



THE STUDY OF RISK FACTORS OF HEPATITIS B INFECTIONS AMONG PATIENTS WHO INFECTED WITH RENAL FAILURE IN DIYALA CITY, IRAQ

Biological Science

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ABSTRACT

Background : The infection of Hepatitis B virus is mutual between patients of dialysis. The dangerous factors and local propagation of hepatitis disease are different from region to another. This search is to specifying of propagation of HBV infection and its related to dangerous factors between hemodialysis patients in the center of hemodialysis in diyala city.

Material and methods: The patient's notes on going hemodialysis were checked when the researchers scanned the patients collection of data whereby serology case of these patients and dangerous factors which could be associated with hepatitis B virus and hepatitis C virus infection. Hepatitis B mean inflammation of the liver that caused by Hepatitis B virus (HBV) which is DNA virus that corrupts people and some kinds of animals such as chimpanzees and birds.

Results : Total 100 patients were attended the hem dialysis unit. Out of this 60 were males and 40 were females (60%,40%) , most of the patients were lie between 10 to 60 years of age. One hundred patients who infected with HBV virus fifty-five of them gave positive results using ELISA test , and forty-five gave negative results. Ten of them gave positive result using rt-PCR only . HBV DNA virus was checked in 10 samples that were positive too for HBsAg 8 of them were females and 2 were a male . while the remaining samples were negative.

Conclusion: vaccination of Hepatitis B is pivotal significance in averting the infection of hepatitis B in hemodialysis patients. Harsh adherence to infection control guidelines is primary to reduction the rate of infection of hepatitis B.

KEYWORDS

Hepatitis B Virus , Hemodialysis, Pcr, Renal Failure

Introduction

Hepatitis B is a disease caused by Hepatitis B Virus (HBV). Hepatitis B virus considered as the main of disease and a dangerous health problem.

Hepatitis B virus (HBV) is a member of *Hepadnaviridae* and a main causative agent of chronic and acute hepatitis, liver cirrhosis and hepatocellular carcinoma (Neisi *et al.*,2011).

Hepatitis B virus may be revealed in blood as well as in saliva ,semen ,vaginal secretion and exudates from coriaceous ulcers. also diagnosis of Chronic hepatitis B is based on the existing hepatitis B surface antigen (HBsAg) and HBV DNA in blood. HBV infection is less common than hepatitis C virus (HCV) in dialysis units (Amen *et al.*,2013). Hemodialysis is the major renal commute treatment in patients who suffering from final-stage kidney disease. .furthermore, patients who suffer from hemodialysis have low level of immunity that inspire them more vulnerable to procure like viruses and have lower rate of response to hepatitis B vaccine (AlSaran *et al.*,2014) The increased risk for hepatitis may be explained by the exposure to viruses from other patients, by the use of mutual equipment and by the management of multiple blood transfusions before the obtainable of recombinant human erythropoietin. Insutiable infection control methods in the hemodialysis units and the repressed immunity of patients may also increase the risk both HBV and HCV synergize in precipitation the advancement of disease to hepatic anomalies, including cirrhosis and cancer. (Tajbaksh,2015).

Numeral of risk factors are embroiled including blood transfusion, period of dialysis, dialysis instrument sterilization and preparation and the use of common drug carts. Studies deduce that the transmission of virus to anemic and repeated blood transfusion, and invasive execution more than the general population which as well put them at higher risk of being infected with these viruses (Kosaraju *et al.*,2013)

HBV infection is less prevalent than HCV in hemodialysis units. The introduction of HBV vaccination, isolation of HBV positive patients, use of devotion dialysis machines and systemic surveillance for HBV infection has dramatically diminutive the spread of HBV in this setting. There are very few survey on the prevalence of such paired infections in hemodialysis patients from this region. subsequently , the

current study was undertaken to evolution the prevalence of HBV and HCV co-infection among hemodialysis patients. (Malhotra *et al.*,2016). End-stage renal disease (ESRD) is a presumed problem in almost all countries and the prevalence has increased deem in developing countries. For example, in Iran its prevalence has increased from 238 per million population in 2000 to 357 per million in 2006 (Mahdavi,2008), with about fifty percent of these patients undergoing hemodialysis. Among the hemodialysis group, regardless of location, HBV infection is a considerable co-morbid event sometimes leading to outbreaks of hepatitis B (Reddy,2005) Hepatitis B virus Quantification DNA perform a significant role in the treatment of chronic HBV infections. HBV have high levels of genetic diversity , and antibiotic-resistant mutations have protrude by using of antiviral drugs. To determine if commercial kits, can be used only one pair of primers and a single probe.

Materials and methods

This study was carried out from September to October 2017 in hemodialysis unit in Baquba hospital in Diyala city, include 100 patients and recorded the age of patients sex and period of time on hemodialysis. Ten ml of blood was taken from the patients and placed in plane blood tubes, it saved to stand at temperature lie between (20-25c^o), that lead to allow clot formation, later the sera were discrete by centrifugation at 3000 rpm for 15 minutes, and splitted into aliquots (250 µl) after that they stored at (-20c^o)until examination . Each part of the serum used at least one time to preclude thawing and freezing. before use in the test the all sera and reagent were kept to stand at room temperature. Serological markers for HBV were determined with (ELISA) , and used Polymerase Chain Reaction (PCR).

Polymerase Chain Reaction (PCR).

PCR test demonstrated highly sensitive in detecting HBV DNA. The PCR was implemented by dealing the DNA extraction of plasma together with primers that are specific for the HBsAg gene of HBV The primers applied consisted of forward primer 5'- TCGGAAATACA CCTCCTTCCATGG3' (HBV genome1353-1377) and reverse primer, 3'GCCTCAAGGTCGGTCGTTGACA-5' (HBV genome1 702-1681). This reaction was implemented with 20 µl volume by using Solis Bio dyne master mix. This volume contained: 4 µl master mix, 1 µl forward primer, 1 µl reverse primer, 5 µl extracted DNA and 9 µl distilled water.

magnification the DNA by thermo-cycling conditions using PCR machine Techno (Japan) as follow: first denaturation at temperature 94°C for 5 min, followed up by recycling 35 cycles of denaturation at 94°C for 1 min, annealing at 62°C for 1 min and extension at 72°C for 1 min, with a final extension 72°C for 7 min.

Serology

For detecting HBsAg and HB core antibodies (precek Bio, Inc, USA) using Commercial ELISA kits (Biorex, United Kingdom). ELISA is a type of solid phase immunoassay in which antigens or antibodies are covalently bound by enzymes and spur the diversion of a substrate into chromatic manufacture. This is a more effectiveness method to examine various serological markers. In this method employ novel monoclonal antibodies as seize stratum with a polyclonal biotinylated antibody as reveal juncture to promote new system of ELISA. Susceptibility of the detection were 98.98% and 99.6% of them when contrast to confirmed mercantile kit. The execution of ELISA test may be depends on coating antibody concentration, conjugates and sera. And using different concentrations by the checkerboard titration method (Fatema *et al.*,2013)

All patients were affected with kidney failure. Total 100 patients were attended the hem dialysis unit. Out of this 60 were males and 40 were females (60%,40%) , most of the patients were lie between 10 to 60 years of age . The results revealed that the number of patient's serum infected with HBV was 55 % by using the techniques Enzyme Linked Immuno Sorbent Assay. The patients had markers named HBsAg with percentages 55% . One hundred patients infected with HBV fifty-five of them with positive results by using ELISA techniques, and forty-five gave negative results table (1)

Table (1) Frequency of HBs Ag in haemodialysis patient in diyala hospital

Gender	Elisa HBs Ag		
	Positive %	Negative %	Total %
male	30	25	55
Female	25	20	45
Total	55	45	100

Ten of them gave positive result using rt-PCR only . HBV DNA was checked with 10 samples that also positive for HBsAg of which 8were males and 2was a females .while the rest were negative. Figure (1).

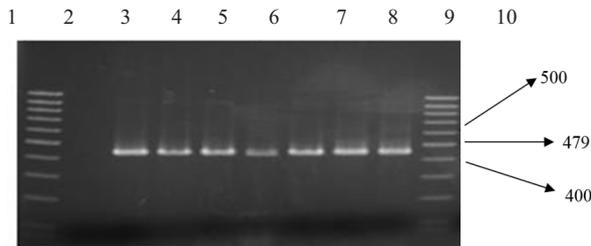


Fig. 1. PCR pattern of HBV positive patients; 1 and 10 Ladder; 2. Negative control; 3. Positive control; 4-9. Patient samples

hepatitis B infection have two risk factors may be associated with this disease . Dialysis that outside the special unit carries a high risk for hepatitis B infection .The result of present study explained that there was no any HBV positive hemodialysis patients less than 10 years. A statistically indication relationship was found among HBV disease and the patient's age, in which patients were older than 50 years old found more volatile to HBV than less 50 years because, a partial immunosuppressant in hemodialysis patients resulting in a poor antibody response to Hepatitis viruses' infection . long with other immune reactions, HBsAb can enucleate the invading HBV from the body. Either HBV vaccinations can cause HBsAb marker to be positive. This antibody can exit in the blood for a long time and progressively decreases with age. negative for HBsAg must be tested in molecular ways such as real – time PCR to know if the patients enter in recovery phase and HBsAg level declined or disappeared from the serum The patients who gave positive results for HBsAb and Enzyme Linked Immuno Sorbent Assay technique depend on the presence of HBsAg in the serum samples, if HBsAg level was high at that time gave positive results but, if HBsAg level was very low at that time gave negative results therefore, we used PCR which is very sensitive technique and can detect few numbers of particles /ml. patients who

gave positive results for HBV must be tested to detect the presence of HBV markers to identify and know the stage of HBV infection,

Discussion

This research reverberate the propagation of hepatitis B include ELISA and PCR techniques to specify HBV values in hemodialysis unit in Baquba hospital in Diyala city . significance for existence of viral infection among Iraqi blood donors including males and females. Meantime, comparison the effectiveness of ELISA technique as replacement. This study assured that the propagation of HBV disease in Diyala city which assented when 100 sample collected from malse and females society (blood donors with suspicious patient belonging to the central blood bank . Recently, HBV infection has truly become a problem which we still face. In Diyala city, the number of patients has become noticeably medium. Worth noting is that all cases were detected accidentally during blood transfusion or other undirected tests, noticeably at Oncology unit, Hemodialysis unit, Central blood bank and Public health laboratory in Diyala city .It is evaluated that 350 million HBV carriers in the world and more than this numbers of patients (Lavanchy *et al.*,2004) .The prevalence of HBV carriers in the general population varies from less than 2% in low-prevalence areas to 8% in high-prevalence areas (Lok and McMahon,2007).

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