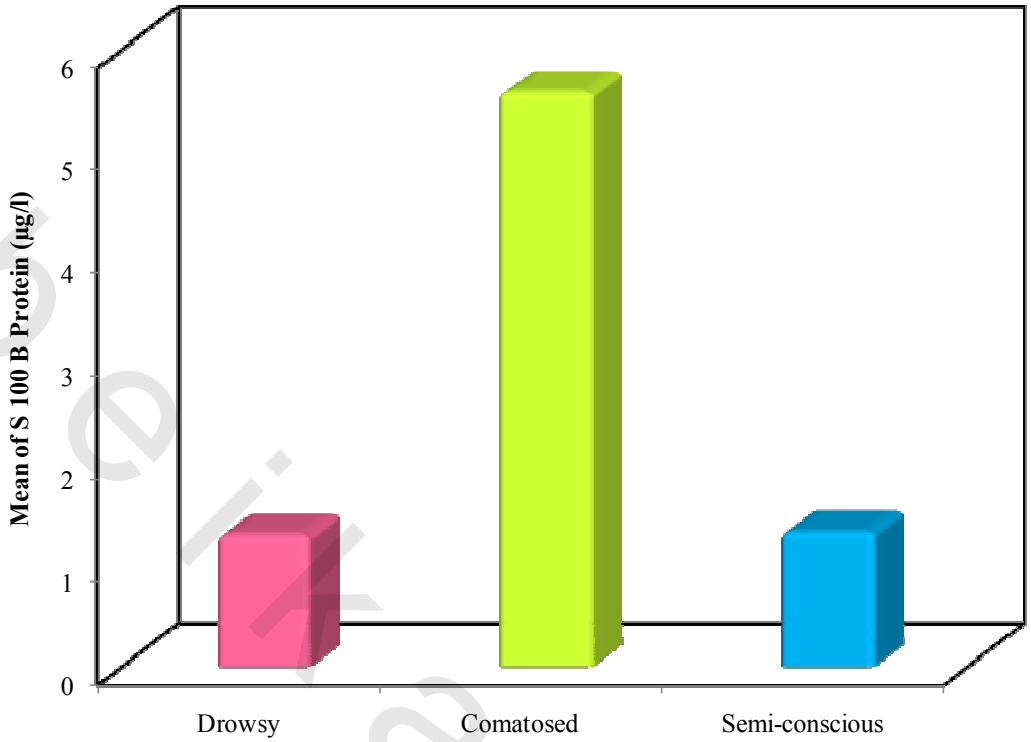

Figure (32): Relation between conscious level with S 100 B protein in cases group

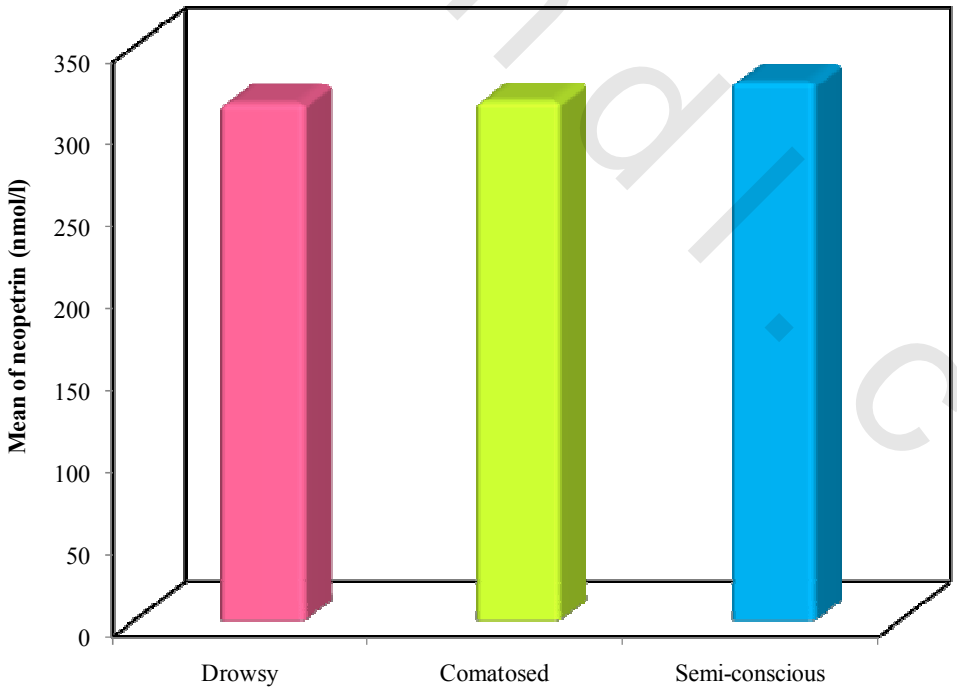

Figure (33): Relation between conscious level with neopetrin in cases group

Table (21) show relation between MRI criteria with S 100 B protein and neopetrin in patients.

Regarding to MRI criteria, 13 patients had unremarkable criteria with mean of S100 B protein 2.16 ± 2.74 while 7 patients had remarkable criteria with mean of S100 B protein 0.92 ± 0.67. There were no statistical significant difference between patients had unremarkable criteria and patient had remarkable criteria regarding to S 100 B protein (P= 0.332).

Regarding to MRI criteria, 13 patients had unremarkable criteria with mean of neopetrin 276.80 ± 88.62 while 7 patients had remarkable criteria with mean of neopetrin 400.90 ± 23.37. The mean value of serum neopetrin was significantly higher in those patients with remarkable criteria than patients with unremarkable criteria.

Table (21): Relation between MRI criteria with S 100 B protein and neopetrin in patients.

	MRI criteria		**Test of sig.**	**p**
	Unremarkable (n = 13)	**Remarkable (n =7)**		
S 100 B Protein (µg/l)				
Min. - Max.	0.22 – 10.0	0.30 – 2.10		
Mean ± SD	2.16 ± 2.74	0.92 ± 0.67	Z= 0.991	0.332
Median	1.0	0.85		
Neopetrin (nmol/l)				
Min. - Max.	162.02 – 399.21	367.59 – 434.78		
Mean ± SD	276.80 ± 88.62	400.90 ± 23.37	t = 4.751*	<0.001*
Median	237.15	399.21		

p: p value for comparing between MRI criteria categories
Z: Z for Mann Whitney test
t: Student t-test
*: Statistically significant at $p \leq 0.05$

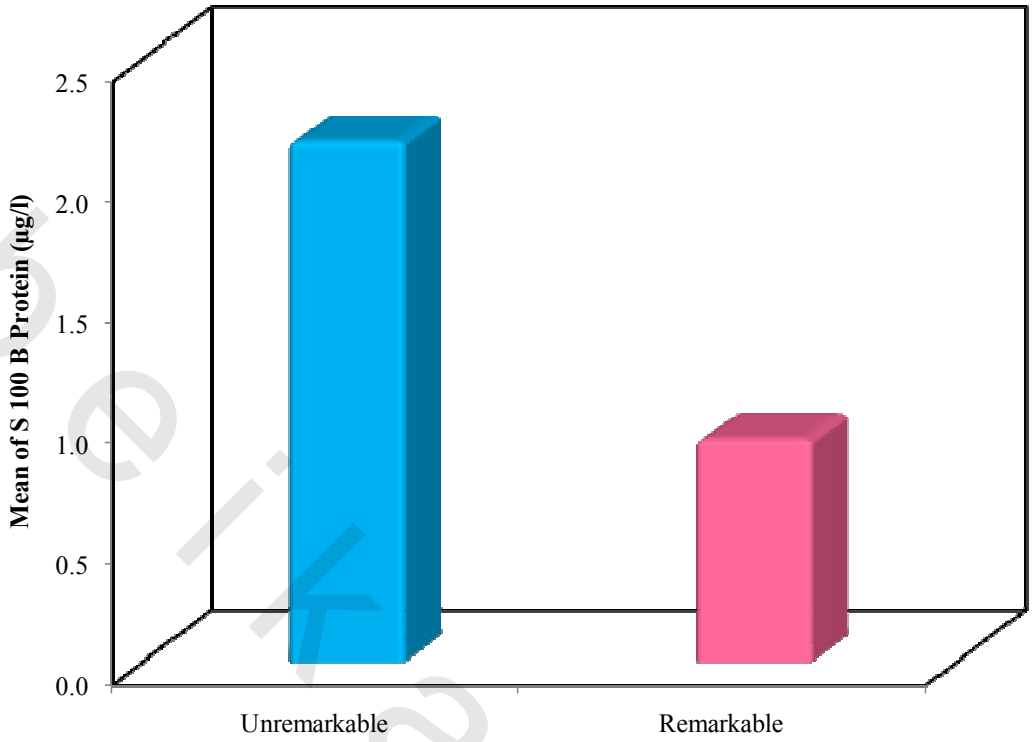

Figure (34): Relation between MRI criteria with S 100 B protein in patients

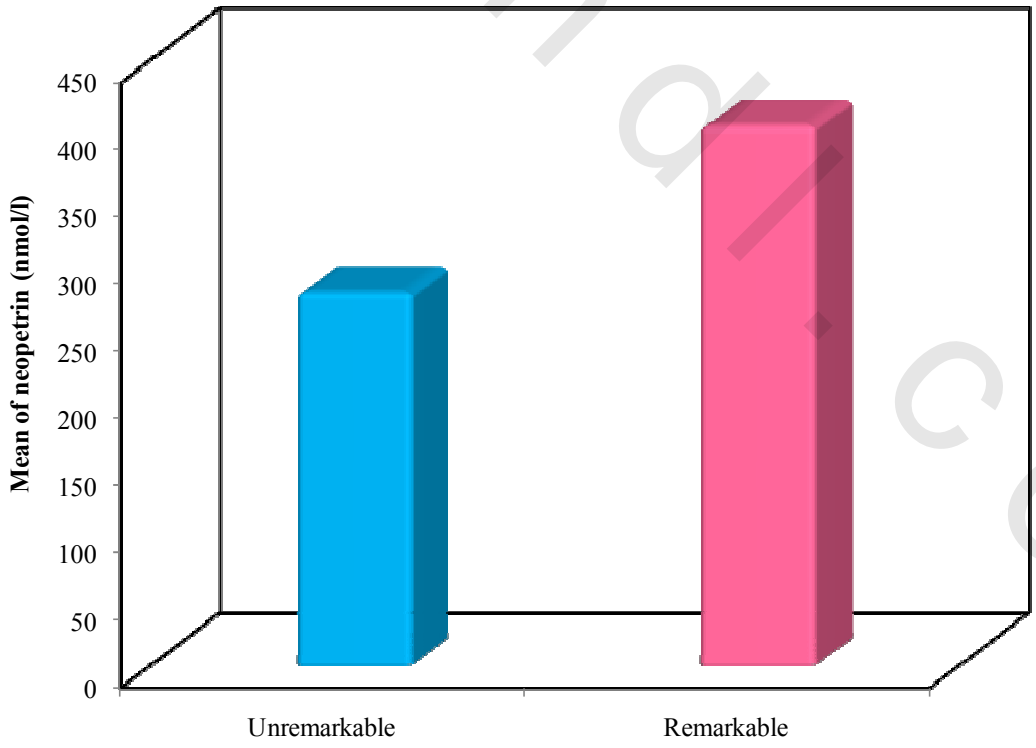

Figure (35): Relation between MRI criteria with neopetrin in patients.

Table (22) show relation between patients ventilated or not with S 100 B protein and neopetrin.

Regarding to patients ventilated, 16 patients had not been ventilated with mean of S100 B protein 1.13 ± 1.24 while 4 patients had been ventilated with mean of S100 B protein 4.06 ± 4.04. The mean value of S100 B protein was significantly higher in those patients had been ventilated than patients who had not been ventilated.

Regarding to patients ventilated, 16 patients had not been ventilated with mean of neopetrin 305.19 ± 95.36 while 4 patients had been ventilated with mean of neopetrin 380.43 ± 66.01. There were no statistical significant difference between patients had not been ventilated and patient had been ventilated regarding to neopetrin (P= 0.110).

Table (22): Relation between patients ventilated or not with S 100 B protein and neopetrin.

	Put on ventilator or not		**Test of sig.**	**p**
	No (n = 16)	**Yes (n =4)**		
S 100 B Protein (µg/l)				
Min. - Max.	0.22 – 5.40	1.0 6 - 10.0		
Mean ± SD	1.13 ± 1.24	4.06 ± 4.04	Z= 2.269*	0.023*
Median	0.84	2.60		
Neopetrin (nmol/l)				
Min. - Max.	162.06 – 422.92	284.58 – 434.78		
Mean ± SD	305.19 ± 95.36	380.43 ± 66.01	t = 1.848	0.110
Median	318.18	401.19		

p: p value for comparing between no put on ventilator and put on ventilator
Z: Z for Mann Whitney test
t: Student t-test
*: Statistically significant at $p \leq 0.05$

Figure (36): Relation between patients ventilated or not with S 100 B protein.

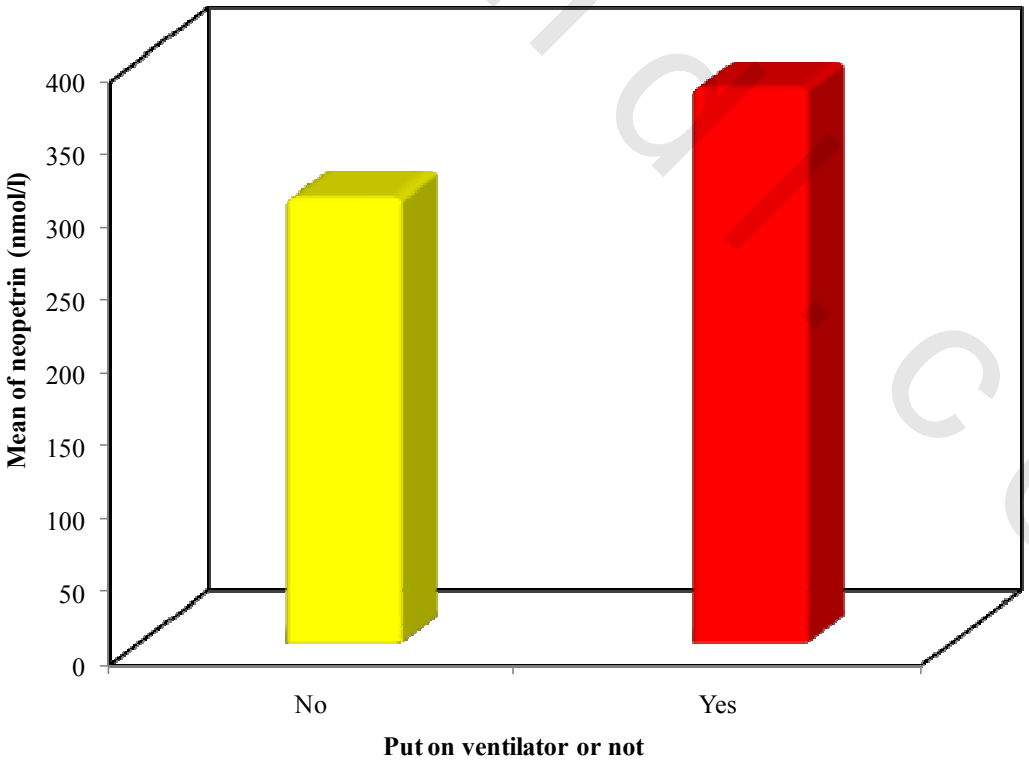

Figure (37): Relation between patients ventilated or not with neopetrin.

Table (23) show relation between complications with S 100 B protein and neopetrin in patients.

Regarding to patients had complications, 10 patients had no complications with mean of S100 B protein 1.62 ± 1.59 while 10 patients had complications with mean of S100 B protein 1.83 ± 2.92. There were no statistical significant difference between patients had no complications and patient had complications regarding to S 100 B protein (P= 0.734).

Regarding to patients had complications, 10 patients had no complications with mean of neopetrin 251.14 ± 77.25 while 10 patients had complication with mean of neopetrin 389.34 ± 45.19. The mean value of neopetrin was significantly higher in those patients with complications than patients without complications.

Table (23): Relation between complications with S 100 B protein and neopetrin in patients.

	Complications		Test of sig.	p
	No (n = 10)	Yes (n =10)		
S 100 B Protein (µg/l)				
Min. - Max.	0.22 – 5.40	0.30 – 10.0		
Mean ± SD	1.62 ± 1.59	1.83 ± 2.92	Z= 0.340	0.734
Median	0.98	0.96		
Neopetrin (nmol/l)				
Min. - Max.	162.06 – 399.21	268.77 – 434.78		
Mean ± SD	251.14 ± 77.25	389.34 ± 45.19	t = 4.883*	<0.001*
Median	227.28	395.33		

p: p value for comparing between no complications and complications
Z: Z for Mann Whitney test
t: Student t-test
*: Statistically significant at p ≤ 0.05

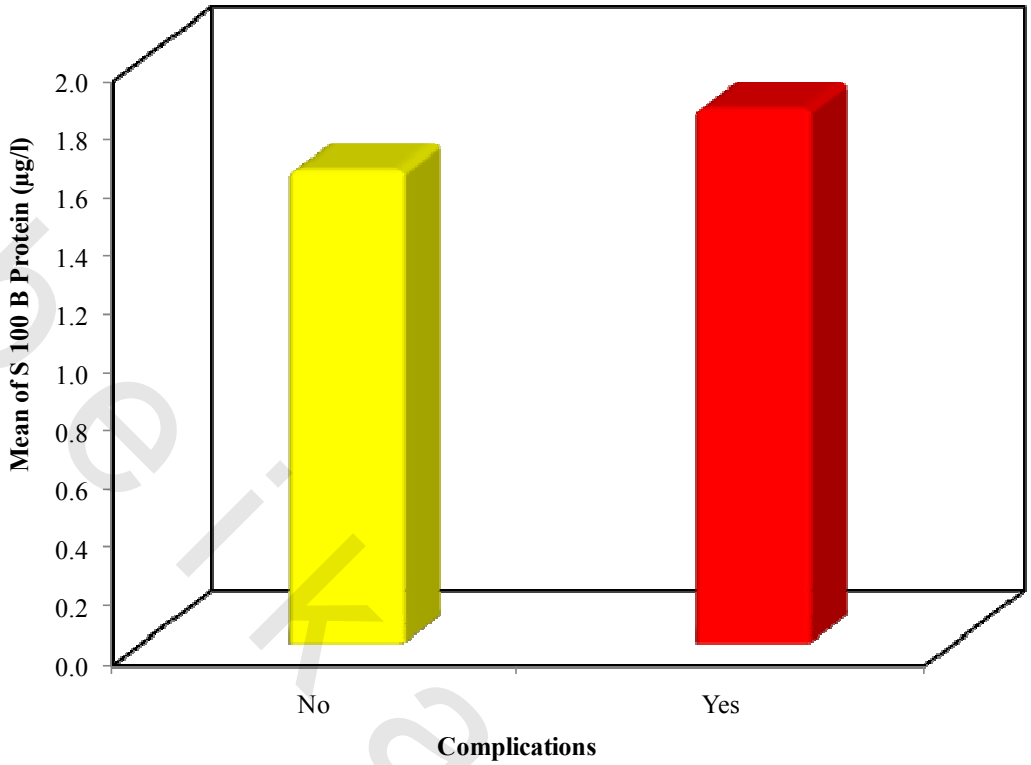

Figure (38): Relation between complications with S 100 B protein in patients.

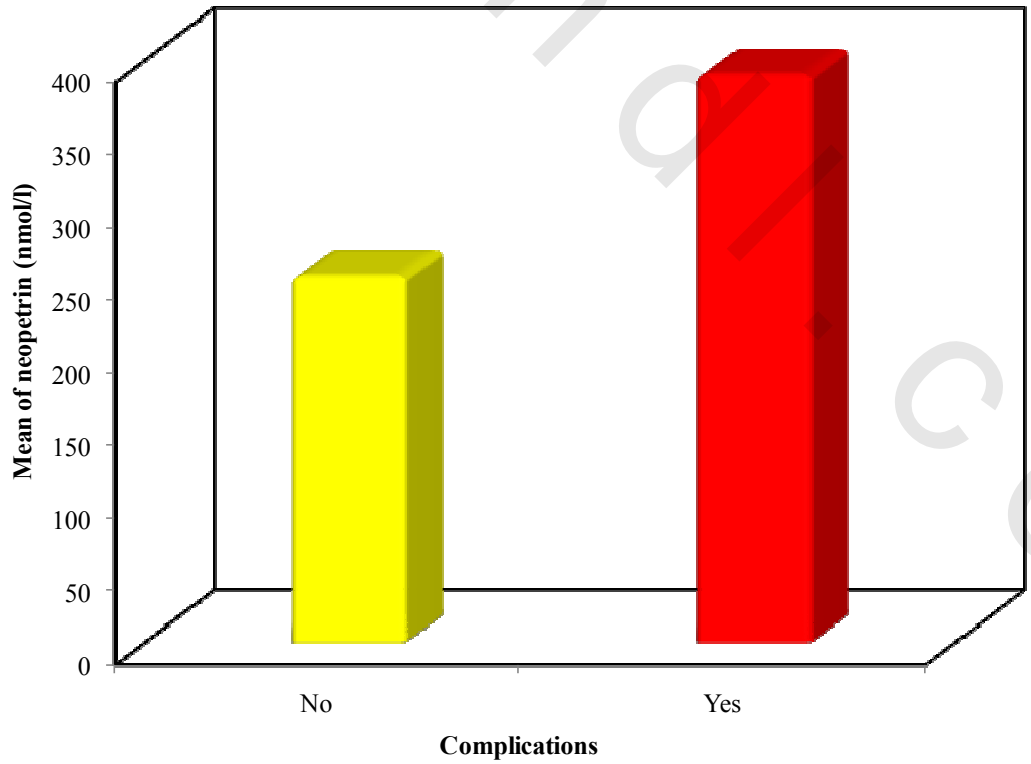

Figure (39): Relation between complications with neopetrin in patients.

Table (24) show relation between death with S 100 B protein and neopetrin in patients.

Regarding to death, 16 patients lived with mean of S100 B protein 1.28 ± 1.34 while 4 patients died with mean of S100 B protein 3.52 ± 4.35. There were no statistical significant difference between patients lived and died regarding to S 100 B protein (P= 0.130).

Regarding to death, 16 patients lived with mean of neopetrin 298.27 ± 92.35 while 4 patients died with mean of neopetrin 408.14 ± 18.61. The mean value of neopetrin was significantly higher in those patients died than lived.

Table (24): Relation between Death with S 100 B protein and neopetrin in patients.

	Death		**Test of sig.**	**p**
	No (n = 16)	**Yes (n =4)**		
S 100 B Protein (µg/l)				
Min. - Max.	0.22 – 5.40	0.91 – 10.0		
Mean ± SD	1.28 ± 1.34	3.52 ± 4.35	Z= 1.521	0.130
Median	0.85	1.58		
Neopetrin (nmol/l)				
Min. - Max.	162.06 – 422.92	395.26 – 434.78		
Mean ± SD	298.27 ± 92.35	408.14 ± 18.61	t = 4.414*	<0.001*
Median	276.67	401.25		

p: p value for comparing between no death and death
Z: Z for Mann Whitney test
t: Student t-test

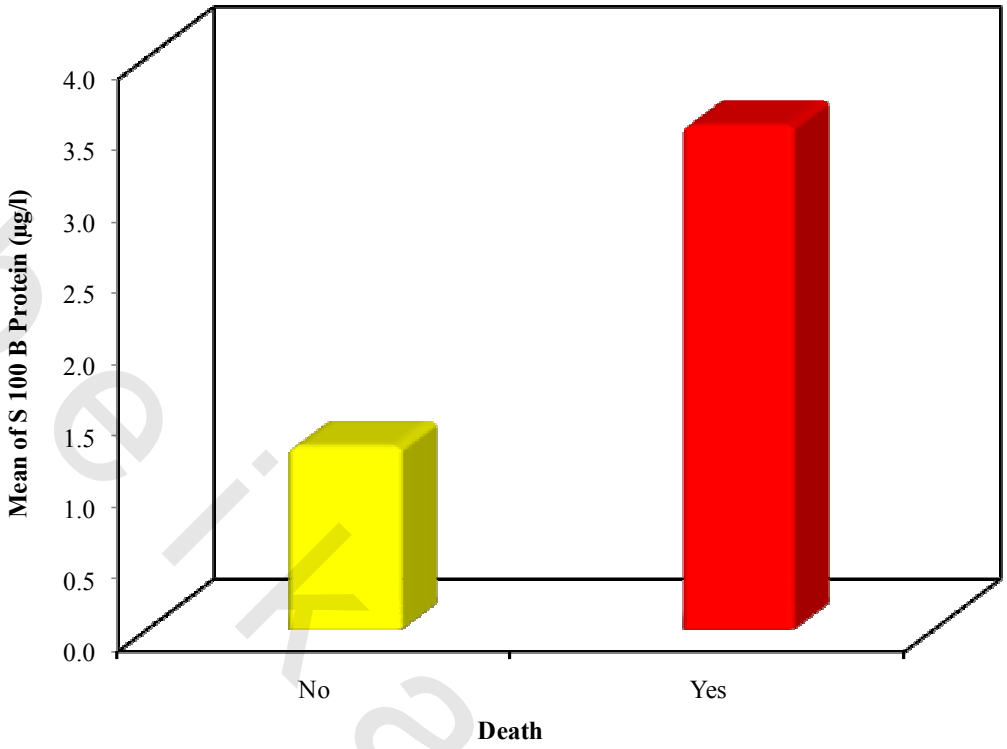

Figure (40): Relation between Death with S 100 B protein in patients.

Figure (41): Relation between Death with neopetrin in patients.

Table (25) show agreement (sensitivity, specificity and accuracy) for Neopetrin with Abdominal gait

Receiver Operating Characteristic (ROC) curve showing relationship between sensitivity and specificity of particular cut-off value of serum neopterin concentrations to detect abnormal gait and its Area Under Curve (AUC = 0.882); (p = 0.039)

Using 379.45 (nmol/l) as a cut-off value for serum neopterin concentrations, it was shown that sensitivity were 100.0 and specificity were 64.71 to detect abnormal gait with positive predictive value (PPV) 33.33 and negative predictive value 100.0 and accuracy 70.0.

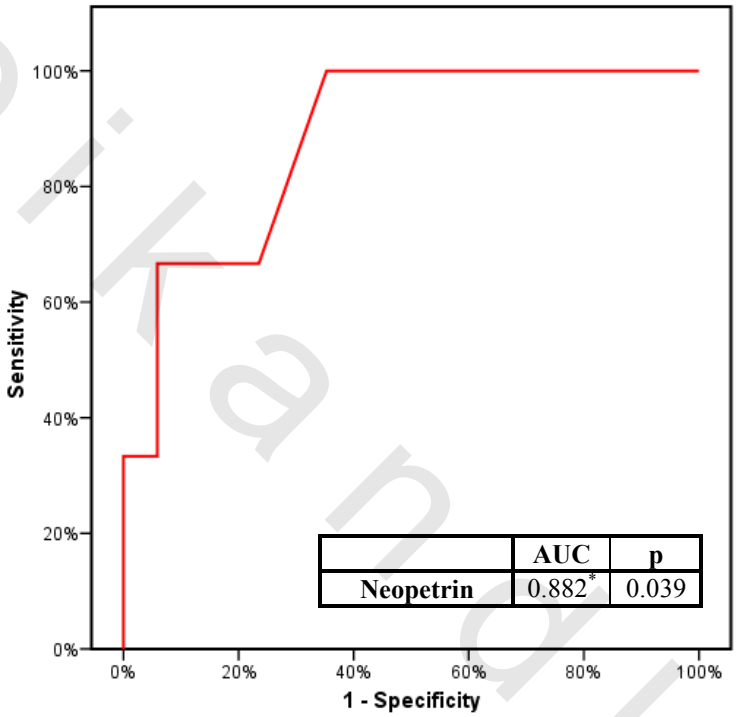

Figure (42): ROC curve for Neopetrin with Abdominal gait

Table (25): Agreement (sensitivity, specificity and accuracy) for Neopetrin with Abdominal gait.

		Abnormal gait (-ve)	Abnormal gait (+ve)	Sensitivity	Specificity	PPV	NPV	Accuracy
Neopetrin (nmol/l)	≤379.45	11	0	100.0	64.71	33.33	100.0	70.0
	>379.45	6	3					

Table (26): show agreement (sensitivity, specificity and accuracy)for Neopetrin with Delayed speach

Receiver Operating Characteristic (ROC) curve showing relationship between sensitivity and specificity of particular cut-off value of serum neopterin concentrations to detect delayed speech and its Area Under Curve (AUC = 0.882); (p = 0.039)

Using 379.45 (nmol/l) as a cut-off value for serum neopterin concentrations, it was shown that sensitivity 100.0 and specificity 64.71 to detect delayed speech with positive predictive value (PPV) 33.33 and negative predictive value 100.0 and accuracy 70.0.

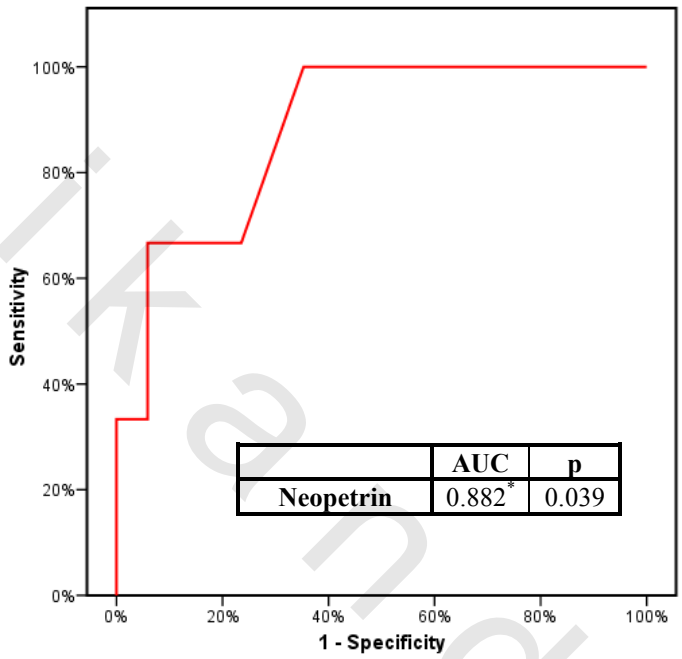

Figure (43): ROC curve for Neopetrin with Delayed speech

Table (26): Agreement (sensitivity, specificity and accuracy) for Neopetrin with Delayed speach

		Delayed speach (-ve)	Delayed speach (+ve)	Sensitivity	Specificity	PPV	NPV	Accuracy
Neopetrin (nmol/l)	≤379.45	11	0	100.0	64.71	33.33	100.0	70.0
	>379.45	6	3					

Table (27): show agreement (sensitivity, specificity and accuracy) for Neopetrin with Eye Symptoms

Receiver Operating Characteristic (ROC) curve showing relationship between sensitivity and specificity of particular cut-off value of serum neopterin concentrations to detect eye symptoms and its Area Under Curve (AUC = 0.882); (p = 0.039)

Using 268.77(nmol/l) as a cut-off value for serum neopterin concentrations, it was shown that sensitivity 100.0 and specificity 53.33 to detect eye symptoms with positive predictive value (PPV) 41.67 and negative predictive value 100.0 and accuracy 65.0.

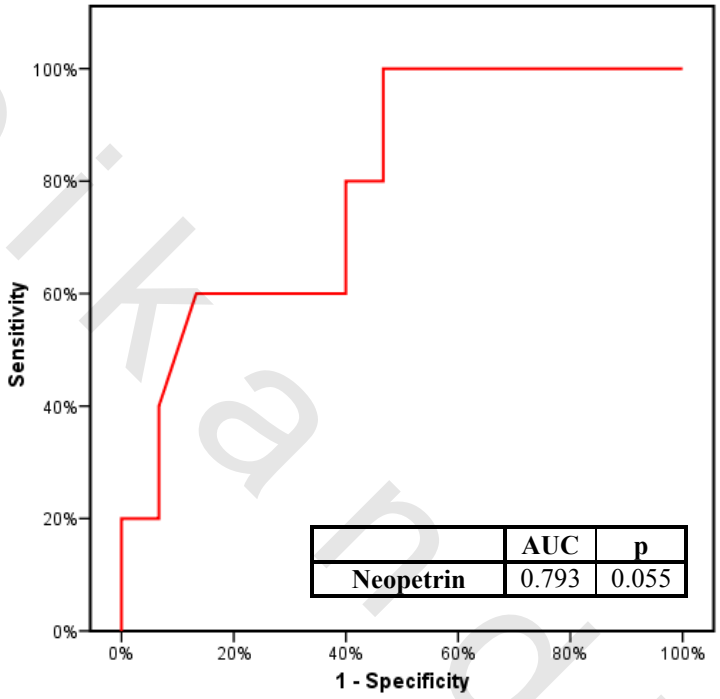

Figure (44): ROC curve for Neopetrin with Eye Symptoms

Table (27): Agreement (sensitivity, specificity and accuracy) for Neopetrin with Eye Symptoms

		Eye Symptoms (-ve)	Eye Symptoms (+ve)	Sensitivity	Specificity	PPV	NPV	Accuracy
Neopetrin (nmol/l)	≤268.77	8	0	100.0	53.33	41.67	100.0	65.0
	>268.77	7	5					

Table (28): show agreement (sensitivity, specificity and accuracy)for Neopetrin with MRI crieteria

Receiver Operating Characteristic (ROC) curve showing relationship between sensitivity and specificity of particular cut-off value of serum neopterin concentrations to detect MRI crietria and its Area Under Curve (AUC = 0.874); (p = 0.007)

Using 284.58 (nmol/l) as a cut-off value for serum neopterin concentrations, it was shown that sensitivity 100.0 and specificity 69.23 to detect MRI crietria with positive predictive value (PPV) 63.64 and negative predictive value 100.0 and accuracy 80.0.

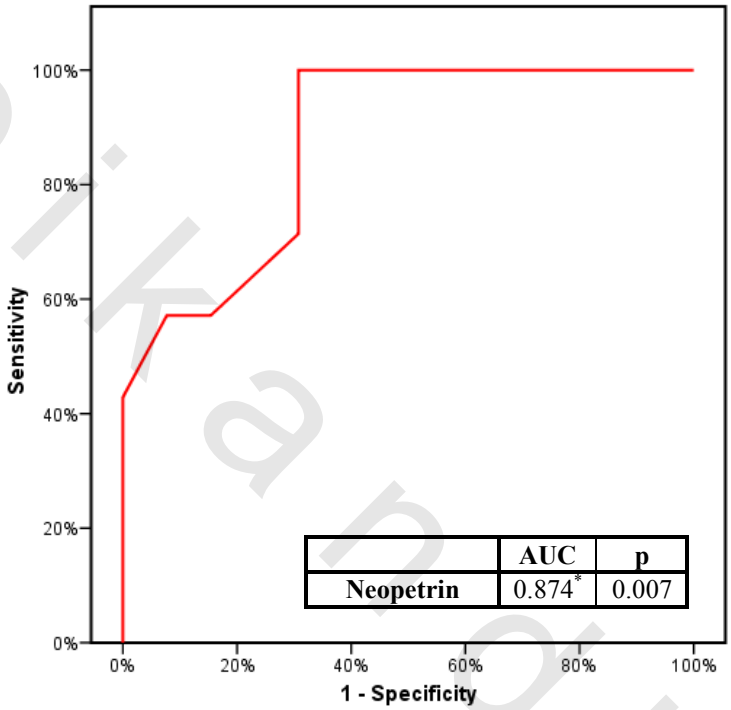

Figure (45): ROC curve for Neopetrin with MRI crieteria

Table (28): Agreement (sensitivity, specificity and accuracy) for Neopetrin with MRI crieteria

		MRI crieteria (-ve)	MRI crieteria (+ve)	Sensitivity	Specificity	PPV	NPV	Accuracy
Neopetrin (nmol/l)	≤284.58	9	0	100.0	69.23	63.64	100.0	80.0
	>284.58	4	7					

Table (29): show agreement (sensitivity, specificity and accuracy)for Neopetrin with Complication

Receiver Operating Characteristic (ROC) curve showing relationship between sensitivity and specificity of particular cut-off value of serum neopterin concentrations to detect complication and its Area Under Curve (AUC = 0.915); (p = 0.002)

Using 367.59 (nmol/l) as a cut-off value for serum neopterin concentrations, it was shown that sensitivity 90.0 and specificity 90.0 to detect complication with positive predictive value (PPV) 90.0 and negative predictive value 90.0 and accuracy 90.0.

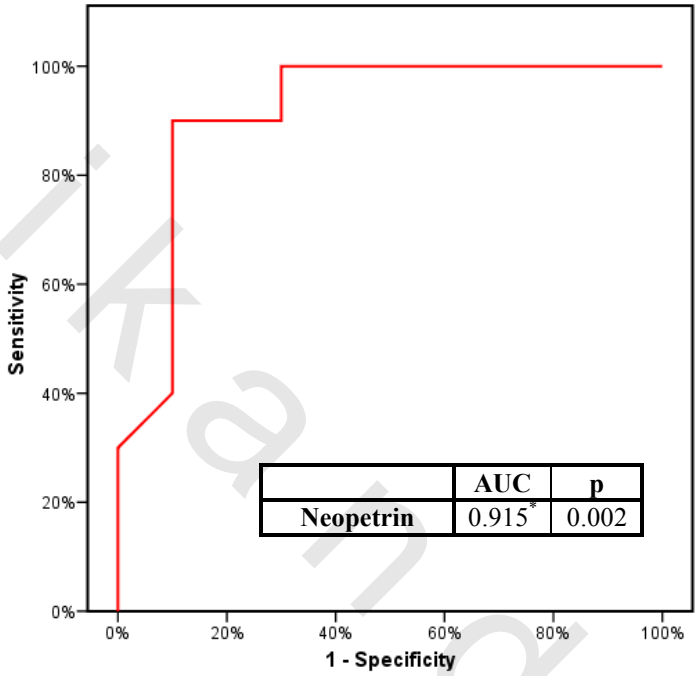

Figure (46): ROC curve for Neopetrin with Complication

Table (29): Agreement (sensitivity, specificity and accuracy) for Neopetrin with Complication

		Complication (-ve)	Complication (+ve)	Sensitivity	Specificity	PPV	NPV	Accuracy
Neopetrin (nmol/l)	≤367.59	9	1	90.0	90.0	90.0	90.0	90.0
	>367.59	1	9					

Table (30): show agreement (sensitivity, specificity and accuracy)for Neopetrin with outcome of patients

Receiver Operating Characteristic (ROC) curve showing relationship between sensitivity and specificity of particular cut-off value of serum neopterin concentrations to detect outcome of patients and its Area Under Curve (AUC = 0.875); (p = 0.023)

Using 379.45 (nmol/l) as a cut-off value for serum neopterin concentrations, it was shown that sensitivity 100.0 and specificity 68.75 to detect outcome of patients with positive predictive value (PPV) 44.44 and negative predictive value (NPV) 100.0 and accuracy 75.0.

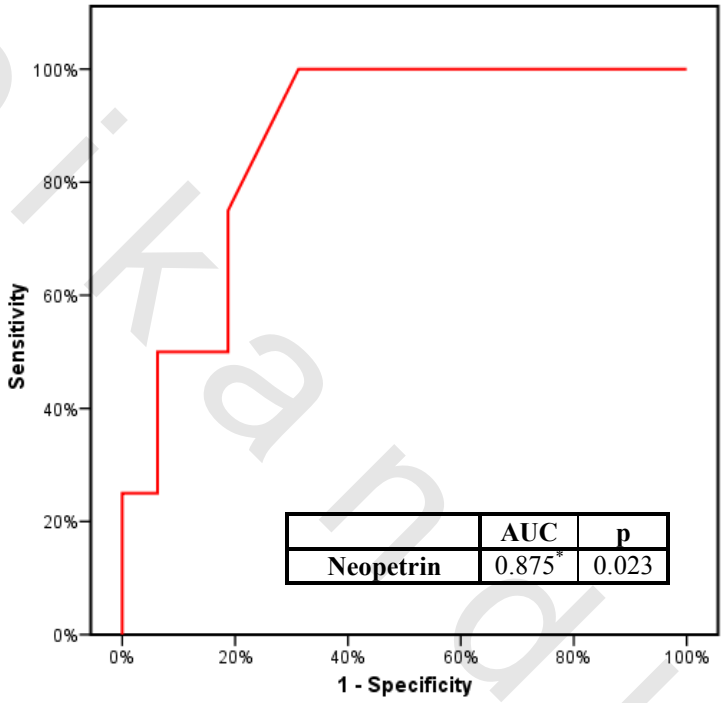

Figure (47): ROC curve for Neopetrin with outcome

Table (30): Agreement (sensitivity, specificity and accuracy) for Neopetrin with outcome

		Survival	Death	Sensitivity	Specificity	PPV	NPV	Accuracy
Neopetrin (nmol/l)	≤379.45	11	0	100.0	68.75	44.44	100.0	75.0
	>379.45	5	4					

Table (31): show agreement (sensitivity, specificity and accuracy) for S 100 B protein with patients were ventilated

Receiver Operating Characteristic (ROC) curve showing relationship between sensitivity and specificity of particular cut-off value of serum S 100 B protein concentrations to detect patients were ventilated and its Area Under Curve (AUC = 0.875); (p = 0.023)

Using 1.0 (µg/l) as a cut-off value for serum S 100 B protein concentrations, it was shown that sensitivity 100.0 and specificity 68.75 to detect outcome of patients with positive predictive value (PPV) 44.44 and negative predictive value 100.0 and accuracy 75.0.

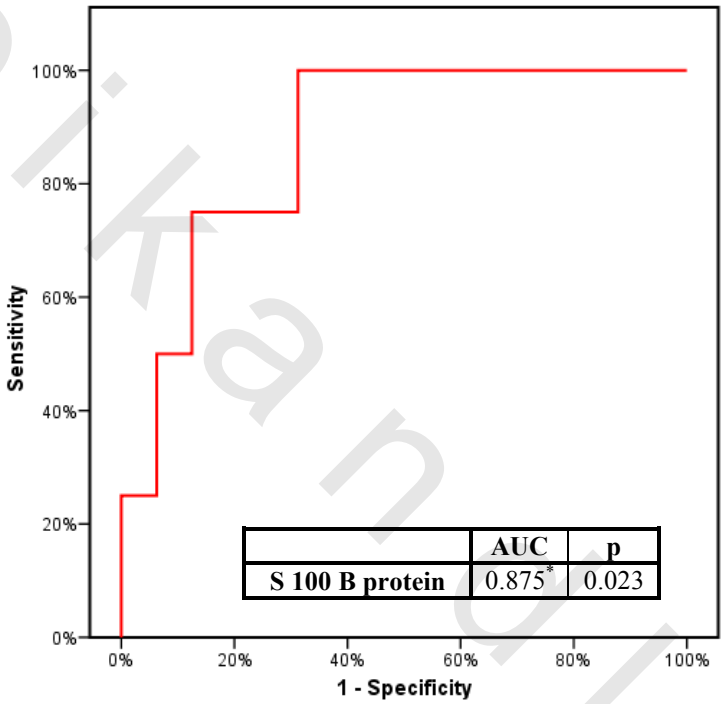

Figure (48): ROC curve for S 100 B protein with patients wereventilated

Table (31): Agreement (sensitivity, specificity and accuracy) for S 100 B protein with patients were ventilated

		Patients were ventilated (-ve)	Patients were ventilated (+ve)	Sensitivity	Specificity	PPV	NPV	Accuracy
S 100 B protein (µg/l)	≤1	11	0	100.0	68.75	44.44	100.0	75.0
	>1	5	4					