Journal Homepage: - www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI: 10.21474/IJAR01/3237
DOI URL: http://dx.doi.org/10.21474/IJAR01/3237

RESEARCH ARTICLE

Prevalence of hepatitis B and C among some high risk groups in Egyptian children attending Benha University hospital.

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Manuscript Info

Manuscript History

Received: 19 December 2016
Final Accepted: 15 January 2017
Published: February 2017

Key words:-
Egypt, children, HCV seropositivity, HCV prevalence, HBV seropositivity, HBV prevalence, real-time PCR, ELISA

Abstract

Background: Hepatitis C virus (HCV) is a global health problem especially in Egypt. Hepatitis B virus (HBV) is a major cause of chronic liver diseases especially cirrhosis and hepatocellular carcinoma. HBV incidence is markedly reduced after mass vaccination programs application.

Objectives: to detect HCV and HBV seroprevalence among some high risk children with molecular confirmation of viremia by real-time PCR for seropositive cases.

Materials and methods: A comparative cross sectional study was conducted on 5 groups of Egyptian children attending Benha University Hospital (diabetes mellitus, thalassemia, hemodialysis, previously exposed to surgery and healthy children). All children were subjected to full history taking, physical examination and laboratory investigations including HCV antibodies by 4th generation ELISA, HBsAg, HBsAb using ELISA, liver function tests and CBC, HCV and HBV viremia detection by real-time PCR for ELISA positive subjects.

Results: HCV seropositivity was detected in 5%, 15%, 30%, 50% and 10% for healthy, diabetic, thalassemia, hemodialysis and previously exposed to surgery, respectively. As regard HBV, only a hemodialysis case was positive for both HBsAg and HBV-DNA. Low social class, blood transfusion, frequent intravenous injection, previous surgery and previous hospitalization are major risk factors for HCV transmission.

Conclusion: The study revealed high HCV seropositivity prevalence among the studied high risk groups. PCR should be done for all HCV seropositive cases to confirm the presence of viremia.

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Introduction:-

Chronic viral hepatitis B (HBV) and C (HCV) infections represent a substantial healthcare burden worldwide with significant global morbidity and mortality; up to 1 million annual deaths are attributable their sequelae including cirrhosis and hepatocellular carcinoma (HCC) [1].

HCV infection has an overall prevalence of 1-3% with 3-4 million new cases every year [2] and 200 million carrier individuals worldwide [3]. Two billion persons have been infected with HBV worldwide and more than 350 million persons have chronic infections [4]. HBV infection and incidence has been markedly reduced after mass vaccination programs [5]. HCV seroprevalence in Egypt has been 14.7 in the year, 2008 [6].

HCV is mainly transmitted by parental route; however about 10% of cases are sporadic without well-defined transmission routes. HCV has rarely been transmitted by transfusion of blood products since the introduction of organ and blood donor screening by antibody testing in 1991 [7].

Intravenous drug users (IDUS) injections and shaving by community barbers have been major risk factors [8]. HCV is suggested to be associated with diabetes mellitus (DM) due to frequent injections [9].

In the last decade, HCV antibodies (HCV-Ab) prevalence in thalassemia varied among countries including Egypt from 12.5 to 100% [10]. HCV-Ab prevalence in Egypt among hemodialysis patients was 35% which may be due to shared dialysis machines, repeated blood transfusion, nosocomial routes, surgery, and multi-dose drug vials [11].

Major surgery also contributes to HCV transmission. HCV viremia prevalence in major surgery was reported to be 8% [12].

Objectives:-

Our study aimed to detect hepatitis C virus and hepatitis B virus seroprevalence with subsequent detection of HCV-RNA and HBV-DNA in seropositive cases among some high risk groups of Egyptian children.

Subject and method:-

This cross sectional study was conducted at the Pediatric Department of Benha University Hospital over the period from 2015 to 2016 upon 100 Egyptian children (6-17 years); 20 apparently healthy children, 20 diabetes mellitus (DM) children on insulin therapy, 20 thalassemia children on regular blood transfusion, 20 chronic renal failure children on regular hemodialysis and 20 children with previous surgery (P surgery).

Patients with clinically suspected or known chronic liver or metabolic diseases were excluded.

Ethical considerations:

A written informed consent was obtained from children' guardians before participation. Also, an approval from the Research Ethics Committee in Benha faculty of medicine was obtained. The study agreed with the Helsinki Declaration of 1975 that was revised in 2000.

All children were subjected to predesigned questionnaire including age, sex, residence and socioeconomic status using Fahmy and Elsherbini score [13] and exposure to some risk factors associated with HCV transmission, thorough clinical examination and laboratory investigations.

Sampling:

Venous blood sample (5ml) was collected from every subject. Two ml were put on EDTA of which 1ml was used for CBC, while the other ml was centrifuged to separate plasma for HBV-DNA virus load quantitation. Three ml blood were centrifuged to separate serum and was kept at (-20oC) until further biochemical investigations.

Laboratory investigations:

CBC was performed by Symex XS-800I cell counter [14]. Liver function tests including serum aspartate amino transferase (AST) [15]; serum alanine aminotransferase (ALT) [15] and total and direct bilirubin were done by biosystem A15 auto-analyzer [16]. HCV-Ab by Innotest HCV-Ab IV ELISA kit (Innogenettics Gmbh, Hanover, Germany) [17].

HBs-Ab by ETI-AB-AUK-3 ELISA kit (DiaSorin, Saluggia, Italy) [18] and HBs-Ag by SURASE B-96 (TMB) ELISA kit (Medical Technology Promedt Consulting Gmbh, Germany) [18] were also performed with absorbance reading on ELISA reader TECAN Infinite F50 (Singapore). According to international standards anti-HBs ≥10 IU/L, was considered protective against HBV infection [19]. Weak response to HBV vaccine after full vaccination dose was defined as 10-100 IU/L while strong response were at a level >100 IU/L [20].

Determination of HCV-RNA and HBV-DNA viral load was performed for ELISA positive cases only.

Determination of HCV-RNA viral load:
QIAamp Viral RNA mini Kits were used for viral RNA extraction from 145µl serum by Qiacube automatic extractor (Qiagen, Germany), then absolute reverse transcription (RT)-PCR quantitation using artus HCV RG RT-PCR Kit with 10µl RNA template (Qiagen, Germany) following the manufacturers' instructions. A standard curve was generated using 4 quantitation standards (QSs) on StepOne real-time PCR system (Applied Biosystems, USA).

Determination of HBV-DNA viral load:
Viral DNA extraction from 200µl plasma by Qiacube automatic extractor using QIAamp DSP virus kit (Qiagen, Germany), then quantitative PCR was performed using Artus HBV PCR kit (Qiagen, Germany) following the manufacturers' instructions on StepOne real-time PCR system using 20µl DNA template. A standard curve was generated using 5 QSs on StepOne real-time PCR system (Applied Biosystems, USA). QSs are defined as IU/µl. Result (IU/ml) = result (IU/µl) x elution volume (µl) / sample volume (ml) for either HCV- or HBV-viral load.

Statistical analysis:-
Collected data were tabulated and analyzed using SPSS version 20 software (SPSS Inc; Chicago; ILL Company). Quantitative data were expressed as mean±standard while categorical data were presented as number and percentage. The difference between two means was statistically analyzed using the student t test. Chi square and Z tests were used as tests of proportion. Each high risk group data were compared to that of the healthy group as a reference group. p value<0.05 was significant.

Results:-

This study included 100 children; 22% of them were HCV seropositive, 51% were male, 50% were below 12 years, 77% were from rural residents and 29% were of low social class.

Table (1) showed non-significant difference between seropositive and seronegative HCV children regarding sex and residence. The majority of seropositive children (81.8%) were of age group (12-17y) versus 41% of the seronegative. Most seropositive HCV children (63.6%) were of low social class while only 13.6% were of high social class versus 19.2% and 28.2% respectively of the seronegatives. These differences were statistically significant (p<0.01 for both).

Risk factors like blood transfusion, frequent IV injection, previous surgery, previous hospitalization, ear piercing, community barber shaving, exposure to blood (p<0.01 for all) and birth attendant's delivery (p<0.05) were statistically significantly higher among seropositive HCV children compared to the seronegatives.

Laboratory investigations; serum AST and ALT were significantly increased among seropositive HCV patients but insignificant other tests (hemoglobin, leucocytes, platelets, total bilirubin and direct bilirubin) compared to the seronegatives.

Table (2) showed non-significant difference as regard sex and residence in the studied groups but significant increased age in hemodialysis and social class in the previous surgery group compared to the healthy children (p<0.05).

Risk factors; frequent IV injection and previous hospitalization and abdominal pain complaint described statistical increase in DM compared to controls (p<0.01 for both).

As regard thalassemia and hemodialysis children; risk factors like blood transfusion, frequent IV injection, previous surgery, previous hospitalization, exposure to blood and complaining of abdominal pain, dark urine, yellow sclera and easy fatigue described statistical increase compared to controls (p<0.01 for all).

As regard the previous surgery group; frequent IV injection, previous surgery, previous hospitalization and abdominal pain complaint showed high statistical increases ($p<0.01$ for all) and statistical increase in HCV family history ($p<0.05$) compared to controls.

HCV seropositivity prevalence was 15%, 30%, 50% and 10% and HCV viremia was 5%, 25%, 50% and 10% for DM, thalassemia, hemodialysis and previous surgery groups respectively compared to 5% in the controls with statistically significant increase in thalassemia and hemodialysis ($p<0.01$ for both).

Only one hemodialysis case was positive for HBs-Ag and HBV-DNA. As regard the child immune state against HBV immunization (HBs-Ab), the negative immune state children were 48%, the weak responders were 25% and the strong responders were 27%. There was non-significant difference in the high risk studied groups compared to the controls.

Discussion:-

HCV infection is a serious medical challenge that is complicated by severe liver disease, including fibrosis, cirrhosis, and HCC [21]. Egypt is considered one of the highest HCV prevalence [4]. HBV is a main reason of chronic liver disease; especially cirrhosis and HCC [5].

Our study revealed that HCV prevalence among controls was 5% that agreed with Barakat and Elbashir, who reported 5.8% for HCV seroprevalence in healthy children, with 4.4% HCV viraemia [22]. Our study showed increased HCV seropositivity among males (54.5%), rural areas (90.9%) and low class (63.6%), this agreed with Mohamoud et al., who reported higher HCV prevalence in males [23], also in agreement with Mostafa et al., who demonstrated higher prevalence in rural areas [24].

In this study, blood transfusion was found in 50% of seropositive children. The Egypt Demographic and Health Survey (EDHS) in 2008 estimated that blood transfusion was identified in 24.3% of the HCV positive cases in a nationwide sample [6], a result that agreed with our study.

In many countries the main drive of HCV incidence and prevalence is intravenous drug using [25]. Surgical including dental procedures were statistically highly significant in this study. HCV transmission in Egypt is primarily associated with inadequate infection control during dental and medical care procedures [26]. Kalil et al. stated that various medical procedures even if minor contribute to HCV susceptibility [27]. Barakat and Elbashir reported that history of previous blood transfusion, circumcision for boys by informal health care providers, surgical intervention, and dental treatment are the most significant risk factors for HCV infection [22].

As regard diabetic group our study reported that, the prevalence of anti-HCV positivity was 15%. El-Karaksy et al. reported a lower rate of anti-HCV of 3.6% among diabetic children attending Cairo Children University Hospital in Egypt [28]. In this study there was highly significant increase in both IV injection and previous hospitalization in DM compared to the healthy. Children with T1DM are usually hospitalized for either diet education (a week) or monitoring and diabetic control (several weeks) they perform self-monitoring of blood glucose by finger puncture many times daily. Particular HCV risk factors in DM include using shared spring-triggered finger-stick device for blood glucose monitoring or contaminated multi-dose insulin vials with the risk of patient to patient transmission [29].

As regard thalassemia in the present study, HCV seropositivity prevalence was 30% similar to a study by Din et al. in which HCV prevalence in thalassemia was strikingly increased as up to 49% [30], and was also similar to another study done in Iran which reported 15.7% to 63.8% for HCV prevalence in thalassemia [31]. All thalassemia patients in this study were subjected to common risk factors such as blood transfusion, exposure to previous surgery, frequent IV injections and previous hospitalization. These risk factors for HCV transmission in thalassemia were highly significant compared to the healthy. Both seropositive and seronegative patients of this group received blood transfusion so we couldn't confirm that blood transfusion was the main risk factor for HCV transmission in thalassemia especially after implementation of blood donors screening, this agrees with a study by Alavian et al. which reported that the risk of HCV transmission has decreased significantly after the introduction of routine anti-HCV screening of blood donors in developed countries and that the main risk factor for acquiring HCV infection before that was blood transfusion [32].

Another study reported that HCV is transmitted primarily by direct contact with infected blood and transfusion of blood products and intravenous and percutaneous drug use [33]. Nemati et al. reported that shaving by community barbers, unsafe injections by health care providers, tattooing and ear piercing, known to be associated with HCV infection [34], but in our study all except IV injection were non statistically significant.

In the current study, HCV seropositivity in hemodialysis was 50%, a similar result was reported by Abed et al. [35] and was nearer to that reported by the Egyptian Renal Registry which reported 49% to 64% for HCV prevalence in Egypt [36]. Bastiani et al. reported 10.17% for HCV prevalence in dialysis which was lower than our result [37]. This study reported that blood transfusions, intravenous injection, previous surgery, previous hospitalization, exposure to blood are significant risk factor for HCV transmission in hemodialysis compared to the healthy. In contrast, another study by Zhao et al. reported no evidence of patient-to-patient HCV transmission in their hemodialysis centers [38]. Halle et al. evidenced that HCV seroprevalence rate in hemodialysis was 11.8% and it was associated with longer duration on dialysis [39].

As regard children previously exposed to surgery in this study, HCV seropositivity rate was 10% and prior hospitalization, prior surgery and IV injection were statistically highly significant compared to the healthy. This agreed with Masood et al. who studied 387 patients admitted for elective surgery. After screening they found that 6% of patients enrolled in their study were positive for both HBV and HCV. HBsAg was positive in 6.5% of patients while 11.3% were positive for HCV. They found that the reuse of contaminated syringes, contaminated surgical instruments and blood products were risk factors. They concluded that HBsAg and anti-HCV prevalence in hospitalized surgical patients was very high. They suggested routine preoperative screening for HBV and HCV [40]. Chaudhry et al. also conducted another study among patients reporting in surgical outpatient department of Fauji Foundation Hospital Rawalpindi during 2006, they screened 2056 patients and found 2.8% and 7.56% for HBV and HCV seropositivity respectively with male predominance in both the groups [41].

As regard prevalence of viremia in seropositive cases, the present study reported 100% for the healthy, 33.3% in diabetes, 83.3% in thalassemia, 100% in dialysis and 100% in the previously exposed to surgery. El-Karaksy et al. reported rates of HCV-RNA positivity by PCR among children with positive HCV-Ab by ELISA as 40% [28]. The decreased frequency of PCR positivity than that of seropositivity by ELISA could be attributed to the following: HCV-Ab positive subjects in absence of HCV-RNA positivity could be attributed to either HCV infection clearance while the patient remains HCV-Ab positive or a false positive ELISA test [42]. HCV-RNA presence in serum is a dependable indicator of ongoing viral reproduction and infectivity where follow-up of infected cases is necessary [43].

RT-PCR is the gold standard technique for the diagnosis of HCV infection, allowing serum HCV-RNA determination; however, obstacles such as technical difficulties, expenses and unavailability may prevent it from being used as a screening test on a large scale of patients on a regular basis [44].

As regard the laboratory investigations among seropositive and seronegative patients in our study there was no significant difference as regard to CBC but there was significant increase in liver enzymes among HCV seropositive than seronegative children. Our result agreed with Bhattacharya et al. who found that HCV can cause asymptomatic infection [45]. Persistently elevated ALT levels were recorded in several Egyptian pediatric and adult studies and consequently, HCV infection is not always benign in Egyptian children [46].

Regarding HBV seropositivity, only one hemodialysis child was positive for HBs-Ag and HBV-DNA viral load. HBV infection has been markedly reduced after mass vaccination programs [5]. In our study, negative HBs-Ab titre group (seroprotected) represented 48%; higher than that of Eldesoky et al. which was 40.5% of the healthy vaccinated children in the age group from 3 to13 years [47], but similar to the result of Khashaba et al., that was 46.2% among 91 screened preschool children [48]. Meanwhile positive HBs-Ab titre group represented 52% which were near the results of Jafarzadeh and montazerifar which reported that 47.9% of Iranian children had protective level of HBs-Ab ≥10mIU/ml at 10 years interval after primary vaccination [49] and that of Khashaba et al. who reported that 53.8% were seroprotective [48]. HBV infection has been documented in hemodialysis patients who have not maintained anti-HBs concentrations >10mIU/ml [50].

Table 1: Socio-demographic data, risk factors and laboratory investigations of the studied children as regard HCV seropositivity.

Variables	Seropositive HCV (n.=22)	Seronegative HCV (n.=78)	Test	p
	n.(%) or mean±SD			
Sex (♂/♀)	12(54.5)/10(45.5)	39(50.0)/39(50.0)	0.14$	0.7
Age (≤12/>12) year	4(18.2)/18(81.8)	46(59.0)/32(41.0)	11.42$	0.001**
Residence (Urban/Rural)	2(9.1)/20(90.9)	21(26.9)/57(73.1)	3.08$	0.07
Social class (H/M/L)	3(13.6)/5(22.7)/14(63.6)	22(28.2)/41(52.6)/15(19.2)	16.45$	0.001**
Blood transfusion (35)	11(50)	24(30.8)	2.37∞	0.008**
Frequency of IV injection (84)	21(95.5)	63(80.8)	5.29∞	0.001**
Prior surgical procedure (42)	10(45.5)	32(41)	3.99∞	0.001**
Prior hospitalization (80)	21(95.5)	59(75.6)	4.83∞	0.001**
Non-medical circumcision (21)	9(40.9)	12(15.4)	0.66∞	0.254
Ear piercing (49)	10(45.5)	39(50)	5.14∞	0.001**
Family history of HCV (11)	0(0)	11(14.1)	-	-
Common barber Shaving (50)	12(54.5)	38(48.7)	4.31∞	0.001**
Exposure to blood (33)	10(45.5)	2(2.6)	3.1∞	0.001**
Birth attendants delivery (34)	12(54.5)	22(28.2)	1.8∞	0.036*
History of schistosomiasis (1)	1(4.5)	0(0)	-	-
Hemoglobin (g/dl)	11.9±3.1	11.1±2.1	1.26#	0.21
Red blood cells ($x10^6$/ml)	4.29±0.96	4.35±0.62	0.24#	0.8
Leucocytes ($x10^3$/ml)	5.6±1.72	6±1.7	0.9#	0.36
Platelets ($x10^3$/ml)	199.5±75.8	248.3±113.3	1.71#	0.09
Serum AST (IU/ml)	50.2±25.1	35±20.2	2.3#	0.02*
Serum ALT (IU/ml)	43.6±24.5	26.7±13.6	3.0#	0.004**
Total bilirubin (mg/dl)	0.96±0.2	1±0.3	0.4#	0.64
Direct bilirubin (mg/dl)	0.4±0.3	0.37±0.3	0.46#	0.64

H: high, M: middle, L: low, IV: intravenous, HCV: hepatitis C virus, AST: Aspartate Aminotransferase, ALT: Alanine aminotransferase, $: Chi square test, #: student test, ∞: Z test, *: significant, **: high significant,

Table 2: Socio-demographic data, risk factors and laboratory investigations of high risk groups compared to healthy.

Variables	Healthy(n.=20)	DM(n.=20)	Thalassemia(n.=20)	Hemodialysis(n.=20)	P surgery(n.=20)
Sex (♂/♀)$	11(55)/9(45)	12(60)/8(40)	11(55)/9(45)	9(45)/11(55)	8(40)/12(60)
Age Y(mean±SD)#	11.3±3.6	11.2±3.6	11.3±3.9	14.8±3**	12.4±3.2
Age Y (≤12/>12)$	12(60)/8(40)	13(65)/7(35)	10(50)/10(50)	4(20)/16(80)	11(55)/9(45)
Urban/Rural$	3(15)/17(85)	5(25)/12(75)	6(30)/14(70)	2(10)/18(90)	7(35)/13(65)
S.Class(H/M/L)$	3(15)/13(65)/4(20)	4(20)/13(65)/3(15)	3(15)/8(40)/9(45)	3(15)/6(30)/11(55)	12(60)/6(30)/2(10)*
Blood transfusion$	0(0)	1(5)	20(100)**	13(65)**	1(5)
IV injection$	7(35)	18(90)**	20(100)**	19(95)**	20(100)**
Previous surgery$	0(0)	2(10)	6(30)**	14(70)**	20(100)**
P hospitalization$	3(15)	17(85)**	20(100)**	20(100)**	20(100)**
NM Circumcision$	5(33.3)	2(11.1)	2(11.1)	11(55)	1(5)
Ear piercing$	9(45)	8(40)	9(45)	11(55)	12(60)
HCV F History$	0(0)	3(15)	2(10)	1(5)	5(25)*
C barber Shaving$	11(55)	11(55)	11(55)	9(45)	8(40)
Common Razors$	1(5)	0(0)	0(0)	1(5)	0(0)
Exposure to blood$	0(0)	1(5)	20(100)**	12(60)**	0(0)
Birth attendants D$	10(50)	3(15)	4(20)	12(60)	5(25)
Schistosomiasis$	0(0)	0(0)	1(5)	0(0)	0(0)
Diarrhea$	9(45)	10(50)	5(25)	9(45)	8(40)
Abdominal pain$	11(55)	19(95)**	18(90)**	17(85)**	17(85)**
Dark urine$	0(0)	0(0)	8(40)**	8(40)**	0(0)
Easy fatigue$	8(40)	17(85)	20(100)**	20(100)**	9(45)
Jaundice$	0(0)	1(5)	8(40)**	8(40)**	0(0)
Poor G health$	5(25)	13(65)	20(100)**	19(95)**	4(20)
School absence$	0(0)	3(15)	18(90)**	19(95)**	0(0)
HCV-Ab (P/N)$	1(5)/19(95)	3(15)/17(85)	6(30)/14(70)**	10(50)/10(50)**	2(10)/18(90)
HCV-RNA in SPC	100%	33.3%	83.3%	100%	100%
HBs-Ag (P/N)$	0(0)/20(100)	0(0)/20(100)	0(0)/20(100)	1(5)/19(95)	0(0)/20(100)
HB-DNA in SPC	-	-	-	100%	-
HBs-Ab (H/W/N)$	6(30)/4(20)/10(50)	5(25)/5(25)/10(50)	5(25)/6(30)/9(45)	4(20)/4(20)/12(60)	7(35)/6(30)/7(35)

DM: diabetes mellitus, P: previous, Y: year, H: high, M: middle, L: low, IV: intravenous, P: previous, S: Social, NM: Non-medical, F: family, C: common, D: delivery, G: general, HCV: hepatitis C virus, RNA: ribonucleic acid, HB: hepatitis B, s: surface, Ag: antigen, SPC: seropositive cases, Ab: antibody, P: positive, N: negative, W: weak, $^{\$}$: chi square test, $^{\#}$: student t test, *: significant, **: high significant.

Conclusion:-

The present study revealed that the HCV seropositivity prevalence in apparently healthy children was 5% and a significant high prevalence of HCV seropositivity in thalassemic children (30%) and hemodialysis children (50%) was reported. HCV seroprevalence in diabetic children was 15% and in children previously exposed to surgery was 90%. Risk factors as blood transfusion frequent IV injections, previous hospitalization and exposure to blood were the most common for HCV transmission. PCR should be done for all HCV seropositive cases to confirm viremia.

Recommendations:-

There must be greater efforts for prevention of hepatitis B and C in Egypt including:

1- Strict emphasis on infection control measures in hemodialysis and hematology units.
2-Routine investigations every 6 months for early detection and treatment of infected persons
3-All children must be vaccinated against hepatitis B with confirmation on booster doses.
4- Molecular screening for blood donors must be done.
5- Confirmation on having personal instruments for each individual.
6- Replacement of injections with oral medications if possible.

ACKNOWLEDGMENT:-

Great thanks to all included children and their guardians for participation in the current study and many thanks to the Molecular Biology Unit, Faculty of Medicine for technical support.

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