AIM OF THE WORK

The aim of the present work was to detect impaired renal functions early in infants and children with congenital heart diseases.

SUBJECTS

Fifty infants and children with CHD from those attending the cardiology outpatient clinic at Alexandria University Children's Hospital were enrolled in this case control study to evaluate renal functions in cyanotic and acyanotic congenital heart diseases.

They were divided to two groups as following:

Group (A): 25 acyanotic CHD patients.

Group (B): 25 cyanotic CHD patients.

In addition to:

Group (C): Twenty five healthy children who came for health maintenance visits were included as a control group**.**

Each group was divided to subgroups according to age as following:

- From the age of one month to one year old and are named subgroups A1 – B1 – C1.
- After the age of one year and up to the age of five years old and are named subgroups A2 – B2 – C2.

Written consent was taken from parents of each child.

METHODS

Every child in the study was subjected to the following:

1. Thorough history taking: including urinary tract infection, having contrast material for any reason before, recently used nephrotoxic drugs e.g.; aminoglycosides, valproate, diuretics and angiotensin converting enzyme inhibitors. Any child met one of these items was excluded.
2. Thorough systemic clinical examination with emphasis on cardiac examination.
3. Detailed echocardiographic study including 2-D, M-mode, PW and CW Doppler and color Doppler to confirm diagnosis and severity of CHD.
4. Abdominal ultrasound to determine morphology, echogenicity and size of kidneys to exclude any pre-existing renal problems.

Blood samples were collected and were allowed to clot for 25 to 30 minutes at room temperature then centrifuged at 3000 RPM for 3 minutes and clear serum obtained and kept frozen at -80°C for estimation of serum β2M and serum creatinine.

Early morning urine samples were collected and urine pH was checked by pH indicator strips. Aliquots of urine were stored at –80°C until they were analyzed for the determination of n-acetyl-beta-D-glucosaminidase activities and albumin. Concentrations of urinary albumin and urinary NAG were expressed as ratios to urinary creatinine (UCr) to correct for variations in urine Concentrations.

Blood and early morning urine samples which were collected from each subject were used to measure:

- Serum creatinine for estimated glomerular filtration rate (eGFR) using:
  a. Schwartz formula for children under 1 year old as follow:
  eGFR (ml/min/1.73m^2)=0.45× (height in cm/serum creatinine in mg/dl).[51]
  b. Revised Schwartz formula for children over 1 year old as follow:
  eGFR (ml/min/1.73m^2)=0.413× (height in cm/serum creatinine in mg/dl).[52]

- Urinary Albumin creatinine ratio (U Alb/Cr) using nephlometry.

- Urinary n-acetyl-beta-D-glucosaminidase / creatinine ratio (U NAG/Cr) using commercially available kit from (Glory Science Co., Ltd, USA) by ELISA technique (FAX STAT 2100). N-acetyl-β-D-glucosaminidase (NAG) was Added to monoclonal antibody Enzyme well which is pre-coated with Human N-acetyl-β-D-glucosaminidase (NAG) monoclonal antibody, incubation for 60 minutes at 37 ℃; then, add N-acetyl-β-D-glucosaminidase (NAG) antibodies labeled with biotin, and combined with Streptavidin-HRP to form immune complex; then carry out incubation again for 10 minutes at 37 ℃ and washing again to remove the uncombined enzyme. Then add Chromogen Solution A then B, the color of the liquid changes into the blue, and at the effect of stop solution, the color finally becomes yellow, within 10min. we measured the optical densit (OD) under 450 nm wave length. According to standards' concentration and the corresponding OD values, calculated out the standard curve linear regression equation, and then applied the OD values of the sample on the regression equation to calculate the corresponding sample's concentration.

- Serum Beta (2)-microglobulin using commercially available kit from (Labor Diagnostika Nord GmbH & Co. KG, Germany) by ELISA technique (FAX STAT 2100). The principle of this test follows the typical competitive binding scenario. Competition occurs between an unlabeled antigen (present in standards, controls and patient samples) and an enzyme-labelled antigen (β2-M-HRP conjugate) for a limited number of antibody binding sites on mouse anti-β2-M antibody coated microwell plate-break apart wells. Then plates incubated on a plate shaker for 1 hour at room temperature. The washing and decanting procedures using wash buffer concentrate – X10 took place for 3 times to remove unbound materials. After the washing step, the enzyme substrate tetramethylbenzidine and hydrogen peroxide is added. The enzymatic reaction is terminated by addition of the stopping solution 1M sulfuric acid. The absorbance is measured on a microtiter plate reader. The intensity of the colour formed is inversely proportional to the concentration of β2-M in the sample. A set of standards is used to plot a standard curve on semi-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis, from which the amount of β2-M in patient samples and controls can be directly read.

Statistical analysis of the data [53]

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. [54] Qualitative data were described using number and percent. Quantitative data were described using Range (minimum and maximum), mean, standard deviation and median. Comparison between different groups regarding categorical variables was tested using Chi-square test. When more than 20% of the cells have expected count less than 5, correction for chi-square was conducted using Fisher's Exact test or Monte Carlo correction. The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test, Shapiro-Wilk test and D'Agstino test, also Histogram and QQ plot were used for vision test. If it reveals normal data distribution, parametric tests was applied. If the data were abnormally distributed, non-parametric tests were used. For normally distributed data, comparison between two independent populations was done using independent t-test while more than two populations were analyzed F-test (ANOVA) to be used and Post Hoc test (LSD). For abnormally distributed data, comparison between two independent population were done using Mann Whitney test while Kruskal Wallis test was used to compare between different groups and pair wise comparison was assessed using Mann-Whitney test. Significance of the obtained results was judged at the 5% level.

We defined the cut off value of each biomarker concentration as mean ± 2 SD in the corresponding control subgroup (mean – 2 SD for eGFR and mean + 2 SD for U Alb/Cr, U NAG/Cr and ß2M).

RESULTS

This study was carried out on 50 children who were diagnosed to have congenital heart disease; half of them with acyanotic lesions (group A) while the second half with cyanotic lesions (group B), attending the Outpatient Clinics of Alexandria Pediatric Hospital. Also other 25 normal children were taken as control group (group C). Each group divided to 2 subgroups.

Table (1): Comparison between the studied groups A1, B1 and C1 according to demographic data

	A1		B1		C1		Test of sig.	p
	No.	%	No.	%	No.	%		
Sex								
Male	7	53.8	8	61.5	7	53.8	$\chi^2 = 0.209$	0.901
Female	6	46.2	5	38.5	6	46.2		
Age months								
Min. – Max.	2.0 – 12.0		2.0 – 9.0		1.0 – 9.0		$^{KW}\chi^2 =$ 1.305	0.521
Mean ± SD.	4.19 ± 2.56		4.04 ± 2.38		4.81 ± 2.48			
Median	3.50		3.0		5.0			

χ^2: Chi square test
$^{KW}\chi^2$: Chi square for Kruskal Wallis test
*: Statistically significant at $p \leq 0.05$

Table (1), shows the sex and age distribution among the studied subgroups A1, B1 and C1, males in group A1 represented 53.8% while females were 46.2%. In group B1 males represented 61.5% and females were 38.5%. In group C1 males represented 53.8% and females were 46.2%. There was no significant difference between the studied groups regarding sex. On the other hand from this table it was found that the age ranged from 2 – 12 months in group A1, with a mean of 4.19 ± 2.56, while in group B1 ranged from 2 – 9 months with a mean of 4.04 ± 2.38. In group C1 ranged from 0.5 – 9 months with a mean of 4.81 ± 2.48. There was no significant difference between the studied groups regarding age.

Table (2): Comparison between the studied groups A2, B2 and C2 according to demographic data

	A2		B2		C2		Test of sig.	p
	No.	%	No.	%	No.	%		
Sex								
Male	5	41.7	11	91.7	7	58.3	$\chi^2 = 6.840$	0.051
Female	7	58.3	1	8.3	5	41.7		
Age months								
Min. – Max.	13.0 – 60.0		18.0 – 47.0		13.0 – 60.0		$^{KW}\chi^2 =$ 3.085	0.214
Mean ± SD.	41.58 ± 18.62		27.42 ± 11.11		35.83 ± 19.10			
Median	44.0		21.50		36.0			

χ^2: Chi square test
$^{KW}\chi^2$: Chi square for Kruskal Wallis test
*: Statistically significant at $p \leq 0.05$

Table (2), shows the sex and age distribution among the studied subgroups A2, B2 and C2, in group A2 males represented 41.7% and females were 58.3%. In group B2 males represented 91.7% and females were 8.3%. In group C2 males represented 58.3% and females were 41.7%. There was no significant difference between the studied groups regarding sex. On the other hand from this table it was found that the age in group A2 ranged from 13 – 60 months with a mean of 41.58 ± 18.62. In group B2 ranged from 18 – 47 months with a mean of 27.42 ± 11.11. In group C2 ranged from 13 – 60 months with a mean of 35.83 ± 19.10. There was no significant difference between the studied groups regarding age.

Table (3): Comparison between the studied groups according to diagnosis

Diagnosis	No.	%
Group (A) Acyanotic CHD		
ASD	8	32
VSD	9	36
PDA	3	12
AVC	1	4
Congenital valvular disease	4	16
Total	25	100
Group (B) Cyanotic CHD		
TOF	11	44
TGA	8	32
VSD,PS	3	12
DORV	1	4
Truncus arteriosus	1	4
TAPVR	1	4
Total	25	100

ASD = Atrial septal defect; VSD = ventricular septal defect; PDA = patent ductus arterious; AVC = atrioventricular canal; TOF = tetralogy of Fallot; TGA = transposition of great arteries; PS = pulmonary stenosis; DORV= double outlet right ventricle; TAPVR, total anomalous pulmonary venous Return.

Table (4): Comparison between the studied groups A1, B1 and C1 according to eGFR

	A1	B1	C1	Test of sig.	P
eGFR					
Min. – Max.	48.0 – 87.0	26.50 – 72.0	39.75 -109.50		
Mean ± SD.	65.36 ± 11.70	55.13 ± 15.74	72.83±21.85	$F = 3.573^{*}$	0.038^{*}
Median	66.38	59.40	66.94		
Sig. bet. grps.	$p_1 = 0.133$, $p_2 = 0.268$, $p_3 = 0.012^{*}$				
Normal	13 (100.0%)	11 (84.6%)	13 (100.0%)	$\chi^2 = 2.842$	$^{MC}p = 0.315$
#Abnormal	0 (0.0%)	2 (15.4%)	0 (0.0%)		

eGFR = estimated glomerular filteration rate
#Abnormal value <29.13(ml/min/1.73m^2)
χ^2: Chi square test
MC: Monte Carlo test
F: F test (ANOVA), Sig. bet. grps was done using Post Hoc Test (LSD)
p_1 : p value for comparing between A1 and B1
p_2 : p value for comparing between A1 and C1
p_3 : p value for comparing between B1 and C1
*: Statistically significant at $p \leq 0.05$

Table (4), shows comparison between the studied subgroups A1, B1 and C1 according to eGFR, eGFR in group A1 ranged from 48.0 – 87.0 ml/min/1.73 m^2 with a mean of 65.36 ± 11.70 ml/min/1.73 m^2. In group B1 eGFR ranged from 26.50 – 72.0 ml/min/1.73 m^2 with a mean of 55.13 ± 15.74 ml/min/1.73 m^2. In group C1 eGFR ranged from 39.75 - 109.50 ml/min/1.73 m^2 with a mean of 72.83±21.85 ml/min/1.73 m^2. There was a significant difference between the studied subgroups B1 and C1 regarding eGFR.

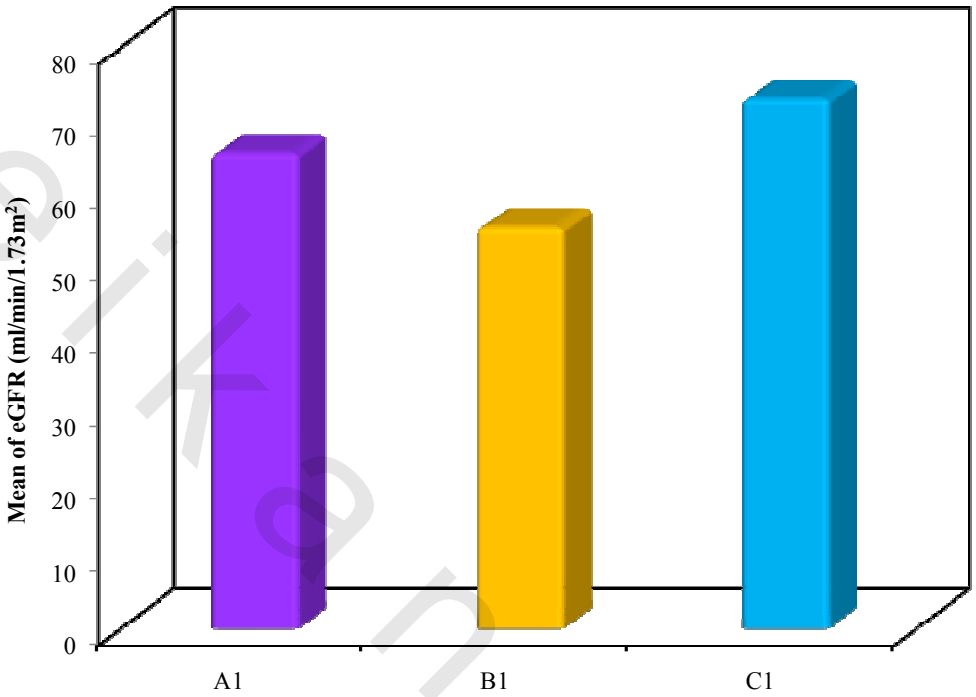

Figure (2): Comparison between the studied groups A1, B1 and C1 according to eGFR

Table (5): Comparison between the studied groups A2, B2 and C2 according to eGFR

	A2	B2	C2	Test of sig.	P
eGFR					
Min. – Max.	73.65 – 156.94	53.0 – 113.58	89.21- 154.87		
Mean ± SD.	116.62 ± 21.07	92.86 ±17.27	120.92 ± 19.71	$F = 7.270^*$	0.002^*
Median	114.09	99.81	118.05		
Sig. bet. grps.	$p_1 = 0.005^*$, $p_2 = 0.590$, $p_3 = 0.001^*$				
Normal	11 (91.7%)	9 (75%)	12 (100.0%)	$\chi^2 = 3.293$	$^{MC}p = 0.289$
$^{\#}$Abnormal	1 (8.3%)	3 (25%)	0 (0.0%)		

eGFR = estimated glomerular filteration rate
$^{\#}$A bnormal value <81.5(ml/min/1.73m^2)
F: F test (ANOVA), Sig. bet. grps was done using Post Hoc Test (LSD)
χ^2: Chi square test
MC: Monte Carlo test
p_1 : p value for comparing between A2 and B2
p_2 : p value for comparing between A2 and C2
p_3 : p value for comparing between B2 and C2
*: Statistically significant at $p \leq 0.05$

Table (5), shows comparison between the studied subgroups A2, B2 and C2 according to eGFR, eGFR in group A2 ranged from 73.65 – 156.94 ml/min/1.73 m^2 with a mean of 116.62 ± 21.07 ml/min/1.73 m^2. In group B2 eGFR ranged from 53.0 – 113.58 ml/min/1.73 m^2 with a mean of 92.86 ± 17.27 ml/min/1.73 m^2. In group C2 eGFR ranged from 89.21 - 154.87 ml/min/1.73 m^2 with a mean of 120.92 ± 19.71 ml/min/1.73 m^2. There was a significant difference between the studied subgroups A2 and B2, B2 and C2 regarding eGFR.

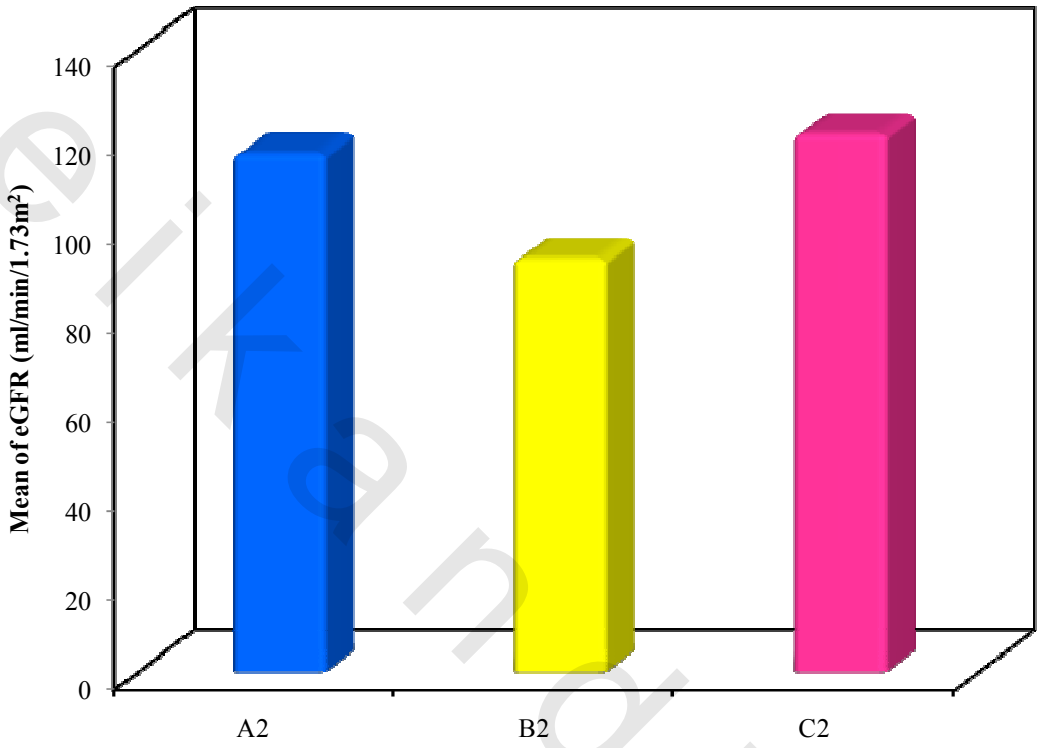

Figure (3): Comparison between the studied groups A2, B2 and C2 according to eGFR

Table (6): Comparison between the studied groups A1, B1 and C1 according to U Alb/Cr

	A1	B1	C1	Test of sig.	P
U Alb/Cr					
Min. – Max.	26.45 – 66.18	28.58 – 71.19	23.10 – 52.47	$^{KW}\chi^2$ = 3.665	0.160
Mean ± SD.	38.84 ± 10.66	46.56 ± 13.85	36.96 ± 9.51		
Median	36.27	46.0	38.0		
Normal	12 (92.3%)	10 (76.9%)	13 (100.0%)	χ^2 = 3.257	^{MC}p = 0.298
#Abnormal	1 (7.7%)	3 (23.1%)	0 (0.0%)		

U Alb/Cr = urinary albumin / creatinine ratio
#Abnormal value >55.98(mg/g)
χ^2: Chi square test
MC: Monte Carlo test
$^{KW}\chi^2$: Chi square for Kruskal Wallis test

Table (6), shows comparison between the studied subgroups A1, B1 and C1 according to U Alb/Cr, U Alb/Cr in group A1 ranged from 26.45 – 66.18 mg/g with a mean of 38.84 ± 10.66 mg/g. In group B1 U Alb/Cr ranged from 28.58 – 71.19 mg/g with a mean of 46.56 ± 13.85 mg/g. In group C1 U Alb/Cr ranged from 23.10 – 52.47 mg/g with a mean of 36.96 ± 9.51 mg/g. There was no significant difference between the studied subgroups regarding U Alb/Cr.

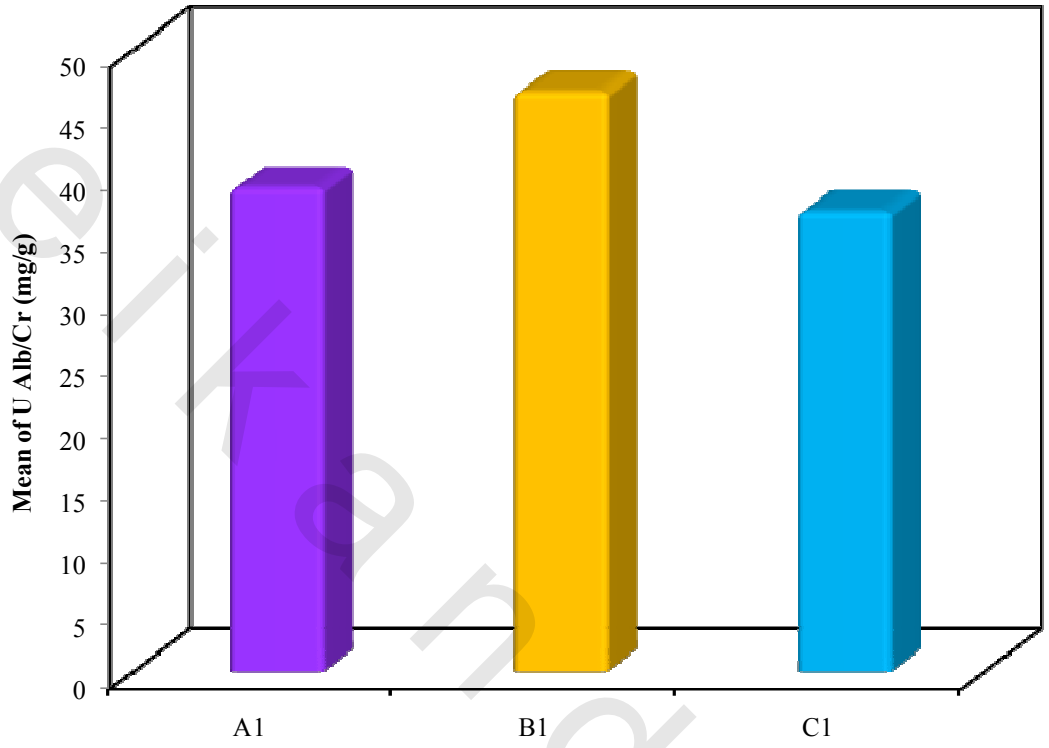

Figure (4): Comparison between the studied groups A1, B1 and C1 according to U Alb/Cr

Table (7): Comparison between the studied groups A2, B2 and C2 according to U Alb/Cr

	A2	B2	C2	Test of sig.	P
U Alb/Cr					
Min. – Max.	28.62 – 78.43	27.59 – 81.24	24.82 – 61.78	$^{KW}\chi^2$ = 1.410	0.494
Mean ± SD.	44.48 ± 15.07	49.34 ± 19.69	38.65 ± 10.84		
Median	41.48	43.11	35.74		
Normal	10 (83.3%)	8 (66.7%)	11 (91.7%)	χ^2 = 2.277	^{MC}p = 0.440
#Abnormal	2 (16.7%)	4 (33.3%)	1 (8.3%)		

U Alb/Cr = urinary albumin / creatinine ratio
#Abnormal value >60.33(mg/g)
χ^2: Chi square test
MC: Monte Carlo test
$^{KW}\chi^2$: Chi square for Kruskal Wallis test

Table (7), shows comparison between the studied subgroups A2, B2 and C2 according to U Alb/Cr, U Alb/Cr in group A2 ranged from 28.62 – 78.43 mg/g with a mean of 44.48 ± 15.07 mg/g. In group B2 U Alb/Cr ranged from 27.59 – 81.24 mg/g with a mean of 49.34 ± 19.69 mg/g. In group C2 U Alb/Cr ranged from 24.82 – 61.78 mg/g with a mean of 38.65 ± 10.84 mg/g. There was no significant difference between the studied subgroups regarding U Alb/Cr.

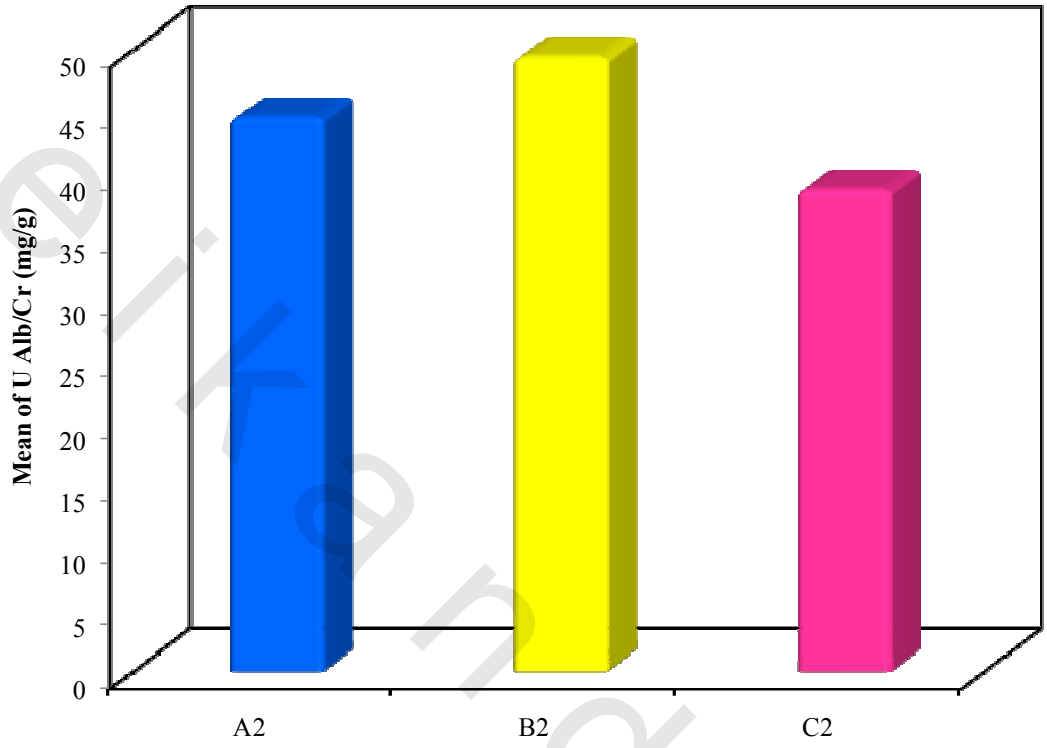

Figure (5): Comparison between the studied groups A2, B2 and C2 according to U Alb/Cr

Table (8): Comparison between the studied groups A1, B1 and C1 according to U NAG/Cr

	A1	B1	C1	Test of sig.	p
U NAG/Cr					
Min. – Max.	2.33 – 8.01	2.42 – 8.11	3.37 – 7.91		
Mean ± SD.	5.34 ± 1.35	5.70 ± 1.90	5.10 ± 1.36	F= 0.496	0.613
Median	5.33	5.78	5.21		
Normal	12 (92.3%)	10 (76.9%)	12 (92.3%)	χ^2 = 1.631	0.587
#Abnormal	1 (7.7%)	3 (23.1%)	1 (7.7%`)		

U NAG/Cr = Urinary n-acetyl-beta-D-glucosaminidase/creatinine ratio
#Abnormal value >7.82(U/g)
χ^2: Chi square test
F: F test (ANOVA)
*: Statistically significant at $p \leq 0.05$

Table (8), shows comparison between the studied subgroups A1, B1 and C1 according to U NAG/Cr, U NAG/Cr in group A1 ranged from 2.33 – 8.01 U/g with a mean of 5.34 ± 1.35 U/g. In group B1 U NAG/Cr ranged from 2.42 – 8.11 U/g with a mean of 5.70 ± 1.90 U/g. In group C1 U NAG/Cr ranged from 3.37 – 7.91 U/g with a mean of 5.10 ± 1.36 U/g. There was no significant difference between the studied subgroups A1, B1 and C1 regarding U NAG/Cr.

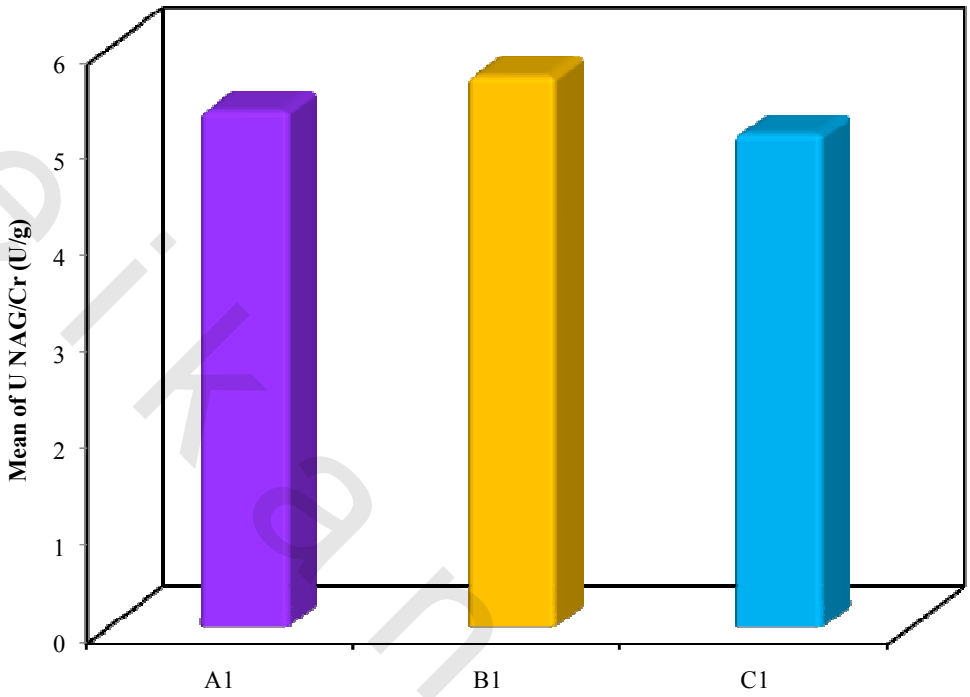

Figure (6): Comparison between the studied groups A1, B1 and C1 according to U NAG/Cr

Table (9): Comparison between the studied groups A2, B2 and C2 according to U NAG/Cr

	A2	B2	C2	Test of sig.	p
U NAG/Cr					
Min. – Max.	2.20 – 8.0	2.23 – 10.77	2.63 – 6.79		
Mean ± SD.	5.07 ± 1.83	6.01 ± 3.53	4.61 ± 1.32	F = 1.058	0.359
Median	4.89	5.49	4.89		
Normal	9 (75.0%)	6 (50.0%)	12 (100.0%)	χ^2= 8.077*	MC p = 0.023*
#Abnormal	3 (25.0%)	6 (50.0%)	0 (0.0%)		
Sig. bet. grps.	p_1 = 0.400, p_2 = 0.217, p_3 = 0.014*				

U NAG/Cr = Urinary n-acetyl-beta-D-glucosaminidase/creatinine ratio
#Abnormal value >7.25(U/g)
χ^2: Chi square test, Sig. bet. grps was done using Fisher Exact test
MC: Monte Carlo test
p_1 : p value for comparing between A2 and B2
p_2 : p value for comparing between A2 and C2
p_3 : p value for comparing between B2 and C2
*: Statistically significant at $p \leq 0.05$

Table (9), shows comparison between the studied subgroups A2, B2 and C2 according to U NAG/Cr, U NAG/Cr in group A2 ranged from 2.20 – 8.0 U/g with a mean of 5.07 ± 1.83 U/g. In group B2 U NAG/Cr ranged from 2.23 – 10.77 U/g with a mean of 6.01 ± 3.53 U/g. In group C2 U NAG/Cr ranged from 2.63 – 6.79 U/g with a mean of 4.61 ± 1.32 U/g. There was a significant difference between the studied subgroups B2 and C2 regarding U NAG/Cr.

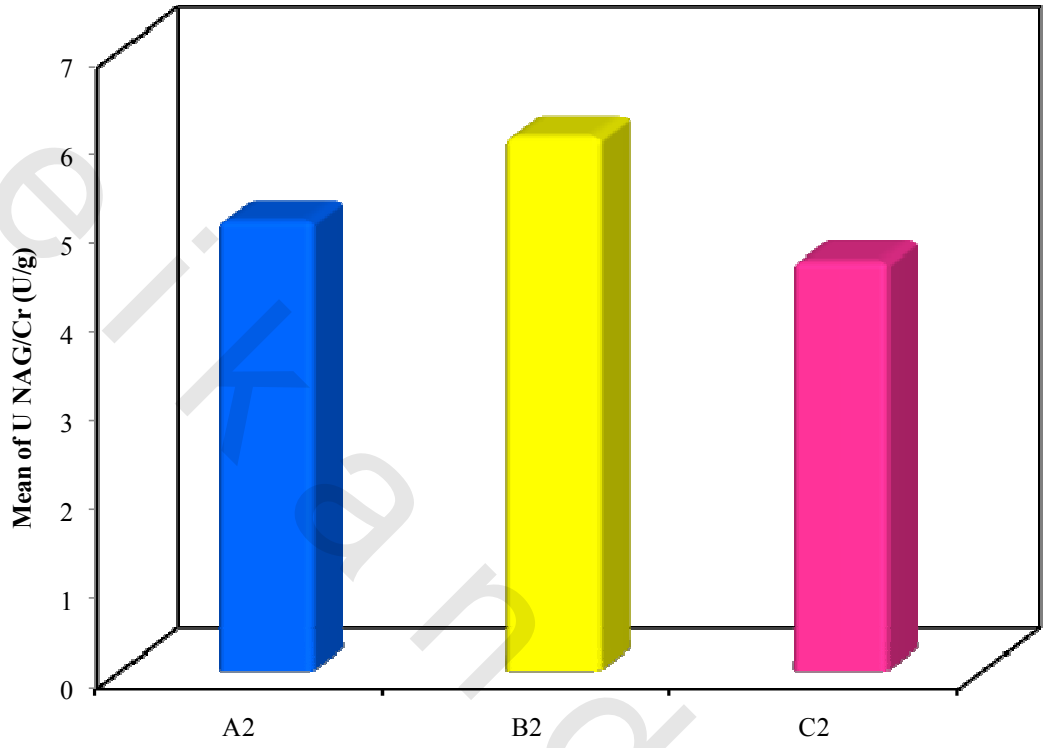

Figure (7): Comparison between the studied groups A2, B2 and C2 according to U NAG/Cr

Table (10): Comparison between the studied groups A1, B1 and C1 according to serum B2M

	A1	B1	C1	Test of sig.	p
B2M					
Min. – Max.	0.50 – 10.0	1.20 – 10.0	0.60 – 6.50	$^{KW}\chi^2$ = 2.110	0.348
Mean ± SD.	3.68 ± 2.62	4.94 ± 2.57	3.80 ± 1.80		
Median	3.0	5.0	4.0		
Normal	12 (92.3%)	11 (84.6%)	13 (100.0%)	χ^2 = 1.980	^{MC}p = 0.756
#Abnormal	1 (7.7%)	2 (15.4%)	0 (0.0%)		

B2M = Serum Beta (2)-microglobulin
#Abnormal value >7.4(mg/l)
χ^2: Chi square test
MC: Monte Carlo test
$^{KW}\chi^2$: Chi square for Kruskal Wallis test

Table (10), shows comparison between the studied subgroups according A1, B1 and C1 to serum B2M, B2M in group A1 ranged from 0.50 – 10.0 mg/l with a mean of 3.68 ± 2.62 mg/l. In group B1 B2M ranged from 1.20 – 10.0 mg/l with a mean of 4.94 ± 2.57 mg/l. In group C1 B2M ranged from 0.60 – 6.50 mg/l with a mean of 3.80 ± 1.80 mg/l. There was no significant difference between the studied subgroups regarding serum B2M.

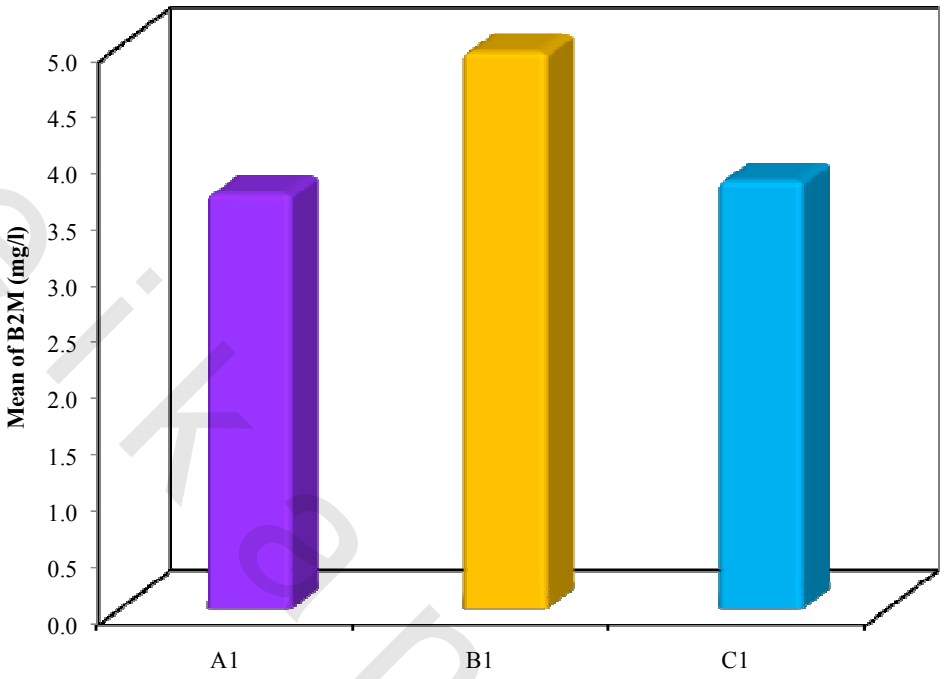

Figure (8): Comparison between the studied groups A1, B1 and C1 according to serum B2M

Table (11): Comparison between the studied groups A2, B2 and C2 according to serum B2M

	A2	B2	C2	Test of sig.	p
B2M					
Min. – Max.	0.60 – 10.0	1.50 – 10.0	0.40 – 10.0	$^{KW}\chi^2$ = 8.079*	0.018*
Mean ± SD.	5.68 ± 2.59	5.57 ± 3.02	2.81 ± 3.07		
Median	5.50	5.0	1.50		
Sig. bet. grps.	$p_1 = 0.749$, $p_2 = 0.13$, $p_3 = 0.017^*$				
Normal	11 (91.7%)	8 (66.7%)	11 (91.7%)	$\chi^2 = 3.017$	^{MC}p = 0.316
#Abnormal	1 (8.3%)	4 (33.3%)	1 (8.3%)		

B2M = Serum Beta (2)-microglobulin
#Abnormal value >8.95(mg/l)
χ^2: Chi square test
MC: Monte Carlo test
$^{KW}\chi^2$: Chi square for Kruskal Wallis test
p_1 : p value for comparing between A2 and B2
p_2 : p value for comparing between A2 and C2
p_3 : p value for comparing between B2 and C2
*: Statistically significant at $p \leq 0.05$

Table (11), shows comparison between the studied subgroups A2, B2 and C2 according to serum B2M, B2M in group A2 ranged 0.60 – 10.0 mg/l with a mean of 5.68 ± 2.59 mg/l mg/l. In group B2 B2M ranged from 1.50 – 10.0 mg/l with a mean of 5.57 ± 3.02 mg/l. In group C2 B2M ranged from 0.40 – 10.0 mg/l with a mean of 2.81 ± 3.07 mg/l. There was a significant difference between the studied subgroups B2 and C2 regarding serum B2M.

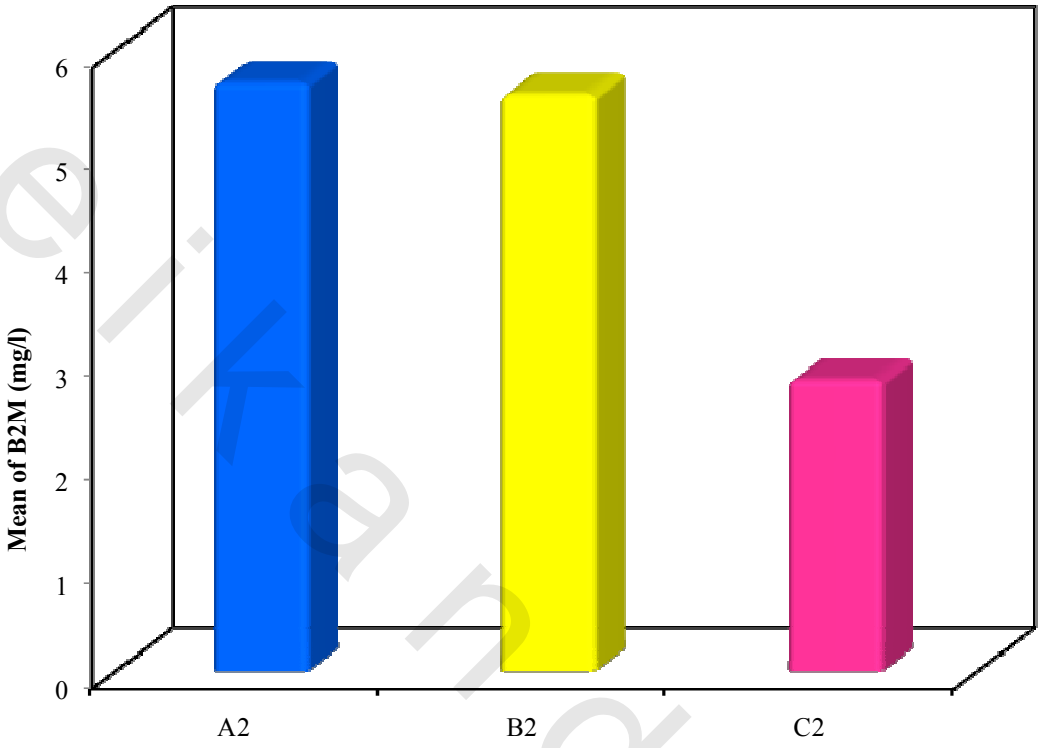

Figure (9): Comparison between the studied groups A2, B2 and C2 according to serum B2M