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Contents

Prevalence of Non Organ-Specific Auto Antibodies and its Effect on Response to Antiviral Therapy in Patients with Chronic Hepatitis C Virus Genotype 4

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Background and Study Aim: Immunological disorders have been frequently described in the course of hepatitis C virus (HCV)–related chronic hepatitis. Our aim was to determine the prevalence of non-organ-specific autoantibodies (NOSAs) and evaluate its impact on the response to combined antiviral therapy in patients with chronic HCV genotype-4.

Patients and Methods: A total of 134 adult patients with chronic HCV genotype-4 were investigated for the presence of serum Antinuclear antibody (ANA), anti–smooth muscle antibody (SMA), and anti liver/kidney microsomal antibody type 1 (LKM1). 109 out of 134 HCV patients were treated naive and received combined antiviral therapy (pegylated interferon–ribavirin). The presence of these autoantibodies was studied in relation to the patient's characteristics and the outcome of antiviral therapy.

Results: Thirty-six (26.9%) patients were positive for at least one autoantibody. Various autoantibodies were presented as follows: ANA in 29 (21.6%) patients, SMA in 9 (6.7%) and anti-LKM-1 in 2 (1.5%). In two patients, both ANA and anti-SMA were positive, and in other two cases both ANA anti-LKM-1 were positive. Female patients had a higher prevalence of positive autoantibodies (P=0.005). Chronic hepatitis C (CHC) patients with positive autoantibodies had higher serum ALT, AST and GGT levels. The rate of sustained virological response to combined antiviral therapy was similar between autoantibody-positive and -negative groups (46.9% vs. 53.2%).

Conclusion: Autoantibodies can be induced in the course of CHC. Autoantibody-positive CHC patients are older and have higher disease activity and severity. However, the presence of these autoantibodies did not influence the response to combination antiviral therapy.

INTRODUCTION

Hepatitis C virus (HCV) is among the leading causes of chronic liver disease worldwide and affects approximately 170 million people [1]. Egypt has the highest prevalence of HCV infection of any country in the world, the situation is quite worse ,the overall prevalence (percentage of people) positive for antibody to HCV was 14.7% [2]. Immunological disorders have been frequently described in the course of HCV-related chronic hepatitis, and non–organ-specific autoantibodies (NOSAs) in particular are common examples of autoreactivity associated with HCV infection [3].

HCV has six major genotypes according to its viral genome, numbered one to six. These viral types and sub-types differ in their geographical distribution and anti-genicity [4]**.** Particular genotypes are associated with different courses and outcome of liver diseases, and also with different responsiveness to interferon therapy. Results of the studies to clarify the relationship between HCV genotype and autoimmune manifestations are controversial.

A majority of them failed to confirm the association between clinical course of HCV infection, autoimmune disorders and particular HCV genotypes. Genotype 4 is the predominant genotype of HCV in Egyptian patients (up to 91%) [5].

To date, combination of pegylated interferon alpha (PEGIFN) and ribavirin is the treatment of choice for chronic HCV patients [6] with an (SVR) of 42%–52% in patients with genotype 1 [7, 8] and in 42-68 % in those with genotype 4 (9-12). The achievement of the SVR in patients with chronic hepatitis C (CHC) has been associated with improvements in liver histology as well as a reduced risk of hepatocellular carcinoma (HCC) and liver-related mortality[13-15] . However, several side-effects have been published in patients treated with IFN-α including the development or exacerbation of underlying autoimmune diseases and the development of a variety of organ and non-organ specific autoantibodies (NOSAs) .The association between these antibodies and either HCV per se or IFN-a related therapy is mainly based on epidemiological surveys [16-21] . Moreover, available data on the relationship between autoantibody seropositivity and the response to antiviral therapy in CHC patients are limited and controversial [22,23] .

In this study, we aimed to assess the prevalence of serum NOSAs in CHC patients. In addition, to evaluate its impact on the response to combined antiviral therapy (IFN or pegylated IFN plus ribavirin) in patients with HCV genotype 4-related chronic hepatitis and to identify clinical, biochemical, or immunological features predictive of response to antiviral treatment.

PATIENTS AND METHODS

The study was conducted into two stages:

Stage I: a comparative cross sectional study among patients with chronic hepatitis C virus Genotype 4

Stage II: a case control study between patients with chronic hepatitis C virus Genotype 4 and healthy cross matched control

Sample size and power of the study

The sample size was calculated by Medcalc program available at www. Medcalc.be. At a level of 95% confidence with alpha error 0.05. and the power of the study was settled at 80 and beta error .02. The prevalence of auto-antibodies was supposed to be ranged from 20% to 10%. The estimated sample is 86 patients. We try to increase the sample of patients to 134 patients to increase the power of the study. Limitation of our resources enforce us to have a control group of 60 subjects

A total of 134 consecutive CHC patients were admitted to this study during the period of July 2009 to January 2012 who visited clinics (inpatients and outpatients) of Mansoura University Hospital. They were 78 males and 56 females, with a mean age of 48.4 ± 3.2 years and 60 healthy controls with matched age and sex. All patients had positive HCV antibody with enzyme-linked immunosorbent assay (ELISA) (Murex anti-HCV (version 4.0) 7F51-06/-07, DiaSorin South Africa (Pty) Ltd, Republic of South Africa) and detectable HCV RNA (Appliedbiosystems, StepOne Real-time PCR system, USA) in the serum. Out of 134 HCV patients, 109 were treated with combined antiviral therapy (peg IFN plus ribavirin), while the remaining patients were missed during the treatment period.

The exclusion criteria included human immunodeficiency virus coinfection, hepatitis B virus infection, autoimmune hepatitis (using the simplified criteria for the diagnosis of AIH) [24], patients who showed evidence of alcohol, illicit drug, or potentially hepatotoxic medication use and major contraindications to IFN or ribavirin therapy. Informed consent was obtained from all patients, and the research protocols were approved by the Medical Ethics Committee of Mansoura University Hospital.

Detection of NOSA:

Serum ANA was detected by ELISA (ORG 538, ORGENTEC Diagnostika GmbH, Germany), ASMA was detected by ELISA (QUANTA LiteTM Actin IgG ELISA 708785, INOVA Diagnostics, Inc.USA) and anti-LKM-1 was also detected by ELISA (QUANTA LiteTM LKM-1 ELISA 708745, INOVA Diagnostics, Inc.USA).

Among the laboratory parameters measured at baseline serum levels of alanine-aminotransferase (ALT), aspartate-aminotransferase (AST), total and direct bilirubin, albumin, alkaline phosphatase, γ-glutamyl transpeptidase (GGT) and α-fetoprotein (AFP) were recorded and included in the analysis. Samples positive for HCV-RNA by real time PCR were subjected to genotyping of HCV,

by RT-PCR for the core domain using the primers modified by **Ohno et al. (1997)** [25] **.**

Histological assessment:

Liver biopsy was done for all patients before the initiation of therapy. The histological evaluation was assessed using the modified Knodell histology index and the Metavir scoring system reflecting the degree of hepatic inflammation and fibrosis [26,27]. Before treatment, informed consent was obtained from each patient.

Treatment regimens and outcomes.

A total of 32 (88.9%) of 36 autoantibodies positive patients and 77 (78.6%) of 98 autoantibodies negative patients had been treated with a combination therapy (either pegylated-IFN alfa-2a 180 µg subcutaneously once a week or pegylated-IFN alfa-2b 1.5 µg/kg subcutaneously once a week plus oral ribavirin 1000 or 1200 mg/day for subjects weighing <75 or ≥75 kg, respectively). According to HCV genotype, the predetermined duration of treatment was 48 weeks with a final efficacy evaluation at week 24 of follow-up.

Patients were regularly followed–up for physical examination, blood tests and virological assays. Treatment outcome was assessed as follows: sustained virological response (SVR) was defined as undetectable HCV RNA 24 weeks after treatment discontinuation; relapse was defined as HCV RNA clearance during treatment and reappearance during follow-up; and nonresponse was defined as a failure to clear HCV RNA at any time during treatment [28] .

Statistical Analysis

The data were collected and entered the computer. **The data were statistical analyzed by using Statistical Package of Social Science (SPSS).** The qualitative data were presented in the form of number and percentage. Chi-square with Yates correction was used as a test of significance for qualitative data when the expected cell less than 5. Chi-square test was used as test of significance for qualitative data when the expected cell more than 5. Significance was considered when p value less than 0.05.

The quantitative data were presented in the form of mean and standard deviation. Student t test was used as a test of significance for quantitative data of two groups. The fibrosis score was presented in the form of median and range. Mannwhitney u test was used as a test of significance for fibrosis score. Significance was considered when p value less than 0.05.

RESULTS

Prevalence of NOSA in patients with chronic hepatitis C:

Table (1) shows the prevalence of NOSAs in patients with chronic hepatitis C and control groups. Among total of 134 patients with chronic hepatitis C, thirty-six (26.9%) were positive for at least one autoantibody. ANA was present in twenty-nine (21.6%) patients, anti-SMA in nine (6.7%) patients , and anti-LKM-1 was found in two (1.5%) patients. In two of the patients, both ANA and anti–SMA were positive, and in other two cases both ANA anti-LKM-1 were positive. The prevalence of serum autoantibodies in patients with chronic hepatitis C was significantly higher than in healthy control (p< 0.05).

Clinical significance of NOSAs in patients with chronic hepatitis C:

Table (2) compares the clinical, laboratory and histological parameters between CHC patients with and without autoantibodies.

As regard demographic data, female patients had a higher frequency of positive autoantibodies (*P*=0.005) and age was significantly higher in the autoantibody-positive CHC patients (51.4 ±2.3) vs (45.4 ±4.1) (P <0.001).

The CHC patients with and without serum autoantibodies were analyzed with comprehensive clinical and biochemical examinations: autoantibodies-positive patients had significantly higher serum levels of ALT (102±20.3) vs (90±22.4) (*P*=0.013), AST (96±15.13) vs (72±16.7) (*P*=0.023), GGT (76.3±15.2) vs (50.9±12.7) (P<0.001) and AFP (18±4.5) vs (13±3.9) (P= 0.012). Autoantibodies-positive patients had also higher fibrosis scores and significantly lower platelet counts (144 ±30.2) vs (186 ±25.12) (P=0.004). No significant difference in HCV viral load between both groups.

Response to combined antiviral therapy:

Table (3) show the response of chronic HCV-infected patients to combined antiviral treatment (peg-IFN plus ribavirin). In autoantibodies positive patients, 15 (46.9%) of 32 HCV-infected patients had a sustained virological response (SVR), whereas 9 patients (28.1%) experienced

nonresponse and 8 (25%) experienced relapse. In their counterpart, autoantibodies negative HCV patients, the response rate was as follow: 53.2% SVR, 24.7% nonresponse and 22.1% relapse. The SVR rates were comparable between autoantiboies positive *vs.* autoantibodies negative patients (46.9% vs 53.2%).

As regard the systemic autoimmune manifestations, one patient with positive serum autoantibodies developed hypothyrodism while in autoantibodies negative group, one patient developed diabetes mellitus, and another one developed hypothyroidism. These complications were controlled on therapy and did not required withdrawal of combination therapy.

Predictors of response to antiviral therapy

In this study, we compared patients with and without SVR (Table 4) in order to predict the factors associated with a favorable response to combined antiviral therapy. Among the clinical, biochemical, and histological parameters studied, our results showed that younger age (P<0.001), lower body mass index (BMI) (P<0.001), higher serum ALT (P<0.001), lower GGT (P<0.001), lower HCV viral load (P<0.001) levels and lower fibrosis score were significantly associated with SVR. In comparison serum ANA, ASMA and LKM-1 were not significantly different between patients with and without SVR.

Table (1) Prevalence of NOSAs in 134 patients with chronic hepatitis C and the control group:

	Case (n)	**Autoantibodies** (n (%))	**ANA** (n (%))	**ASMA** (n (%))	**Anti-LKM-1** (n (%))
Patients with CHC	134	36 (26.9%)	29 (21.6%)	9 (6.7%)	2 (1.5%)
Control group	60	7 (11.7%)	7 (11.7%)	0 (0%)	0 (0%)
Test of significance		0.018*	0.098	.031*	.47

Table (2): Clinical, laboratory and histological parameters of patients with chronic hepatitis C who did or not test positive for non-organ specific autoantibodies.

Parameters	**Autoantibody positive N= 36**	**Autoantibody negative N= 98**	**P value**
Gender Male Female	 14 22	 64 34	P=0.005**
Age (year)	51.4 ±2.3	45.4 ±4.1	<0.001***
Body mass index	26.1±2.1	27.2±2.4	.059
Hb level (g/dl)	13.6 ±1.2	13.9 ± 1.5	.28
WBCs	5.9±2.1	6.2 ±2.3	.19
Platlet count (×10^9/l)	144 ±30.2	186 ±25.12	0.004**
Albumin (g/dl)	4.03 ±0.75	4.2±0.32	.49
Total Bilirubin (mg/dl)	1.04±0.5	0.9±.41	.101
ALT (IU/ml)	102.3 ±20.3	90.5 ±22.4	0.013*
AST (IU/ml)	96.4 ± 15.13	72.9 ±16.7	0.023*
ALP (U/l)	247.08±42.3	238.67±36.8	.37
GGT (IU/l)	76.3±15.2	50.9±12.7	<0.001***
HCV RNA(×10^6 IU/ml)	0.58±.2	0. 66 ± .3	0.141
AFP	18.2 ±4.5	13.3 ±3.9	0.012*
Fibrosis score (0-2/3,4)	21/15 1(1-4)	70/28 2 (1-4)	<0.001***

* SIGNIFICAT P LESS THAN 0.05

** HIGHLY SIGNIFICANCE LESS THAN .01

**** EXTREMLY SIGNIFICANCE LESS LESS THAN .001

Table (3): Response of chronic hepatitis C patients with and without autoantibodies to combined antiviral therapy

	Autoantibodies positive patients N= 32	Autoantibodies negative patients N=77	P value
SVR	15 (46.9%)	41 (53.2%)	**0.83**
Non responder	9 (28.1%)	19 (24.7%)	
Relapse	8 (25%)	17 (22.1%)	

Table (4): Comparison between patients with SVR and Non-SVR

	SVR n= 56	Non-SVR n= 53	P Value
Gender Male/ Female	31/25	33/20	0.46
Age (year)	44±5.21	52±4.2	<0.001***
Body mass index	25.3±2.31	28.5±1.41	<0.001***
ALT (IU/ml)	116±25.7	86±50.1	<0.001***
AST (IU/ml)	87.7±17.2	82.5±19.2	0.15
GGT (0-40IU/l)	47.7±5.7	82.3±6.2	<0.001***
ANA (+/-)	13/43	16/37	.41
ASMA (+/-)	4/52	5/48	.51
Anti-LKM-1	1/55	1/52	.96
HCV RNA ($\times 10^6$ IU/ml)	0.49±0.56	0.86±0.72	<0.001***
Fibrosis score	1 (1-3)	3 (2-4)	0.003**

DISCUSSION

Patients chronically infected by HCV present various immune-mediated phenomena mainly due to B lymphocyte dysfunction as mixed cryoglobulinemia and non-organ-specific autoantibodies (NOSAs) production [29]. Previous studies have shown that serum autoantibodies are commonly found in CHC patients [30]. In this study, the global prevalence of NOSAs among patients with chronic hepatitis C was 26.9%. ANA was the most commonly found autoantibodies being present in 21.6% of patients. The prevalence of ANA is higher than that reported by studies from some countries [31], while it is comparable to that reported from some other countries. Lenzi et al., demonstrated the occurrence of ANA in 16% of patients with chronic hepatitis C [21]. In Estonia, Zusinaite et al., reported 14.4% prevalence of ANA in patients with chronic hepatitis C [32] . As regard the prevalence of ASMA in patients with chronic hepatitis C, it was found to be 6.7%. This result is lower than that reported in some studies [16,21,32]. Anti-LKM-1 autoantibodies are detected worldwide in approximately 0-7% of patients with chronic hepatitis C [33,34] . Available data on the prevalence of anti-LKM-1 in Egyptian patients with CHC are relatively uncommon. Here we reported that the positive rate of anti-LKM-1 was 1.5%. These results confirm that AIH-related autoantibodies can exist in CHC patients.

Molecular mimicry between the HCV polyprotein and "self" proteins may account for the production of autoantibodies in chronic HCV infection. A sequence homology between the HCV polyprotein and cytochrome p450 2D6 (CYP 2D6), the antigenic target of anti-LKM1, was previously reported [35] . The reactivity against the viral protein would induce the production of anti-LKM1 in HCV-related CLD. Gregorio and colleagues documented molecular mimicry between HCV polyprotein and three nuclear host antigens including matrin, histone H2, and replication protein as a mechanism for the emergence of ANA [36]. Polyclonal B cell activation by persistent HCV infection has been proposed as another mechanism for the production of autoantibodies. In determining one

of the mechanisms for polyclonal B cell activation, Pileri and colleagues documented that HCV envelope protein (E2) represented a co-stimulatory signal to B cells by binding to CD 81 (tetraspanin) and thereby facilitated the production of autoantibodies [37] . B-lymphocyte activating factor (BAFF) appeared to play a crucial role in HCV-induced autoimmunity [38].

Variations in the prevalence of autoantibodies may be attributed to several factors. First, there may be differences in viral strains causing these differences [3]. Secondly, the differences in detection methods, ethnic background and geographic distribution of the study cohort [39] .

In our study, patients with positive autoantibodies were significantly older. This is in agreement with the findings of Squadrito et al., [16], who found that NOSAs positive HCV patients were older than those with negative autoantibodies. This phenomenon might result from functional defects in suppressor T cells in older patients [40,41]. However, other studies found no age difference between the two groups [42,43] . The positive rate of autoantibodies was higher in females, which is in accord with reports by other groups [31,44] . This may reflect the difference in autoimmune reactions between males and females after CHC infection, suggesting that hormones, such as estrogen, may play an important role in infection [45] .

As regard the biochemical finding, this study showed that autoantibody-positive CHC patients had significantly higher serum ALT and AST levels than those without autoantibodies. This is in agreement with previous reports by Lenzi, et al., who reported that NOSAs were significantly prevalent in patients with HCV-related chronic liver disease, and were especially so when the alanine aminotransferase activity was higher [21]. Moreover, Cassani, et al., showed in a prospective series of patients with HCV related chronic liver disease who were positive for autoantibodies, a biochemical and histological activity were higher than that of patients with no markers of autoimmunity [46]. In controversy, Stroffolini et al., showed no correlation between the positivity of autoantibodies and liver damage [43]. Muratori, et al., showed that in the absence of active liver disease the prevalence of non-organ specific autoantibodies was similar in HCV positive individuals and negative controls [3]. This suggests that the presence of non-organ-specific autoantibodies is more likely associated with increased patient's age, duration and severity of chronic liver disease. Thus, reactivity against self-antigens can be related to the severity of liver damage without any independent pathogenic role.

Our finding also demonstrated that NOSA-positive CHC patients had low platelet count and more advanced fibrosis scores than seronegative CHC patients. These finding are in agreement with most published data, suggesting that HCV-infected autoantibody- positive patients have higher disease activity and severity than those who are autoantibody-negative [46,47] .

IFN-alfa is the treatment of choice for patients with chronic hepatitis C, but its immunomodulatory activity may also favor the appearance or amplification of autoimmune reactions [48]. The response to IFN-α in patients with HCV infection and autoimmune markers continue to be controversial [42]. In our study, we found that the presence of serum NOSA in CHC patients did not influence the response to combined antiviral therapy, which was similar in both serum NOSA-positive and -negative patients (46.9 % vs 53.2%). This results is in agreement with other studies who reported that the presence of autoantibodies such as ANA or anti-LKM1 in patients with CHC is less likely to affect the response to antiviral treatment [46,49] . In contrast, the favourable pridectors of SVR were younger age, lower body mass index (BMI), higher serum ALT, lower GGT, lower HCV viral load levels and lower fibrosis score. These results are in agreement with other reports as in all large prospective studies of (PEG) IFN and RBV combination therapy younger age correlated significantly with an SVR when assessed by univariate and multivariate analyses and patients younger than 40–45 years showed the best response rates [50] . GGT has been identified as a prognostic factor in other studies [51,52] . In this study, we found that low GGT level had a favorable prediction of SVR. This is in accordance with previous reports in which low pre-treatment serum GGT levels were significantly and independently associated with SVR in multivariate regression analysis[53,54] . The pathogenetic background of GGT elevation in chronic hepatitis C is not fully understood. However a close relationship between serum GGT levels and hepatic steatosis, advanced fibrosis, and insulin resistance has been described [55,56] . Moreover, GGT levels are

related with an increased expression of TNFα in the liver that seems to reduce the efficacy of antiviral therapy [57] . We also confirmed previous reports signaling that a low viral load is predictor of SVR. A low baseline viral load (<600,000–800,000 IU/ ml or less) was shown to be an independent predictor of SVR regardless of genotype in numerous studies[50,53,58,59].

In conclusion, serum $NOSA_S$ were frequently found in HCV-infected patients. Patients with positive serum autoantibodies were older, and have higher disease activity and advanced fibrosis scores than their negative counterparts. The positivity of autoantibodies did not influence the response to combination antiviral therapy. Combined antiviral treatment is safe and effective in autoantibodies-positive patients with CHC. Routine testing of autoantiantibodies may be needed to monitors the progress and severity of disease that might be areas for further research.

Limitation of the study:

Some limitations should be considered when interpreting our findings. First, detection of autoantibodies was based on ELIZA method, and there was no record of the distribution type of NOSAs. Whether the distribution type of autoantibodies has clinical relevance is worthy of future study. Second, the external validity of this study is questionable, since the sample of the patients may not be representative of all Egyptian population due to cost variable, long duration of follow up and the interferon therapy is not available for most of Egyptian patients; therefore, it is possible that our findings cannot be extrapolated to all CHC patients in Egypt.

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REFERENCES

1. Lavanchy D. The global burden of hepatitis C. *Liver Int* 2009; 29: 74–81.
2. El-Zanaty, Fatma and Ann Way. Egypt Demographic and Health Survey 2008. Cairo, Egypt: Ministry of Health, El-Zanaty and Associates, and MacroInternational. 2009.
3. Muratori P, Muratori L, Stroffolini T, Pappas G, Terlizzi P, Ferrari R, et al. Prevalence of non organ specific autoantibodies in HCV-infected subjects in the general population. *Clin Exp Immunol* 2003; 131:118–21.
4. .Sy T, Jamal MM. Epidemiology of hepatitis C virus (HCV) infection. *Int J Med Sci* 2006;3 (2):41-6.
5. Ray SC, Arthur RR, Carella A, Bukh J, Thomas D. Genetic epidemiology of hepatitis C virus throughout Egypt. *J Infect Dis*. 2000;182:698–707.
6. Strader DB, Wright T, Thomas DL, Seeff LB . Diagnosis, management, and treatment of hepatitis C. *Hepatology*2004; 39: 1147–71.
7. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001;358:958-965.
8. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Gonçales FL Jr, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002;347:975-982.
9. Esmat G, Abouzied A, Abdel-Aziz F . Treatment with PEG-IFN alfa-2b plus ribavirin compared to interferon alfa-2b plus ribavirin in subjects with chronic hepatitis Cinfected with HCV genotype 4 .*Hepatology* 2002;36: 364A.
10. Alfaleh FZ, Hadad Q, Khuroo MS . Peginterferon alpha-2b plus 40. ribavirin compared with interferon alpha-2b plus ribavirin for initial treatment of chronic hepatitis C in Saudi patients commonly infected with genotype 4. *Liver Int*.2004; 24(6): 568-574.
11. Derbala M, Amer A, Bener A . Pegylated interferon-alpha 2b-ribavirin combination in Egyptian patients with genotype 4 chronic hepatitis. *J Viral Hepat*.2005; 12(4): 380-385.
12. El-Zayadi A, Attia M, Barakat E . Response of hepatitis C genotype-4 naïve patients to 24 weeks of Peg-interferon-alpha2b/ribavirin or induction-dose interferon alpha2b/ribavirin/amantadine: a non-randomized controlled study. *Am J Gastroenterol.*2005; 100(11): 2447-2452.
13. Berenguer J, Alvarez-Pellicer J, Martín PM, López-Aldeguer J, Von-Wichmann MA, Quereda C, et al. Sustained virological response to interferon plus ribavirin reduces liver-related complications and mortality in patients coinfected with human immunodeficiency virus and hepatitis C virus. *Hepatology* 2009;50:407-413.
14. George SL, Bacon BR, Brunt EM, Mihindukulasuriya KL, Hoffmann J, Di Bisceglie AM. Clinical, virologic, histologic, and biochemical outcomes after successful HCV therapy: a 5-year follow-up of 150 patients. *Hepatology* 2009;49:729-738.
15. Hung CH, Lee CM, Lu SN, Wang JH, Hu TH, Tung HD, et al. Long-term effect of interferon alpha-2b plus ribavirin therapy on incidence of hepatocellular carcinoma in patients with

hepatitis C virus-related cirrhosis. *J Viral Hepat* 2006; 13:409-414.
16. Squadrito G, Previti M, Lenzi M, Le Rose EP, Caccamo G, Restuccia T, et al. High prevalence of non- organ-specific autoantibodies in hepatitis C virus-infected cirrhotic patients from southern Italy. *Dig. Dis. Sci*.2003; 48: 349-353.
17. Wu YY, Hsu TC, Chen TY, Liu TC, Liu GY, Lee YJ, et al. Proteinase 3 and dihydrolipoamide dehydrogenase (E3) are major autoantigens in hepatitis C virus (HCV) infection. *Clin Exp Immunol* 2002; 128: 347–52.
18. Monti V, Aghemo A, Rumi MG, Donato MF, Del Ninno E, Colombo M. The prevalence, clinical features and response to antiviral therapy of patients with chronic hepatitis C who are seropositive for liver-kidney microsome type 1 antibodies. *Antivir Ther* 2005; 10(6): 715–20.
19. Dalekos GN, Kistis KG, Boumba DS, Voulgari P, Zervou EK, Drosos AA, et al. Increased incidence of anti-cardiolipin antibodies in patients with hepatitis C is not associated with aetiopathogenetic link to anti-phospholipid syndrome. *Eur J Gastroenterol Hepatol* 2000; 12(1): 67–74.
20. Fattovich G, Giustina G, Favarato S, Ruol A. A survey of adverse events in 11,241 patients with chronic viral hepatitis treated with alfa interferon. *J Hepatol* 1996; 24: 38–47.
21. Lenzi M, Bellentani S, Saccoccio G, Muratori P, Masutti F, Muratori L, et al. Prevalence of non-organ-specific autoantibodies and chronic liver disease in general population: a nested case-control study of the Dionysos cohort. *Gut* 1999; 45: 435-441
22. Wasmuth HE, Stolte C, Geier A, Dietrich CG, Gartung C, Lorenzen J, et al. The presence of nonorgan- specific autoantibodies is associated with a negative response to combination therapy with interferon and ribavirin for chronic hepatitis C. *BMC Infect Dis* 2004;4:4.
23. Muratori P, Muratori L, Guidi M, Granito A, Susca M, Lenzi M, et al. Clinical impact of non-organ-specific autoantibodies on the response to combined antiviral treatment in patients with hepatitis C. *Clin Infect Dis* 2005;40:501-7.
24. Hennes EM, Zeniya M, Czaja AJ, Parés A, Dalekos GN, Krawitt EL. et al. Simplified criteria for the diagnosis of autoimmune hepatitis. Hepatology. 2008;48:169–176.
25. Ohno O, Mizokami M, Wu RR, Saleh MG, Ohba K, Orito E, et al. New hepatitis C virus (HCV) genotyping system that allows for identification of HCV genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a, and 6a. *J Clin Microbiol*. 1997 Jan;35(1):201-7.
26. Bedossa P, Poynard T and French METAVIR Cooperative Study Group. An algorithm for grading activity in chronic hepatitis C. *Hepatol* 1996; 24: 289-93.
27. Ishak K, Baptista A, Bianchi L, Callea F, Groote JD, Gudat F, et al. Histological grading and staging of chronic hepatitis. *J Hepatol* 1995; 22: 696-699.
28. Feld JJ, Hoofnagle JH. Mechanism of action of interferon and ribavirin in treatment of hepatitis C. *Nature* 2005; 436:967–72.
29. Dustin LB, and Rice CM. Flying under the radar: the immunobiology of hepatitis C. *Annu Rev Immunol*. 2007; 25:71-99.
30. Boyer N, Marcellin P. Pathogenesis, diagnosis and management of hepatitis C. *J Hepatol* 2000;32 Suppl 1:98 112.
31. Yee LJ, Kelleher P, Goldin RD, Marshall S, Thomas HC, Alberti A, et al. Antinuclear antibodies (ANA) in chronic hepatitis C virus infection: correlates of positivity and clinical relevance. *J Viral Hepat* 2004; 11: 459-464.
32. Zusinaite E, Metsküla K, Salupere R. Autoantibodies and hepatitis C virus genotypes in chronic hepatitis C patients in Estonia World J *Gastroenterol* 2005;11(4):488-491
33. Czaja AJ, Carpenter HA, Santrach PJ, Moore SB, Taswell HF and Homburger HA (1993): Evidence against hepatitis viruses as important causes of severe autoimmune hepatitis in the United States. *J Hepatol*; 18: 342-352.
34. Nishioka M, Morshed SA, Kono K, Himoto T, Parveen S, Arima K, et al. (1997): Frequency and signifcance of antibodies to P450IID6 protein in Japanese patients with chronic hepatitis C. *J. Hepatol*.; 26(5):992-1000.
35. Bogdanos DP, Choudhuri K, Vergani D. Molecular mimicry and autoimmune liver disease: virtuous intentions, malign consequences. *Liver*. 2001;21(4):225-32.
36. Gregorio GV, Choudhuri K, Ma Y, Pensati P, Iorio R, Grant P, et al. Mimicry between the hepatitis C virus polyprotein and antigenic targets of nuclear and smooth muscle antibodies in chronic hepatitis C virus infection. *Clin Exp Immunol*. 2003;133(3):404-13.
37. Pileri P, Uematsu Y, Campagnoli S, Galli G, Falugi F, Petracca R, et al. Binding of hepatitis C virus to CD81. *Science*. 1998;282(5390):938-41.
38. Sene D, Limal N, Ghillani-Dalbin P, Saadoun D, Piette JC, Cacoub P. Hepatitis C virus-associated B-cell proliferation- -the role of serum B lymphocyte stimulator (BLyS/BAFF). *Rheumatology (Oxford)*. 2007;46(1):65-9.
39. Pawlotsky JM, Roudot-Thoraval F, Simmonds P, Mellor J, Ben Yahia MB, Andre C, et al. Extrahepatic immunologic manifestations in chronic hepatitis C and hepatitis C virus serotypes. *Ann Intern Med* 1995; 122: 169-173.
40. Tomer Y, Shoenfeld Y. Ageing and autoantibodies. *Autoimmunity* 1988;1:141-9.

41. Antel JP, Oger JJ, Dropcho E, Richman DP, Kuo HH, Arnason BG. Reduced T-lymphocyte cell reactivity as a function of human aging. Cell *Immunol* 1980;54:184-92.
42. Clifford BD, Donahue DG, Smith L, Cable E, Luttig B, Manns M et al. High prevalence of serologic markers of auto-immunity in patients with chronic hepatitis C. *Hepatology 1995*; 21: 613-619.
43. Stroffolini T, Colloredo G, Gaeta GB, Sonzogni A, Angeletti S, Marignani M, et al. Does an 'autoimmune' profile affect the clinical profile of chronic hepatitis C? An Italian multicentre survey. *J. Viral. Hepat*.2004; 11: 257-262.
44. Hsieh MY, Dai CY, Lee LP, Huang JF, Tsai WC, Hou NJ, et al. Antinuclear antibody is associated with a more advanced fibrosis and lower RNA levels of hepatitis C virus in patients with chronic hepatitis C. *J Clin Pathol* 2008; 61: 333-337.
45. Whitacre CC. Sex differences in autoimmune disease. *Nat Immunol* 2001; 2: 777-780.
46. Cassani F, Cataleta M, Valentini P, Muratori P, Giostra F, Francesconi R, et al. Serum autoantibodies in chronic hepatitis C: comparison with autoimmune hepatitis and impact on the disease profile. *Hepatology* 1997;26:561-6.
47. Noda K, Enomoto N, Arai K, Masuda E, Yamada Y, Suzuki K, et al. Induction of antinuclear antibody after interferon therapy in patients with type-C chronic hepatitis: its relation to the efficacy of therapy. *Scand J Gastroenterol* 1996;31:716-22.
48. Garcia-Buey L, Garcia-Monzon C, Rodriguez S, Borque MJ, Garcia-Sanchez A, Iglesias R, et al. Latent auto-immune hepatitis triggered during interferon therapy in patients with chronic hepatitis C. *Gastroenterology* 1995; 108(6): 1770-1777.
49. Iijima Y, Kato T, Miyakawa H, Ogino M, Mizuno M, Sugihara K, et al. Effect of interferon therapy on Japanese chronic hepatitis C virus patients with anti-liver/kidney microsome autoantibody type 1. *J Gastroenterol Hepatol*. 2001;16(7):782-8.
50. Shiffman ML, Suter F, Bacon BR, Nelson D, Harley H, Sola R, et al. Peginterferon alfa-2a and ribavirin for 16 or 24 weeks in HCV genotype 2 or 3. *N Engl J Med* 2007;357:124–134.
51. Mihm U, Herrmann E, Sarrazin C, Zeuzem S. Peredicting response in hepatitis C virus therapy. *Aliment Pharmacol Ther* 2006; 23: 1043-54.
52. Hernandez A, Domper F, Leon A, Lorente R, Lopez B, de la Santa E, et al. Viral kinetics during the first month of treatment in patients with genotype 1 chronic hepatitis C. *Rev Esp Enferm Dig* 2009; 101: 671-9.
53. Berg T, von Wagner M, Nasser S, Sarrazin C, Heintges T, Gerlach T, et al. Extended treatment duration for hepatitis C virus type 1: comparing 48 versus 72 weeks of peginterferon-alfa- 2a plus ribavirin. *Gastroenterology* 2006;130:1086–1097.
54. von Wagner M, Huber M, Berg T, Hinrichsen H, Rasenack J, Heintges T, et al. Peginterferon-alpha-2a (40KD) and ribavirin for 16 or 24 weeks in patients with genotype 2 or 3 chronic hepatitis C. *Gastroenterology* 2005;129:522–527.
55. Hwang SJ, Luo JC, Chu CW, Lai CR, Lu CL, Tsay SH, et al. Hepatic steatosis in chronic hepatitis C virus infection: prevalence and clinical correlation. *J Gastroenterol Hepatol* 2001;16:190–195.
56. Silva IS, Ferraz ML, Perez RM, Lanzoni VP, Figueiredo VM, Silva AE. Role of gamma-glutamyl transferase activity in patients with chronic hepatitis C virus infection. *J Gastroenterol Hepatol* 2004;19:314–318.
57. Taliani G, Badolato MC, Nigro G, Biasin M, Boddi V, Pasquazzi C, et al. Serum concentration of γGT is a surrogate marker of hepatic TNF-α mRNA expression in chronic hepatitis C. *Clin Immunol* 2002; 105: 279-85.
58. Jacobson IM, Brown Jr RS, Freilich B, Afdhal N, Kwo PY, Santoro J, et al. Peginterferon alfa-2b and weight-based or flatdose ribavirin in chronic hepatitis C patients: a randomized trial. *Hepatology* 2007;46:971–981.
59. Zeuzem S, Buti M, Ferenci P, Sperl J, HorsmansY, Cianciara J, et al. Efficacy of 24 weeks treatment with peginterferon alfa-2b plus ribavirin in patientswithchronic hepatitisCinfectedwith genotype 1 and low pretreatment viremia. *J Hepatol* 2006;44:97–103.

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Prevalence and Predictors of Diabetes Mellitus in Chronic Hepatitis C Patients with and without Cirrhosis

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Background and study aim: The highest Hepatitis C Virus (HCV) prevalence in the world occurs in Egypt. The frequency of type 2 diabetes mellitus tends to be high in patients infected with HCV especially those with cirrhosis. We conducted this study to define the prevalence and predictors of diabetes mellitus (DM) in chronic hepatitis C patients with and without liver cirrhosis.

Patients and methods: Four hundred patients with HCV were enrolled (200 without cirrhosis [group I] and 200 with cirrhosis [group II]).Two hundred non hepatic disease subjects were enrolled as a control group [group III]. All patients were subjected to thorough history taking and physical examination. Investigations include liver function tests, viral markers (anti-HCV anti-bodies and polymerase chain reaction (PCR) for HCV), liver biopsy and abdominal ultrasound. Multiple logistic regression analysis was used to adjust for potential confounders.

Results: Prevalence of Type 2 diabetes was 30.25% in HCV patients as opposed to only 6.5 % of control group (p <0.0001, odds ratio [OR] = 2.7).Moreover the prevalence is significantly higher in HCV patients with cirrhosis than in non cirrhotic patients (51% vs. 9.5%), (OR= 9.9, P < 0.001). Partial correlation of prevalence of diabetes mellitus in HCV patients remain highly significant after adjustment for age, sex, family history of diabetes and BMI (r=0.291, P< 0.0001). Using logistic regression; older age, positive family history of diabetes, higher BMI, lower serum albumin level, higher activity and fibrosis score (OR= 1.3,19.4,1.8, 6.6,1.3 and 2.1 respectively) in patients with chronic hepatitis C were found to be associated with higher prevalence of DM (P <0.05), while activity by fibrosis was insignificant.

Conclusion: Chronic HCV is associated with increased risk of diabetes to 2.7-fold.Development of cirrhosis in patients with chronic HCV increases risk of diabetes to 10-folds. This association seems not to be related to the known risk factors for diabetes. Potential predictors for this association might include older age, positive family history of diabetes, higher BMI, lower serum albumin level, higher activity and fibrosis score.

INTRODUCTION

Chronic HCV infection is associated with an increased risk for the development of type 2 diabetes mellitus (DM) [1]. Therefore, type 2 diabetes is more prevalent among patients with chronic HCV compared to those with other liver diseases and the general population, irrespective of the presence or absence of liver cirrhosis [2–4].

An increased prevalence of glucose intolerance and diabetes mellitus among patients with chronic hepatitis C virus (HCV) infection has been reported in several studies [5-9]. An Egyptian study showed that the incidence of type 2 DM is increased two folds in patients who had HCV infection compared with those who did not, and reported that HCV-infected persons with type 2 DM were more likely to need insulin [10]. On the other hand, a higher prevalence of HCV infection has been reported in Spain in diabetic patients (11.5%) in comparison with non-diabetic blood donors (2.5%) [11].

Insulin resistance and progressive pancreatic β-cell dysfunction have been identified as the two fundamental features in the pathogenesis of type 2 DM in those patients [12]. Clinical and

experimental data suggested a direct role of HCV in the disturbance of glucose metabolism. Moreover, HCV can disturb glucose homeostasis via indirect mechanisms including cytokine stimulation [13]. HCV infection itself is a more important predictor of glucose intolerance than cirrhosis, and the combination of both factors further increases the risk of diabetes [14].

Type 2 DM has been suggested to enhance the development of HCC and to be associated with poorer prognosis of liver transplantation [15-17]. Thus, early intervention to prevent or improve type 2 DM seems necessary [18-21].

At the present time, investigating prevalence of type 2 DM in HCV patients in Egypt and whether potential risk factors as age, family history of diabetes, BMI, activity and fibrosis score contribute to it is not yet clear. So, the aims of this study were: *firstly*, to evaluate type-2 diabetes prevalence in chronic hepatitis C patients with and without liver cirrhosis; *secondly*, to investigate whether this relationship between HCV infection and type-2 diabetes could be modified by some risk factors of diabetes including age and obesity, and *thirdly* to study possible risk factors that may predict diabetes in such patients.

PATIENTS AND METHODS

This cross-sectional study was carried out on 400 patients with chronic HCV who were attending the outpatient clinic at Mansoura Specialized Medical Hospital over a 9-month period (January 2012 to September 2012). They were subdivided into two groups; group I which comprised 200 chronic HCV patients without liver cirrhosis and group II which comprised 200 chronic HCV patients with liver cirrhosis. A control group composed of 200 apparently healthy non-hepatic individuals were chosen to look for prevalence of DM.

Exclusion criteria:

- Associated other hepatic disorders as HBV, autoimmune liver disease, etc.
- Patients who had diabetes before or at the onset of diagnosing HCV.
- Patients with HCC or pancreatic tumor.
- Patients with systemic diseases as chronic renal failure.
- Patients using drugs known to alter glycemic state including, systemic steroids, and interferon.

All cases were subjected to the following:

1. Thorough history taking including family history of diabetes.
2. Physical examination including body weight and height to calculate body mass index.
3. Routine laboratory investigations including full blood count, prothrombin time, and liver function tests and blood glucose levels.
4. Diagnosis of HCV infection was based on positive testing for serum anti-HCV markers (Anti-body against HCV was detected with a third-generation enzyme-linked immunoassay).
5. Commercially available polymerase chain reaction assay was done to detect serum HCV RNA (Cobas Amplicor HCV Monitor Test, v2.0, Roche, Tokyo, Japan).
6. Diabetes mellitus was diagnosed according to the American Diabetes Association guidelines (ADA updated criteria, 2008& 2012) [22&23].
7. Abdominal ultrasonography.
8. Liver biopsy was available from all patients except those with advanced liver disease (Child B and Child C). Liver samples were scored for activity and fibrosis using METAVIR score [24&25].

Statistical Analysis

Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS, Chicago, IL, USA, version 17.0 for Windows). All quantitative variables were expressed as mean ± standard deviation. Comparison between groups was done using one-way ANOVA or the Student's t-test, whichever was applicable. Qualitative or categorical variables were described as proportions. Proportions were compared by use of the Chi-squared test or Fisher's exact test, whichever was applicable. Correlation was determined by Pearson's linear regression analysis. All P values are based on a two-sided test of statistical significance. P value of ≤ 0.05 will be considered as significant. Logistic regression analyses were used to evaluate the predictive variables that could be associated with the presence of diabetes.

RESULTS

Table (1): Clinical characteristics of the studied groups.

		Group 1 (n= 200)	Group2 (n=200)	Group 3 (n=200)	F	P value
Quantitative data		Mean ± S.D.				
Age (years)		38.33±9.75	51.25±10.51	46.08±9.87	83.652	<0.0001
BMI (kg/m^2)		21.7±2.55	24.9±2.75	23.6±2.98	70.457	<0.0001
Nominal data		Number (%)			X^2	P value
Sex	Male	131(65.5%)	169(84.5%)	106(53%)	19.253	<.0.0001
	Female	69(34.5%)	31(15.5%)	94(47%)		
FH of DM No. (%)		7 (3.5%)	2 (1%)	2 (1%)	4.630	>0.05

Group 1= HCV without cirrhosis Group2= HCV with cirrhosis

Group 3= control BMI= body mass index FH= Family history

The table showed a statistically significant difference between the 3 groups regarding age, sex, and BMI. On the other hand, positive family history of DM was insignificantly different between groups.

Table (2) : Frequency of diabetes mellitus in HCV & control patients.

Comparison groups		Diabetes mellitus		Chi square test $\chi 2$, p value	Binary logistic regression OR, p value
		N	%		
HCV vs control	HCV (*n* = 400)	121	30.25%	43.359, <0.0001	2.7, **<0.0001**
	Control (*n* = 200)	13	6.5%		
Group (1) vs Group (2)	Group (1) (*n* = 200)	19	9.5%	81.626, <0.0001	9.9, **<0.0001**
	Group (2) (*n* = 200)	102	51%		
Group (1) vs control	Group (1) (*n* = 200)	19	9.5%	1.223, >0.05	0.66, >0.05
	Control (*n* = 200)	13	6.5%		
Group (2) vs control	Group (2) (*n* = 200)	102	51%	96.671, <0.0001	15.6, **<0.0001**
	Control (*n* = 200)	13	6.5%		

OR= odds ratio

The table showed highly significant increased prevalence of diabetes mellitus in HCV patients versus control group, in group 2 versus control group and in group 2 versus group 1 (OR= 2.7, 15.6& 9.9 respectively). Partial correlation of prevalence of diabetes mellitus in all groups remain highly significant after adjustment for age, sex, family history of diabetes and BMI (r=0.291, P< 0.0001). A non significant increased prevalence was found on comparing group 1 with control group (P>0.05).

Table (3): Predictors for development of diabetes mellitus in HCV patients.

Factor	B	P value	OR
Age	0.299	< 0.01	1.35
FH. of DM	2.965	< 0.01	19.4
BMI	0.610	< 0.01	1.84
Serum albumin	0.76	<0.0001	6.6
Activity score	0.262	<0.05	1.3
Fibrosis score	0.755	< 0.001	2.1

B= Regression coefficient

The table showed that each one year more in age , positive family history of diabetes , each one unit higher of BMI , each one gram lower in serum albumin level than 4 grams / dl, each one grade of activity up, and each one stage of fibrosis up increase risk of diabetes by 1.3, 19.4, 1.8, 6.6, 1.3 and 2.1 folds respectively. Age by BMI is still significant (P <0.05{logistic regression}), while activity by fibrosis became insignificant.

DISCUSSION

Several studies have suggested a possible link between HCV infection and an increased prevalence of Type 2 DM [26, 27]. Diabetes mellitus was found to be more prevalent in patients with chronic hepatitis C than in patients with other liver disease [28]. Liver cirrhosis had a strong, independent association with Type 2 diabetes [29].

The present study showed that 30.25% of HCV patients had diabetes as opposed to only 6.5 % of control group (p <0.0001, OR 2.7). Prevalence of Type 2 diabetes was significantly higher in HCV patients with cirrhosis (51%) than in control group (6.5%), (OR 15.6, P < 0.001).Prevalence is also significantly higher in cirrhotic HCV patients (51%) versus non-cirrhotic patients (9.5%), (OR 9.9, P < 0.001). Non significant difference was found on comparing non-cirrhotic HCV patients with control group. This is consistent with previously published results of case-control studies, where the prevalence of DM had been reported in 21% to 50% (a two to ten fold increase in prevalence) among patients with chronic HCV infection, which was significantly higher than that in the general population or among patients with other forms of liver diseases [26,27&29]. However in our study a relatively larger number of patients were used to define the prevalence and predictors of DM in chronic hepatitis C patients. Also, in the present study we tried to define the effect of development of liver cirrhosis on diabetes prevalence in HCV patients. Prevalence was found to be significantly higher in cirrhotic versus non- cirrhotic patients.

Zein et al. [30] also reported that the prevalence of diabetes was significantly greater among patients with hepatitis C compared with those with cholestatic liver disease. They also mentioned that patients with cholestatic liver cirrhosis had a prevalence of diabetes similar to that of an age- and sex-matched general population suggesting that the mechanism of diabetes in patients with liver cirrhosis is related more closely to HCV. An association of type 2 DM and HCV was reported by Bahtiyar et al. [4] in a cohort of 100 patients with cirrhosis; 50% of those with HCV-related cirrhosis had type 2 DM as opposed to only 9% of those with cirrhosis from other etiologies (odds ratio = 10:5). In another cohort, diabetes was observed in 21% of patients with HCV infection, as compared with only 12% of HBV-infected patients [31].

However, the previous data were not adjusted for risk factors for Type- 2 diabetes such as family history of diabetes or BMI. Petit et al. [32] suggested that among individuals with HCV infection, those with diabetes are more likely to have traditional risk factors of diabetes. So, we examined whether this relationship between HCV infection and type 2diabetes could be modified by known risk factors of diabetes. In our study, it was found that the strong association between HCV and DM was not attributable to age, sex, family history of diabetes or BMI. This is comparable to that found by Wlazlo et al. [29] who reported that, classical risk factors such as family history of diabetes and BMI could not explain the association between HCV infection and Type 2 diabetes.

In our study, the frequency of diabetes in control group was 6.5%. This goes in line with that reported by Arafa & Amin [33] in Egyptian adults in 2010 (6.4%) and higher than that in 2008 (4.07%) However, the frequency in the present study might be lower than that expected by speculation in 2012 probably due to exclusion of patients with liver disease in our control group.

In this study, logistic regression analysis confirmed that age, family history of diabetes, BMI, serum albumin level, hepatitis activity and fibrosis score were independent predictors for diabetes mellitus. The study showed that positive family history of diabetes, each one year increase in age, each one unit higher in BMI, each one g/dl lower in serum albumin level, each one grade of activity up, and each one stage of fibrosis up increase risk of diabetes in HCV patients by 19.4, 1.3, 1.8, 6.6, 1.3, 2.1 folds respectively. However this effect disappeared on testing activity by fibrosis. In another study [34] age and residency in urban regions were the predictive variables that could be associated with the presence of diabetes in HCV patients.

Alavian et al. [31] also confirmed that age and HCV infection were independent predictors for diabetes mellitus. However, they found that HCV infection is a more important predictor of glucose intolerance than cirrhosis, and that the combination of both factors further increases the risk of diabetes. This is contradictory to our study which confirmed that cirrhosis is an even more important for the development of diabetes in HCV patients.

CONCLUSION

Chronic HCV is associated with increased risk of diabetes to 2.7-fold.Development of cirrhosis in patients with chronic HCV increases the risk to 10-fold. This association seems not to be related to the known risk factors for diabetes. Potential predictors for this association might include older age, positive family history of diabetes, higher BMI, lower serum albumin level, higher activity and fibrosis score. Further prospective trials are required to confirm these results.

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REFERENCES

1. Negro F, Alaei M .Hepatitis C virus and type 2 diabetes. *World J Gastroentrol*. 2009; 15:1537–1547.
2. Mehta SH, Brancati FL, Sulkowski MS, Strathdee SA, Szklo M, Thomas DL. Prevalence 2 diabetes mellitus among persons with hepatitis C virus infection of type in the United States. *Ann Intern Med* 2000; 133(8):592–9.
3. El Zayadi AR, Selim OE, Hamdy H, Dabbous H, Ahdy A, Moniem SA. Association of chronic hepatitis C infection and diabetes mellitus. *Trop Gastroenterol* 1998; 19(4):141–4.
4. Bahtiyar G, Shin j, Aytaman A, Sowers JR, McFarlane SI. Association of diabetes and hepatitis c infection: Epidemiologic evidence and pathophysiologic insight. *Current Diabetes Reports* 2004; 4:194-198.
5. Arao M, Murase K, Kusakabe A, Yoshioka K, Fukuzawa Y, Ishikawa T, et al. Prevalence of diabetes mellitus in Japanese patients infected chronically with hepatitis C virus. *J Gastroenterol.* 2003; 38:355—60.
6. Mason A. Is type II diabetes another extrahepatic manifestation of HCV infection? *Am J Gastroenterol* 2003; 98: 243—6.
7. Chehadeh W, Sarkhouh HA, Al-Nakib W. Predictors of glucose intolerance in HCV-infected patients with no family history of diabetes. *Diabetes Res Clin Pract* 2007; 77: 157—8.
8. Chehadeh W, Abdella N, Ben-Nakhi A, Al-Arouj M, Al-Nakib W. Risk factors for the development of diabetes mellitus in chronic HCV genotype 4 infection. *J Gastroenterol Hepatol* 2009; 24:42—8.
9. Chehadeh W, Al-Nakib W. Severity of liver disease predicts the development of glucose abnormalities in patients with chronic hepatitis B or C following achievement of sustained virological response to antiviral therapy. *J Med Virol* 2009; 81:610—8.
10. Pyrsopoulos NT, Reddy KR: Extrahepatic manifestations of chronic viral hepatitis. *Curr Gastroenterol Rep* 2001, 3(1):71-8, Review.
11. Simó R, Hernández C, Genescà J, Jardí R, Mesa J. High prevalence of hepatitis C virus infection in diabetic patients. *Diabetes Care* 1996; 19:998—1000.
12. Song Y, Manson JE, Tinker L, Howard BV, Kuller LH, Nathan L, Rifai N, Liu S. Insulin sensitivity and insulin secretion determined by homeostasis model assessment and risk of diabetes in a multiethnic Cohort of women: the women's health initiative observational study. *Diabetes Care* 2007; 30(7):1747–52.

13. Huang J. F., Dai C. Y., Yu M. L., Hsieh M. Y., and Chuang W. L. Abnormal liver function test predicts type 2 diabetes: community-based prospective study .*Diabetes Care* 2008; 31(6).
14. Mason AL, Lau JY, Hoang N, Qian K, Alexander GJ, Xu L, et al. Association of Diabetes Mellitus and Chronic Hepatitis C Virus Infection. *Hepatology* 1999; 29(2) 328-333.
15. Imazeki F, Yokosuka O, Fukai K , Kanda T, Kojima H, Saisho H. Prevalence of diabetes mellitus and insulin resistance in patients with chronic hepatitis C: comparison with hepatitis B virus-infected and hepatitis C virus-cleared patients. *Liver Int* 2008; 28: 355– 62.
16. Arao M, Murase K, Kusakabe A, Yoshioka K, Fukuzawa Y, Ishikawa T et al. Prevalence of diabetes mellitus in Japanese patients infected chronically with hepatitis C virus. *J Gastroenterol* 2003; 38: 355–60.
17. Arase Y, Suzuki F, Suzuki Y, Akuta N, Kobayashi M, Kawamura Y, et al . Sustained virological response reduces incidence of onset of type 2 diabetes in chronic hepatitis C. *Hepatology* 2009; 49: 739–44.
18. Rouabhia S, Malek R, Bounecer H, Dekaken A, Bendali Amor F, Sadelaoud M et al . Prevalence of type 2 diabetes in Algerian patients with hepatitis C virus infection. *World J Gastroenterol* 2010; 16: 3427–31.
19. Kawamura Y, Arase Y, Ikeda K, Hirakawa M, Hosaka T, Kobayashi M, et al. Diabetes enhances hepatocarcinogenesis in noncirrhotic, interferon-treated hepatitis C patients. *Am J Med* 2010; 123: 951–6.e1.
20. Veldt BJ, Chen W, Heathcote EJ, Wedemeyer H, Reichen J, Hofmann WP et al .Increased risk of hepatocellular carcinoma among patients with hepatitis C cirrhosis and diabetes mellitus. *Hepatology* 2008; 47: 1856– 62.
21. Imai K, Takai K, Nishigaki Y, Shimizu S, Naiki T, Hayashi H et al. Insulin resistance raises the risk for recurrence of stage 1 hepatocellular carcinoma after curative radiofrequency ablation in hepatitis C virus-positive patients. *Hepatol Res* 2010; 40: 376–82.
22. ADA American Diabetes Association: Diagnosis and classification of Diabetes Mellitus. *Diab Care* 2008, 31, S1, S55-S60
23. ADA American Diabetes Association: Diagnosis and classification of Diabetes Mellitus. *Diab Care* 2012, 35, S1, S51-S52.
24. Friedman SL. Liver fibrosis – from bench to bedside. J Hepatol 2003; 38(Suppl 1):S38–53.
25. Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR and DOSVIRC groups. *Lancet* 1997; 349(9055):825–32.
26. Kaabia N, Ben Jazia E, Slim I, Fodha I, Hachfi W, Gaha R, Khalifa M, Hadj Kilani A, Trabelsi H, Abdelaziz A, Bahri F, Letaief A: Association of hepatitis C virus infection and diabetes in central Tunisia. *World J Gastroenterol* 2009, 15(22):2778-81.
27. Chehadeh W, Kurien SS, Abdella N, Ben-Nakhi A, Al-Arouj M, Almuaili T et al: Hepatitis C virus infection in a population with high incidence of type 2 diabetes: Impact on diabetes complications. *Journal of Infection and Public Health* 2011; 4:200-206.
28. Grimbert S, Valensi P, Levy-Marchal C, Perret G, Richardet JP, Raffoux C, Trinchet JC, Beaugrand M: High prevalence of diabetes mellitus in patients with chronic hepatitic C: a case-control study. *Gastroenterol Clin Biol* 1996; 20:544-48.
29. Wlazlo N, Beijers H. J. B. H, Schoon E. J , Sauerwein H. P, Stehouwer and Bravenboer C: High prevalence of diabetes mellitus in patients with liver cirrhosis. *Diabet. Med* 2010; 27: 1308–1311.
30. Zein NN, Abdulkarim AS, Wiesner RH, Egan KS, Persing DH. Prevalence of diabetes mellitus in patients with end-stage liver cirrhosis due to hepatitis C, alcohol, or cholestatic disease. *J Hepatol* 2000; 32 : 209–217
31. .Alavian SM, Hajarizadeh B, Nematizadeh F, Larijani B. Prevalence and determinants of diabetes mellitus among Iranian patients with chronic liver disease. *BMC Endocr Disord* 2004; 4:4.
32. Petit JM, Bour JB, Galland-Jos C, Minello A, Verges B, Guiguet M, Brun JM, et al. Risk factors for diabetes mellitus and early insulin resistance in chronic hepatitis C. *J Hepatol* 2001; 35:279-283.
33. Arafa N, Amin G. The Epidemiology of Diabetes Mellitus in Egypt: Results of a National Survey. *The Egyptian Journal of Community Medicine* 2010; 28 (3):29-43.
34. Elhawary E, Gamal F Mahmoud G , El-Daly M , Mekky F , Esmat and Abdel-hamid M . Association of HCV with diabetes mellitus: an Egyptian case-control study. *Virology Journal* 2011; 8:367-376.

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Ascitic Fluid Lactoferrin as a Diagnostic Marker for Spontaneous Bacterial Peritonitis

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Background and study aim: Spontaneous bacterial peritonitis (SBP) is an important cause of morbidity and mortality in patients with cirrhosis and ascites. The diagnosis of SBP is established when the ascitic fluid polymorphonuclear leukocyte (PMN) counts is ≥250 cells/ mm3 with or without a positive ascitic fluid culture but this test lack sensitivity. The objective of this study was to evaluate the possible role of lactoferrin in diagnosis of SBP.

Patients and Methods: The study included seventy patients with liver cirrhosis and ascites admitted to hospital. Patients were classified into SBP group and control group by ascetic fluid PMN count. Aspirated ascitic fluid samples were examined for bacteriological culture, biochemical assay, and cytological count. Ascitic fluid lactoferrin was measured by ELISA technique.

Results: Ascitic fluid lactoferrin was significantly increased in SBP patients compared to control group. There was a statistically significant positive correlation between lactoferrin levels and PMN counts in SBP patients ($p<0.001$). ROC curve was used to determine a cutoff value for lactoferrin in diagnosis of SBP. At lactoferrin level ≥270 ng/ml, the sensitivity was 96%, specificity was 95%, positive predictive value was 97.96%, negative predictive value was 90.5%, and accuracy was 95.7% in diagnosis of SBP.

Conclusion: Measurement of ascitic fluid lactoferrin could serve as a rapid and reliable screening tool for diagnosis of SPB.

INTRODUCTION

Cirrhotic ascites forms as the result of a particular sequence of events. Development of portal hypertension is the first abnormality to occur. [1]. Hypoalbuminemia and reduced plasma oncotic pressure favor the extravasation of fluid from the plasma to the peritoneal fluid and thus ascites is infrequent in patients with cirrhosis unless both portal hypertension and hypoalbuminemia are present [2].

It is an important cause of morbidity and mortality in patients with cirrhosis and ascites, which identified in 10%-30% of hospitalized ascitic patients [3] and mortality can approach 30% [4]. Bacteria participating in SBP come from the digestive tract. Extra-intestinal bacteria are much less frequent. The development of SBP thus depends on the antibacterial capacity of ascitic fluid which is positively correlated to the content of the total protein in ascitic fluid and the immune-competence of the patient. The organism reacts to the infection by activating neutrophilic granulocytes which migrate into the peritoneal cavity and trigger a complex cytokine cascade. So, there are four key elements of SBP pathogenesis: small intestinal bacterial overgrowth, increased intestinal permeability, bacterial translocation and immune-suppression. These key elements are not separate, but interlinked [5].

The diagnosis of SBP is established when the ascitic fluid polymorphonuclear leukocyte (PMN) count is ≥250 cells/ mm3 with or without a positive ascitic fluid culture [6]. Lysis of the PMNs during transport to the laboratory may lead to false negative results. Manual measurement of the ascitic fluid PMN count is operator–dependant makes quality control difficult and can delay the diagnosis [7]. The use of urinary reagent strips has been proposed for rapid diagnosis of SBP. The urinary strips identify leukocytes by detecting their esterase activity via a colorimetric reaction. However, a large multicenter study suggested a lack of sensitivity of strip tests for the diagnosis of SBP and indicated an absence of diagnostic efficacy for this test [8].

Lactoferrin is an iron binding protein that is found mainly in external secretions such as breast milk and in PMNs and is released on degranulation [9]. Previous studies showed that lactoferrin in stool provide a reliable marker of inflammatory diarrhea [10]. The measurement of ascitic fluid lactoferrin could provide a reliable biomarker for the presence of PMNs and detection of SBP in patients with cirrhosis [3]. Our objective was to evaluate the possible role of lactoferrin in diagnosis of SBP.

PATIENTS AND METHODS

Seventy cirrhotic patients with ascites who were admitted to Tropical Department, Zagazig University Hospitals were included in this study. The diagnosis of liver cirrhosis and ascites was based on clinical, biochemical and ultrasonographic findings. Patients were classified into 20 patients with ascitic fluid PMN count <250 cells/mm³ (control group) and 50 patients with ascitic fluid PMN count ≥250 cells/mm³ (SBP group). SBP group was further subdivided into culture negative SBP& culture positive SBP. Bacteriological culture using aerobic and anaerobic standard blood culture bottles containing brain-heart infusion broth, which were inoculated with 10 mL of ascitic fluid and incubated for 48 hours at 37°C. None of the patients had received antibiotics for ten days prior to hospital admission. Patients with evidences of secondary bacterial peritonitis, tuberculous peritonitis or malignant ascites were excluded. Further exclusion criteria were ascites due to other causes e.g. cardiac, renal diseases or Budd-Chiari syndrome. All participants provided written informed consent after receiving oral and written information concerning the study.

All studied patients were subjected to medical history taking, clinical examination, routine laboratory investigations and abdomino-pelvic ultrasonographic examination. Aspirated ascitic fluid samples were immediately examined for bacteriological culture and identification of microorganisms, cytological count (manual), and biochemical assays (Cobas 501, Roch Diagnostics). Ascitic fluid albumin was measured using Albumin Latex Biosystem kit. Ascitic fluid lactoferrin was determined using Assay Max Human Lactoferrin ELISA Kit (Endomedx). This assay employs a quantitative sandwich enzyme immunoassay technique. The minimum detectable level is 0.1 ng/ml. Intra-assay and inter-assay coefficient of variation are 4.1% and 7.1% respectively.

Statistical Analysis

Statistical analysis was performed with SPSS software (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for the Microsoft Windows. Kolmogorov-Smirov test was used to verify the normality of distribution of continuous variables. Data are presented as mean ± standard deviation (SD) for continuous variables, frequency and percentage for categorical ones. Differences between the studied two groups were evaluated by independent sample t test and $\chi 2$ respectively. Fisher exact- test was used for comparisons between qualitative categories when there is an observed cell < 5. Bivariate correlations were performed using the Pearson correlation to determine correlation of lactoferrin to the different studied variables. The test results were considered significant when P value < 0.05. Receiver operator characteristic (ROC) analysis, area under curve (AUC) and 95% confidence interval (CI) were used to determine the optimum cutoff value of lactoferrin in diagnosis of SBP. Diagnostic performance was represented using the terms sensitivity, specificity, positive predictive value, negative predictive value, and accuracy.

RESULTS

Demographic and clinical manifestations of the studied groups are presented in table 1. Most of patients had Hepatitis C virus (HCV) infection which represent 75% in control group and 84% in SBP group, followed by bilharzias (15% in

control group and 10% in SBP group), then mixed HCV and bilharzias (10% in control group and 6% in SBP group), with no statistically significant difference between the two groups (p>0.0.05). Table 2 represents cytological and biochemical characteristics of the studied groups. Ascitic fluid lactoferrin was significantly increased in SBP patients compared to control group (p=0.001).

Most of SBP patients showed negative culture results (70%), while 30% showed positive results of E.coli, Klebsiella, Staphylococcus and Pseudomonas (18%, 8%, 2% and 2% respectively). Culture results were negative in control group (100%). There was no statistically significant difference in the studied laboratory parameters including lactoferrin between culture positive and culture negative SBP patients (p>0.05).

Correlations between ascitic fluid lactoferrin and the other studied laboratory parameters in SBP group are represented in table 3, PMN counts, glucose A/S ratio, and LDH A/S showed significant correlations. Ascitic fluid lactoferrin levels were significantly correlated with PMN counts in SBP patients (r=0.56, p<0.001) (figure 1). Analysis of ROC-AUC revealed AUC of 0.995 (95% CI: 0.985-1.005) (figure 2). At cutoff value ≥ 270 ng/ml, lactoferrin can detect 48 out of 50 SBP cases. Nineteen out of 20 control subjects had lactoferrin levels <270 ng/ml. Ascitic fluid lactoferrin had 96% sensitivity, 95% specificity, 97.96% positive predictive value, 90.5% negative predictive value, and 95.7% accuracy in diagnosis of SBP.

Table (1): Demographic and clinical manifestations of the studied groups.

Parameter	Control Group N=20	SBP Group N=50	P
Age (years)	55.4±7.2	52.8±7.1	0.17
Sex (M/F)	12/8(60/40)	25/25(50/50)	0.31
No symptoms	13(65)	8(16)	< 0.001 *
Fever	2(10)	24(48)	0.003 *
Abdominal Pain	2(10)	23(46)	0.005 *
Encephalopathy	2(10)	21(42)	0.008 *
Jaundice	2(10)	10(20)	0.265
Splenomegaly	3(15)	30(60)	0.001 *

N: number of subjects. Data are represented as numbers (frequencies) or mean ± SD *Significant.

Table (2): Cytological and laboratory characteristics of the studied groups.

Parameter	Control Group N=20	SBP Group N=50	P
Leucocytes (/mm^3)			
Total	159.5±72.4	4150.7±1202.3	<0.001*
PMN	33.2±13.9	3451.2±1148.2	<0.001*
Ascitic protein (g/dL)	2.41±0.66	1.64±0.54	<0.001*
Albumin (mg/dL)			
Ascitic	10.86±2.6	3.65±0.36	0.002*
Serum	2.18±0.35	2.51±0.45	0.047*
SAAG	2.3±0.48	2.14±0.36	0.128
Glucose (mg/dL)			
Ascitic	154.6±51.3	101.5±32.8	0.005*
Serum	152.3±42.6	114.2±31.9	0.062
A/S ratio	1.1±0.36	0.9±0.29	0.048*
LDH (IU/L)			
Ascitic	106.1±37.3	424.9±154.8	0.002*
Serum	481.6±136.8	562.6±126.2	0.148
Ratio	0.23±0.14	0.67±0.21	<0.001*
Ascitic lactoferrin (ng/mL)	162.5±65.3	3400.5±1177.1	0.001

N: number of subjects. Data are represented as mean ± SD. *Significant. A/S: ascitic fluid/serum

Table (3): Correlations between ascitic lactoferrin and other studied laboratory parameters in SBP patients.

Parameter	r	P
PMNs	0.56	<0.001*
Ascitic total protein	0.25	0.086
SAAG	-0.40	0.004*
Glucose A/S ratio	-0.61	<0.001*
LDH A/S ratio	0.79	<0.001*

*Significant.

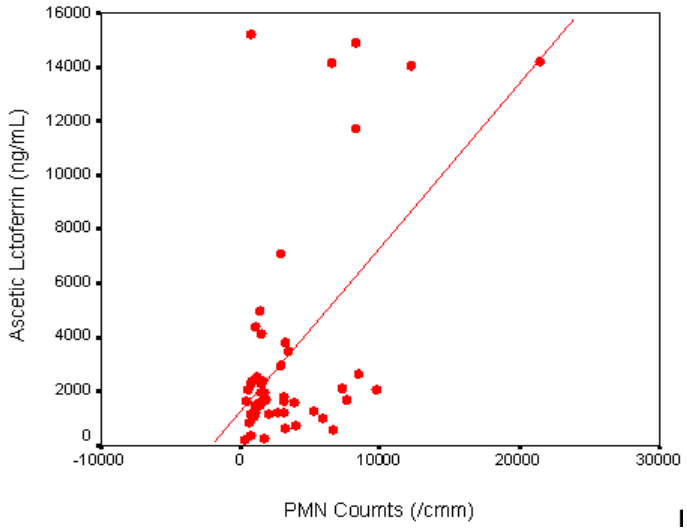

Figure (1): Correlation between ascitic fluid lactoferrin and PMN count in SBP group.

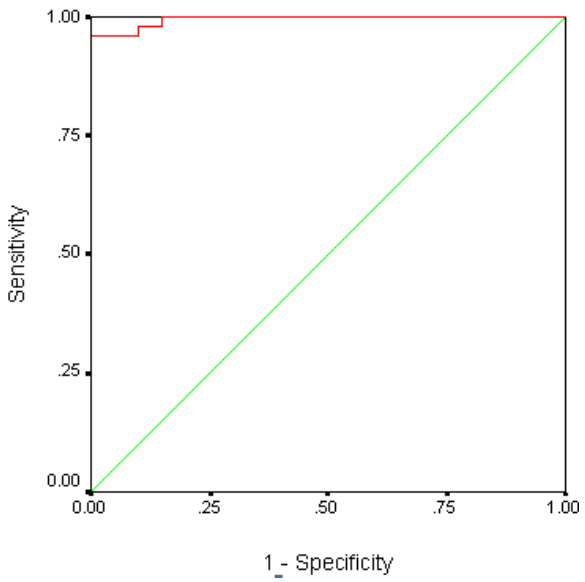

Figure (2): ROC curve analysis of ascitic lactoferrin for diagnosis of SBP. AUC of 0.995 (95% CI: 0.985-1.005)**.**

DISCUSSION

The clinical picture of SBP is non-specific and variable, mainly depending on the stage at which SBP is diagnosed [11]. The absence of clinical manifestations in some patients with SBP makes the dependence on a reliable marker is an important target taking into consideration that SBP is one of the most frequent and important complications found in cirrhotic patients with ascites [12]. In hospital, mortality rate can reach 30% in spite of infection control measures [13]. In the present study, the clinical features among the patients of SBP were not specific and asymptomatic patients constitute a relatively high percentage (16%). Presence of ascites usually prevents the development of a rigid abdomen by separating the visceral from the parietal peritoneal surfaces [14].

A positive bacterial culture is obtained in the minority of the patients with SBP and results are delayed for several days [7]. In the present study 70% of SBP patients had negative bacterial culture. Currently the diagnosis of SBP is based on PMN count but this test sometimes lack sensitivity and can delay the diagnosis [15]. A delay in antibiotic therapy entails a high mortality rate. On the basis of these considerations, considerable efforts have been made in the recent years to develop an alternative test for more rapid diagnosis.

Lactoferrin is an iron- binding protein contained in PMNs that is released on degranulation [16]. Titers of lactoferrin correlate with absolute neutrophil count in blood samples, and with the presence of neutrocytic inflammation in body fluid such as sputum samples [17]. Similar to the proposed utility of lactoferrin in the diagnosis of SBP, measurements of fecal lactoferrin was evaluated as a mean to diagnose inflammatory diarrhea in a community setting where cell lysis and specimen transport might result in false negative results [10]. Lactoferrin also has been shown to be remarkably stable and resistant to degradation when left at room temperature for extended periods of time. This property makes this marker attractive for clinical use [18].

In this study, ascitic fluid lactoferrin was assessed in cirrhotic ascitic patients with or without SBP to evaluate its role in the diagnosis of SBP. The mean ascitic fluid lactoferrin level was significantly elevated in SBP patients. Our results confirm the previous results reported by Parsi et al. [16]. The elevation of lactoferrin level in patients with SBP could be explained as had been described that lactoferrin is a major component of specific granules of human PMN leukocytes to be actively secreted by these cells into the environment in response to inflammation, bacterial infection and cytokine stimulation [19]. Ascitic fluid lactoferrin was significantly correlated with ascetic PMNs count ($r=0.56$, $p<0.001$) in this study and also with LDH A/S ratio($r=0.79$, $p<0.001$) . In SBP bacteremia with subsequent bacterial localization in the ascitic fluid would make the amount of bacterial DNA which stimulates the immunological response more pronounced in the ascitic fluid rather than blood, , this fact explains rising of lactoferrin level in ascitic fluid more than blood [20]. Lactoferrin plays a role in the first line of defense against microbial infections to prevent invading pathogens from utilizing host iron supplies for multiplication [21, 22].

ROC curve analysis identified an optimal ascitic lactoferrin level of 270 ng/ml for diagnose SBP. Ascitic lactoferrin concentration ≥270 ng/ml had 96%sensitivity and 95% specificity, 97.96% positive predictive value, 90.5% negative predictive value, and 95.7% accuracy in diagnosis of SBP. Parsi et al. [16] demonstrated 95.5% sensitivity and 97% specificity at a cutoff 242 ng/ml. The high sensitivity and specificity suggest that lactoferrin could act as a surrogate marker for PMN count in ascitic fluid in diagnosis of SBP.

The results concerning ascitic fluid lactoferrin in SBP represent an interesting and promising area of investigation, which could determine the further optimization of SBP management and further improvement in its prognosis. An early start of antibiotic therapy is important for the successful treatments of SBP. The development of bedside test that can diagnose SBP rapidly might facilitate patient selection for further diagnostic tests or admission to hospital and improve the cost effectiveness of SBP diagnosis. Qualitative and rapid tests are already commercially available for bedside measurements of lactoferrin concentration in stool. . In those centers in which PMN count and lactoferrin in ascitic fluid can not be measured, a reagent strip for leukocyte esterase designed for the testing of urine is a rapid, easy to use, and inexpensive tool for diagnosis of ascitic fluid infection [23]. A study done on two types of reagents, however, concluded that the negative predictive value for strips for be a high mortality

disease is not enough to discard SPB. In terms of the severity of SPB, the rate of false negative results could be considered high [24].It was previously hypothesized that qualitative tests able to detect lactoferrin levels in excess of a predetermined level for bedside diagnosis can easily be developed with limited costs [16].

In conclusion, measurement of ascitic fluid lactoferrin may serve as a rapid and reliable screening tool for SPB in patients with cirrhosis. Further studies are recommended to compare lactoferrin to other possible diagnostic markers.

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REFERENCES

1. Runyon BA: Management of adult patients with ascites due to cirrhosis: An update. *Hepatology* 2009; 49: 2087-2107.
2. Gines P, Cardenas A. The management of ascites and hyponatremia in cirrhosis. *Semin Liver Dis* 2008; 28(1):43-58.
3. Rimola A, Navasa M, Garcia-Tsao G, Piddock LJ, Planas R, Bernard B et al. Diagnosis, treatment and prophylaxis of spontaneous bacterial peritonitis: a consensus document. *J Hepatol* 2000; 32:142–153.
4. Thuluvath PJ, Morss S, Thompson R. Spontaneous bacterial peritonitis in hospital mortality, predictors of survival, and health care costs from 1988 to 1998. *Am J Gastroenterol* 2001; 96:1232-1236.
5. Fernandez J, Bauer T, Navasa M, Rodés J. Diagnosis, treatment and prevention of spontaneous bacterial peritonitis. *Baillieres Best Pract Res Clin Gastroenterol* 2000; 14: 975-990.
6. Runyon BA. Management of adult patients with ascites due to cirrhosis. *Hepatology* 2004; 39: 841–856.
7. Runyon BA. Strips and tubes: refining the diagnosis of spontaneous bacterial peritonitis. *Hepatology* 2003; 37: 745–747.
8. Noubaum JB, Cadranel JF, Nahon P, Khac EN, Moreau R, Thévenot T, et al. Diagnostic accuracy of multistix 8 SG reagent strip in diagnosis of spontaneous bacterial peritonitis. *Hepatology* 2007; 45:1272-1281.
9. Lyer S, Lonnerdal B. Lactoferrin, lactoferrin receptors and iron metabolism. *Eur J Clin Nut* 1993; 47:232-241.
10. Langhorst J, Elsenbruch S, Koelzer J, Rueffer A, Michalsen A, Dobos GJ. Noninvasive markers in the assessment of intestinal inflammation in inflammatory bowel diseases: performance of fecal lactoferrin, calprotectin, and PMN-elastase, CRP, and clinical indices. *Am J Gastroenterol* 2008; 103(1):162-169.
11. Parsi M, Atreja A, Zein N. Spontaneous bacterial peritonitis: Recent data on incidence and treatment. *Cleveland Clinic Journal of Medicine* 2004; 71: 565-57.
12. Gines P, Arroyo V, Rodes J. Therapy of ascites and spontaneous bacterial peritonitis. In: Cohen S, Davis GL, Gianella RA, et al., eds. Therapy of digestive disorders: A Companion to Sleisenger and Fortran's Gastrointestinal and Liver Disease 2000; Philadelphia: WB Saunders: 373–384.
13. Garcia-Tsao G. Current management of the complications of cirrhosis and portal hypertension: variceal hemorrhage, ascites, and spontaneous bacterial peritonitis. *Gastroenterology* 2001; 120(3):726-48.
14. Akriviadis E, Runyon B. Utility of an algorithm in differentiating spontaneous from secondary bacterial peritonitis. *Gastroenterology* 1990; 98:127-133.
15. Riggo O, Angeloni S. Ascitic fluid analysis for diagnosis and monitoring of spontaneous bacterial peritonitis. *World J Gastroentrol* 2009; 15(31): 3845-3850.
16. Parsi M, Saadeh S, Zein N, Davis GL, Lopez R, Boone J et al. Ascitic fluid lactoferrin for diagnosis of spontaneous bacterial peritonitis. *Gastroenterology* 2008; 135: 803-807.
17. Martin CA, Fonteles MG, Barrett LJ, Guerrant RL. Correlation of lactoferrin with neutrophilic inflammation in body fluids. *Clin Diagn Lab I mmunol* 1995; 2: 763-765.
18. Kayazawa M, Saitoh O, Kojima K, Nakagawa K, Tanaka S, Tabata K et al. Lactoferrin in whole gut lavage fluid as a marker for disease activity in inflammatory bowel disease: comparison with other neutrophil-derived proteins. *Am J Gastroenterol* 2002; 97:360– 369.
19. Birgens HS. Lactoferrin in plasma measured by and ELISA technique: evidence that plasma lactoferrin is an indicator of neutrophil turnover. *Scand J Hematol* 1985; 34: 326-331.
20. Lonnerdal B, Iyer S. Lactoferrin: Molecular structure and biological functions. *Annual Review of Nutrition* 1995; 15:93-110.
21. Masson P, Heremans J. Studies on lactoferrin, the iron binding protein of secretions. *Prot Biol Fluids* 1966; 14: 115-124.
22. Jeremy H. The physiology of lactoferrin. *Biochem Cell Biol* 2002; 80:1-6.
23. Castellote J, López C, Gornals J, Tremosa G, Fariña ER, Baliellas C, et al. Rapid diagnosis of spontaneous bacterial peritonitis by use of reagent strips. *Hepatology*. 2003;37(4):893-6.

24. Téllez-Ávila FI, Chávez-Tapia NC, Franco-Guzmán AM, Uribe M, Vargas-Vorackova F. Rapid diagnosis of spontaneous bacterial peritonitis using leukocyte esterase reagent strips in emergency department: uri-quick clini-10SG® vs. Multistix 10SG®.*Ann Hepatol* 2012;11(5):696-9.

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Utilization of Abdominal Ultrasonography in AIDS Patients

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Background and study aim: The acquired immunodeficiency syndrome AIDS is a destructive disease ,which become a major out-break in the community affecting both adult and young people, causing not only ill-health but affect both the economy, and the psychology of the patient. The abdomen is the largest cavity in the body. Ultrasound is most cheap, available and non invasive tool that can be used in diagnosing abdominal diseases. The aim of the current study was to detect the ultrasonographic findings of prevalent non-specific abdominal abnormalities associated with AIDS.

Patients and Methods: 200 patients with confirmed positive HIV were scanned at the Voluntary and Counseling Testing Centre in Omdurman Teaching Hospital, Sudan. with an international scanning guideline and protocols for abdominal ultrasonography. A two ultrasound scanners (Shimadzu SDU-350XL and KIAXIN) were used for scanning.

Results: The most affected age group was the 4th decade (36 %) which were in the active and product period and most of them (72%) were married. The most frequent abdominal ultrasonography findings were splenomegaly, enlarged lymph nodes, hepatomegaly and increased liver echogenicity (31.25%), (13.54%), (43.75%), (26.04%) respectively. The low frequent occurring were focal lesions in parenchymal of the organs and nephromegaly (1.04%), ascites (4.17%), portal hypertension (1.04%) and abscesses (1.04%).

Conclusion: Abdominal ultrasonography revealed that (48.0%) of the patients developed abnormal abdominal findings supporting the use of abdominal ultrasonography as a diagnostic tool in AIDS.

INTRODUCTION

The acquired immunodeficiency syndrome AIDS is a retroviral disease caused by the human immunodeficiency virus HIV and is characterized by the profound immuno suppression leading to opportunistic infections, secondary neoplasms, and neurologic manifestation [1,2].

In the past decade, AIDS has become a major health problem throughout the world. The world Health Organization WHO statistics showed that on 1st of July 1989 there were 167373 patients reported to have full blown AIDS. Of these, 95561 were in the United State of America USA, 29906 in Africa and 22708 in Europe. It estimated that there are 5 million people in the world who are infected [3].

In 2009, 33.3 million people were living with HIV worldwide, 2.6 million people newly infected with HIV worldwide, and 1.8 million people died of AIDS –related illness worldwide[4,5].

In the united states, the typical adult patient with AIDS present with fever, weight loss, diarrhea, generalized lymphadenopathy, multiple opportunistic infections, neurologic disease, and (in many cases) secondary neoplasms [1].

Although the largest number of infections are in Africa, the most rapid increase in HIV infection in past decade are in south east Asian countries, including Thailand, India and Indonesia[1].

The annual number of new HIV infections has been steadily declining since the late 1990s and there are fewer AIDS-related deaths due to the significant scale up of antiretroviral therapy over the past few years. Although, the number of new infections has been falling, level of new infections overall is still high, and with significant reductions in mortality the number of people living with HIV worldwide has increase[6].

In Sudan (September 2008) estimated number of adults and children living with HIV whether or not they have developed symptoms of AIDS as follows: adults > 15 years represented 270.000 VS children < 14 years represented 20.000 [4].

Patients with AIDS have a high incidence of certain tumors, particularly Kaposi sarcoma, non-Hodgkin lymphomas, and cervical cancer in women [1].

From the epidemiologic and subsequent laboratory investigations the transmission of HIV occurs under conditions that facilitate the exchange of blood or body fluids that contain the virus or virus-infected cells. Thus, the three major routes are sexual contact, parenteral inoculation, and passage of virus from infected mothers to their newborn [1].

Diagnostic ultrasonography imaging provides; a dynamic means of evaluating abdominal soft tissue structures in cross section [7] and also provides information concerning the size, shape, and echo pattern, position of the organs and other structure[8].

Areas affected by AIDS include central nervous system (limitation of ultrasound imposed by bones), respiratory system (ultrasound imposed by air in lung), abdominal retroperitoneal and superficial. Sonographic findings of prevalent non-specific abdominal abnormalities associated with AIDS include splenomegaly, hepatomegaly hyperechoic liver parenchyma, gall bladder wall thickening, lymphadenopathy, and nephropathy [9].

Nephropathy associated with HIV infection is an important cause of AIDS morbidity. Sonographically the characteristic findings are enlarged kidneys with increased cortical echogenicity. Additional findings include a globular appearance of the kidneys, decreased renal sinus fat, and heterogeneous parenchyma with echogenic striations [10].

Acute pancreatitis is common in AIDS caused by various opportunistic infections and medication; develop in 25% of patients taking didanosve. If patient is known to have AIDS, careful scanning will sometimes reveal echogenic thickening of the distal duct wall indicative of a stricture due to AIDS cholangitis [11].

The most common splenic ultrasonographic finding in AIDS is moderate splenomegaly, reported with 50% to 70% of patients referred for abdominal ultrasonography. Focal lesions can occur in AIDS. These may be caused by opportunistic infections such as candida, neumocystis, or mycobacterium. There have been reports of disseminated pneumocystis appearing as tiny focal echoes through the liver, spleen, and kidneys. The spleen may also be involved in Kaposi's sarcoma or lymphoma. Although fluid filled, actively peristaltic gut may be seen with infectious viral or bacterial gastroenteritis. Most affected patients do not demonstrate a sonographic abnormality. Also, certain high-risk populations, such as those with AIDS and neutropenia, appear to be susceptible to acute typhlitis and colitis, which also have a highly suggestive sonographic appearance [11].

OBJECTIVES

The aim of the study is to detect the findings of abdominal abnormalities associated with AIDS.

MATERIALS AND METHODS

This is a descriptive study concerns with the abdominal ultrasonographic findings in AIDS. The study was conducted at the Voluntary and Counseling Testing Centre in Omdurman Teaching Hospital, Sudan. The center is supplied by WHO and concern with HIV/AIDS offering psychological treatment, antiretroviral therapy, laboratory investigations and follow up.

A simple random sampling technique selected a number of two hundred confirmed positive HIV with signs and symptoms of AIDS were scanned by ultrasound. Those patients who have not developed AIDS signs and symptoms were excluded from participation. All participants were infectious and treated with different anti viral drugs and medications as well. All patients

were scanned twice according to the international protocol and guidelines of abdominal ultrasonography scanning [9]. The first scan was performed by the researchers and the findings were confirmed by a consultant radiologist.

The abdomen was completely evaluated in at least two scanning planes. Surveys were used to set correct imaging techniques, to rule out pathologies, and to recognize any normal variants. Typically, full abdominal surveys begin with aorta, followed by the inferior vena cava and the liver, and then the rest of the abdominal organs and associated structures. Only if they are well visualized, survey the aorta along the left lobe of the liver and the inferior vena cava with right lobe followed by the rest of the abdominal organs and associated structures. If an abnormality is identified, it is surveyed in at least two scanning planes following the completed survey of the abdominal organs.

The following criteria were utilized to assess the abdominal organs: Hepatomegaly- longitudinal dimension at mid-claviular line > 15cm; splenomegaly- longitudinal dimension > 13 cm; thickened gallbladder wall- dimension > 3mm at the anterior wall ; pancreatic enlargement- dimension > 1.5 cm, 2,5 cm or 2.0 cm for the head, body or tail respectively; renomegaly- longitudinal dimension > 12 cm. thickened bowel wall- thickness >4 mm; biliary dilatation- intrahepatic biliary ducts luminal diameter > 2mm, common bile duct > 6mm; lymphadenopathy- visualized lymph nodes (para-aortic) measured >10mm. The ultrasonographic criteria of enlargement of mesenteric lymphnodes has been variably defined as the detection of nodes larger than 4mm in the short axis and larger than 10mm in the long axis.

For scanning a Shimadzu SDU- 350XL (Japan) ultrasound machine with multi-frequency curvilinear probe (3.5 – 5 MHz) which has variable focal zone and frequency capability, and KIAXIN (China) with two probes curvilinear multi-frequency (2 MHz – 5 MHz) and linear high frequency 6.5 MHz probe have been used. High frequency probe 6.5 MHz was used to evaluate the gallbladder, the abdominal wall, appendix and other superficial structures. Shimadzu SDU-350 Xl curvilinear probe was used for the other abdominal organs. Proper setting of the overall gain system and time gain or depth gain compensation (TGC/ DGC) was adjusted to optimally visualize each organ.

To ensure combined validity and reliability the ultrasound results was verified by consultant radiologist who had expertise in performing ultrasound scanning. To ensure generalizability all patients were scanned by the same ultrasound machine using the same international guidelines and protocol for performing ultrasound and with using the same room preparation, then it is assumed that all patients offered the same level performing ultrasound.

Especial consideration was given to the right confidentially and anonymity of all research participants. Anonymity was achieved by using numbers for each research participant that would provide link between the information collected and the participants. In addition confidentiality was ensured by making the collected data accessible only to the researcher and the consultant radiologist. The right to equality will be ensured by giving each patient the same facilities, and the privacy of each patient was considered, so no individual patient's details throughout this study. Justice and human dignity was observed by treating selected patients equally when telling them to participate in the research as a sample of this study. The patients were free to decide whether to participate or not. Permission to conduct study was obtained from the hospital director of Omdurman Teaching Hospital. Data has been analyzed using SPSS (V.16) and presented as number and percentages.

RESULTS

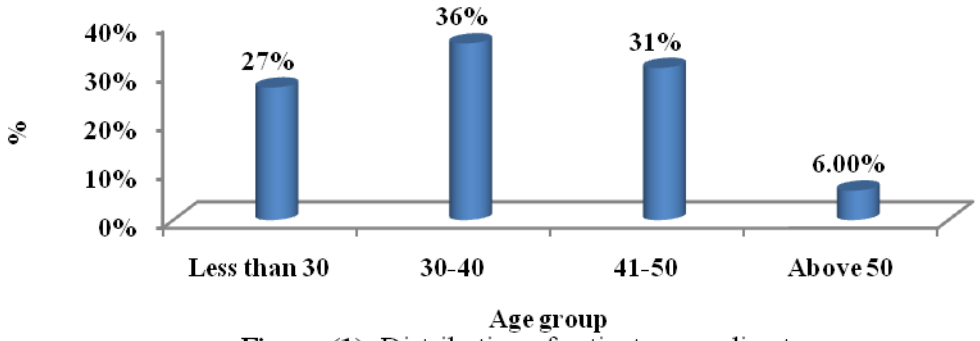

Figure (1): Distribution of patients according to age

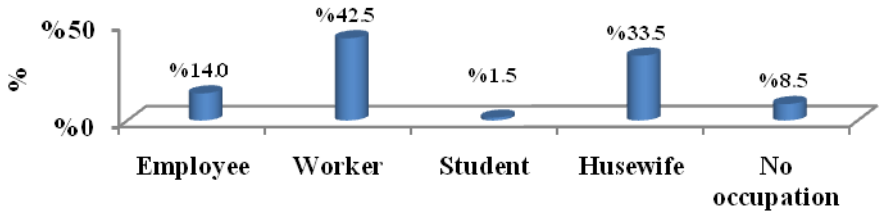

Figure (2): Distribution of patients according to occupation

Table (1): Frequency distribution of ultrasonography concerning liver size

Liver size	No.	Percentage %
Normal	54	56.25
Enlarged (Hepatomegaly)	42	43.75
Total	96	100

Table (2): Frequency distribution of ultrasound findings concerning liver in AIDS patients

Findings	No.	Percentage %
Paraenchyma echotexture	1	1.04
Hemangioma	1	1.04
Pyogenic abscess	1	1.04
Calcification	1	1.04
Simple cyst	2	2.04
Coarse liver parenchyma echotexture, hemangioma, liver abscess and calcification	90	93.76
Total	96	100

Table (3): Frequency distribution of ultrasound findings concerning size of spleen

Spleen size	No.	Percentage %
Normal	66	68.75
Splenomegaly	30	31.25
Total	96	100

Table (4): Frequency distribution of ultrasound findings concerning spleen echogenicity

Spleen echogenicity	No.	Parentage %
Normal	88	91.67
Decreased	3	3.12
Increased	5	5.21
Total	96	100

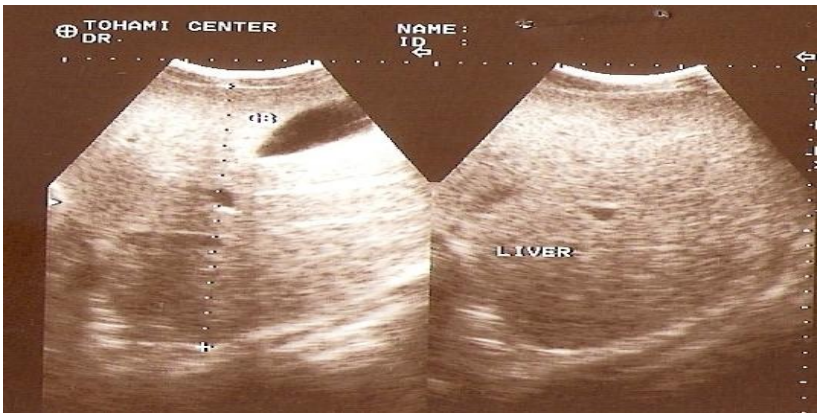

Image (1): A longitudinal gray scale sonogram of the liver of 42 years old male AIDS patient showing hepatomegaly.

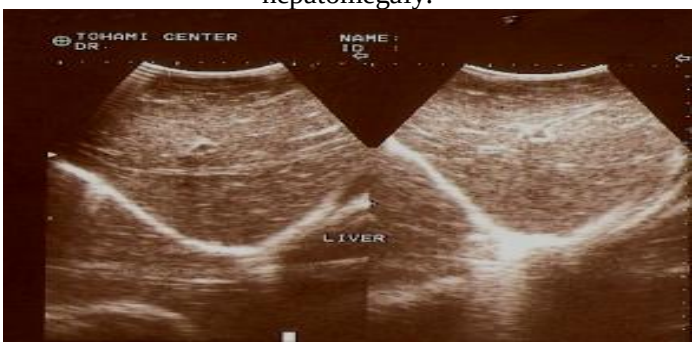

Image (2): A longitudinal sonogram of the liver of 40 years old male AIDS patient showing coarse liver parenchyma.

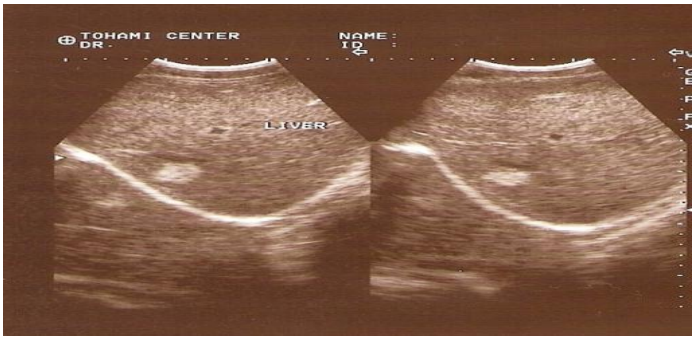

Image (3): A longitudinal sonogram of the liver of 33 years old male AIDS patient showing solid hyperechoic mass (hemangioma).

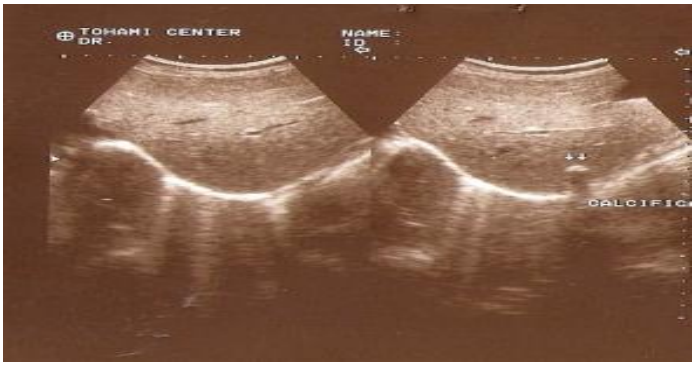

Image (4): A longitudinal gray scale sonogram of the liver showed small calcification.

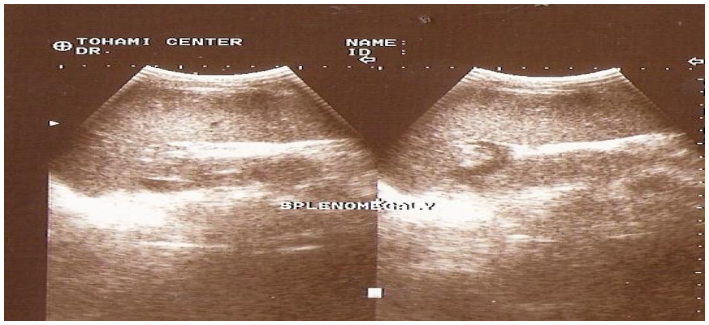

Image (5): A longitudinal sonogram of a 22 years old male AIDS patient showing splenomegaly

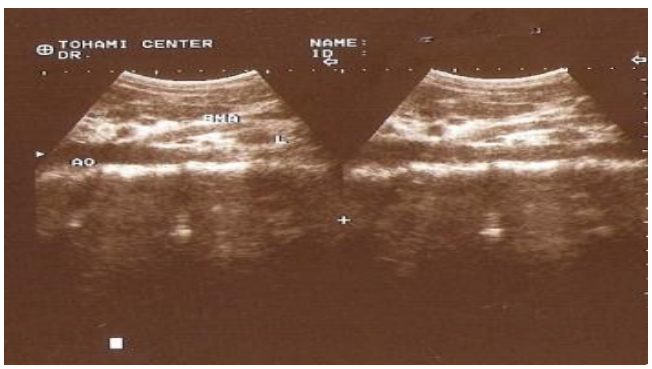

Image (6): A sagittal sonogram of 35 years male AIDS patient showing enlarged paraortic lymph nodes.

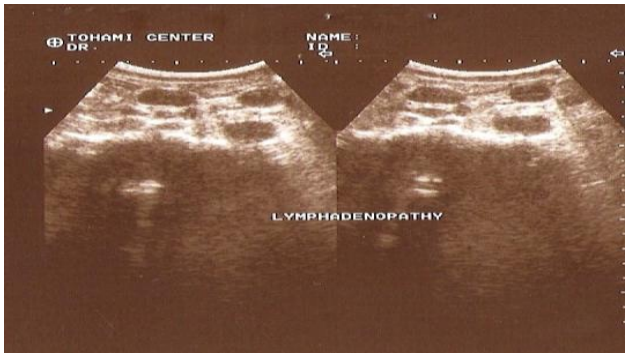

Image (7): A gray scale sonogram of 25 years old female AIDS patient showing enlarged lymph nodes.

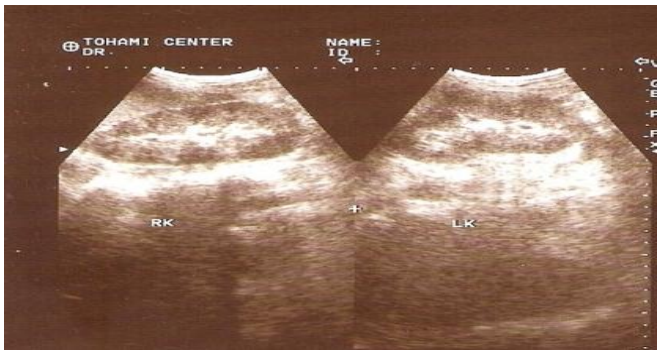

Image (8): A longitudinal sonogram of a 25 years old male AIDS patient showing bilateral increased renal cortex echogenicity.

DISCUSSION

Two hundred patients were scanned , 109 patients were male forming (54.50%). This showed slight difference from Obajami et al who studied abdominal ultrasonography in HIV/AIDS patients in southern Nigeria [2]. Obajami et al [2] revealed that (66.5 %) were females and (33.5%) were males, this difference may be due to the tradition state of our community and how people were dealing with AIDS patient.

The age parameter was divided into four groups in this study < 30years, between 31 up to 40 years, between 41 up to 50 years, and > 50 years. AIDS was most prevalent in the 4th decade or the most effected age group was between 31 - 40 years, this forms about (36 %). The finding matches with Obajimi et al [2] who reported that the prevalence of the disease was in the 4th decade confirming the theory that prove the most common cause of HIV transmission increases due to increasing sexual activity in the 3rd and 4th decade [2].

The majority of the patients were workers forming (42.5 %) of the participants. This clearly explain that low level of education increases the possibility of an individual to be infected by HIV.

In this study positive ultrasonography findings represent (48%) of the two hundred participants suggests that this record support the use of ultrasonography in AIDS management program and make it a reliable modality as an imaging tool.

Hepatomegaly was the most liver finding by ultrasonography. It was demonstrated in (43.75%) of the patients showing mostly non specific findings such as increased liver parenchyma echogenicity (26.04%) compatible

with fatty infiltration of the liver. Tshibwabwa et al [12] recorded that patients had hepatomegaly were (35%). Slightly lowering percentage by Tshibwabwa et al may be due to increased risk factors of hepatomegaly in our area because of malaria.

Other liver findings include coarse liver parenchyma echotexture, hemangioma, liver abscess, and calcification representing the same incidence (1.04%). Furthermore, (2.04%) patients had simple cysts. The only solid hyperechoic mass recorded was not biopsied for lack of appropriate facility in the centre.

Hepatosplenomegaly in AIDS patients in the absence of hepatic focal lesions may suggest infection (M. avium intracellular, malaria or histoplasmosis) rather than lymphoma [13].

Yee et al attributed the diffuse infiltration or increased hepatic echogenicity mostly to fatty infiltration or hepatic granulomatosis [14]. However, hepatitis from infection or drugs can cause the observed hyperechoic and also hypoechoic hepatic parenchyma changes [15]. This finding is at variance with the study from central Africa where intrinsic mass lesions, namely AIDS- related lymphoma, Kaposi sarcoma of the liver, diffuse nodular regenerative hyperplasia, multiple hyperechoic nodules from extra pulmonary pneumocystic carinii, and mycetic abscess were found. The absence of theses hepatic changes in our patients may suggest an improved quality of life consequence to the administration of highly-Active Antiretroviral Therapy HAART. There was however sonographic evidence of increased liver parenchyma echogenicity (26.04%) of these patients. This latter is compatible with well documented fatty changes in AIDS patients.

Splenomegaly was demonstrated in (31.25%) of the patients. Other abnormalities of the spleen in this study occurred far less common. The four cases of focal hypoechoic splenic areas may have been due to splenic lymphoma and small abscesses. Focal splenic lymphomas are commonly depicted as a hypoechoic lesion and were often seen in association with splenomegaly as in the cases identified. There was one case of echogenic foci may due to splenic calcification. There were 3 (3.12%) hypoechoic splenic parenchyma, and 5 (5.21%) cases of increased echogenicity of splenic parenchyma. These cases of splenic hyperechogenicity could not be attributed to any particular disease entity.

The frequency of splenomegaly was compared with that recorded by Tshibwabwa et al [12] in which (35%) of their patients had splenomegaly and this agreed with the findings of this study. Also slightly matched the results of Objimi et al[2] in which identified splenomegaly in (45%) of all samples.

Splenomegaly without focal lesion is relatively common in the tropics and could have myriads of causes including malaria, septicaemia, typhoid, schistosomiasis, portal hypertension, haemolytic anaemia and tropical splenomegaly [16].

Lymphadenopathy was diagnosed in 13 patients (13.54%) in this study enlarged lymphnodes were seen as multiple and were greater than 1 cm, mostly oval shaped with an echogenic hilum and a narrow symmetric cortex suggesting that they were benign. An ultrasound guided fine needle aspiration could have further characterized these nodes, but this could not be carried out in the centre because of unavailability of appropriate needles and other facilities. This record disagrees with Langer who studied [17] abdominal sonographic findings in patients with AIDS, and he found that lymphadenopathy was (21%), N'Zi Pk et al recorded [18] that lymphadenopathy was (17.2%), and this approximately agreed with this study.

The gallbladder wall was thickened in 4 (4.17%) patients; cases of cholelithiasis were not seen. These patients had no symptoms referable to the biliary system and the cause of this finding remains unknown.

Enlargement of the kidney was seen in 1 case bilaterally and a small kidney also in 1 case. The kidney size is not significant in our study. This disagreed with the study carried by Blessing et al [19]which showed that renomegaly was seen in (18.7%). The difference may due to the difference in the sample size which was less in our study.

There were 4 (4.17%) of patients had decreased renal cortical echogenicity and 5 (5.21%) of patients had increased renal cortical echogenicity. Other renal findings included 4 (4.17 %) case of AIDS nephropathy and 10 (10.41%) case of renal stones mainly right side, this study showed significantly increased renal stone, but still the detection of nephrolithiasis is a coincidental finding because there was no correlation between HIV infection and formation of renal stones. This strongly agreed with the

findings of Tshibwabwa et al [13] and Objimi et al [2].

The pattern of increased renal cortical echogenicity seen in this study was similar to that described by Herald et al [20] as AIDS nephropathy.

Conclusion

A wide range of abnormal abdominal organs can be seen on ultrasonography in patients with AIDS. The most frequent findings include splenomegaly, enlarged lymph nodes, hepatomegaly and increased liver echogencity. Focal lesions of parenchyma of the organs, nephromegaly, ascites, portal hypertension and abscesses are uncommon. The study's data supports the fact that the ultrasound is useful diagnostic tool for AIDS patient.

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REFERENCES

1. Vinay Kumar, Ramzi S. Cortan, Stanley L. Robbins. Basic Pathology. 7th ed., *Saunders; Philadelphia; Pennsylvania*: 2003; 156-147
2. Millicent O Obajimi, Mojisola O Atalabi, Godwin Ogbole, Adenike T Adeniji-Sofoluwe, Atinuke M Agunloye, Ademola J Adekanmi ; et al Abdominal Ultrasonography in HIV/AIDS Patients in Southwestern Nigeria, *BMC medical imaging*: 2008;8,1-6
3. Roderick N M Mac Sween and Keith Whaley, Muir's Textbook of Pathology, 13th ed., *Arnold; London:* 1992;3 p.p 243
4. Epidemiological Fact Sheets on HIV and AIDS, www.who.int.globalatlas/predefined reports. 2008. (Accessed 12 November 2011).
5. www.who.int. 2010 report.(Accessed 10 October 2011).
6. www.who.int/hiv/data. 2009.(Accessed 25 October 2011).
7. http://www.unaids.org/en/ (Accessed 12 December 2012).
8. Kathryn A. Gill. Abdominal ultrasound. W.B. Saunders; USA: 2002 chap 2, 35-56
9. Syed Amir Gilani. Guidelines and Protocols for Medical Diagnostic Ultrasound. 1st ed., *The Burwin Institute of Ultrasound. Lahore, Pakistan:* 2002; 44-46
10. Miller F, Patrikh S, Gore R, Nemcek A, Fitzgerald S, Vogelzang R: Renal Manifestations of AIDS. RadioGraphics 1993; **13**:587-596.
11. Devin Dean. Ultrasonography of the Abdomen and Small Parts. *The Burwin Institute of Diagnostic Medical Ultrasound; Lunenburg, Canada:* 2005; **44**
12. Carol M. Rumack, Stephanie R. Wilson, J. William charponeau. Diagnostic Ultrasound. Vol. 1,2; *Mosby; USA*: 1991; 10-230
13. ET .Tshibwabwa , P. Mwaba, J Bogle-Taylor, A, http://www.ncbi.nlm.nih.gov/pubmed?term=Zumla%20A%5BAuthor%5D&cauthor=true&cauthor_uid=10823454 . Four Years Study of Abdominal Ultrasound in 900 Central Africa Adults with AIDS referred for Diagnostic Imaging. *Pub Med Abdom Imaging*: 2000; **25**:290-296.
14. Townsend RR. CT of AIDS-related Lymphoma. *Am J Roentgenol*: 1991; **156**:969-974.
15. Yee JM, Raghavendra BN, Horii SC, Ambrosino M. Abdominal Sonography in AIDS: a review. *J Ultrasound Med* 1989; **8**: 705-714.
16. Schneiderman DJ. Hepatobiliary Abnormalities of AIDS. *Gastroenterrol Clin North Am:* 1988; 615-630.
17. Richard M. Gore, Frank H. Miller, Vahid Yaghami. Seminars in Ultrasound, CT and MRI. Science direct. 1998. **19**:175-189.
18. Blessing Ose-Emenim Igbinedion. Journal of radiology. *Benin, Nigeria*: 2009.
19. Herald T. Lutz & Hassen A. Gharbi. Manual of Diagnostic Ultrasound in Infectious Tropical Diseases. *Springer-verlag; Berlin Heidelberg, Germany*: 2006; **10**, 72-80.
20. Langer R, Langer M, Schütze B, Zwicker C, Wakat JP, Felix R . Abdominal Sonographic Findings in Patients with AIDS, *Rontgenblatter,PubMed* : 1989;**22**:121-125
21. N'Zi PK, Coulibaly A, N'Dri K, Quattara ND, Diabate SA, Zunon-kipre E, Djedje AT: Ultrasound aspects of abdominal involvement in adults with HIV infections in the Ivory Coast: apropos of 146 cases. Sante 1999; **9**:85-88.

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Prevalence of HCV Antibodies and HBV Surface Antigen among Workers of Zagazig Faculty of Medicine and its Hospitals

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Key words: Hepatitis C virus antibodies (HCV Abs), Hepatitis B surface antigen (HBsAg), Intensive care unit (ICU)

Background and study aim: Viral hepatitis is a serious global public health problem affecting billions of people globally, and both hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are rapidly spreading in the developing countries including Egypt due to the lack of health education, poverty, and and illiteracy. So this study was conducted to determine the prevalence of HCV antibodies and HBsAg and possible risk factors of transmission of these infections among workers of Zagazig Faculty of medicine and its hospitals.

Patients and Methods: This study was conducted on two hundred workers (non medicals) All were randomly selected from all clinical and academic Departments of Zagazig Faculty of Medicine and its Hospitals. Their age was above 18 years and up to 60 years. Possible associated factors with infections by the viruses were collected from patient using questionnaire Rapid diagnostic test kits were used to screen for Hepatitis B surface antigen (HBsAg) and anti-Hepatitis C virus (HCV) antibodies.

Results: Out of two hundred 39 (19.5%)of workers had Hepatitis C virus (HCV) antibodies and 7 (3.5%) of workers had Hepatitis B surface antigen and one worker 1 ((0.5%). had dual hepatitis B and C virus coinfection . The prevalence of chronic HCV and HBV is higher among males (20.2% and 4% respectively) than females (17.3% and 1.92%) and both HBsAg and HCV Abs was (0.7)% in males and negative in females. The prevelance of HCV Abs was high among workers with past history of barbering (69.23%) while prevalence of HBV was high in workers with a history of blood transfusion (28.57%).Multivariate regression was used to estimate independent effects of risk factors on seropositivity of both virsus . A highly significant association was found between workers with history barbering (OR 4.58) and those with seroposiotivity of HCV abs. While there was no significant association between workers with a history of dental procedure(OR 1.44), operation (OR 1.2) and blood transfusion(OR 1.5) and those with acquired seropositivity of HCVAbs. And a highly significant association was found between workers with history of blood transfusion and those with acquired seropositivity of HBV (OR 8.18), while there was no significant association between workers with a history of dental procedure(OR 0.2), barbering(OR 0.59) and operation (OR 0.63) and those with acquired seropositivity of HBs.

Conclusion: We can conclude that he prevalence of HBV and HCV infections among workers of Zagazig Faculty of Medicine and its hospitals in this study is high .Barbering has 4 times risk of HCV infection and blood transfusion has 8 times HBV infection. and working in operation units was associated with increase the risk of HBV and HCV infections, but working in other units like surgical and non surgical , administration and ICU was associated with increase HCV transmission. For prevention the spread of HBV and HCV, people must be educated about these infections and modes of transmission , better infection control practices in hospitals, healthcare and barbering facilities.

INTRODUCTION

Chronic hepatitis C virus (HCV) infection remains a global health threat with 175 million carriers worldwide. Approximately 3% of the worldwide population is infected with the hepatitis C virus (HCV) [1]. The prevalence of HCV infection varies throughout the world Egypt has the highest prevalence of hepatitis C virus (HCV) in the world[2].

Estimated nationally at 14.7%. This nation has weathered the largest iatrogenic transmission epidemic of blood-borne pathogens in human history during the era of parenteral antischistosomal therapy and circumcision among males administered by health-care workers using improperly sterilized glass syringes [3]. HCV transmission is ongoing in Egypt, and incidence rates have been estimated at 2.4 per 1,000 person-years (165,000 new infections annually) [4]. overall, an estimated 6 million Egyptians had chronic HCV infection in 2008. Primary modes of HCV transmission include unsafe injections, other inadequate infection control practices, and unsafe blood transfusions[6,7]. HCV transmission also occurs among injection-drug users in Egypt [8].

Hepatitis B infections are major health problems in Egypt and the entire continent of Africa. Egypt is considered to be a region of intermediate prevalence for HBV infection with a reported 3%–11% [8] The Hepatitis B Virus (HBV) is transmitted hematogenously and sexually. The outcome of this infection is a complicated viral host interaction that results in either an acute symptomatic disease or an asymptomatic disease.

Patients may become immune to the Hepatitis B Virus (HBV), or they may develop a chronic carrier state. Later consequences are cirrhosis and the development of hepatocellular carcinoma [9] It is approximately 90% for an infection acquired perinatally, and is as low as 5% (or even lower) for adults [10].

The incidence of new infections has decreased in most developed countries, most likely due to the implementation of vaccination strategies [11]. Therefore, transmission from healthcare workers to patients is rare event, while the risk of transmission from an HBV-positive patient to a healthcare worker seems to be higher. Healthcare workers positive for hepatitis B are not generally prohibited from working. However, the individual situation has to be evaluated in order to decide on the necessary measures. The highest risk factor in most instances is injection drug use [9]. Although direct percutaneous inoculation is the most direct mode of transmission of HCV and HBV, several studies have demonstrated that sexual, household, occupational and vertical transmission may also be of importance[12]. Blood is one of the major sources of transmission of HBV, HCV, and physicians and patients are becoming more concerned about safe transfusion of blood [13]. The aim of the present study was to measure the prevalence of anti-HCV and HBsAg and possible risk factors of transmission of these infections among workers of Zagazig Faculty of medicine and its hospitals.

MATERIALS AND METHODS

The people recruited to the study were informed about the objectives of the study and that they were free to refuse participation. A verbal witnessed consent was obtained from each study participant. The study included a total number of two hundred workers. All workers (non medicals) were randomly selected from all clinical and academic Departments of Zagazig Faculty of Medicine and its Hospitals including surgical departments (general, cardiothoracic, gynecological and obstetric and surgical emergencies) and non surgical departments (Internal medicine, Pediatric and academic departments). The age of all workers was above 18 years and up to 60 years. All workers were selected to be non diabetic patients, non smokers, free from hepatic disorders, lung disease, renal disorders, acute inflammatory conditions, or acute infections and not receiving any drugs that are known to affect the liver.

All the subjects included in the study were subjected for full history taking , physical examination and Possible associated factors with infections by the viruses were collected from patient using questionnaire The questionnaire included socio-demographic data ,type of health care providers they consulted for health problems, history of jaundice, history of taking injections, previous surgical procedures, frequency of dental visits, receiving blood or blood products, history of current/past use of intravenous drugs, and visiting community barbers for shaving in males. and serological testes for HBs Ag and HCV antibodies had been performed by rapid acting test [14].

Statistical analysis

Data were entered checked and analyzed using Epi-Info version 6 and SPP for Windows version 8 [15] X^2 (chi-squared) used for difference between two or more qualitative variable. Correlation between variables was done using correlation coefficient "r". in all tests ($p < 0.05$) is significant and ($p< 0.001$) is highly significant.

RESULTS

Table (1) showed prevalence of HCV Abs and HBs Ag among workers of Zagazig Faculty of Medicine Departments and its Hospitals. Hepatitis C virus (HCV) antibodies was detected in 39 (19.5%) and Hepatitis B surface antigen 7 (3.5%) and both HBsAg and anti-HBc were positive in 1 ((0.5%).

Table (2) showed prevalence of HCV Abs and HBs Ag among workers of Zagazig Faculty of Medicine Departments and its Hospitals in relation to Sex. HCV and HBV prevalence was higher among males 30/148 (20.27) % and 6/148(4%) compared to females 9/52(17.3%) and 1/52(1.92% respectively) and the Prevalence of both HBs Ag and HCV Abs was 1/148 (0.7)% in males and negative in females .

Table (3) showed correlation between Prevalence of HCV Abs among workers of Zagazig Faculty of Medicine Departments and its Hospitals and risk factors of transmission, 46.15% of HCV seropositivity was reported in workers with past history of dental procedure (OR 1.44), 23.08% had history of operation (OR 1.2) 7.69% had history of blood transfusion (OR 1.59) but 69.23 % had past history of barbering (OR 4.58) which gives four times risk of HCV infection .

Table (4) showed correlation between Prevalence of HCV Abs among workers of Zagazig Faculty of Medicine Departments and its Hospitals and occupational exposure. The highest risk was reported with workers in operation units and surgical departments OR >3.36 .

Table (5) showed correlation between prevalence of HBs Ags among workers of Zagazig Faculty of Medicine Departments and its Hospitals and risk factors of transmission. The highest risk was recorded in workers with past history of blood transfusion (OR 8.18) compared with history of dental procedure,(OR0.29),barbering(OR0.59) and operation (OR1.58).Blood transfusion gives eight times risk of HBV infection..

Table (6) showed correlation between Prevalence of HBs Ags among workers of Zagazig Faculty of Medicine Departments and its Hospitals and occupational exposure. The highest risk was reported with workers in operation units OR 4.42 and to less extent in surgical units OR1.17.

Table (1): Prevalence of HCV Abs and HBs Ag among workers of Zagazig Faculty of Medicine Departments and its Hospitals

		Number (200 workers)	Percentage %
HCV Abs	positive	39	19.5%
	negative	161	80.5%
HBs Ag	positive	7	3.5%
	negative	193	96.5%
Both HBs Ags & HCV Abs +ve workers		1	0.5%

Table (2): Prevalence of HCV Abs and HBs Age in relation to sex

	Male (148)	**Female (52)**	**X^2**	**P**
HBs Ags (+ve) workers	6 (4%)	1 (1.92%)	Fisher	0.67
HCV Abs (+ve) workers	30 (20.27%)	9 (17.3%)	0.22	0.64
Both HBs Ags & HCV Abs +ve workers	1 (0.7%)		Fisher	1.0

Table (3): Correlation between Prevalence of HCV Abs and risk factors of transmission (dental procedure, Barbering, operation , and blood transfusion).

		HCV ab		O.R	X^2	p
		positive	negative			
Dental history	Yes	18 (46.15%)	60 (59.41%)	1.44 (0.67-3.09)	1.04	0.31
	No	21 (53.85%)	161 (40.59%)			
History of barbering	Yes	27 (69.23%)	53 (33.125%)	4.58 (2.03-10.48)	17.25	0.000*
	No	12 (30.77%)	108 (66.875%)			
History of operation	Yes	9 (23.08%)	32 (19.88%)	1.21 (0.48-2.99)	0.20	0.65
	No	30 (86.92%)	129 (80.12)			
History of blood Transfusion	Yes	3 (7.69%)	8 (4.97%)	1.59 (0.32-7.08)	Fisher Exact	0.45
	No	36 (92.31%)	153 (95.03%)			

Table (4): Correlation between Prevalence of HCV Abs and occupational exposure

Department		**HCV Abs**		**Total**	**OR**	**X^2**	**P**
		Positive 7	Negative 193				
Administration		8 (10.8%)	66 (89.2%)	74 (100%)	0.37(0.15-0.91)	5.65	0.01*
Operation units		12 (37.5%)	20 (62.5%)	32 (100%)	3.13(1.27-7.71)	7.86	0.005*
ICU		3 (20%)	12 (80%)	15 (100%)	1.03(0.22-4.24)	FISHER	1.0
Departments	**Surgical**	10 (40%)	15 (60%)	25 (100%)	3.36(1.25-8.93)	FISHER	0.01*
	Non surgical	4 (8.7%)	42 (91.3%)	46 (100%)	0.32(0.09-0.99)	4.44	0.03*
Out Patients		2 (25%)	6 (75%)	8 (100%)	1.4(0.19-8.12)	FISHER	0.65

Table (5): Correlation between Prevalence of HBs Ags and and risk factors of transmission (dental procedure, Barbering, operations, blood transfusion).

		HBs Ag		O.R	X^2	p
		positive	negative			
Dental History	Yes	2 (28.57%	106 (54.92	0.29 (0.04-1.75)	Fisher exact	0.24
	No	5 (71.43%)	87 (45.08%)			
History of Barbering	Yes	2 28.57%)	78 (40.41%)	0.59 (0.08-3.54)	Fisher exact	0.70
	No	5 (71.43%)	115 (59.59%)			
History of operation	Yes	2 (28.57%)	39 (20.20%)	1.58 (0.2-9.70)	Fisher exact	0.63
	No	5 (71.43%	154 (79.80%)			
History of blood Transfusion	Yes	2 (28.57%	9 (4.66 %)	8.18 (1.0-59.5)	Fisher exact	0.04*
	NO	5 (71.43%)	184 (95.34%)			

Table (6): Correlation between Prevalence of HBs Ags and occupational exposure

Department		**HBs Ag**		**Total**	**OR**	**X^2**	**P**
		Positive 7	Negative 193				
Administration		2 (2.7%)	72 (97.3%)	74 (100%)	0.67(0.09-4.05)	FISHER	1.0
Operation units		3 (9.4%)	29 (90.6%)	32 (100%)	4.24(0.71-24.1)	FISHER	0.08
ICU		0 (0.0%)	15 (100%)	15 (100%)	0.0(0.0-10.4)	FISHER	1.0
Departments	**Surgical**	1 (4%)	24 (96%)	25 (100%)	1.17(0.5-9.3)	FISHER	1.0
	Non surgical	0 (0.0%)	46 (100%)	46 (100%)	0.0(0.0-2.6)	FISHER	0.35

DISCUSSION

In comparison to rate of HBsAg and HCV infection, in general population of Egypt , The current study presented the prevalence of HBV surface antigens and HCV antibodies among workers of Zagazig Faculty of Medicine and its Hospitals. In our study, HCV Abs was detected in 19.5 percent of the workers of Zagazig Faculty of medicine and its hospitals. Our findings were consistent with El-Zanaty [5] who mentioned that the prevalence is as high as 20% in Egypt. In our study, the prevalence of HCV Abs in males is 20.27 percent and in females is 17.3 percent. Males had considerably higher rates of HCV antibodies than females. Similar results were reported in a cross-sectional survey in Upper Egypt, in which the prevalence of HCV Abs was higher among males than females (12% and 8%, respectively and it was also highest among those > 30 years of age [16].The most likely explanation for the higher prevalence among males than females is that males make more frequent visits to barber shops than females and may share shaving equipment, and circumcision for boys by informal health care providers was marginally associated with HCV infection [17]. In our study, the prevalence of HCV Abs is 27 (69.32) of 39 HCV seropositive patients with past history of barbering, Razor sharing and shaving in barber shops has been identified as a key risk factor of transmission of HCV.Many workers consider infection with HCV to be an

occupational hazard for barbers [26] Other researchers consider barbers a source of infection to their clients, especially when there is reuse of razor blades that may transmit infection through micro-trauma [26] ,howeve, others found no relation between shaving by community barbers and infection with viral hepatitis [17]. The current study reported that 18 (46.15 %) of 39 HCV-seropositive patients with a past history of dental procedure with an O.R. 1.44 (0.67-3.09). An analysis of data on acute viral hepatitis collected by an Italian surveillance system found that 9 percent of all cases of acute HCV infection had only a history of dental work as a risk factor [18].

In our study, the prevalence of HCV Abs is 9 (23.08 %) of 39 HCV-seropositive patients with a past history of operation with an O.R. 1.21 (0.48-2.99). There is no significant association between seropositivity of HCV and past history of operation. The risk of acquiring hepatitis C by needle-stick injury is extremely low, ranging from 0 to 10.3 % [19].

In our study, the prevalence of HCV Abs 3 (7.69%) of 39 of workers with a past history of blood transfusion O.R.1.59 (0.32-7.08). There is no significant association between sero-positivity of HCV and past history of blood transfusion the result is an agreement with Khattab [20] who found that 13.6% of Egyptian blood donors were serologically confirmed to be infected with HCV. Infection with HCV is reduced due to effective blood screening before blood transfusion [12]. Incidence of transfusion related hepatitis C is still higher in some areas of the world. In a study of 147 Chilean patients with chronic hepatitis C, the most common risk factor was blood transfusion in 54% [21]. In our study, the prevalence of HCV Abs is 3 (10.2%) of 15 workers of ICU with an O.R1.03(0.22-4.24) and the prevalence of HCV Abs is 2 (25%) of 8 workers of out patients with an O.R 1.4(0.19-8.12) and the seropositvity of HCV due to frequent contact with infected people. There is no significant association between seropositivity of HCV and working in these Departments. This is due to good infection control program. Neal [22] reported that healthcare workers are at greater risk of exposure to the hepatitis C virus than the rest of the general population.

HBV infection is one of the most important infectious diseases worldwide. Around one million persons die of HBV-related causes annually. Egypt is considered as intermediate prevalence area (3-11%) (20). In our study, HBs Ag was detected in 3.5 percent of the workers of Zagazig Faculty of medicine and its hospitals. Our findings were consistent with the prevalence (3-5%) in the Mediterranean countries, Japan, Central Asia, the Middle East, and Latin and South America [11].

In our study, the prevalence of HBs Ag Abs in males is 4 percent and in females is 1.92 percent. Males had considerably higher rates of HBs Ags than females. Similar figures were reported from a study in Pakistan in which the prevalence was 2.5% for HBs Ag, and among them the majority of cases were males [23].

In our study, the prevalence of HBs Ag is 2 (28.57 %) of 7 workers with a past history of dental procedure with an O.R. 0.29 (0.04-1.75). There is no significant association between seropositivity of HBV infection and history of dental procedure. Vectors of infection with HBV in dental practice are blood, saliva and nasopharyngeal secretions [24]. In our study, the prevalence of HBs Ag is 2 (28.57 %) of 7 of workers with history of barbering with an O.R. 0.59 (0.08-3.54).There is no significant association between sero positivity of HBV infection and history of barbering. Facial shaving from barbers has been repeatedly documented as a risk factor for transmission of HBV in various countries and is well known to cause abrasions and small cuts [25]

In our study, the prevalence of HBs Ag is 2(28.57 %) of 7 of workers with history of operation with an O.R. 1.58 (0.2-9.70). There is no significant association between seropositivity of HBV infection and history of operation, Due to the implementation of routine vaccination of health care workers the incidence of HBV infection among them is lower than in the general population. Therefore, transmission from healthcare workers to patients is a rare event, while the risk of transmission from an HBV-positive patient to a healthcare worker seems to be higher [26]. In our study, the prevalence of HBs Ag is 2 (28.57 of 72 of workers of Administration with an O.R. 0.67 (0.09-4.05) .The prevalence of HBs Ag is 1 (28.57 %) of 7 of workers of Out Patients with an O.R. 4.0(0.5-29.1). The prevalence of HBs Ag is 1 (28.57 %) of 24 of workers of surgical departments with an O.R. 1.17 (0.5-9.3). There is no significant association between seropositivity of HBs Ag

infection and occupational exposure, although the risk of transmission increased 4 fold in operation units and out patient due to exposure to blood, blood products and surgical instruments. Exposure to blood and body fluids remains an important concern for healthcare workers, especially those who sustain a percutaneous injury. The risk of acquiring hepatitis B infection following a needle stick injury is estimated at approximately 30%[27].

We conclude that the prevelance of HCV Abs among workers was 19.5% with males affection more than females .In HCV Abs positive workers 46.15% had history of dental procedure, 69.27% had history of barbering, 23.08% had history of operation,7.6% had history of blood transfusion, Barbering increase the risk of HCV transmission 4 times .Workers of surgical departments showed 40% for positivity of HCV Abs and in operation units 37.5%, both increase the risk of transmission more than 3 times , Also workers in non surgical department and administration showed significant association with HCV Abs positivity. The prevelance of HBV Ag among workers was 3.5% with males affection more than females, HBVAg positivity was significantly associated with history of blood transfusion ($p<0.05$), with 8 fold increase the risk of transmission, the prevelance of HBS Ag was 9.4 % among workers in operation units, with increase risk of transmission 4.24 times. All of the above reflect the importance of infectious control measures not only in ICU, but also in all departments, including operation surgical and non surgical and even administration. Increase puplic awareness of these infections , modes of transmission, ways of prevention may help to reduce the prevelance of both HCV and HBV.

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REFERENCES

1. WHO: WHO Int. World Health Organization fact sheet, hepatitis C. Available via: Fact sheet N°204 July 2012.
2. Sievert W, Altraif I, Razavi H, . A systematic review of hepatitis C virus epidemiology in Asia, Australia and Egypt. *Liver Int* ,2011; 31 (Suppl 2):61–80.
3. Strickland G. Liver disease in Egypt: hepatitis C superseded schistosomiasis as a result of iatrogenic and biological factors .*Hepatology*, 2006; 43:915–22.
4. Talaat M, Kandeel A, Rasslan O . Evolution of infection control in Egypt: achievements and challenges. *Am J Infect Control* 2006 ; 34:193–200.
5. El Zanaty F , Way A . Knowledge and prevalence of hepatitis C .*EDHS* 2009: 251-258.
6. Paez Jimenez A, Sharaf Eldin N, Rimlinger F. HCV iatrogenic and intrafamilial transmission in greater Cairo,Egypt. Gut 2010 ; 59:1554–60.
7. Mostafa A, Taylor S, El-Daly M. . Is the hepatitis C virus epidemic over in Egypt? Incidence and risk factors of new hepatitis C virus infections. *Liver Int* 2010 ;31:560–6.
8. Nelson, PK; Mathers BM, Cowie B."Global epidemiology of hepatitis B and hepatitis C in people who inject drugs: results of systematic reviews" .*Lancet* 2011 ;378 (9791): 571–83.
9. Gomaa AI, Khan SA, Toledano MB. Hepatocellular carcinoma: epidemiology, risk factors and pathogenesis.*World J Gastroenterology* 2008 ; 14(27): 4300.
10. Wasley A, Grytdal S and Gallagher K. Surveillance for acute viral hepatitis: United States. MMWR *Surveill Summ* 2008; 57(2):1.
11. Gilca V, De Serres G, Boulianne N..Antibody kinetics among 8-10 years old respondents to hepatitis B vaccination in a low endemic country and the effect of a booster dose given five or ten years later. *Vaccine* 2009 ;27(43):6048-53.
12. Busch MP, Glynn SA, Stramer SL, Strong DM. A new strategy for estimating risks of transfusion-transmitted viral infections based on rates of detection of recently infected donors.*Transfusion* 2005;45: 254-264
13. Nico Lelie, Evangelia Walker, Charl Coleman, Mark Keyter; Hepatitis B virus transmission by blood transfusion during 4 years of individual-donation nucleic acid testing in South Africa: estimated and observed window period risk. *Transfusion* 2012; 52: 880–892.
14. Hoofnagle JH, di Bisceglie AM. Serologic diagnosis of acute and chronic viral hepatitis. *Semin Liver Dis* 1991.;11:73-83
15. Dean A., Dean J. and Brendele K.Epi-Info version 6.02 ward processing data base and statistics program for epidemiology Microcomputer CDC, Atlanta, Gerogia USA 2006. 620.
16. Darwish MA, .: Prevalence of HCV and HBV antibodies among intravenous drug adults and associated risk factors. *Egyptian Journal of Medical Laboratory Sciences* 2005; 14.
17. El –Raziky MS.: Prevalence and risk factors of asymptomatic hepatitis C virus infection in Egyptian children. *World Journal of Gastroenterology* 2007; 13:1828–1832.

18. Mele, Tosti, Marzolini, Moiraghi. :Prevention of hepatitis C in Italy: lessons from surveillance of type-specific acute viral hepatitis.J. *of Viral Hepatitis* 2000; V 7, P 30–35.
19. Lowenfals A, Mehta V, Levi D.. Reduced frequency of percutaneous injuries in surgeons: 1993 versus 1988. *AIDS* 1995; 9:199–202.
20. Khattab MA, Eslam M, Sharwae MA, HamdyL(2010). Seroprevalence of hepatitis C and B among blood donors in Egypt: Minya Governorate, 2000-2008. *Am J Infect Control.* 2010; 38(8):640-1.
21. Soza A, Arrese M Gonzalez R, Alvarez M. Clinical and epidemiological features of 147 Chilean patients with chronic C. *Ann Hepatology* 2004 ; 3 (4):146–51.
22. Neal KR, Dornan J, Irving WL. Prevalence of hepatitis C antibodies among healthcare workers of two teaching hospitals. Who is at risk? *BMJ* 2004 7;314:179–80.
23. Amin J, Yousuf H, Mumtaz A, Iqbal M, Ahmed R, Adhami SZ, et al.,. Prevalence of hepatitis B surface antigen and anti hepatitis C virus among general population in Lahore. *Prof Med J* 2004; 11:334–7
24. Kidd-Ljunggren K, Holmberg A, Bläckberg J, Lindqvist B. "High levels of hepatitis B virus DNA in body fluids from chronic carriers". *The Journal of Hospital Infection* 2006 (4): 52–7.
25. Jokhio AH, Bhatti TA, and Memon S. Knowledge, attitudes and practices of barbers about hepatitis B and C transmission in Hyderabad. *Pakistan East Mediterr Health J,* 2010;16(10):1079-84.
26. Duseja A, Arora L, Masih B. Hepatitis B and C virus: Prevalence and prevention in health care workers. *Trop Gastroenterology*2002 ;23(3):125.
27. Hanafi MI, Mohamed AM, Kassem MS, Shawki M. Needle stick injuries among health care workers of University of Alexandria Hospitals. *Eastern* Mediterranean *Health Journal*. 2011 ;17(1):26–35.]

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Lyme Borreliosis - a Multisystem Disease

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Lyme borreliosis, due to the tick-borne spirochete *Borrelia burgdorferi sensu lato* (Bb.sl), causes significant morbidity throughout the world. Preliminary studies have indicated the presence of the arthropod vector and the pathogen in North Africa. A few clinical cases have been reported. Our objective is to evaluate whether Lyme borreliosis constitutes a threat to public health. To access our objective, we plan to establish a map of the tick distribution, to evaluate the prevalence of Borrelia infection in ticks, to identify Borrelia strains and to determine their genetic diversity, to identify reservoirs used to maintain Bb.sl, and to evaluate the incidence of the disease in the human population. The knowledge of the natural enzootic cycle involving arthropods and wild vertebrates in the maintenance of Bb.sl should permit to develop prevention strategies to counter any public health threat.

INTRODUCTION

Lyme borreliosis is a multisystem disease involving the skin, nervous system, joints and heart [1]. The causative agent *Borrelia burgdorferi* is transmitted by tick bite [2]. The prevalence of specific antibodies of blood donors living in endemic areas as described in south Lower Saxony (Germany) has been 7% and of forestry workers 21%, indicating that this is the most prevalent arthropod-borne infection in this geographic region [3,4]. One third of flagged Ixodes ricinus contain *B.burgdorferi* as demonstrated by polymerase chain reaction (PCR) [5]. Therefore reinfection should not be a rare event. Forestry workers with a high risk of repeated tick bites often have a high prevalence and high titers of specific antibodies but only sporadic corresponding clinical symptoms of Lyme borreliosis [6-8]. Thus it has been concluded that a natural protection existed in most cases after an infection with the spirochete.

HISTORY

In 1980, Steere et al., began to test antibiotic regimens in adult patients with Lyme disease [9]. In the same year, New York State Health Dept. epidemiologist Jorge Benach provided Willy Burgdorfer, a researcher at the Rocky Mountain Biological Laboratory, with collections of *I. dammini* from Shelter Island, NY, a known Lyme-endemic area as part of an ongoing investigation of Rocky Mountain spotted fever. In examining the ticks for rickettsiae, Burgdorfer noticed 'poorly stained, rather long, irregularly coiled spirochetes'. Further examination revealed spirochetes in 60% of the ticks. Burgdorfer subsequently confirmed his discovery by isolating from patients with Lyme disease spirochetes identical to those found in ticks [10]. In June 1982, he published his findings in Science, and the spirochete was named *Borrelia burgdorferi* in his honor [2].

PATHOPHYSIOLOGY

Borrelia burgdorferi can spread throughout the body during the course of the disease, and has been found in the skin, heart, joint, peripheral nervous system and central nervous system. Many of the signs and symptoms of Lyme disease are a

consequence of the immune response to the spirochete in those tissues [11]. *B.burgdorferi* is injected into the skin by the bite of an infected Ixodes tick. Tick saliva, which accompanies the spirochete into the skin during the feeding process, contains substances that disrupt the immune response at the site of the bite [12]. This provides a protective environment where the spirochete can establish infection. The spirochetes multiply and migrate outward within the dermis. Days to weeks following the tick bite, the spirochetes spread via the blood stream to joints, heart, nervous system, and distant skin sites, where their presence gives rise to the variety of symptoms of disseminated disease. The spread of *B.burgdorferi* is aided by the attachment of the host protease plasmin to the surface of the spirochete. If untreated, the bacteria may persist in the body for months or even years, despite the production of *B.burgdorferi* antibodies by the immune system. In the brain, *B.burgdorferi* may induce astrocytes to undergo astrogliosis, which may contribute to neurodysfunction [13]. The spirochetes may also induce host cells to secrete products toxic to nerve cells, including quinolinic acid and the cytokines IL-6 and TNF-alpha, which can produce fatigue and malaise [14,15].

In Lyme encephalopathy, diffuse white matter pathology can disrupt grey matter connections, and could account for deficits in attention, memory, visuospatial ability, complex cognition, and emotional status. White matter disease may have a greater potential for recovery than gray matter disease, perhaps because neuronal loss is less common.

EPIDEMIOLOGY

In northern Africa, *B.burgdorferi* sensu lato has been identified in Morocco,Algeria, Egypt and Tunisia [16-18]. Lyme disease in Sub-Saharan Africa is presently unknown, but evidence indicates it may occur in humans in this region. The abundance of hosts and tick vectors would favor the establishment of Lyme infection in Africa. In East Africa, two cases of Lyme disease have been reported in Kenya [19].

SIGNS AND SYMPTOMS

Lyme disease can affect multiple body systems and produce a range of symptoms. Not all patients with Lyme disease will have all symptoms, and many of the symptoms are not specific to Lyme disease, but can occur with other diseases as well. The incubation period from infection to the onset of symptoms is usually one to two weeks, but can be much shorter, or much longer. Symptoms most often occur from May through September, because the nymphal stage of the tick is responsible for most cases. Asymptomatic infection may be much more common among those infected in Europe.

DIAGNOSIS

Patients with *B. burgdorferi* sensu lato infection may experience one or more clinical syndromes of early or late LB. Usually, early infection consists of localized erythema migrans (EM), which may be followed within days or weeks by clinical evidence of disseminated infection that may affect the skin, nervous system, heart, or joints and subsequently, within months, by late infection [20-23]. EM is the characteristic sign of early infection with *B. burgdorferi* sensu lato and the clinical hallmark of LB. In recent series it is recognized in at least 80% of patients with objective clinical evidence of *B. burgdorferi* sensu lato infection who meet the CDC surveillance definition of LB [24]. The rash begins at the site of the tick bite as a red macule or papule, rapidly enlarges, and sometimes develops central clearing. The clinical diagnosis of early LB with EM relies on recognition of the characteristic appearance of a skin lesion of at least 5 cm in diameter. Hematogenous dissemination of *B. burgdorferi* sensu lato to the nervous system, joints, heart, or other skin areas, and occasionally to other organs, may give rise to a wide spectrum of clinical manifestations of what is called early LB. Usually, patients with objective evidence of dissemination experience one or more of the following syndromes: multiple EM lesions, atrioventricular conduction defects, myopericarditis, arthritis, facial palsy, meningitis, and meningoradiculoneuritis (Bannwarth's syndrome) [25-27].

Labotary Diagnosis

A variety of laboratory techniques have been developed for direct detection of *B. burgdorferi* sensu lato. These assays provide evidence for the presence of intact spirochetes or spirochete components such as DNA or protein in tick vectors, reservoir hosts, or patients.

Four different approaches have been used in the clinical laboratory: microscope-based assays, detection of *B. burgdorferi*-specific proteins or nucleic acids, and culture. Of these, culture of *B.*

burgdorferi sensu lato undoubtedly offers the best confirmation of active infection and has been increasingly used as a diagnostic modality by many researchers on both sides of the Atlantic. The availability of cultured organisms has also allowed investigation of the structural, molecular, antigenic, and pathogenetic properties of the different *B. burgdorferi* sensu lato species.

Direct microscopic detection of *B. burgdorferi* sensu lato has limited clinical utility in laboratory confirmation of LB due to the sparseness of organisms in clinical samples [28-33] Antigen detection assays (aside from PCR) also suffer from the same limitations as microscopic detection. Although antigen capture tests have been used to detect *B. burgdorferi* sensu lato antigens in CSF of patients with neuroborreliosis [34,35] and in urine samples from patients with suspected LB [36], their reliability is poor or at best questionable [37].

Prevention of tick bites

The ticks that can transmit Lyme disease are found in wooded areas, high grasses, marshes, gardens, and beach areas. In endemic residential areas, clearing brush and trees, removing leaf litter and woodpiles, and keeping grass mowed may reduce tick exposure by removing habitats suitable for ticks and their reservoir hosts [38]. Area application of pesticides to residential properties is effective for suppressing vector ticks but may be harmful to other wildlife and people [39]. Exclusion of deer from residential yards by fencing and maintaining tick-free pets also may reduce tick exposure [40]. Heavily infested tick habitats, such as wooded areas, should be avoided if possible. If not possible, then use of wide trails, not straying off the trail, and not sitting on the ground may decrease exposure. Careful attention also should be given to clothing worn in these areas. Clothing should be light-colored to make tick identification easier. Long sleeves and long pants that are tight at the wrists, ankles, and waist, and long pants tucked into light colored socks are preferable. A hat should be worn in densely wooded areas. Persons should be taught to inspect themselves and their children's bodies and clothing daily after possible tick exposure. Special attention should be given to the exposed hairy regions of the body where ticks often attach, including the heads and necks of children.

Because animal studies indicate that transmission of *B. burgdorferi* from infected ticks usually requires a prolonged duration of attachment (≥48 hours), ticks should be removed promptly [41,42]. The body of the tick should not be squeezed during removal. It should be grasped with a fine tweezers as close to the skin as possible and removed by gently pulling the tick straight out without twisting motions. If fingers are used to remove ticks, they should be protected with gloves or facial tissue and washed after removal of the tick. Analysis of ticks to determine if they are infected is not indicated because the predictive values of such tests in relation to the development of human disease are unknown.

NEUROLOGIC MANIFESTATIONS

Myositis is rare and may occur in the early or late stage. It is diagnosed by muscle enzymes, electromyography or biopsy. Myositis is associated with pains and paresis in proximal muscles in particular. Neuritis cranialis is an acute manifestation and may affect all the cranial nerves including the N. olfactorius. Unilateral or bilateral paresis may occur and recur. Mono-or polyradiculitis and meningopolyradiculitis are the neurologic symptoms of borreliosis that occur most frequently, corresponding to the descriptions by Garin, Bujadoux and Bannwarth [3,4]. Radiculitis is connected to an elevated protein concentration , and meningitis is accompanied by lymphocystic pleocytosis.

PROGNOSIS

For early cases, prompt treatment is usually curative. However, the severity and treatment of Lyme disease may be complicated due to late diagnosis, failure of antibiotic treatment, and simultaneous infection with other tick-borne diseases, including ehrlichiosis, babesiosis, and immune suppression in the patient. A meta-analysis published in 2005 found some patients with Lyme disease have fatigue, joint or muscle pain, and neurocognitive symptoms persisting for years, despite antibiotic treatment [7]. Patients with late stage Lyme disease have been shown to experience a level of physical disability equivalent to that seen in congestive heart failure.

Lyme Disease Vaccine

Animal studies have demonstrated that purified recombinant proteins, particularly of certain outer surface proteins (Osp) of *B burgdorferi,*

such as Osp A, B, and C, induce antibody responses that are highly protective [43]. The most extensively studied [44] of the single Osp vaccines and the one currently licensed contains recombinant OspA (rOspA), which is highly protective in the mouse model when the challenge strain is homologous or closely related to the isolate from which the OspA was derived [45,46]. However, when the challenge strain is different from the isolate from which the OspA was derived, protection to challenge is minimal or nonexistent.

Route of administration, immunization schedule, and dose:

Three doses of 0.5 mL (30 μg) of rOspA vaccine administered by intramuscular injection are required for optimal protection; the second dose is given 1 month later, and a third dose is given 12 months after the first dose. Dosages should be timed so that the second and the third doses are given several weeks before the start of the Lyme disease transmission season, which usually begins in April.

Preliminary data suggest that other immunization schedules (e.g., 0, 1, 6 months) are safe and induce antibody responses similar to the 0, 1, 12 month schedule [46]. However, at this time, only the 0, 1, 12 month schedule is approved by the US Food and Drug Administration.

CONCLUSION

Borrelia is transmitted to humans by the bite of infected ticks belonging to a few species of the genus Ixodes. Delayed or inadequate treatment can lead to the more serious symptoms, which can be disabling and difficult to treat. At present the major problem in the early diagnosis of borreliosis is the high percentage of seronegativity of 20-50% depending on the duration of the erythema migrans. Another problem is the persistence of elevated IgM antibodies after therapy. Misinterpretations of serology contribute to the over diagnosis and over treatment of chronic Lyme disease and irrational Lyme anxiety. The diagnosis of Lyme borreliosis should primarily be based on clinical and epidemiologic evidence.

REFERENCES

1. Steere AC. Medical progress-Lyme disease. *N.Engl. J.Med*. 1989; 321: 586-596.
2. Burgdorfer W, Barbour AG, Hayes SF, Benach JL, Grunwaldt E, Davis JP. Lyme disease-a tick-borne spirochetosis? *Science*. 1982 Jun 18;216(4552):1317-9.
3. Eiffert H, Ohlenbusch A, Fehling W, Lotter H, Thommssen R. Nucleotide sequence of the ospABoperon of a Borrelia burgdorferi strain expressing OspA but not OspS. *Infect Immun.* 1992; 60 :1864-1868.
4. Christen HJ, Hanefeld F, Eiffert H, Thomssen R. Epidemiology and clinical manifestations of Lyme borreliosis in childhood. A prospective multicentre study with special regard to neuroborreliosis. *Acta Paediatr. Scand.*1993; Suppl. 386: 1-75.
5. Eiffert H, Ohlenbusch A, Christen HJ, Thomssen R, Spielman A, Matuschka FR. Nondifferentiation between Lyme disease spirochetes from vector ticks and human cerebrospinal fluid. *J Infect Dis.* 1995;171(2):476-9.
6. Hassler D, Maiwald M. Re-Infektionen mit Borrelia burgdorferi bei einem immunkompetensen Patient. *Dutche Med. Wochenschr.* 1994 ; 119 :338-342
7. Fahrer H, Sauvain MJ, vd Linden S, Zhioua E, Gern L, Aeschlimann A. [Prevalence of Lyme borreliosis in a Swiss population at risk]. *Schweiz Med Wochenschr.* 1988 16;118(2):65-9.
8. Münchhoff P, Wilske B, Preac-Mursic V, Schierz G. Antibodies against Borrelia burgdorferi in Bavarian forest workers. *Zentralbl. Bakteriol Mikrobiol Hyp*.A.1987; 263:412-419.
9. Steere AC, Hutchinson GJ, Rahn DW, Sigal LH, Craft JE, DeSanna ET. Treatment of the early manifestations of Lyme disease. *Ann Intern.Med* 1983 ;99(1): 22-26.
10. Burgdorfer W. Discovery of the Lyme disease spirochete and its relation to tick vectors. *Yale J Biol Med.* 1984;57(4):515-20.
11. Auwaerter PG, Aucott J, Dumler JS. Lyme borreliosis (Lyme disease) molecular and cellular pathobiology and prospects for prevention, diagnosis and treatment. *Expert Rev Mol. Med.* 2004; 6(2): 1-22.
12. Fikrig E, Narasimhan S. Borrelia burgdorferi-traveling incognito? *Microbes. Infect.* 2006; 8(5) : 1390-9.
13. Ramesh G, Alvarez AL, Roberts ED, Dennis VA, Lasater BL, Alvarez X. Pathogenesis of Lyme neuroborreliosis. Borrelia burgdorferi lipoproteins induce both proliferation and apoptosis in rhesus monkey astrocytes. *Eur. J. Immunol*. 2003; 33(9) : 2539-50.

14. Halperin JJ, Heyes MP. Neuroactive kynurenines in Lyme borreliosis. *Neurology* 1992; 42(1) : 43-50.

15. Papanicolaou DA, Wilder RL, Manolagas SC, Chrousos GP. The pathophysiologic roles of interleukin-6 in human disease. *Ann. Intern. Med* 1998; 128(2) : 127-137.

16. Bovattour A, Ghorbel A, Chabchoub A, Postic D. Lyme borrerliosis in North Africa. *Arch Inst Pasteur Tunis.* 2004;81(1-4):13-20.

17. Dsouli N, Younsi-Kabachii H, Postic D, Nouiras S, Gerne L. Reservoir role of lizard Psammodromus algirus in transmission cycle of Borrelia burgdorferi sensu lato in Tunisia. *J.Med.Entomol*. 2006; 43(4) : 737-742.

18. Helmy N. Seasonal abundance of Ornithodoros, savignyi and prevalence of infection with Borrelia spirochetes in Egypt. *J Egypt Soc Parasitol.* 2000;30(2):607-19.

19. Javi JO, Gathua SN. Lyme disease report of two cases. *East Afr Med J*. 2005;82(5):267-9.

20. Nadelman RB, Wormser GP. Lyme borreliosis. *Lancet* 1998; 352:557-565.

21. Stanek G, Strle F. Lyme borreliosis. *Lancet* 2003; 362:1639-1647.

22. Steere AC. Lyme disease. *N. Engl. J. Med*. 2001; 345:115-125.

23. Steere, AC, Batsford WP, Weinberg M, Alexander J, Berger HJ, Wolfson S et al. Lyme carditis: cardiac abnormalities of Lyme disease. *Ann. Intern. Med.* 1980; 93:8-16.

24. Steere AC, Sikand VK, Meurice F, Parenti DL, Fikrig E, Schoen RT, et al. Vaccination against Lyme disease with recombinant Borrelia burgdorferi outer-surface lipoprotein A with adjuvant. *N. Engl. J. Med*. 1998; 339:209-215.

25. Oschmann P, Dorndorf W, Hornig C, Schäfer C, Wellensiek HJ, Pflughaupt KW. Stages and syndromes of neuroborreliosis. *J. Neurol*. 1998; 245:262-272.

26. Strle F, Nelson JA, Ruzic-Sabljic E, Cimperman J, Maraspin V, Lotric-Furlan S, et al. European Lyme borreliosis: 231 culture-confirmed cases involving patients with erythema migrans. *Clin. Infect. Dis.* 1996; 23:61-65.

27. van der Linde MR. Lyme carditis: clinical characteristics of 105 cases. Scand. *J. Infect. Dis.* 1991; Suppl. 77:81-84.

28. Berger BW, Clemmensen OJ, Ackerman AB. Lyme disease is a spirochetosis. A review of the disease and evidence for its cause. *Am. J. Dermatopathol.* 1983; 5:111-124.

29. Bergler-Klein J, Sochor H, Stanek G, Globits S, Ullrich R, Glogar D. Indium 111-monoclonal antimyosin antibody and magnetic resonance imaging in the diagnosis of acute Lyme myopericarditis. *Arch. Intern. Med*. 1993; 153:2696-2700

30. de Koning J, Bosma RB, Hoogkamp-Korstanje JA. Demonstration of spirochaetes in patients with Lyme disease with a modified silver stain. *J. Med. Microbiol.* 1987; 23:261-267.

31. de Koning J, Duray PH. Histopathology of human Lyme borreliosis 1993, p. 70-92. In K. Weber and W. Burgdorfer (ed.), Aspects of Lyme borreliosis. *Springer-Verlag*, Berlin, Germany.

32. de Koning J, Hoogkamp-Korstanje JA, van der Linde MR, Crijns HJ. Demonstration of spirochetes in cardiac biopsies of patients with Lyme disease. *J. Infect. Dis.* 1989; 160:150-153.

33. Hoffmann JC, Stichtenoth DO, Zeidler H, Follmann M, Brandis A, Stanek G, et al. Lyme disease in a 74-year-old forest owner with symptoms of dermatomyositis. *Arthritis Rheum.* 1995; 38:1157-1160

34. Courtney JW, Massung RF. Multiplex Taqman PCR assay for rapid detection of Anaplasma phagocytophila and Borrelia burgdorferi. *Ann. N. Y. Acad. Sci.* 2003; 990:369-370.

35. Coyle PK, Deng Z, Schutzer SE, Belman AL, Benach J, Krupp LB, et al. Detection of Borrelia burgdorferi antigens in cerebrospinal fluid. *Neurology* 43:1093-1098.

36. Dorward DW, Schwan TG, Garon CF. Immune capture and detection of Borrelia burgdorferi antigens in urine, blood, or tissues from infected ticks, mice, dogs, and humans. *J Clin Microbiol*. 1991;29(6):1162–1170.

37. Klempner MS, Schmid CH, Hu L, Steere AC, Johnson G, McCloud B, et al. Intralaboratory reliability of serologic and urine testing for Lyme disease. *Am. J. Med.* 2001; 110:217-219.

38. Schulze TL, Jordan RA, Hung RW. Suppression of subadult Ixodes scapularis (Acari: Ixodidae) following removal of leaf litter. *J Med Entomol*. 1995; 32:730–733.

39. Curran KL, Fish D, Piesman J. Reduction of nymphal Ixodes dammini (Acari: Ixodidae) in a residential suburban landscape by area application of insecticides. *J Med Entomol*. 1993; 30:107–113.

40. Hayes EB, Maupin GO, Mount GA, Piesman J. Assessing the effectiveness of local Lyme disease control. *J Public Health Manage Pract.* 1999; 5:86–94.

41. Piesman J. Dynamics of Borrelia burgdorferi transmission by nymphal Ixodes dammini ticks. *J Infect Dis.* 1993; 167:1082–1085.

42. Piesman J, Mather TN, Sinsky RJ, Spielman A. Duration of tick attachment and Borrelia burgdorferi transmission. *J Clin Microbiol*. 1987; 25:557–558.

43. Wormser GP. Lyme disease vaccine. Infection. 1996; 24:203–207.

44. Fikrig E, Barthold SW, Kantor FS, Flavell RA. Protection of mice against the Lyme disease agent by immunizing with recombinant Osp A. *Science*. 1990; 250:553–556.

45. Schaible UE, Kramer MD, Eichmann K, Modolell M, Museteanu C, Simon MM. Monoclonal antibodies specific for the outer surface protein A (OspA) of Borrelia burgdorferi prevent Lyme borreliosis in severe combined immunodeficiency (SCID) mice. *Proc Natl Acad Sci* U S A. 1990; 87:3768–3772.

46. Van Hoecke C, Lebacq E, Beran J, Perenti D. Alternative vaccination schedules(0, 1, 6 months versus 0, 1, 12 months) for a recombinant OspA Lyme disease vaccine. *Clin Infect Dis*. 1999; 28:1260–1264.

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How Morocco Succeeds in Eliminating Schistosomiasis

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Schistosomiasis is an endemic parasitic infection very linked to poverty. It is considered a world health problem since it infects people from 76 different countries especially tropical and subtropical regions. For many years, Morocco was endemic for shistosome infection. The unique form is urinary schistosomiasis and the first case was described in 1914. After an alarming prevalence rate (13 416 cases) in 1973, Morocco decided to establish a strategy to control and then to eliminate schistosomiasis through three major phases (control, elimination and consolidation). Since 2004 and until now, it isn't reported any new indigenous case. Morocco achieves the goal and succeeds in decreasing the prevalence of infection to a level of zero so eliminating schistosomiasis in all endemic geographic areas.

INTRODUCTION

Schistosomiasis or bilharzia is a tropical parasitic infection due to blood-dwelling fluke worms of the genus Schistosoma [1]. It is widespread in 76 countries and territories in tropical and subtropical zones and is approximated to infect about 200 million people worldwide, leading to critical morbidity [2,3,4]. Schistosomiasis continue to be truly disregarded in some countries by reason of the close link with poverty, geographical isolation, underappreciated global problem, stigmatization, absence of political voice of those infected and the lack of an developed global financing system[5,6]. Schistosomiasis control strategies should be founded on four principal goals for interventions: 1) screening for infected people and killing adult worm by chemotherapy 2) acting on intermediate host "snail" by biological or chemical control and environmental management 3) preventing water contamination by using people information, education and communication (IEC), 4) prevention of human contamination by using IEC, sanitation and hygiene. The ultimate aim of all schistosomiasis interventions efforts is required to be the elimination of this infection [7]. Elimination has been accomplished in several epidemiological locations and control progress in numerous endemic areas are now in the position to work for elimination after they have efficiently reduced morbidity relating to schistosome infections. Recognition of the public health implications of schistosomiasis, political will and motivation, and continual application of the established national control programs achieved with success wherever tried as in Brazil, the People's Republic of China (P.R. China) and Egypt [8,9]. In this article we will take the example of Morocco to illustrate the efforts of responsible authorities in combination with World Health Organization (WHO) to fight and eliminate schistosomiasis and to achieve the goals drawn through different programs.

DISCUSSION

Schistosomiasis was endemic to rural areas in southern Morocco for many years, and the first cases were identified in 1914 [10]. The only form of schistosomiasis in Morocco is urinary schistosomiasis due to Schistosoma haematobium [11]. During the last three decades, the progression of irrigation resulted in the spread of the disease, transmitted

by B truncatus, to the north and center of the country leading to ecologically diverse endemic foci: oases and arid areas; periodical streams in mountainous areas; modern irrigation strategies; coastal plains, swamps and rivers [12].

The epidemiological situation of the disease was viewed as alarming in 1973 (13 416 cases) thing witch push, three years later, the Ministry of Health to invest in a national control program. This program was launched in 1976 by a preparatory phase which lasted three years (1977-1981), followed by a test phase in three pilot provinces [13]. The preparatory phase intended for defining an appropriate strategy and operational approach to develop the national control program, and not until 1982 that it became operational in all provinces at risk of disease transmission [14].

The operational phase covering the years 1982–1993, where the adopted strategy designed to control morbidity, infection and transmission by: case-detection (selective passive detection, selective active detection, exhaustive detection, mass screening; malacological observation (snail monitoring, molluscicide); chemotherapy (individual and mass treatment) and health education [15]. After the introduction and spread of the program in all provinces exposed, the number of cases decreased from 6,582 in 1982 to 3,887 in 1989, with a peak of 10,645 cases in 1983[16].

This favorable evolution of the epidemiological situation of the disease was due in large part to the continuous actions of the control program, reinforced in 1987 by the introduction of praziquantel, very effective drug and administered as a single dose. By the end of 1992, certain foci of transmission had been totally inactivated; others were widely under control and prevalence was progressively falling. In 1993, a schistosomiasis elimination program (SEP) was developed [15].

The elimination phase has been started since 1994; the objective is to intensify the effort to eliminate all disease transmission foci at the end of 2004. It is based on: improving case-detection in high-risk areas; providing treatment of all detected cases and wide coverage in the case of mass chemotherapy; improving snail surveillance and molluscicide where necessary; supporting health education; developing intersectoral action and improving intersectoral coordination; motivating community participation [15]. Moreover there was monitoring and continual evaluation of the efficiency of the interventions. Parasitological monitoring was intensive with 149,718 samples being tested in 2000, 130,826 in 2004 and 90,470 in 2006 [12].

Since 2005, the program moved into in the consolidation phase, which will continue until 2010. This has been characterized by (1) upkeep of the surveillance activities with a aim for detection in previous endemic locations and schools (children under 10 years of age enrolled or non-enrolled) to identify replaced transmission (2) epidemiological surveys around this sort of cases and mass treatment (3) Prolonged surveillance of water bodies (323 water bodies were analyzed in 2006) and control of snail hosts [12].

Since the start of the SEP, the number of cases of schistosomiasis in Morocco has been steadily reduced. In 1999, 231 cases were recorded, of which 83% reported in four provinces, and in 2002 this figure was reduced to 42 cases. No indigenous case has been noticed in the country since 2004[14]. During the period of 2005-2009, epidemiologic observations mentioned that there was an interruption of transmission at the national level. No active focus of transmission was noticed, in spite of intensified surveys within the at risk provinces. Only 13 and 4 sporadic cases were found in 2005 and 2006, respectively. Epidemiologic investigations executed around these cases affirmed that nine cases were imported, and eight cases were residual cases [10].

The epidemiological situation of Schistosomiasis in 2010 was marked by control of the situation at all foci, no cases from active transmission has been identified for the sixth consecutive year. The ministry reported just the detection of imported case from Mauritania and a residual case native of Taroudant [17]. In 2012 no indigenous or imported case was reported [18]. Figure 1 shows the evolution of the number of cases during 1994-2012.

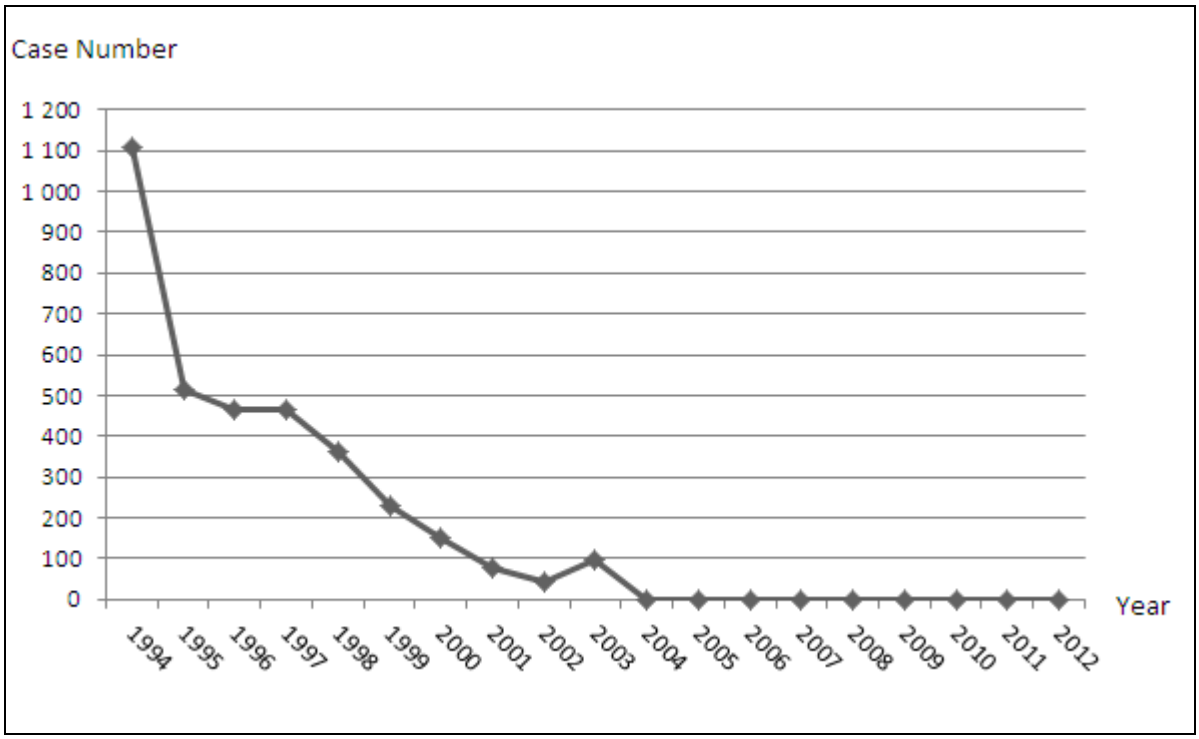

Figure 1: Evolution of schistosomiasis case number during the period of 1994-2012

We can say that Moroccan strategy was based on three major phases witch are control phase, eliminating phase and consolidation phase. The course of the Moroccan strategy is summarized in figure 2.

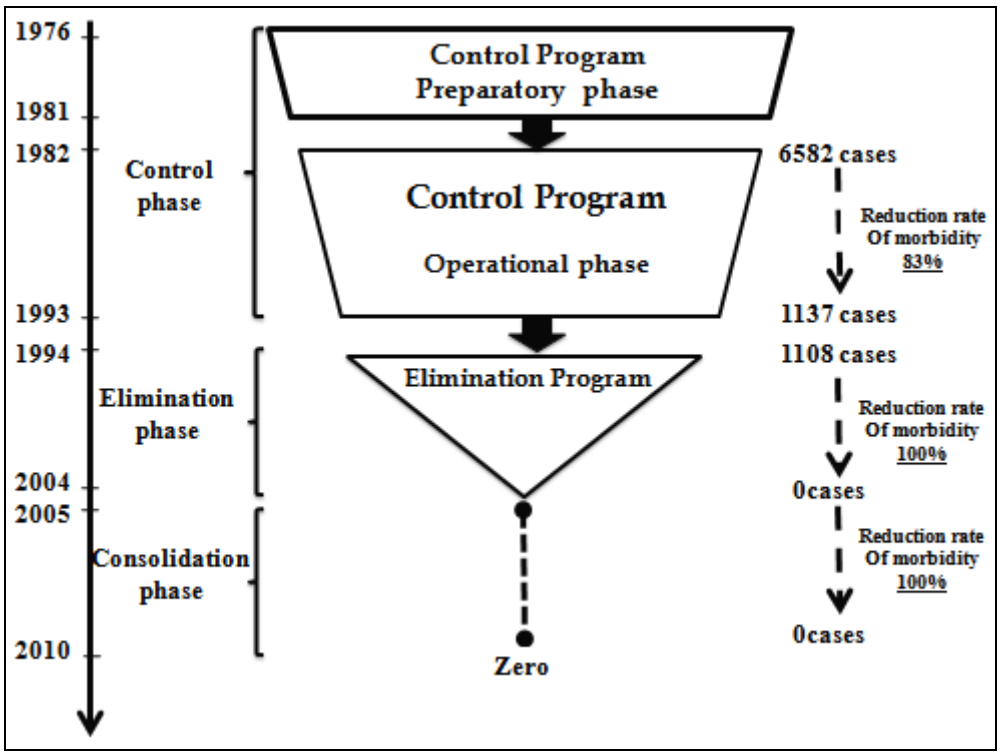

Figure 2: Moroccan strategy to control and eliminate schistosomiasis

Another factor that influenced the schistosomiasis incidence rate was the rainfall deficit (> 20%), which happened during 1990–2000 in Morocco and resulted in the natural drying of irrigation canals and a reducing in snail habitats. Moreover, infrastructure developments, including safe water supplies as a means of excreta disposal and an improved primary health care system in rural zones, presented crucial roles in schistosomiasis elimination. All of these factors contributed to decrease the prevalence and intensity of infection to a level of zero [10]. Given these results, the relevant officials at the Ministry of Health began the process of applying for certification of disease elimination from WHO.

CONCLUSION

To conclude Morocco achieves the goal and succeeds in eliminating schistosomiasis in all defined endemic geographic areas. The author acknowledges all the efforts established by Moroccan authorities and suggest to all endemic countries, with very high rate of shistosomiasis morbidity, to follow such strategy in order to control and eliminate this disease.

REFERENCES

1. Gryseels B, Polman K, Clerinx J, Kesten L: Human schistosomiasis. *Lancet* 2006, 368: 1106-1118.
2. Hodges MH, Soares Magalhaes RJ, Paye J, Koroma JB, Sonnie M, Clements A, Zhang Y: Combined Spatial Prediction of Schistosomiasis and Soil-Transmitted Helminthiasis in Sierra Leone: A Tool for Integrated Disease Control. *PLoS Negl Trop Dis* 2012, 6(6): e1694. doi:10.1371.
3. Chitsulo L, Engels D, Montresor A, Savioli L: The global status of schistosomiasis and its control. *Acta Trop* 2000, 77: 41-51.
4. Steinmann P, Keiser J, Bos R, Tanner M, Utzinger J: Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. *Lancet Infect Dis* 2006, 6: 411-425.
5. Gray DJ, McManus DP, Li YS, Williams GM, Bergquis R, Ross AG: Schistosomiasis elimination: lessons from the past guide the future. *Lancet Infect Dis* 2010, 10: 733-736.
6. Payne L, Fitchett JR: Bringing neglected tropical diseases into the spotlight. *Trends Parasitol* 2010, 26: 421-423.
7. Stothard JR: Improving control of African schistosomiasis: towards effective use of rapid diagnostic tests within an appropriate disease surveillance model. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2009, 103:325-332.
8. Engels D, Chitsulo L, Montresor A, Savioli L: The global epidemiological situation of schistosomiasis and new approaches to control and research. *Acta Trop* 2002, 82: 139-146.
9. Wang L, Utzinger J, Zhou XN: Schistosomiasis control: experiences and lessons from China. *Lancet* 2008, 372: 1793-1795
10. Amarir F , El Mansouri B, Fellah H, Sebti F, Lakranbi M , Handali S, Wilkins P , Laamrani El Idrissi A, Sadak A, and Rhajaoui M: National Serologic Survey of Haematobium Schistosomiasis in Morocco: Evidence for Elimination. *Am. J. Trop. Med. Hyg* 2011, 84(Suppl 1):15-19
11. Doumenge JP, Mott KE: Global distribution of schistosomiasis: CEGET/WHO atlas. *World Health Stat* 1984, Q 37: 186 -199.
12. Anonymous: EMRO Report of Schistosomiasis. *Inter-Country Meeting on Strategies to Eliminate Schistosomiasis from the Eastern Mediterranean Region* . Oman . November 6-8, 2007.
13. Anonyme : Guide de la lutte contre la schistosomiase. Rabat, Ministère de la Santé, Direction des Affaires techniques, 1982.
14. Barkia H, Barkia A, Nhammi H et Belghyti D : La schistosomiase au Maroc : de sa découverte à l'après-élimination. *Eastern Mediterranean Health Journal* 2011, 17(3):250-6
15. Anonymous: Report of a WHO Informal Consultation, Elimination of schistosomiasis from low-transmission areas. *World Health Organization* Brazil 2008, (WHO/HTM/NTD/PCT/2009.2)
16. DELM : Etat d'Avancement des Programmes de Lutte Contre les Maladies Parasitaires. *Rapport Annuel d'Activités* 1982 à 2002.
17. DELM : Etat d'Avancement des Programmes de Lutte Contre les Maladies Parasitaires. *Rapport Annuel d'Activités* 2010.
18. DELM : Etat d'Avancement des Programmes de Lutte Contre les Maladies Parasitaires. Rapport Annuel d'Activités 2012.

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Video Case: Granulommatous Esophagitis: Uncommon Cause of Iron Deficiency Anemia

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Comment

A 29-year old female patient presented by microcytic hypochromic anemia with parameters of iron deficiency. She was examined by diagnostic upper GIT endoscopy 12 months earlier and it showed GERD grade B. The patient continued to experience anemia and began to feel burning sensation behind the lower end of the sternum besides manifestations of reflux. Then she was re-examined by upper endoscopy and the lower esophagus showed diffuse ulcerations and marked tissue necrosis in a circumferential pattern, multiple biopsies were taken for histopathological examination (video) that revealed granulommatous inflammation of the esophagus.

Image Case: Irregular Stricture of The Lower Esophagus: Differential Diagnosis

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A 56 years old male patient presented by progressive dysphagia to solids and fluids. Barium swallow shows long segment lower esophageal irregular stricture (Figure1). The differential diagnosis of this condition includes cancer esophagus, corrosive stricture and esophageal motility disorder. Further investigations are needed to reach the final diagnosis mainly upper GI endoscopy. In case of cancer esophagus the patient is usually of old age, may be young but with history of long standing GERD, usually associated with anorexia, cachexia and weight loss and may also bleeds, endoscopy usually clinch the diagnosis and take biopsy. Corrosive stricture usually follows an acute insult after ingestion of corrosive material. Motility disorder may occur at any age and upper endoscopy as was seen in this case may be irrelevant.

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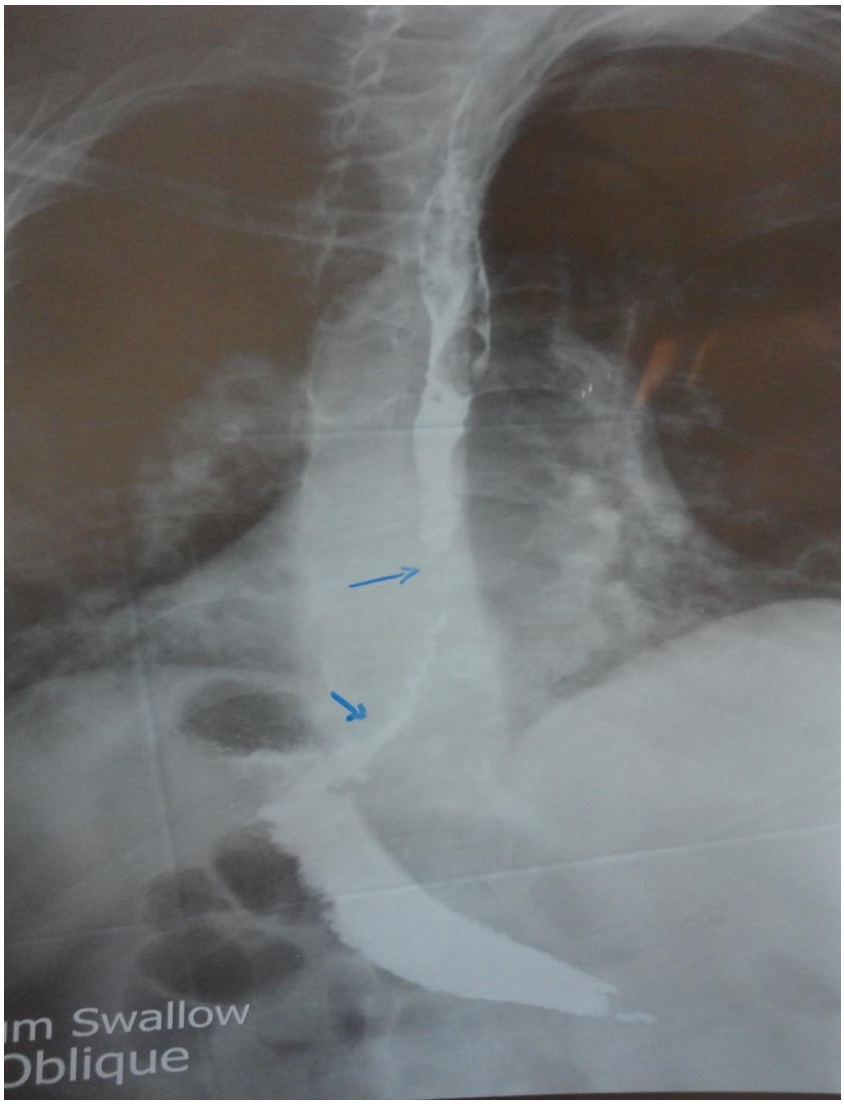

Figure 1: Barium swallow showing long segment lower esophageal irregular stricture

Case 1-2013: Filarial Lymphoedema of Upper and Lower limbs

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Case records of Endemic and Tropical Medicine Department, Zagazig University Hospitals, Zagazig, Egypt

Case presentation:

Twenty two-year-old house wife from Sharkeya, Egypt presented to the outpatient clinic of Tropical Medicine Department with fatigue and bilateral heaviness and swelling in her lower limbs as well as her left upper limb that started at the dorsum of her feet and progressed gradually to affect both limbs asymmetrically (the left lower limb was swollen more than the right) (Figure 1) up to the knee and then appeared in the left upper limb starting from the hand and gradually progressing to reach level of the elbow. The patient had normal general examination.

Examination of the affected limbs revealed normal color, temperature and hair distribution. No visible or dilated veins were noted. The examination revealed no ulceration or any skin lesions in the affected limbs. The oedema was partially pitting with spongy sensation (stage 2). The circumferences of the affected limbs were measured to observe the response to therapy. The oedema level was up to knee in both lower limbs and up to elbow in the upper limb (grade 1). The pulsation of dorsalis pedis, posterior tibial, popliteal, and femoral arteries in both lower limbs were intact as well as radial, ulnar, brachial and axillary arteries pulsations in the affected upper limb. There were no palpable inguinal or axillary lymph nodes. The patient was admitted to Tropical Medicine Department, Zagazig University Hospitals for evaluation of her condition. The patient received broad spectrum antibiotic and diuretic therapy and was advised to elevate her lower limbs and wear an elastic band over the affected upper limb. The previous measures gave minimal improvement of the swelling. The patient's routine laboratory investigations were normal. The patient performed Doppler evaluation for the venous systems in the affected limbs which was normal. The nocturnal peripheral blood film was negative for *W. bancrofti* microfilaria. The lymphiscintigraphy for lower limbs revealed patent lymphatics.

Differential diagnosis of lymphoedema:

1- Primary congenital lymphoedema.

2- Secondary to injury of lymphatics by dissection, irradiation or obstruction by malignancy.

3- Long standing untreated venous insufficiency.

4- Filariasis.

5- Podoconiosis

6- Leprosy

7- Mycetoma pedis.

Staging of lymphoedema: [1, 2]

According to the consistency and oedema response to elevation of the limb:

- **Stage 0 (latent)**: The lymphatic vessels have sustained some damage which is not yet apparent. Transport capacity is still sufficient for the amount of lymph being removed. Lymphedema is not present.
- **Stage 1 (spontaneously reversible)**: Tissue is still at the "non-pitting" stage: when pressed by the fingertips, the tissue bounces back without any indentation. Usually upon waking in the morning, the limb or affected area is normal or almost normal in size.
- **Stage 2 (spontaneously irreversible)**: The tissue now has a spongy consistency and is considered "pitting": when pressed by the fingertips, the affected area indents and holds the indentation. Fibrosis found in stage 2 lymphedema marks the beginning of the hardening of the limbs and increasing size.
- **Stage 3 (lymphostatic elephantiasis)**: At this stage, the swelling is irreversible and usually the limb(s) or affected area is very large. The tissue is hard (fibrotic) and unresponsive; some patients consider undergoing reconstructive surgery, called "debulking". This remains controversial, however, since the risks may outweigh the

benefits, and the further damage done to the lymphatic system may in fact make the lymphedema worse.

Grading for lymphoedema: [1, 2]

According to the extent of involvement in the affected body parts.

- **Grade 1** (mild edema): Lymphedema involves the distal parts such as a forearm and hand or a lower leg and foot. The difference in circumference is less than 4 cm, and other tissue changes are not yet present.
- **Grade 2** (moderate edema): Lymphedema involves an entire limb or corresponding quadrant of the trunk. Difference in circumference is more than 4 but less than 6 cm. Tissue changes, such as pitting, are apparent. The patient may experience erysipelas.
- **Grade 3a** (severe edema): Lymphedema is present in one limb and its associated trunk quadrant. The difference in circumference is greater than 6 centimeters. Significant skin alterations,
  suchas cornification or keratosis, cysts and/or f istulae, are present. Additionally, the patient may experience repeated attacks of erysipelas.
- **Grade 3b** (massive edema): The same symptoms as grade 3a, except two or more extremities are affected.
- **Grade 4** (gigantic edema): Also known as elephantiasis, in this stage of lymphedema, the affected extremities are huge due to almost complete blockage of the lymph channels. Elephantiasis may also affect the head and face.

DISCUSSION

The most probable diagnosis of this condition is filariasis. Filarial disease is endemic in Egypt in some villages of Nile Delta governorates where it is transmitted by *Culex pipiens* female. [3] The prevalence of filariasis in Egypt is 10-50 case/ 100000 populations. The prevalence of asymptomatic microfilaraemia is higher. [4]

Filariasis is considered endemic in tropical and subtropical regions of Asia, Africa, Central and South America, and Pacific Island nations, with more than 120 million people infected and one billion people at risk for infection. [4] In communities where lymphatic filariasis is endemic, as many as 10% of women can be afflicted with swollen limbs, and 50% of men can suffer from mutilating genital symptoms. [5]

The most important landscape elements associated with high prevalence of filariasis in Egyptian countryside are water, clay soil and different vegetation. Knowing this association not only helps mapping of the high prevalence areas but also helps predicting high risk of transmission. [3]

Filariasis is usually diagnosed by identifying microfilariae on Giemsa stained, thin and thick blood film smears, using the "gold standard" known as the finger prick test. The finger prick test draws blood from the capillaries of the finger tip; larger veins can be used for blood extraction, but strict windows of the time of day must be observed. Blood must be drawn at appropriate times, which reflect the feeding activities of the vector insects. Most cases of elephantiasis are amicrofilaremic in a condition called (occult filariasis). [6]

In conditions where microfilaria can't be seen in blood film, various concentration methods are applied: membrane filter, Knott's concentration method, and sedimentation technique. Polymerase chain reaction (PCR) and antigenic assays, which detect circulating filarial antigens, are also available for making the diagnosis. The latter are particularly useful in amicrofilaraemic cases. Spot tests for antigen are far more sensitive, and allow the test to be done any time, rather in the late hours. [7]

Lymph node aspirate and chylus fluid may also yield microfilariae. Medical imaging, such as CT or MRI, may reveal "filarial dance sign" in chylus fluid; X-ray tests can show calcified adult worms in lymphatics. The DEC provocation test is performed to obtain satisfying numbers of parasites in daytime samples. [6, 7]

A panoramic look up on this case can help you exclude most causes of lympoedema. The primary congenital type rarely presents late in the 3rd decade of life (lymphoedema tarda). [8] However, the normal lymph flow excludes this possibility. The long standing venous insufficiency was excluded by the Doppler study the possibility of cancer is excluded by absence of lymphadenopathy, however further investigations and follow up will be necessary later to exclude the possibility of hidden malignancy. The injury of the lymphatic vessels isn't supported by history of surgery or fractures in the affected limbs. Podoconisis is a type of lymphoedema that is caused by continuous

exposure to irritant soil in genetically predisposed individuals that occurs exclusively in the lower limbs. The mycetoma pedis is associated with multiple sinus formation on the skin of the affected limb with sulpher granules-like discharge, so it was excluded because of the healthy skin overlaying oedema. Leprosy is excluded because the sensations in the affected parts were preserved and the absence of the leprosy disfiguring lesions elsewhere.

The recommended treatment for filariasis is albendazole (a broad-spectrumthis patient is anthelmintic) combined with ivermectin. A combination of diethylcarbamazine and albendazole is also effective. All of these treatments are microfilaricides; they have no effect on the adult worms. [9]

In 2003, the common antibiotic doxycycline was suggested for treating elephantiasis. [10]Filarial parasites have symbiotic bacteria in the genus *Wolbachia*, which live inside the worm and seem to play a major role in both its reproduction and the development of the disease. Clinical trials in June 2005 by the Liverpool School of Tropical Medicine reported an eight-week course almost completely eliminated microfilaraemia. [11]

REFERENCES

1. The WHO Expert Committee on Filariasis: Lymphatic filariasis: The disease and its control. Fifth report *World Health Organization technical report series* 1992,821: 1–71.
2. Tretbar LL, Morgan CL, Lee BB, Blondeau B, Simonian SJ: Lymphedema: Diagnosis and Treatment. *Springer*. 2007, ISBN 1-84628-548-8.
3. Sowilem MM, Bahgat IM, el-Kady GA, el-Sawaf BM: Spectral and landscape characterization of filarious and non-filarious villages in Egypt. *J Egypt Soc Parasitol*.2006;36(2):373-88.
4. The Carter Center: Summary of the Third Meeting of the International Task Force for Disease Eradication, 5the crater center. 2002.
5. Ottesen EA, Hooper PJ, Bradley M, Biswas G: The Global Programme to Eliminate Lymphatic Filariasis: Health Impact after 8 Years, in De Silva, *Nilanthi*, *PLoS NTDs*. 2008(10): 317.
6. Centers for Disease Control and Prevention: Lymphatic Filariasis. CDC. 2008.
7. Hopkins DR: Disease Eradication. *N Engl J Med.* 2013 (368): 54–63
8. Brorson H, Ohlin K, Olsson G, Svensson B, Svensson H: Controlled Compression and Liposuction Treatment for Lower Extremity Lymphedema. *Lymphology*, 2008, 41: 52–63.
9. World Health Organization: Progress report 2000-2009 and strategic plan 2010-2020 of the global programme to eliminate lymphatic filariasis: halfway towards eliminating lymphatic filariasis, , 2010.
10. Hoerauf A, Mand S, Fischer K, Kruppa T, Marfo-Debrekyei Y, Debrah AY, et al: Doxycycline as a novel strategy against bancroftian filariasis-depletion of Wolbachia endosymbionts from Wuchereria bancrofti and stop of microfilaria production. *Med Microbiol Immunol* (Berl). 2003,192 (4): 211–6.
11. Taylor MJ, Makunde WH, McGarry HF, Turner JD, Mand S, Hoerauf A: Macrofilaricidal activity after doxycycline treatment of Wuchereria bancrofti: a double-blind, randomised placebo-controlled trial. *Lancet.* 2005,365(9477): 2116–21.

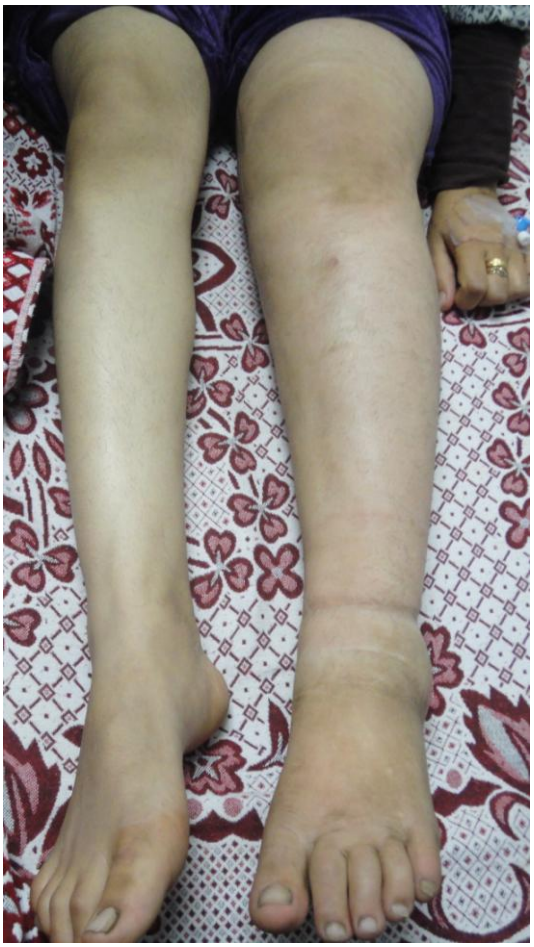

Figure 1: Asymmetrical swelling of the lower limbs (more swelling is noticed in the left side).