

Prevalence of Viral Infection among Egyptian Children with End Stage Renal Disease

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Abstract: Background: Viral infections are frequent in hemodialysis patients, notably those due to hepatitis C virus (HCV), hepatitis B virus (HBV), hepatitis G virus (HGV) cytomegalovirus (CMV) and human immunodeficiency virus (HIV). **Objective:** The aim of this work is to study the prevalence of viral infections among Egyptian children with end stage renal disease whether on conservative management or on hemodialysis and to identify the possible associations between viral infections and some clinical parameters. **Subjects:** This cross-section study included 50 patients with end stage renal disease. They were divided into two groups; the first group consisted of 20 patients on conservative management. The second group consisted of 30 patients on regular hemodialysis. **Inclusion criteria:** Children below 18 years, both gender, end stage renal disease patients whether on conservative management or on hemodialysis. **Exclusion criteria:** Patients on immunosuppressant treatment for any particular disease. **Methods:** All patients were subjected to full history, thorough clinical examination and laboratory investigations in the form of complete blood count, renal function tests, and serum electrolytes. Abdominal ultrasound and echocardiography were also done. In addition virological screen was assessed (conventional PCR for HCV, HGV and CMV and ELISA for HBV, HCV and HIV). **Results:** The comparative study between patients on conservative management (group I) and patients on hemodialysis (group II) showed no statistical significant difference between the two groups as regard to gender and age. 60% of the examined patients in each group were male. The mean age in group I was 11.48 ± 4.06 year while in group II it was 10.27 ± 3.20 year ($P=0.253$). However, great statistical differences was found between both groups in the mean onset of chronic renal failure it was 5.93 ± 2.67 year in group I and 3.40 ± 1.30 year in group II ($P=0.002$). Also significant statistical differences were found between both groups as regard to the presence of anemia, ($P=0.000$) and history of blood transfusions ($P=0.002$). 100% of hemodialysis patients had anemia and 86.7% of them received blood. Hypertension was present in 35% of group I and in 63.3% of group II patients ($P=0.049$). Stunted growth was found in 50% of patients on conservative management and in 83.3% of patients on hemodialysis ($P=0.012$). Laboratory studies showed no statistical significant differences between both groups as regard to hemoglobin, hematocrit, ALT, AST, calcium, phosphorus and alkaline phosphatase enzyme. Also no statistical significant difference was found in blood urea between both groups. Whereas there was a significant statistical difference in creatinine level between the two groups. The mean creatinine level in group I patients = 3.49 ± 1.72 mg/dl while in hemodialysis patients = 7.16 ± 1.41 mg/dl ($P=0.000$). Abdominal ultrasound showed that the common cause of chronic renal failure (CRF) in patients on conservative management was obstructive uropathies (60% of cases). While congenital malformations were the commonest cause of CRF in hemodialysis patients (60%). Echocardiography showed that 50% and 10% of hemodialysis patients had left ventricular hypertrophy and pericardial effusion respectively. The virological studies showed that the commonest viral infection in both groups was HCV. It was detected by PCR in 35% of group I and in 50% of group II patients as single infection or as coinfection with other viruses. Cytomegalovirus was present in 20% of group I and in 10% of group II patients. HGV was only present in hemodialysis patients (13.3% of cases). HCV antibodies were detected by ELISA test in 15% of patient on conservative management and in 43.3% of patients on hemodialysis therapy $P=0.035$. No antibodies for HBV and HIV were detected in our patients. Significant association was found between viral infections and patient's age ($P=0.043$). Also significant association was found between viral infections and duration of hemodialysis ($P=0.015$). But no significant associations were found between viral infections and both frequency of dialysis settings ($P=0.485$) and patient's gender ($p=0.361$). **Conclusion and Recommendations:** Viral infections are frequent in hemodialysis patients.

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Strict infection control measures in dialysis units may help in decreasing the risk of infection. Both PCR and ELISA tests are required to maximize HCV diagnostic sensitivity. We also recommended more researches to explore the prevalence of viral infections among children with end stage renal disease in the different nephrology departments and renal dialysis units.

Key words: Chronic renal failure-hemodialysis-viral infections.

INTRODUCTION

Chronic renal failure (CRF) is defined as an irreversible reduction in glomerular filtration rate (GFR). The prevalence of CRF in the pediatric population is approximately 18 per 1 million [Vogt and Avner, 2004]. In CRF, almost every system in the body eventually becomes compromised (Williams *et al.*, 2002).

Hyperfiltration injury is proposed to be an important final common pathway of glomerular destruction. As nephrons are lost, the remaining nephrons undergo structural and functional hypertrophy with increased glomerular blood flow. Although this compensatory hyperfiltration temporarily preserves total renal function, it is theorized to cause progressive damage to the surviving glomeruli, possibly by a direct effect of the elevated hydrostatic pressure on the integrity of the capillary wall and /or the toxic effect of increased protein traffic across the glomeruli leading to sclerosis and vicious cycle (Williams *et al.*, 2002). The rate of nephron destruction differs from one case to the other, ranging from a few months to several years (Yu, 2003).

If the diagnosis is established and the appropriate treatment instituted, accelerated deterioration of renal function could be reversed (Williams *et al.*, 2002). Patients with CRF should be treated in conjunction with a medical centre capable of supplying medical, nursing, social service, nutritional and psychological support as they progress to end stage renal failure (Bergstein, 2000).

Technologic advances in renal replacement therapy have improved markedly the outcome for children with CRF and due to the entitlement programs of dialysis and transplantation, the number of patients with CRF surviving their disease in adding year after year. In the USA, the number of patients treated with dialysis and transplantation is projected to increase from 340.000 in 1999 to 651000 in 2010 (Levey *et al.*, 2003).

The first dialysis in Egypt was performed in 1958 in Ain-Shams university dialysis centre using artificial kidney (El-Said, 1985). Most end stage renal disease patients in Egypt are undergoing intermittent hemodialysis (97.1%), while a minority (2.9%) is treated by peritoneal dialysis. The lack of continuous ambulatory peritoneal dialysis (CAPD) is due to shortage in training for this kind of treatment and the need to import CAPD systems from other countries (Barsoum, 2002).

Hemodialysis is a method for removing waste products such as potassium and urea, as well as free water from the blood when the kidneys are incapable of this (i.e. in renal failure) (Allon and Robbin 2002). Chronic dialysis is usually an interim measure to allow time to prepare for kidney transplantation (Goldstein *et al.*, 2006). Viral infections in immuno-compromised patients is a problem for clinicians (Ocak *et al.*, 2006).

Aim of the Work:

The objective of this work is to study the prevalence of viral infections among Egyptian children with end stage renal disease patients whether on conservative management or on hemodialysis and to identify the presence of any associations between viral infections and some clinical parameters.

Subjects:

This cross-section study included 50 patients with end stage renal disease who were divided into two groups, the first group consisted of 20 patients receiving conservative management and they were selected from the outpatients of the nephrology clinic in Abou El Resh Hospital Cairo University. The second group consisted of 30 patients on regular hemodialysis (HD) selected from the center of Egyptian Society of pediatric dialysis and transplantation (ESPDT) within the same hospital.

Inclusion Criteria:

Children below 18 years, both gender, end stage renal disease patients whether on conservative management or on hemodialysis.

Exclusion Criteria:

Patients on immunosuppressant treatment for any particular disease

Methods:

All Patients were subjected to the following

- 1- Full history which includes age of the patient, cause of renal failure, onset of chronic renal failure and history of blood transfusion.
- 2- Thorough clinical examination including assessment of blood pressure, signs of stunted growth and any signs suggesting active infection.
- 3- Investigations in the form of:

- Complete blood picture
- Renal function tests
- Serum electrolytes
- Virology screen (conventional PCR for HCV, HGV and CMV and ELISA for HBV, HCV and HIV)
- Abdominal ultrasonography
- Echocardiography

Detection of Hepatitis B Surface Antigens and HCV Antibodies:

Markers for both HBV and HCV were tested by ABBOTT AXSYM, which is Microparticle Enzyme Immunoassay (MEIA). MEIA is a variation of the enzyme immunoassay (EIA) principal. Solid phase EIA uses antigens and/or antibodies coated on a surface to bind complementary analysts. The bound analyze is detected by a series of antigen-antibody reaction. In the AXSYM final reaction, an antibody couple to an enzyme act upon a substrate to produce a fluorescent end product. The fluorescence produced by the enzyme reaction is measured (Miyamura *et al.*, 2001).

Serum Polymerase Chain Reaction (PCR):

Extraction of total DNA and RNA was done using INVITEK viral DNA and RNA extraction kit, Germany. Extraction was done according to the manufacturer instructions, (200 µl serum was added to a tube containing extraction buffer, 200 µl H₂O was added, tube placed at 80°C (in thermomixer) for 15 min, then 95°C for 10 min, 400 µl binding buffer were added and were vortexed for 30 sec, the contents of the tube were transferred to spin column and centrifuged at 14000 RPM for 1 min, after washing 2 times and centrifugation 60 µl elution buffer was added then centrifuged at 14000 RPM. The eluted buffer contain RNA and DNA.

Reverse Transcription-Nested PCR for HCV and HGV:

Reverse transcription for preparation of complementary DNA (cDNA) with reverse transcriptase (Moloney Murine leukemia virus [MMULV]) using ready to go RT-PCR beads (Pharmacia or Amersham biosciences). The outer and inner primer sequences of HGV for nested PCR were selected from NS3 helicase region of HGV genome, the primer sequences were as follows:

- Outer primers: Sense S1: 5'-GGC ACC TCG TGT TCT GCC A-3'
Antisense A1: 5'-AGG TCT CCG TCC TTG ATG AT-3'
Inner primers: Sense S2: 5'-CAT TC (AC) AAG GCG GAG TGC GAG-3'
Antisense A2: 5'-(AG) TC (CT) TT GAT GAT GGA ACT GTC-3'

The outer and inner primer sequences of HCV for nested PCR were 5'UTR-specific primers of HCV genome, the primer sequences were as follows:

- Outer primers: Sense NF5(5'-GTG AGG ACC TAC TGT CTT CAC GCA G)
Antisense NR5(5'-TGC TCA TGG TGC ACG GTC TAC GAG A)
Inner primers: Sense KF2 (5'-TTC ACG CAG AAA GCG TCT AG)
Antisense 211(5'-CAC TCT CGA GCA CCC TAT CAG GCA GT)

Reverse Transcription-PCR Mixture:

5 µl extracted sample, 1 µl of sense and 1 µl of antisense primers of outer primers (50 pmol each), 43ml H₂O, the 50 µl were put on tubes containing the beads or mastermix and amplification was done in Perkin Elmer 9600 thermalcycler. The second round of PCR using pure Taq ready to go PCR beads (Pharmacia), 2 µl of the first round PCR, 1 µl of each inner primers, and 21 µl H₂O, the total 25 µl were added to the kit tube containing the beads and put in the thermalcycler for second amplification.

PCR protocol consisted of one cycle of RT at 57°C for 3 min, 42°C for 30 min, 95°C for 3 min followed by first PCR round at 94°C for 30 sec, 51°C for 30sec, 72°C for 30 sec, this is repeated for 35 cycles, then 72°C for 5 min. The second round PCR (nested) at 95°C for 5 min, 94°C for 30sec, 53°C for 30 sec, 72 °C for 30 sec, repeated 30 cycles then 72°C for 5 min for 1 cycle.

DNA amplification for CMV detection:

Extracted DNA first prewarmed at 95°C, then quickly chilled on ice.

CMV was amplified by PCR with CMV specific primers

Forward 5-ACGTGTTACTGGCGGAGTCG-3

Reverse 5-AGCACTGGCCAGCTCATATC-3

PCR amplification was done by using ready to go PCR beads (Amersham Pharmacia biosciences), the following were added to the ready to go PCR beads, 30 pmol of each primers, 5 µl of extracted DNA and complete with sterile H₂O to total volume of 25 µl, thermocycling conditions 95°C for 5 min, 94 °C for 30 sec, 55 °C for 30 sec, 72°C for 60 sec, for 35 cycle, 1 cycle at 72 °C for 7 min. All amplification products were separated by agarose gel electrophoresis in 2% agarose gel stained with ethidium bromide, and photographed under ultraviolet light. In each PCR assay true negative control, and one positive control were tested (Sigma chemical co) genomic DNA, RNA were interpreted against the pattern of a molecular size marker (500 bp DNA ladder).

HGV genomic RNA had a size of 101bp.

HCV genomic RNA had a size of bp.

CMV genomic DNA had a size of 368 bp.

Avoidance of PCR Contamination:

To avoid PCR contamination we used separate airflow cabinets for RNA, DNA extraction and RT-PCR. In addition individual sets of micropipettes and aerosol-resistant tips were used for each procedure. Agarose gel electrophoresis was carried out in a separate room. We strictly followed the recommendation of Kwok and Higuchi (1997).

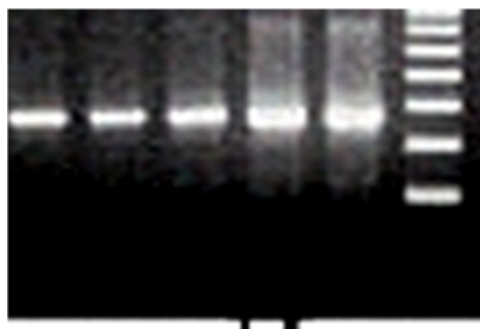


Fig. 1: CMV gel electrophoresis showing 368 bp of CMV

Statistical Analysis:

Data were statistically described in terms of mean \pm standard deviation (\pm SD), frequencies (number of cases) and relative frequencies (percentages) when appropriate. Comparison of quantitative variables between the study groups was done using Mann Whitney U test for independent samples when comparing 2 groups and Kruskal Wallis analysis of variance (ANOVA) test when comparing more than 2 groups. For comparing categorical data, Chi square (X^2) test was performed. Yates correction equation was used instead when the expected frequency is less than 5. A probability value (p value) less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs Microsoft Excel version 7 (Microsoft Corporation, NY, USA) and SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) statistical program for Microsoft Windows.

RESULTS AND DISCUSSION

Table (1a&b): Show the comparative study between patients on conservative management (group I) and patients on hemodialysis (group II) as regard to the history. 60% of patients in each group were male (P=1.000). The mean age in group I was 11.48±4.06 year while in group II it was 10.27±3.20 year, p-value =0.253 which indicated that there were no significant differences in gender and age between both groups.

However the mean onset of CRF in group I was 5.93 ± 2.67 year and in group II it was 3.4 ± 1.30 year (P=0.002) which indicates a significant difference in the onset of CRF. This table shows also that there were significant differences between both groups as regard to the history of the presence of anemia and blood transfusion. 35% of patients on conservative management and 100% of hemodialysis patients had anemia (P=0.000). Blood transfusions were given to 45% of group I patients and it was given to 86.7% of group II patients (P=0.002).

Table (2): Shows the comparative study between patients on conservative management and patients on hemodialysis as regard to some clinical pictures. Hypertension was present in 35% of patients on conservative management and it was present in 63.3% of patients on hemodialysis therapy (P=0.049) which means a significant statistical difference between both groups. Significant statistical difference was also found between both groups as regard to stunted growth. This clinical finding was present in 50% of group I patients and it was present in 83.3% of hemodialysis patients (group II) P=0.012.

Table (3): Shows the comparison between conservative and hemodialysis groups as regard to laboratory data. The mean ± SD of urea level in group I was 52.48 ± 22.54 mg/dl while in group II it was 63.67± 15.87 mg/dl, P-value = 0.140 which indicated that there was no significant difference between both groups. Whereas there was a highly significant statistical difference between both groups as regard to the creatinine level. The mean creatinine level ± SD in group I was 3.49 ± 1.72 mg/dl while in group II it was 7.16 ± 1.41 mg/dl, the p-value=0.000. The rest of the laboratory data showed that there were no significant differences between both groups as regard to the hemoglobin, hematocrit, alanine aminotransferase (ALT), aspartate aminotransferase (AST), calcium, phosphorus and alkaline phosphatase enzyme.

Table (4): Shows the abdominal ultrasonography and the echocardiography findings in both groups. Congenital renal malformations were present in 30% of group I patients and in 60% of hemodialysis patients. Obstructive uropathies were found in 60% of patients on conservative management and in 26.7% of hemodialysis patients. Echocardiography showed that 50% and 10% of hemodialysis patients had left ventricular hypertrophy and pericardial effusion respectively.

Table (5): Shows the prevalence of viral infections in end stage renal disease. The commonest viral infection was HCV which was detected by PCR in 35% of group I and in 50% of group II patients as a single infection or as coinfection with other viruses. This table showed also an association between patients on regular hemodialysis and the presence of positive HCV antibodies.

Table (6): Shows that there was significant statistical differences between PCR positive and PCR negative cases as regard to the duration of hemodialysis (P=0.015) and age of patients (P=0.043) but there was no statistical significant difference between them as regard to the frequency of settings.

Table (7): Shows the association between gender and PCR results in hemodialysis patients. No significant association was found between gender and PCR positive cases and PCR negative cases P= 0.361.

Table (8): Shows that there was no significant association between the cause of end stage renal disease whether obstructive or non obstructive and PCR results in hemodialysis patients.

Table (9): Shows the association between HCV antibodies and PCR in all patients whether on hemodialysis or on conservative management. All cases with positive HCV antibodies had also positive PCR but there were 6 cases with negative HCV antibodies had positive PCR i.e not all viral antibody negative cases were negative by PCR testing.

Table 1a: Comparative study between patients on conservative management and patients on hemodialysis as regard to the history.

Patients	Mean	Std deviation	Median	Minimum	Maximum	P-value
Patient's age (year)						
Conservative	11.48	4.06	12.00	3.00	17.00	0.253
Hemodialysis	10.27	3.20	10.25	4.00	16.00	
Onset of CRF (year)						
Conservative	5.93	2.67	5.50	1.50	10.00	0.002*
Hemodialysis	3.40	1.30	3.50	1.00	5.50	
Paternal age (year)						
Conservative	43.85	10.20	42.50	26.00	60.00	0.684
Hemodialysis	42.07	6.24	43.00	32.00	60.00	
Maternal age (year)						
Conservative	38.80	8.87	36.50	22.00	53.00	0.512
Hemodialysis	36.93	6.03	36.50	24.00	50.00	

Table 1b: Comparative study between patients on conservative management and patients on hemodialysis as regard to the history.

	Group			P-value
	Conservative	Hemodialysis	Total	
Gender				
Male count	12	18	30	1.000
% within group	60%	60%	60%	
Female count	8	12	20	40%
% within group	40%	40%	40%	
History of anemia				
Negative count	13	0	13	0.000**
%	65%	0%	26%	
Positive count	7	30	37	74%
%	35%	100%	74%	
History of bl. transfusion				
Negative count	11	4	15	0.002*
%	55%	13.3%	30%	
Positive count	9	26	35	70%
%	45%	86.7%	70%	
History of Surgery				
Negative count	10	16	26	0.817
%	50%	53.3%	52%	
Positive count	10	14	24	48%
%	50%	46.7%	48%	

P > 0.05 = insignificant

* P < 0.05 = significant, ** P < 0.001= highly significant

Table 2: Comparative study between patients on conservative management and patients on hemodialysis as regard to some clinical findings.

	Group			P- value
	Conservative	Hemodialysis	Total	
Hypertension				
Negative count	13	11	24	0.049*
%	65%	48%	60%	
Positive count	7	19	26	63.3%
%	35%	52%	63.3%	
Stunted growth				
Negative count	10	5	15	0.012*
%	50%	30%	40%	
Positive count	10	25	35	83.3%
%	50%	70%	83.3%	

P > 0.05 = insignificant

* P < 0.05 = significant, ** P < 0.001= highly significant

Table 3: Comparative study between patients on conservative management and patients on hemodialysis as regard to laboratory data.

Patients	Mean	SD	Median	Minimum	Maximum	P-value
Urea (mg/dl)						
Conservative	52.48	22.54	58.00	12.00	88.00	0.140
Hemodialysis	63.67	15.87	62.00	35.00	99.00	
Creatinine (mg/dl)						
Conservative	3.49	1.72	3.90	0.50	6.30	0.000**
Hemodialysis	7.16	1.41	6.85	4.60	10.40	
Hb (gm/dl)						
Conservative	9.40	1.70	9.05	5.80	13.30	0.098
Hemodialysis	10.45	2.02	10.25	7.00	14.60	
Hematocrit (%)						
Conservative	26.39	5.88	25.95	13.00	36.00	0.208
Hemodialysis	28.78	6.89	28.65	17.60	39.00	
ALT (µ/L)						
Conservative	50.98	63.49	25.00	12.00	280.00	0.148
Hemodialysis	33.29	38.49	20.00	4.00	200.00	
AST (µ/L)						
Conservative	51.85	61.77	36.00	17.00	289.00	0.151
Hemodialysis	43.00	68.88	24.50	11.00	391.00	

Table 3: Continue

Calcium (mg%)						
Conservative	9.28	1.34	9.30	6.70	12.50	
Hemodialysis	8.84	1.51	8.80	6.20	13.40	0.098
Phosphorus (mg %)						
Conservative	6.03	1.55	6.10	3.60	10.00	0.127
Hemodialysis	5.11	1.69	5.25	1.30	8.10	
ALP (%)						
Conservative	660.80	363.54	606.50	121.00	1235.00	0.546
Hemodialysis	751.70	441.67	666.00	192.00	1843.00	

Hb = Hemoglobin, ALT = Alanine aminotransferase, AST= Aspartate aminotransferase, ALP= Alkaline phosphatase.

P > 0.05 = insignificant

* P < 0.05 = significant, ** P< 0.001= highly significant

Table 4: Comparative study between patients on conservative management and patients on hemodialysis as regard to abdominal ultrasound and echocardiographic findings.

Diagnostic data	Group	
	Conservative	Hemodialysis
US findings		
Congenital renal malformations	6(30%)	18(60%)
Obstructive uropathies	12 (60%)	8(26.7%)
Medical renal disease	2(10%)	4(13.3%)
Echo findings		
Negative	13(65%)	15 (50%)
LVH	7(35%)	15 (50%)
Pericardial effusion	2(10%)	3(10%)

US = Ultrasonography, LVH= Left ventricular hypertrophy

Table 5: Prevalence of viral infections in end stage renal disease patients.

	Group		
	Conservative	Hemodialysis	Total
HCV Antibodies			
-ve count	17	17	34
% within group	85%	56.7%	68%
+ve count	3	13	16
% within group	15%	43.3%	32%
PCR:			
-ve count	10	12	22
% within group	50%	40%	44%
HCV			
+ve count	6	12	18
% within group	30%	40%	36%
CMV			
+ve count	3	0	3
% within group	15%	0%	6%
HCV +CMV			
+ve count	1	2	3
% within group	5%	6.7%	6%
HGV			
+ve count	0	3	3
% within group	0%	10%	6%
HCV +HGV +CMV			
+ve count	0	1	1
% within group	0%	3.3%	2%

HCV= Hepatitis C virus, HGV= Hepatitis G virus, CMV= Cytomegalovirus

PCR= Polymerase chain reaction

• = Significant association between hemodialysis patients & positive HCV antibodies,P= 0.035.

Table 6: Statistical difference between PCR negative and PCR positive as regard to the frequency of setting, duration of dialysis and age of the patients.

	Mean	Std deviation	Median	Minimum	Maximum	P-value
Frequency						
PCR -ve	156.00	33.03	160.50	106.00	211.00	0.485
PCR +ve	156.00	37.71	166.00	64.00	231.00	
Duration (year)						
PCR -ve	2.50	1.09	3.00	1.00	4.00	0.015*
PCR +ve	3.58	1.10	3.50	1.00	5.00	
Patient's age (year)						
PCR -ve	9.00	2.66	9.00	4.00	13.00	
PCR +ve	11.11	3.31	11.50	4.00	16.00	0.043

*P > 0.05 = insignificant * P < 0.05 = significant

Table 7: Association between gender and PCR results in hemodialysis patients.

	PCR			P-value	
	Negative	Positive	Total		
Gender					
Male	count	6	12	18	0.361
	%	50.0%	66.7%	60.0%	
Female	count	6	6	12	40.0%
	%	50.0%	33.3%	40.0%	

Table 8: Association between the cause of ESRD (obstructive and non obstructive) and PCR results in hemodialysis patients.

Cause	PCR			P-value	
	Negative	Positive	Total		
Non obstructive					
	count	10	11	21	0.193
	%	83.3%	61.1%	70.0%	
Obstructive					
	count	2	7	9	30.0%
	%	16.7%	38.9%	30.0%	

ESRD = End stage renal disease, P> 0.05 = Not significant

Table 9: Association between HCV antibodies and PCR in all patients whether on hemodialysis or on conservative management.

	PCR (HCV)			P-value	
	Negative	Positive	Total		
HCV Antibodies					
- ve					
	count	28	6	34	< 0.001
	% within Abs	82.35%	17.65%		
	% within PCR	100.0%	27.3%		
+ve					
	count	0	16	16	
	% within antibodies	0%	100.0%		
	% within PCR	0%	72.7%		

P-value < 0.001 highly significant.

Figure 2: Shows the association between both groups and HCV antibody results.

Figure 3: Shows the association between both groups and PCR results.

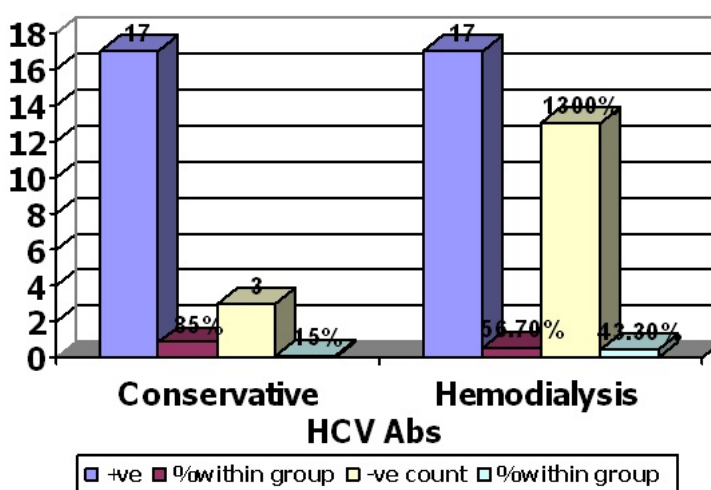


Fig. 2: Association between both groups and HCVAbs results.

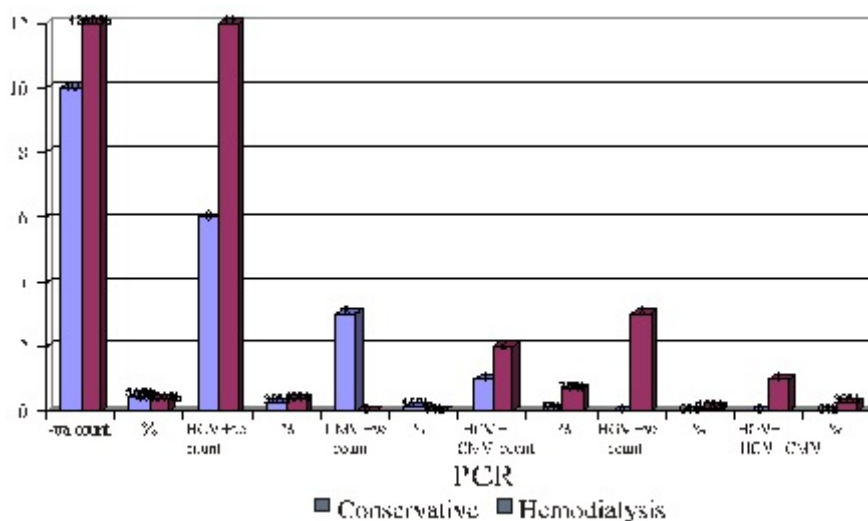


Fig. 3: Association between both groups and PCR results.

Discussion:

Viral infections are frequent in hemodialysis patients, notably those due to hepatitis C virus (HCV), hepatitis B virus (HBV), cytomegalovirus (CMV) and human immunodeficiency virus (HIV) (Boulaajaj *et al.*, 2005).

Hepatitis G virus (HGV) infection was frequent in hemodialysis patients related to transfusions and independent of HCV prevalence (Fernandez *et al.*, 2000).

Kidney diseases are important complications of human immunodeficiency virus infection, particularly in African-American populations (Wyatt *et al.*, 2007). Some of these disorders can result in end-stage renal disease (ESRD). HIV may occur in patients on dialysis, its prevalence varies by method of detection, center location, and demographics of the local ESRD population (Klptman *et al.*, 2008).

The aim of this work is to study the prevalence of viral infections among children with end stage renal disease and to identify the possible associations between viral infections and some clinical parameters.

The present study was conducted on 50 patients with ESRD who were divided into two groups, the first group consisted of 20 patients received conservative management and the second group consisted of 30 patients on regular hemodialysis (HD). 60% of cases in both groups were males, the age of the patients in the first group ranged from 3 to 17 year with mean \pm standard deviation (SD) 11.48 ± 4.06 while in the second group the age of the patients ranged from 4 to 16 year with mean \pm SD 10.27 ± 3.20 . No statistical significance difference was found in the age between both groups ($P=0.253$). Also no statistical significance difference were detected between both groups as regard to gender, maternal or paternal age ($P=1.000$), ($p=0.512$), ($P=0.684$) respectively. Whereas there was a significant statistical difference between both groups as regard to the onset of chronic renal failure (CRF) ($P=0.002$). The mean onset of CRF in group I patients was (5.93 ± 2.67 year) while in group II it was (3.40 ± 1.30 year). Patients on regular hemodialysis therapy have shorter onset of CRF as they subjected earlier to hemodialysis.

CRF can result from a variety of conditions that lead to permanent loss of nephrons such as glomerulonephritis with its different forms, polycystic kidneys, renal dysplasia, obstructive uropathies, hypertension, diabetes and metabolic disorders such as hyperoxaluria (Parmar, 2002, Yu 2003, Vogt and Avner 2004).

The abdominal ultrasound showed in our study that the most common cause of CRF was obstructive uropathy (60%) of patients on conservative management while congenital renal malformations were the commonest cause of CRF (60%) of patients on regular hemodialysis therapy, medical renal diseases were the least cause of CRF in both groups.

In the present work a significant past history of anemia was found in patients on regular hemodialysis. (Past history of anemia was reported in 35% of patients on conservative management whereas it was found in 100% of patients on regular hemodialysis). Great association was present between anemia and CRF ($P<0.001$).

Our results was in agreement with Thomas, (2007) who stated that approximately 20 million people in the United States population have chronic kidney disease (CKD) and 2-4 million of them have anemia. Vogt and Avner, (2004) explained the cause of anemia in CRF that it is due to decreased erythropoietin production, decrease erythrocyte production, low grade hemolysis and bleeding tendency.

Potential consequences of anemia include cognitive impairment, angina and the cardio-renal anemia syndrome which is a triad of (worsening anemia, worsening chronic kidney disease and worsening congestive heart failure) (Thomas, 2007).

Echocardiographic findings in this study showed that 35% of patients on conservative management and 50% of patients on regular hemodialysis had left ventricular hypertrophy (LVH). In addition pericardial effusion was found in 10% of cases in each group.

Hypertension was observed among our patients with CRF, it was present in 35% of group I and in 63.3% of group II. Williams *et al.*, (2002) stated that sustained hypertension was related to volume overload and /or excessive rennin production.

Features of renal disease that are marked during childhood include severe growth impairment (Liach and Bover, 2000) developmental delay (Yu, 2003) and bone abnormalities (Couttenye *et al.*, 1999). In this study 50% of patients on conservative management (group I) and 83.3% of patients on regular hemodialysis (group II) had stunted growth. Williams *et al.*, (2002) explained the pathophysiology of renal osteodystrophy that there is an impairment of phosphate excretion, which results in elevation of serum phosphate and a reciprocal drop in calcium stimulating the development of secondary hyperparathyroidism.

Significant hypertension and stunted growth were present in patients on regular hemodialysis when compared to those on conservative management (P=0.049) and (P=0.012) respectively.

The biochemical and hematological studies showed that there were no statistical significant differences between patients on conservative management and patients on regular hemodialysis as regard to hemoglobin level, hematocrit, ALT, AST, calcium, phosphorus and alkaline phosphatase enzyme. Also no significant difference in the mean urea level was found between both groups. The mean urea level \pm SD in group I was 52.48 ± 22.54 mg/dl and it was 63.67 ± 15.87 mg/dl in group II (P=0.140).

However the mean \pm SD of creatinine level in group I was 3.49 ± 1.72 mg/dl while in group II it was 7.16 ± 1.41 mg/dl which indicates that there was a highly significant difference between both groups (P-value < 0.001). This denotes the importance of creatinine in the diagnosis of the severity of chronic renal disease.

Another aspect of this study is the prevalence of viral infections among children with end stage renal diseases whether on conservative management or on regular hemodialysis therapy.

Hepatitis B virus (HBV) and Hepatitis C virus (HCV) infections are important causes of morbidity and mortality in hemodialysis patients (Saha and Agarwal, 2001) and pose problems in the management of the patients in the renal dialysis units. Chronic renal failure patients do not clear these viral infections efficiently. Several outbreaks of hepatitis have occurred in these settings (Moreira *et al.*, 2003).

HBV infection is less prevalent than HCV in hemodialysis units (Oesterreicher *et al.*, 1995). Our study showed the absence of HBV infection from all the patients in this work. On the other hand HCV infection was the commonest viral infection and it was present in 35% of cases on conservative management and in 50% of cases on regular hemodialysis; which was detected by PCR.

These results coincided with Delarocque-Astagneau *et al.*, (2002) and Vikrant *et al.*, (2004) who stated that the prevalence of HCV infection among hemodialysis is high and varies between countries (2%-60%) and between dialysis units within a single country. Reddy *et al.*, (2005) found positive HCV in 9.6% of their cases. Our results disagree with Ghosh and Joshi, (2000), Saha and Agarwal, (2001) and Cao *et al.*, (2007) who reported the prevalence of hepatitis B in dialysis population ranges from 3.4% to 42% in various studies and hepatitis C in 12% to 15% .

The severity of liver disease among hepatitis C patients on hemodialysis is controversial. Trevizoli *et al.*, (2008) concluded that the lower biochemical and inflammatory activities observed in hemodialysis patients suggest that hemodialysis and uremia may have a protective role against progression of the disease caused by HCV.

Fabrizi *et al.*, (2002) explained the low prevalence of HBV that the introduction of HBV vaccination, isolation of HBV positive patients, use of dedicated dialysis machines and regular surveillance for HBV infection dramatically reduced the spread of HBV in this setting.

Recently Taziki and Espahbodi (2008) found that the prevalence of hepatitis C virus infection in HD has decreased significantly due to more strict infection control measures in the dialysis units.

In the present investigation HGV infection was not present in any patients on conservative management while it represented 10% of the viral infections in patients on hemodialysis in addition to 3.3% of patients on HD who had coinfection of HGV, HCV and CMV.

Although mixed HCV/HGV infections indicate common risks, the prevalence of HCV in a particular setting doesn't predict prevalence of HGV. As new infections are detected in the absence of blood transfusion. So, HGV may be another marker of viral transmission (Cornu *et al.*, 1997).

CMV infection is more widespread in developing countries and in areas of low socio-economic conditions. Once a person become infected, the virus remains alive, but usually dormant. It rarely causes recurrent disease unless the person's immune system becomes suppressed due to therapeutic drugs or disease (Ocak *et al.*, 2006). It is well known that patients on regular HD treatment are in an immunodeficient state (Eleftheriadis *et al.*, 2004).

The prevalence of CMV in our study was shown in 20% of patients on conservative management and in 10% of patients on regular hemodialysis. Higher prevalence of CMV infection was found in the study of Ocak *et al.*, (2006) who detected the virus in 99.6% of the HD patients. This difference may be explained that our results were diagnosed by qualitative PCR while Ocak *et al.*, (2006) reported their results by ELISA to investigate anti CMV IgG and IgM antibodies.

This study showed the absence of human immunodeficiency virus (HIV) in the patients of both groups. HIV seropositively has been reported in nearly 20% of dialysis patients in some urban centers, particularly those located in communities with high HIV seroprevalence (Winston and Klotman 1996 and Klptman *et al.*, 2008). However dialysis centers in suburban communities or those with a much lower percentage of minority patients may have few or no HIV-infected patients (Vigneau *et al.*, 2005).

Associations between viral infections and some parameters such as age, gender, causes of ESRD, blood transfusion, duration of hemodialysis and frequency of HD settings were studied in this work.

No significant associations were found between viral infections and both gender ($P=0.361$) and etiology of ESRD ($P=0.193$) whereas significant associations were detected between viral infections and both patient's age ($P=0.043$) and duration of dialysis ($P=0.015$).

History of previous blood transfusion was frequent in patients on regular HD when compared to patients on conservative management ($P=0.002$).

Vladutiu *et al.*, (2000) reported that the prevalence of HBV and HCV infections didn't correlate with the age of the patients and depended on the quality of transfused blood.

The link between the duration of hemodialysis and the prevalence of HBV and /or HCV infections proved nosocomial transmission. Our results were not in accordance with some results of Molle *et al.*, (2002) who noticed only an association of HCV infection with length of time on dialysis, but not with age, gender or units of blood transfused. However, Cao *et al.*, (2007) detected that 39.4% of their patients positive for HBV markers had history of blood transfusion.

Our study showed no significant statistical difference between PCR negative and PCR positive cases as regard to the frequency of settings ($P=0.485$); this result disagree with that of El Banna *et al.*, (2001) who found a significant correlation between the frequency of dialysis per week and HCV infections status.

The association between HCV antibodies and PCR was investigated in all patients whether on conservative management or on regular hemodialysis, we observed that all cases with positive HCV antibodies had also positive PCR results but there were 6 cases who had negative HCV antibodies and had positive PCR i.e. not all viral antibody negative cases were negative by PCR testing. These means that PCR is more accurate than ELISA in detecting recent infection and both tests are required to maximize HCV diagnostic sensitivity (Molle *et al.*, 2002).

Conclusion and Recommendations:

Viral infections are frequent in hemodialysis patients. This study showed that HCV infection is the commonest viral infection among Egyptian patients with end stage renal disease whether on conservative management or on regular hemodialysis.

The introduction of HBV vaccination, isolation of HBV positive patients and the use of dedicated machines in patients receiving regular hemodialysis therapy were important measures to eradicate HBV infection. Regular virologic screening of HBV, HCV and HIV in blood banks may eliminate the possible infections by blood transfusion. Adequate sterilization of equipments during procedures and more strict infection control measures in the dialysis units are required to reduce nosocomial transmission. We also recommended the use of both PCR and ELISA to maximize HCV diagnostic sensitivity in patients with end stage renal diseases.

In conclusion, our study can be repeated on a large scale and more researches are needed to investigate the prevalence of viral infections among Egyptian children with end stage renal disease in other nephrology departments and renal dialysis units.

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