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ASSOCIATION OF UREMIC PRURITIS IN HEMODIALYSIS PATIENTS WITH HEPATITIS C VIREMIA: ONE EGYPTIAN CENTRE EXPERIENCE

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ABSTRACT

Pruritis is a common complaint in uremic patients. Various pathogenic factors are claimed to be associated with complaint. Hepatitis C virus infection may be a predisposing factor for such complaint. The aim of the present study was to investigate the prevalence of pruritis in ESRD patients under hemodialysis in one Egyptian center and to correlate its association with the presence of hepatitis C viremia. This cross sectional study was conducted on ninety three patients attending Mansoura University hospital, Mansoura Nephrology and Dialysis Unit, Egypt from January 2015 till August 2015. The patients were subjected to full clinical examination and virological studies for hepatitis C virus (HCV) IgG and detection of HCV viremia by nested reverse transcriptase polymerase chain reaction (nested RT-PCR). Prutitis complaint was present among the majority of patients 86 (92.5%). HCV IgG was positive in more than half of the patients 51 (54.8%) with positive HCV viremia in 28 (30.1%). In comparison between patients with pruritis and patients without, HCV IgG , HCV RNA and duration of dialysis had high significant association with pruritis (P=0.0001 for each). The present study highlights the high prevalence of prutitis in patients under hemodialysis. The distinguished finding of the present study is the high significant association of hepatitis C viremia with pruritis in patients under hemodialysis. Future multicenters studies are recommended to elucidate the significance of this finding.

KEYWORDS: **Hepatitis C viremia, Pruritis, Chronic renal failure**

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INTRODUCTION

Uremic pruritis is the most common problem in patients with end stage renal disease (ESRD) requiring hemodialysis. It was reported in one study in USA that pruritis was prevalent among in 57% of 18,801 hemodialysis (HD) patients. The prevalence of pruritis varies among different studies from 15% up to 90% [1, 2.] The severity of pruritis in ESRD ranges from sporadic discomfort to complete restlessness during day and night. The complaint may be associated with depression, lack of sleep, and low physical and mental activity and a low score of life quality [2-4]. The etiology of pruritis in ESRD is thought to be associated with pro-inflammatory imbalance due to enhance activity of T helper cells type 1 (TH1) with uncontrolled production of cytokines like interleukin 6 and interleukin 2 [5]. Another theory for an explanation of this phenomenon was hypothesized claiming peripheral neuropathy as a cause. Active nerve fibers for specific mediators called a neuron enolase manor were found to spread throughout the epidermis in HD patients, but not in healthy controls [6] these were not found by other researchers [7, 8]. Metabolic disorders associated with increase serum levels of calcium and phosphorus are claimed by some researchers with increased level of calcium in deeper layers of epidermidis [4, 8, 9] .Skin dryness is the most common skin manifestation of ESRD which may be another predisposing factor for this condition [10, 11]. In Egypt, an area highly endemic for hepatitis C virus infection, the assumption of association with hepatitis C infection in ESRD with purities may need to be investigated a role. It is known that infection with hepatitis C virus (HCV) is usually associated with different clinical manifestations in addition to hepatic affection [12, 13]. Among these manifestations are dermatological conditions and systemic disorders affecting the skin [14]. In some cases, cutaneous signs or symptoms may provide a suspicious marker for the presence of an underlying HCV infection [15, 16]. It is thought that the same mechanism is responsible for development of pruritus in both cholestatic and non cholestatic liver diseases. It is assumed that it is caused by substances that are not secreted in the bile, but rather accumulate in the skin, interacting with the nerve endings leading to the sensation of itching. Bile acids and histamine have been claimed to be the principal responsible agents in pruritis associated with hepatitis C infection. However, other factors may contribute like change in central neurotransmitter levels. The aim of the present study was to investigate the prevalence of pruritis in ESRD patients under hemodialysis in one Egyptian center and to correlate its association with the presence of hepatitis C viremia.

MATERIALS AND METHODS

This cross sectional study was conducted on 93 patients attending Mansoura University hospital, Mansoura Nephrology and Dialysis Unit, Egypt from January 2015 till August 2015. They were complaining of ESRD requiring regular hemodialysis. Informedconsent had been obtained from participating subjects according to Ethics Committee of Faculty of Medicine, Mansoura University. A complete medical record was obtained for each patient including age, history of blood transfusion and duration of hemodialysis. All patients were subjected to a complete physical examination. Blood samples were obtained from each subject and sera were separated. A serum for each subject was distributed into three aliquots. One for full biochemical tests for liver, including alanine aminotransferase (ALT) aspartate aminotransferase (AST), bilirubin and albumin. The other aliquot was used for serological studies by enzyme linked immunosorbant assay for hepatitis C virus IgG (HCV IgG (Dia-Pro ANTI-HCV, ITALY). The third sera aliquots were kept frozen at -70^0 for further molecular study of hepatitis C virus RNA by nested reverse transcriptase polymerase reaction (RT-PCR).

Nested RT-PCR, PCR for HCV RNA

Viral RNA extraction

This was performed by QIAamp Viral RNA minikit for purification of viral RNA from serum according to manufacturer instructions (Qiagen, Operon, Colongne, Germany).

Nested RT-PCR

HCV-RNA detection was performed by nested RT-PCR with primers deduced from the conserved 5' non coding region (5'UTR) according to Qiagen protocol. The method could detect as few as 1,000 copies/µl of HCV-RNA, as evaluated by the accuracy sensitivity control system (Boston Bio-medica, *West Bridgewater, MA)* [17]. The following mixture (for each sample) was prepared for each extracted RNA, Qiagen One step RT-PCR buffer 10 ul RNase-free water 16 ul, dNTP mix 2 ul, primer (1CH)5 ul primer (2CH)5 ul Qiagen One step RT-PCR Enzyme Mix 2 ul, and 10 ul extracted RNA . Amplification cycle used was 50°C for 30 min, 95°C for 15 min, (94°C for 1 min 50–68°C 1 min 72°C for 1 min) 40 cycles. Negative control was included with the use of sterile distilled water instead of extracted RNA. For nested HCV PCR The following mixture for each sample was done RNase-free water 12 ul, Taq master mix 25 ul, Primer (4CH) 5 ul, Primer (1TS) 5 ul, cDNA from first step 3 ul.PCR tubes were placed in the thermal cycler under the following conditions, 94^oC for 3 min, (94^oC for 1 min, 55^oC for 1 min, 72^oC for 1 min) 40 cycles, 72^oC for 10 min. Negative control was sterile distilled water instead of cDNA from first step. Qualitative determination was done using agarose gel electrophoresis 1.5% for 20 minutes. Examination of agarose gel was carried out using a UV trans-illuminator for detection of HCV specific band at 187 bp.

Figure 1
Electrophoresis* pattern *of positive HCV PCR samples and negative control

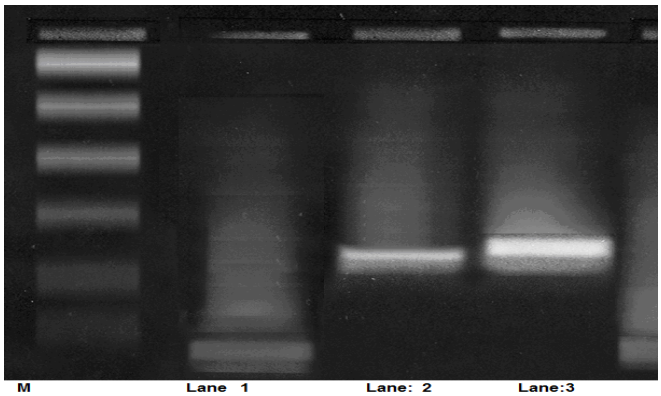

Figure (1): electrophoresis of negative and positive samples for HCV
M molecular marker Lane 1: Negative sample Lanes 2,3 Positive samples

Table (1)
Demographic and laboratory findings in CHD (n=93)

Age	45.2 10.1
Sex	
Male	42 (45.2%)
Female	51 (54.8%)
Duration of dialysis (months)	41.9± 3.1
Presence of purities	86 (92.5%)
Purities score	
Range	0-10
ALT (IU/L)	30.4± 1.5
AST(IU/L)	31.9 ± 1.5
Albumin (gm/dl)	3.6± 0.5
Bilirubin (mg/dl)	1.0± 0.7
HCV IgG	51(54.8%)
PCR for HCV	28 (30.1%)

Table (2)
Comparison between patients with pruritis and patients without pruritis complaint

	Patients with Purities (n=86)	**Patients without Purities (n=7)**	
HCV IgG (No. %)	47(54.7%)	4 (57.1%)	P=0.0001
HCV PCR (No. %)	28(32.6%)	0	P=0.0001
Duration of dialysis (months)	37.5± 3.5	16.5± 1.7	P=0.0001
Blood transfusions			
Range	0-4	0-5	P=0.2
ALT(IU/L)	31.01± 1.5	33.3± 1.2	P=0.1
AST(IU/L)	33.2± 1.6	27.3± 7.5	P=0.5
Bilirubin(mg/dl)	0.95± 0.7	0.8± 0.1	P=0.3
Albumin(gm/dl)	3.6± 0.5	3.4± 0.4	P=0.9

Table (3)
Comparison between HCVIg G and HCV nested RT-PCR

		PCR		**Total**
		Positive N0. (%)	**Negative N0. (%)**	**No. %**
HCV Ig				
	Positive	17(60.7%)	34(52.3%)	**51(54.8%)**
	Negative	11(39.3%)	31 (47.7%)	**42(45.2%)**
		28 (100%)	65(100%)	**93(100%)**

RESULTS

Demographic and laboratory data of the studied patients were summarized in table 1. Among 93 studied ESRD patients the sex distribution was females 51 (54.8%) and males 42 (45.2%) with mean± SD age 45.2±10.1 years. The patients had a long duration of dialysis as the mean± SD of dialysis duration was 41.9± 3.1 months. Prutitis complaint was present among the

majority of patients 86 (92.5%). HCV IgG was positive in more than half of the patients 51 (54.8%) with positive HCV viremia in 28 (30.1%).In comparison between patients with pruritis and patients without, HCV IgG , HCV RNA and duration of dialysis had high significant association with pruritis (P=0.0001 for each), table 2 . Table (3) shows comparison between HCVIg G and HCV nested RT-PCR .There was a highly significant correlation between HCV IgG and nested RT-PCR for HCV RNA. However, there were 39.3% of positive cases for HCV viremia without positive HCVIgG.

DISCUSSION

Pruritis is a common complaint among ESRD. In the present study it was present among the majority of patients 86 (92.5%). Pruritus was the most common cutaneous abnormality reported previously in 55% of Egyptian ESRD on hemodialysis. The rate of pruritis complaints of those patients ranged from 19-90% in previous studies [18-20]. The explanation for difference in the rates can be attributed to the individual tolerance and threshold for itch due to racial and ethical background as the assessment of itching is a subjective description based on perception [21-23].
In the present study the score of pruritis varied from 1-10. Usually, the severity of pruritis in ESRD ranges from just discomfort to complete restlessness during all the time. The complaint may be associated with varieties of psychological disorders like depression, lack of sleep, and low mental activity [2, 3]. Pruritis is not reported in acute renal failure and not relieved with dialysis but it improves after kidney transplantation[7]. The pathogenesis of pruritis in end stage renal disease is not well understood but it is associated with the severity of the diseases[8]. Other factors could be claimed for this complaint is hypervitaminosis a leading to the increase of retinol in the epidermidis. The increase of $Beta_2$ microglobulin, advanced glycosylation end products and parathyroid hormone may be other mediators for this complaint[11]. It worth noticing in the present study, that the duration of dialysis had high significant association with pruritis (P=0.0001).Similar result was noticed by Li et al. 2015 who reported that prolonged peritoneal dialysis duration was associated with severe pruritus and more attention should be given to the pruritus symptoms of those patient to investigate its etiology. In our study, HCV IgG was positive in more than half of the patients 51(54.8%) with positive HCV viremia in 28 (30.1%).Previous reports stated that HCV infection can be detected from 1.9% up to 84.6% ESRD in different countries [24, 25]. Multiple factors increase the susceptibility of those patients to HCV infection such as exposure to infected persons, contaminated equipments and blood transfusions. Some investigators reported that hospital acquired HCV infections could be reduced by isolation of infected HCV patients in special units [26, 27.] An important result in the present study was the highly significant association of HCV IgG and HCV RNA with pruritis (P=0.0001 for each).There are scarce reports about the association of anti- HCV infection with uraemic pruritis [28-31]. It is postulated that hepatitis C virus infection is associated with increase of oxidative stress and production of excessive amount of inflammatory cytokines, chemokines and serum soluble cellular adhesion molecules that lead to presence of pruritis levels in uraemic patients [32, 33]. Other factors that can predispose uremic patients infected with HCV to pruritis is the presence of liver fibrosis, bile duct lesions and cholestasis [34-37]. Previous studies correlated only the presence of anti HCV with pruritis. To the best of our knowledge the present study is the first to correlate HCV viremia with pruritis in uremic patients.In our study about 39.3% of our patients with positive HCV by nested RT-PCR were seronegative by using ELISA which indicates the importance of using nested RT-PCR as the gold standard method for detection of HCV infection in haemodialysis patients matching with the conclusion of Saab et al. (2001) [38.] Nahum et al. in (2004) [39].The present study highlights the high prevalence of prutitis in patients under hemodialysis. The distinguished finding of the present study is the high significant association of hepatitis C viremia with pruritis in patients under hemodialysis. Future multicenter studies are recommended to elucidate the significance of this finding.

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