CHAPTER ONE

INTRODUCTION

1.1. Introduction

Hepatitis E virus (HEV) a spherical, non-enveloped, single-stranded RNA virus is the only member of the genus *Hepevirus* belonging to the family of *Hepeviridae*. It represents the major aetiological agent of enteric non-A viral hepatitis. It is often responsible of acute clinical hepatitis in developing world, specifically the Indian subcontinent, Southeast Asia, the Middle East and North Africa, where it is a common cause of sporadic and outbreaks with high morbidity and mortality rate 15%-20% among pregnant women (Gaetano and Vicenzina, 2013).

Hepatitis E virus (HEV) has been recognized since 2004 as a transfusion-transmissible infectious agent (Juhl, 2014) and has recently been reported in a number of countries whereas the increased HEV incidence raises concern about the safety of blood and blood products. Patients commonly receiving multiple blood transfusions are at risk of fulminant hepatitis or chronic rapidly progressive liver disease from an HEV infection (Kaufmann *et al.*, 2011; Alaaeldeen and Yousif, 2015).

It has been reported that a substantial proportion of blood donors (1.5%) were positive for HEV RNA and are potentially able to cause transfusion-associated hepatitis E in areas of high endemicity. Studies conducted in the Middle East showed that blood transfusion increased the risk of hepatitis E infection compared with hospital controls (Kaufmann *et al.*, 2011).

In the United Arab Emirates (UAE), the hazards based on blood-borne viruses are categorized within mandatory testing for human immunodeficiency virus (HIV), hepatitis A, B and C viruses, Cytomegalovirus and human T cell leukemia virus (HTLV).

The aim of this study was to determine the seroprevalence of Hepatitis E virus (HEV) among blood donors in SKMC Hospital-UAE in light of raised concerns regarding safety of donated blood and blood products, and to identify demographic risks associated with transfusion-transmitted hepatitis E virus.

1.2. Rationale

The reported studies of HEV transmission via blood transfusion and associated clinical complications have emphasized the importance of studies on hepatitis E virus among blood donors.

- Hepatitis E virus (HEV) testing is not a routine test in blood banks in most parts of the world including the UAE. This study provides data on the magnitude of HEV prevalence among blood donors and possible transfusion associated risk.
- Current data on the magnitude of HEV among blood donors is limited.
- Blood donors in the UAE are demographically diverse representing regions in developing world where hepatitis E virus is endemic.
- The study defines the adoption of screening strategies appropriate to the needs, infrastructure and resources of the country.
- The study helps define a possible route of transmission of sporadic HEV infection in the country.

1.3. Objectives:

1.3.1. General Objective

To determine seroprevalence of hepatitis E virus among blood donors in SKMC Hospital-Abu Dhabi, United Arab Emirates.

1.3.2. Specific Objectives

1.3.2.1. To detect presence of antibodies to hepatitis E virus (anti-HEV IgM, IgG and IgA) among donors blood samples in SKMC hospital-UAE.

1.3.2.2. To estimate the rate of Hepatitis E virus seroprevalence among blood donors in SKMC hospital-UAE.

1.3.2.3. To determine demographical potential risks correlated with hepatitis E virus prevalence among blood donors.

CHAPTER TWO

LITERATURE REVIEW

2.1. HISTORY

An enterically transmitted non-A non-B hepatitis (NANBH) virus was first suspected by Khuroo in 1980, during an outbreak of acute viral hepatitis in Kashmir, India. A few months later, he reported the results of the retrospective serological testing of stored sera that had been stored since a widespread epidemic of NANBH in New Delhi in 1955-1956 as a result of fecal contamination of drinking water. More than 29,000 people were infected, that is 2.3% of the population in the affected areas with high incidence among young adults. The peculiarities of this form of hepatitis were its brief prodromal period and the high frequency of fulminant hepatic failure in pregnant women with a high mortality rate. The name "enterically transmitted NANBH virus" was coined. HEV has been first discovered during the Soviet occupation of Afghanistan in the 1980s after an outbreak of unexplained hepatitis at a military camp. Nearly a decade after its initial discovery, Reyes *et al*., (1990) isolated a complementary DNA representing a part of the genome of the virus responsible for enterically transmitted NANBH from bile obtained from an experimentally-infected animal. They also identified similar genomic sequences in clinical specimens obtained from several geographical regions at different time-points. The molecular cloning and sequencing of the entire genome of the virus soon followed in 1991 (Marano *et al*., 2015).

Currently, HEV is the most common cause of acute viral hepatitis globally with an

estimated 20 million HEV infections annually, resulting in 3.4 million cases of acute hepatitis and 70,000 deaths (Rein *et al.*, 2012).

2.2. Classification and Morphology

HEV is currently placed in genus *Hepevirus*, and is the only member of family *Hepeviridae*. HEV is a spherical, non-enveloped virus of about 27–34 nm and have prominent protrusions on their surface. Electron microscopy (EM) analyses (Figure-1) shows spherical particles of possible icosahedral symmetry, with indefinite surface substructure, resembling the Caliciviruses. Morphologically, HEV is similar to Norwalk virus, a member of the calicivirus family, although the sequence of HEV most closely resembles the sequence of rubella virus, a Togavirus (Pischke *et al*., 1996).

HEV isolates are classified into 4 major genotypes which belong to one serotype. Genotypes 1 and 2 infect humans and are often associated with outbreaks and epidemics in developing countries. Genotypes 3 and 4 infect humans, pigs and other animal species and have been responsible for sporadic cases of disease (Aggarwal, 2010).

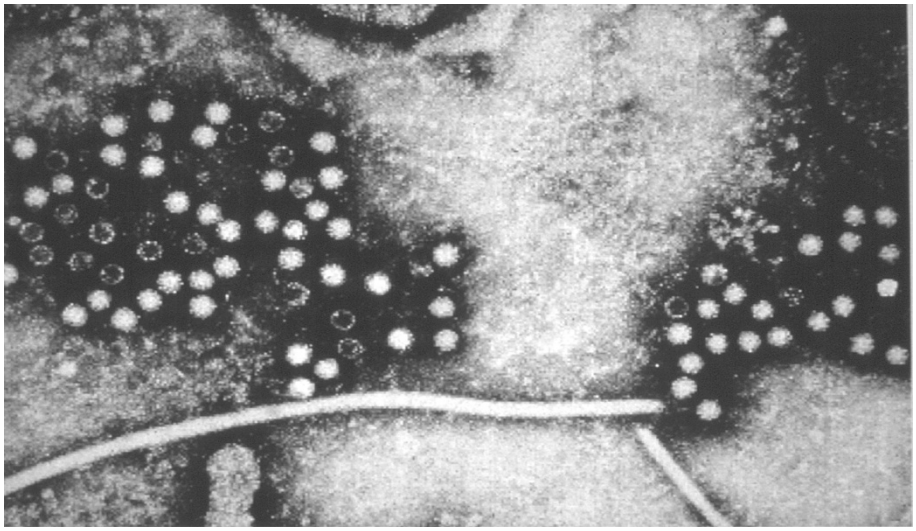

Figure.1. Electron micrograph of hepatitis E virus particles (*CDC public image library*)

2.3. Genome Organization

The viral genome (Figure 2) consists of a single-stranded positive-sense RNA molecule organized into three discontinuous and partially overlapping open reading frames (ORF1, ORF2 and ORF3) flanked by short 5' and 3' untranslated non-coding regions (UTRs). The largest open reading frame known as ORF1 is involved in viral replication and coding for non structural protein processing through RNA-dependent RNA polymerase. ORF2 encodes the viral capsid protein, which is involved in attachment to host cells and induction of neutralizing antibodies. Finally, ORF3 encodes for a small immunogenic phosphorylated protein involved in virion morphogenesis and release (Guu *et al*., 2009).

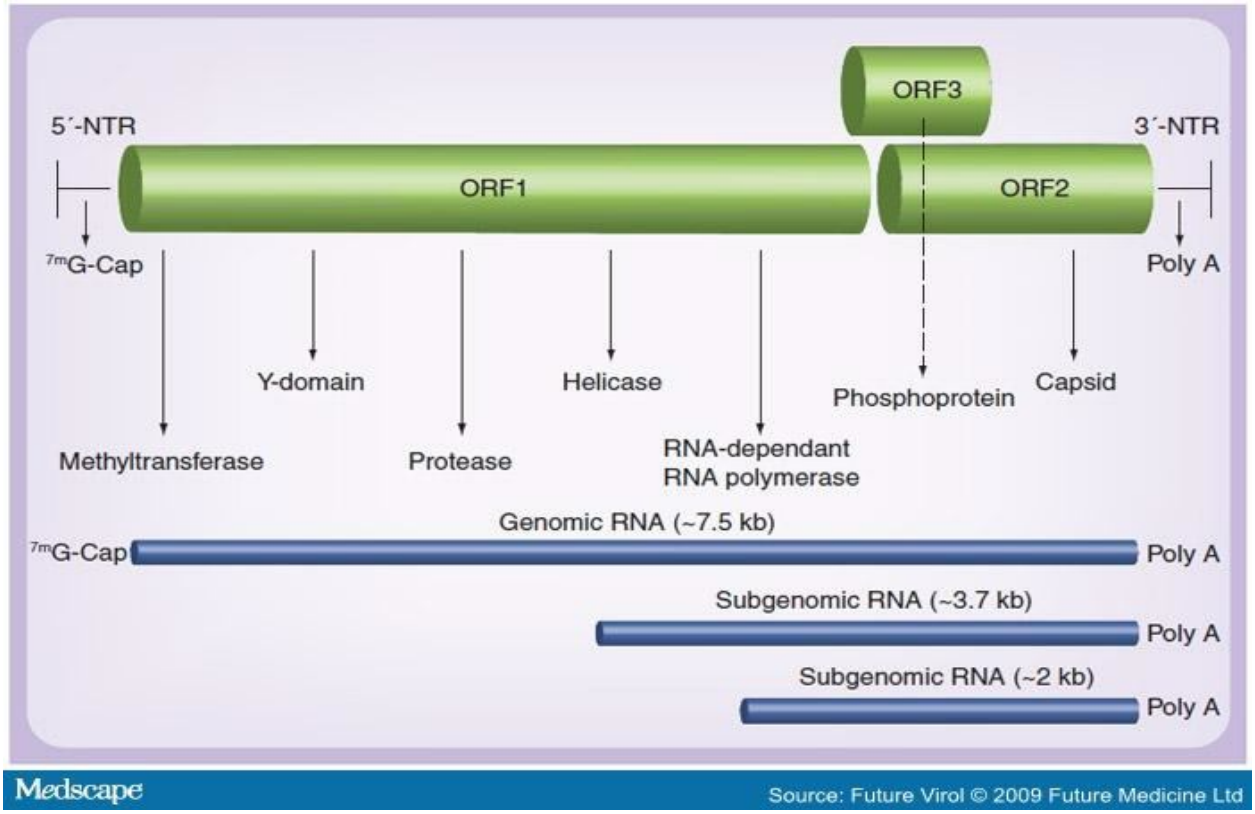

Figure.2. The structure of the hepatitis E virus genome. RNA length: 7.2 kb. It has short 5 and 3 non-coding regions and three overlapping open reading frames (ORFs)

2.4. Replication

HEV replication mechanisms are not well known due to the lack of an efficient cell culture system or animal model. A replication model (Fig-3**)** had been proposed

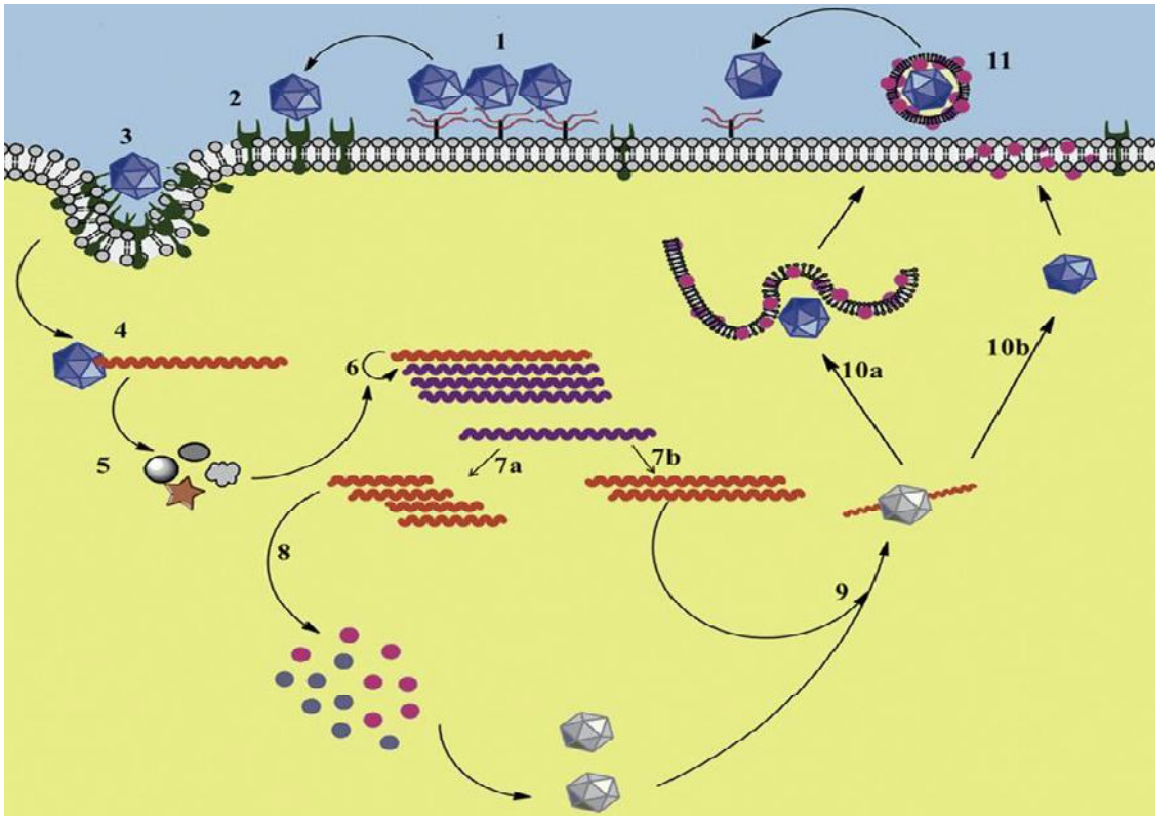

Figure.3. Proposed Replication of HEV (Imran *et al*., 2011; Oliveira 2013).

1. Attachment
2. Binding to cellular proteins
3. Particle internalization by receptor-mediated endocytosis
4. Uncoating
5. RNA translated into nonstructural proteins by host ribosomes
6. Positive sense RNA replicated into negative strands. The RdRp replicates (alone or with aid of cellular proteins) the positive RNA into negative RNA, which will

serve as template for synthesis of the positive sense RNA strand by the viral RNA polymerase.

7. Synthesis of subgenomic (7a) and full-length positive sense RNA (7b);
8. Subgenomic RNA translated into ORF2 and ORF3 proteins
9. genomic RNA packaged by capsid protein
10. ORF3 associated with endomembranes (10a) or plasma membranes (10b)
11. Mature virions associated with ORF3 proteins and lipids released

2.5. Pathogenesis

To date, the reasons for the varying manifestations of HEV infections remain unknown. Although neurologic symptoms and detection of viral RNA in the cerebrospinal fluid have been reported in hepatitis E patients (Kamar *et al*., 2011), the liver is assumed to be the target organ for HEV (Gupta *et al*., 1993).

Histological examination of liver biopsies reveal moderate to severe damage including swollen hepatocytes with giant cell formation, lymphocytic portal infiltration, cholangitis, apoptosis of hepatocytes and parenchymal necrosis, chronic hepatitis with cellular infiltration, periportal activity and fibrosis was observed in organ-transplanted patients suffering from chronic hepatitis E (Malcolm *et al*., 2007; Brost *et al*., 2006).

2.6. Clinical manifestation

Hepatitis E virus causes acute sporadic and epidemic viral hepatitis mostly asymptomatic and anicteric. Symptomatic HEV infection is most common in adults aged 15-40 years generally causing a self-limiting illness which lasts a few weeks. Following an incubation period of 2 to 6 weeks, symptoms of hepatitis develop, with fever and nausea, abdominal

pain, vomiting, anorexia, malaise, and hepatomegaly. Jaundice occurs in about 40% of patients. Excess mortality is seen in pregnant females and individuals with underlying chronic liver disease (Labrique *et al.,* 2010; Naik *et al.,* 2013). Extrahepatic disorders include a range of neurological syndromes, renal injury, pancreatitis, and hematological problems (Dalton *et al.*, 2008).

2.7. Transmission

Hosseini, (2011) described four major documented routes of HEV transmission:

A. Large-scale epidemics of HEV have been associated with fecally-contaminated water or food.

B. Zoonotic transmission (food-borne) is associated with sporadic consumption of raw meat products or by close environmental contact to an infected source.

C. Transfusion transmission has been reported from infected donors presumably resulting from an extended asymptomatic viremic period.

D. Person-to-person spread is relatively uncommon; sexual transmission is unproven and evidence vertical transmission via intra-uterine route had been suggested.

2.8. Diagnosis

2.8.1 Direct methods

2.8.1.1 Detection of viral proteins or nucleic acids in blood and stool samples by immune-electron microscopy

2.8.1.2 The detection of HEV RNA in biologic specimen (serum and/or stools). HEV RNA can be detected in stools 1 week before and up to 6 weeks

after the onset of symptoms and in serum for 3-4weeks from the onset of illness (Kamar *et al.,* 2014).

2.8.2. The Indirect methods

Detection of anti-HEV IgM is considered diagnostic for acute infection. Anti-HEV IgM is detectable 4 days after the onset of jaundice and persists for up to 3–5 months. Shortly after the appearance of IgM, IgG antibodies develop and peak at about 4 weeks after the onset of symptoms and persist for a variable period of 1 to 14 years after infection (Fujiwara *et al.*, 2014; Arends *et al.,* 2014).

2.9. Epidemiology

HEV is classified into four major genotypes 1-4 and sub-types and only one serotype. Genotype 1 is the most frequent cause of epidemic and sporadic hepatitis E in the developing world. HEV genotype 2 was first identified from the 1986 epidemic in Mexico and subsequently from Chad and Nigeria. HEV genotype 3 is prevalent globally in the swine population and is now being increasingly identified in human cases in the developed world. Genotype 4 was first described in Taiwan and subsequently found in China, Japan and India. Genotypes 3 and 4 also have been isolated from swine in the United States, Africa and Asia. There are clear differences in the epidemic potential of the various genotypes and epidemics occur exclusively in developing countries where the predominant circulating human strain is HEV genotype 1. In developed countries, human HEV was considered uncommon, with a prevalence of less than 1%. However, rates of anti HEV antibody within the general population have increased significantly; 2% in Europe and 1%-3% in the United States, with up to 20% prevalence in certain high-risk

groups. (Teshale and Hu, 2011).

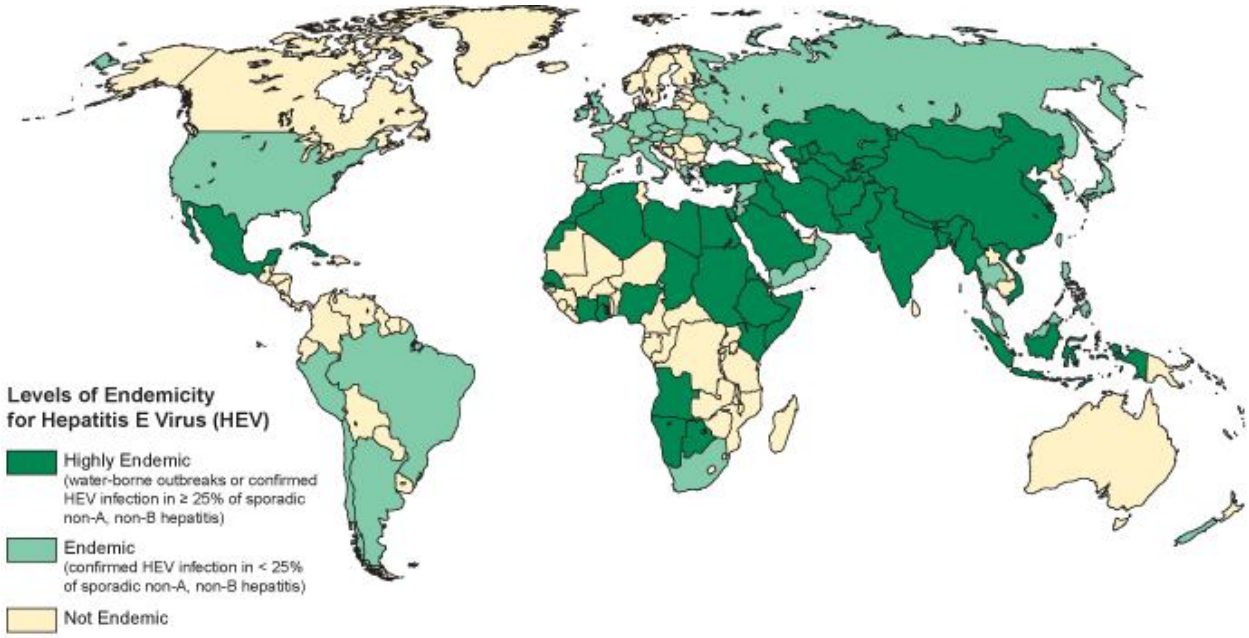

Figure.4. Geographic distribution of HEV infection (Centers for Disease Control and Prevention (2012)

2.10. HEV blood transfusion transmission

Previous Studies

Hepatitis E virus (HEV) has been recognized since 2004 as a transfusion-transmissible infectious agent, and recent epidemiological data suggest that it may pose a safety threat to the blood supply. It has recently become obvious that hepatitis E is endemic in industrialized countries, and that more infections are autochthonous than travel-associated. The seroprevalence and incidence of HEV in the general population and blood donors in European countries indicate an underestimated risk for transfusion transmissions (Dreier and Juhl, 2014).

Investigation studies in Germany with samples from three different groups of blood donors; patient with history of acute hepatitis and patients positive for antibodies against

other hepatitis viruses had shown that 37 % of the HEV seropositives had received a blood transfusion before (Wang *et al.,* 1993). Similar studies reported HEV antibodies in other European countries such as Switzerland (Lavanchy *et al.,* 1994), Italy (Zanetti and Dawson, 1994), Australia (Moaven *et al.,* 1995) and Brazil (Parana *et al.,* 1997).

Abdel Hady *et al*., (1998) determined the frequency of hepatitis E virus (HEV) infection by ELISA techniques among 95 unpaid Egyptian blood donors. The prevalence of anti-HEV IgG was 45.2% (43/95). These findings emphasized that HEV is endemic in Egypt and tends to accumulate in certain groups.

A cross– sectional study to estimate the prevalence of hepatitis E virus seropositivity in Tehran blood donors was performed by the enzyme immunoassay method in a group of 90 blood donors. Seven cases (7.8%) were anti-HEV Ab positive. This figure correlates with the prevalence ratio of endemic parts. There was no association between seropositivity and sex. The commonest age group was 40-49 years. The findings concluded that Iran can be considered as an endemic area for type E hepatitis (prevalence >5%), and it is more common in Iran than Israel and Turkey, but less common than Saudi Arabia, Iraq and Pakistan (Aminiafshar *et al*., 2004).

A retrospective study investigated hepatitis E virus infection transmitted through blood transfusions in an endemic area involved 145 multiple transfused patients and 250 healthy controls. Markers of acute HEV infection was detected in a significantly higher number of multiple transfused patients (13 of 145) compared to controls (two of 250) ($P < 0.001$). In contrast, none of the non-transfused patients developed HEV infection during the follow-up period. Study revealed that multiple blood transfusion places

recipients at risk of HEV transmission and therefore advocates the donor screening policy by blood banks (Khuroo *et al*., 2004).

On other hand, molecular studies of positive HEV serum samples detected by ELISA in American and German blood donors had confirmed that a substantial proportion of blood donors (1.5%) were positive for HEV ribonucleic acid (RNA) and viraemic blood donors are potentially able to cause transfusion-associated hepatitis E. (Dawson *et al.,* 1992; Oncu *et al.,* 2006).

In a cross-sectional study to determine the seroprevalence of hepatitis E virus (HEV) infection among volunteer blood donors at Regional Blood Bank of Londrina, Brazil, anti-HEV IgG was confirmed in 23/996 (2.3%), a ratio similar to previous results obtained in developed countries (Bortoliero *et al*., 2006).

A retrospective study was conducted to evaluate HEV seropositivity on stored serum samples from 399 voluntary male blood donors at a blood transfusion centre in Iran had revealed a prevalence rate (7.8%) of anti-HEV IgG antibodies (Taremi *et al*., 2007).

Another cross-sectional study to determine the seroprevalence of hepatitis E virus (HEV) infection was carried out among 400 volunteer blood donors of the regional blood banks from May to December 2005 in Khuzestan, Iran. Serum samples were tested for IgG anti-HEV antibody using a specific enzyme linked immuno-assay (ELISA) kit. The prevalence of HEV infection was found to be 11.5% (46/400). The data indicated higher prevalence of HEV 14.6% (38/260) among males compared to 5.7% (8/140) of females (Assarehzadegan *et al*., 2008).

Table-1: Global Studies on HEV Seroprevalence among Blood Donors

Country	Sample size	Seroprevalence (%)
Ghana	239	4.6
Burkina Faso	191	16.2
United States of America	1939	18.8
Saudi Arabia	900	18.7
India	262	13.7
Japan	12600	3.4
China	10741	27.4
United Kingdom	333	8.1
Egypt	488	20.9
Switzerland	550	4.9
Germany	116	15.5
France	512	52.3
Italy	151	1.3
Spain	99	4.0
Germany	1019	6.8
Greece	265	9.43
Sudan	90	26.7

(http://www.who.int/immunization/sage/meetings/2014/october/7_summary_HEV_systematic_review.pdf)

In a recent study, anti-HEV IgG antibody by ELISA method was found 25.3% (90/356) among blood donors in East China indicating high prevalence of HEV seropositivity in the male group compared to the female group (17.7%, 23/130) (Zhuang *et al*., 2011).

A similar study on 550 blood donors, 332 men (60.4%) and 218 women (39.6%) was performed for evaluating the presence of HEV IgG, in the region of Lausanne Switzerland. The overall anti-HEV IgG was 27/550 (4.9%), seroprevalence was 5.4% (18/332) in men and 4.1% (9/218) in women.

In a similar study by Scotto *et al*., (2012), seroprevalence of hepatitis E virus among blood donors in Southern Italy was 2/151 (1.3%) which is lower than anti-HEV IgG ratios reported by recent studies in Brazil 2.3%3, France 3.2%4 and Switzerland 4.9%5 and is consistent with data on the seroprevalence of HEV in the USA (1.2%).

A recent study on male blood donors in Makkah Saudi Arabia, Hepatitis E virus IgG antibodies were detected in 168/900 (18.7%) indicating higher prevalence among blood donors in the city compared to the neighboring countries (Johargy *et al*., 2013).

Seroprevalence of hepatitis E virus among blood donors was evaluated on 488 subjects attending blood transfusion Center of Suez Canal University Hospital on 2010. The overall prevalence of anti-hepatitis E virus antibodies was 20.9% (102/488). Seroprevalence increased significantly with age; from 8.3% in subjects below 20 years of age, 16.94% in 20-34 years of age, 34.5% in 35-49 years of age and a slight decline of 33.3% over those of 50 years of age (Endale *et al.,* 2013).

In a similar recent study, serum samples from 200 Serbian volunteer blood donors were tested for the presence of anti-HEV IgG by enzyme-linked immunosorbent assay

(ELISA). A total 15% of the volunteer blood donors were seropositive. The seroprevalence of HEV increased with age; 21.5%, 14.2%, and 5.4% in individuals older than 51 years, between 31 and 50 years, and in those younger than 30 years of age, respectively (Petrović *et al*., 2014).

A recent study was conducted to evaluate the prevalence of HEV among adults in South-West of Iran on 510 Blood donors 206 (40.4%) males and 304 (59.6%) females. The overall anti-HEV Abs prevalence rate was 46.1%. Seroprevalence increased with age from 14.3% in subjects aged 18–30 years to 71.4% in persons over 70 years old, and (90.9%) in ages 61 to 70 years (Farshadpour *et al*., 2015).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study design

3.1.1. Type of study

This is a cross-sectional analytical study

3.1.2. Study population

The study population was both male and female voluntary blood donors at Sheikh Khalifa Medical City, Abu Dhabi - UAE

3.1.2.1. Inclusion criteria

Voluntary healthy non-therapeutic donors who were eligible for blood donation criteria specified by Sheikh Khalifa Medical City

3.1.2.2. Exclusion criteria

Therapeutic blood donations

3.2. Study duration

The study was conducted during the period from February to April 2015

3.3. Study variables

The study included three independent variables (Demographics):

1- Age (three age groups, 15-30 years, 31- 45 years and 46-60 years)

2- Sex

3- Nationality

Dependent variable:

Screening results of hepatitis E virus antibodies (anti-HEV Abs)

3.4. Sample size

A convenience randomized sampling method was used to select 318 donated blood units. Sample size was calculated at confidence level of 95% and precision degree of 0.05 for a known population size (Cochran, 1963) using the following Formula:

$$n = N / (1+ N (d)^2)$$

- n: the sample size
- N: population size
- d: level of precision

Provided that the population (blood donors) size in early 2015 was 2000, the estimated sample size calculated at level of precision 0.05 as follows:

$$n = 2000/ (1+ 2000 (0.05)^2) = 333$$

3.5. Specimen and data collection

Demographic data (age, sex and nationality) were collected according to routine practice and volunteer blood donors were interviewed for history of intravenous drug abuse, jaundice, hospital admission and history of HBV vaccination. About 5 ml of venous blood were collected consecutively from participants in plain tubes during the study period, serum was separated and stored at -20°C till tested.

3.6. Methods

3.6.1. **ELISA kit**

HEV 4.0 ELISA kits (MP diagnostics Asia Pacific Ltd, Singapore) for the detection of total antibodies (IgG, IgM and IgA) against HEV in serum or plasma (Appendix-1).

3.6.2. **Test principle**

The MP diagnostics HEV ELISA 4.0 is a direct enzyme immunoassay. The wells of the polystyrene microplate strips are coated with a proprietary recombinant antigen presenting a conformational epitope that is highly conserved between different HEV strains. The HRP conjugate is produced with the same recombinant antigen labeled with horseradish peroxidase. This conjugate is first diluted appropriately in diluent buffer prior to being dispensed into the antigen-coated wells of the microplates. Serum or plasma samples were then added to the antigen-coated wells containing the diluent buffer and the conjugate. After incubation, HEV specific antibodies (IgG, IgM and IgA), if present, will bind to both the antigens immobilized on the wells and the antigen of the conjugate in the diluent. Subsequently, the wells are thoroughly washed to remove the unbound materials. A substrate solution containing 3,3',5,5'-tetramethylbenzidine (TMB) was then added to each well. The presence of specific antibodies is indicated by the presence of blue color solution after incubation. Reaction is terminated by addition of sulphuric acid.

3.6.3. **Assay performance**

Reagent preparation, samples dilution and test performance was carried out in respective to manufacturer instructions and guidelines. Serum samples were tested for the presence of anti hepatitis E virus total antibodies (IgG, IgM and IgA).

The color intensity of the resulting yellow reaction product was measured at 450nm within 30 minutes of adding the stop solution using microplate reader and its corresponding optical density or absorbance is proportional to the amount of antibodies present in the specimen. Specimens with absorbance values more than the Cut-off Value

were considered initially reactive by MP diagnostics HEV 4.0 ELISA. Specimens with absorbance values within ± 10% of the Cut-off Value were considered to be in the greyzone.

3.7. Statistical analysis

3.7.1. Study variables were coded numerically to enable entering and processing the data systematically and efficiently. Data was analyzed using the Statistical Package for Social Sciences (SPSS) software version 13.0 for windows (SPSS Inc., USA).

3.7.2. Descriptive analysis was used to find out the frequency of independent variables (age group, gender and nationality) within study population.

3.7.3. The prevalence of anti-HEV antibodies at 95% confidence intervals (95% CIs) was calculated for different variables (age group, gender and nationality).

3.7.4. Cross tabulation and Chi-square test were used to detect correlation between HEV seropositivity and the variables (age, gender and nationality) at significance of *p*-value of <0.05.

3.7.5. MS Office was used for summarizing analysis reports and output findings were presented through text and tables.

3.8. Ethical considerations

This study was approved by Institutional Review Board (IRB) of Abu Dhabi Health Authority according to local and international guidelines. Purpose and objective of the research were explained, privacy and confidentiality of participants was maintained.

CHAPTER FOUR

RESULTS

4.1Demographics and general characteristics of study samples

In this study, a total of 318 voluntary blood donors were enrolled during the study period between February 2015 and April 2015. The mean age was 34.7 years and (SD $\pm$ 8.48) with rang from 15-60 years. Study population was categorized into three age groups; 116/318 (36.47%) in the age group of 15-30 years, 154/318 (48.42%) in the age group of 31-45years and 48/318 (15.09%) were in the age group of 46-60 years. Majority were male donors 286/318 (89.93%) and females 32/318 (10.06%). South-East Asians 128/318; (40.25%) comprised the dominant donors, United Arab Emirates nationals 114/318; (35.84%) and Eastern-Mediterraneans 76/318 (23.89%). The baseline demographic characteristics of the study population are summarized in (Table 2).

4.2 HEV screening results and its correlation to donors' demographics

The overall HEV virus seropositivity in blood donations enrolled was 34/318 (10.69%). A remarkable gender disparity in overall seropositivity for HEV was observed as being higher in males 32/318 (10.06%) compared with females 2/318 (0.62%).

Seropositivity-age group correlation for middle aged donors 15-30 years 14/318 (4.40%) and donor group 31-45 years 15/318 (4.71%) was significantly higher compared to age group 46-60 years 5/318(1.57%). Statistically, no significant association was observed between hepatitis E virus seropositivity, sex and age (p=0.39, 0.82) respectively. HEV seropositivity correlation to nationality (Table 3) was significant among South-East

Asians 24/318 (7.54%); *p*-value=0.0006, United Arab Emirates nationals 7/318 (2.20%) and East-Mediterraneans 3/318 (0.94%).

Table-2: Baseline Frequency of HEV Study population Demographics (n=318)

Parameter		**Frequency**	**(%)**
Gender	Male	286	(89.93)
	Female	32	(10.06)
Total		318	
Age Groups	(15-30 years)	116	(36.47)
	(31-45 years)	154	(48.42)
	(46-60 years)	48	(15.09)
Nationality	United Arab Emirates	114	(35.84)
	East Mediterranean	76	(23.89)
	South-East Asia	128	(40.25)

Table 3: Frequency and distribution of HEV seropositivity in correlation to study variables

			Anti-HEVAb		
Variables		**Frequency n (%)**	**Positive n (%)**	**Negative n (%)**	**p-Value**
Gender	Male	286 (89.93)	32(10.06)	254 (79.87)	
	Female	32 (10.06)	2 (0.62)	30 (9.43)	0.39
Age Category	(15-30 years)	116(36.47)	14 (4.40)	102 (32.07)	
	(31-45 years)	154 (48.42)	15 (4.71)	139 (43.71)	
	(46-60 years)	48 (15.09)	5 (10.69)	43 (89.30)	0.82
Nationality Group	United Arab Emirates	114 (35.84)	7 (2.20)	107 (33.64)	
	East Mediterranean	76 (32.89)	3(0.94)	73 (22.95)	
	South-East Asia	128 (40.25)	24 (7.54)	104 (32.70)	0.0006

CHAPTER FIVE

DISCUSSION

Blood-borne transmission of HEV has been recognized since 2004 as a potential transfusion risk by many investigators worldwide (Dreier and Juhl, 2014). To our knowledge, this is the first study evaluating hepatitis E virus seropositivity among blood donors in the United Arab Emirates.

We studied HEV seropositivity in 318 healthy blood donors at Sheikh Khalifa Medical City Blood Bank. The obtained results were discussed in reference to study objectives and statements considering multiplicity of analyses and results from previous similar studies.

5.1 Frequency of HEV seropositivity among blood donors

Compared to regional studies among blood donors, our anti-HEV IgG antibody seroprevalence ratio (10.69%) is consistent with findings obtained by Johargy *et al.,* (2013) in Makkah Saudi Arabia (18.7%) and Assarehzadegan *et al.,* (2008) in Khuzestan Southwest Iran (11.5%). On other hand, our ratio is higher than what was reported in similar studies by Aminiafshar *et al.,* (2012) 8.37% in Riyadh, and 7.8% in Tabriz city reported by Taremi, *et al*., (2007), but apparently lower than seroprevalence among blood donors in Egypt 45.2% (43/95) reported by Tadesse, *et al*., (2013) and among blood donors in China 21.1% and 32.60% ratios respectively in two different studies (Guo *et al*., 2010).

Compared to global studies, our anti-HEV antibody rate was higher than ratios reported in Northern France (3.2%) by Boutrouille, *et al*., (2007) and in South West France

(16.6%) by Mansuy *et al* (2011). Also it is higher than figures obtained from blood donors in Germany (6.8%) Huzly *et al.,* (2013), Spain (2.8%), Ghana (4.6%) and in Brazil (2.3%) (Tadesse *et al*., 2013).

5.2 HEV seropositivity in correlation to study variables (age, gender and nationality)

In the current study, seroprevalence increased significantly with age; from 4.40% in subjects below 30 years of age, 4.7% in 31 to 45years of age and a slight decline of 1.57% over those 46 years of age which may be due to the small study subjects in this age group. Similar finding of seropositivity associated with age group was also reported in studies among persons living in HEV endemic areas and also in non-endemic regions (Arankalle *et al*., 1995).

Our study results are consistent with the previous studies which revealed that hepatitis E virus seroprevalence peaked with age, from 10% in subjects less than or equal 31 years to 16.7% in those more than 31 years, as shown by Johargy *et al.,* (2013), Kaufmann *et al.,* (2011) and Taremi *et al.,* (2007). In contrast to the above findings, some studies have found older age to be a risk factor for anti-HEV positivity; 14.3% in Central Iran among participants with mean age 13-50 years (Ehteram *et al.,* 2013).

With regard to nationality, our findings in this study showed significant seropositivity correlation (p=0.0006) among South East Asians residing in the UAE 24/128; 18.75% which is consistent with similar findings obtained by Bortoliero *et al.,* (2006); 17.8% of the donors from South East Asia were positive for IgG antibody to HEV. These results support the notion that the seroprevalence of hepatitis E virus is higher among donors

from countries with high HEV endemicity.

5.3 Conclusion

Seroprevalence of HEV-antibody among blood donors in the United Arab Emirates as shown in our study is comparable to findings obtained in regional and some global investigations

5.4 Recommendations

Screening of all blood donors for HEV is recommended and a careful surveillance in the general population. Further molecular and epidemiological studies are also required for a better understanding of the etiological role of HEV, transmission and risk groups.

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APPENDICES

Appendix-1

MP Diagnostics HEV ELISA 4.0 kit contents

1. HEV microplate Twelve 8-well strips; each microwell contains adsorbed recombinant HEV proteins
2. Non-Reactive Control; inactivated normal human serum, nonreactive for anti-HCV, anti-HEV, HBsAg and anti- HIV-1contains thiomersal and sodium azide stabilizers
3. Reactive Control; inactivated human serum containing high titer of IgG antibodies specific for HEV 1contains thiomersal and sodium azide stabilizers
4. Sample Diluent; tri based saline solution containing heat treated normal goat serum, stabilizers and preservatives
5. Plate Wash Concentrate; phosphate buffered saline with Tween 2 and preservatives

6. Conjugate; HEV antigen labeled with HRP, contains 0.2% Thimerosal as preservative
7. Substrate Buffer; Buffer containing 3,5, 5,5 Tetramethylbenzidine (TMB)
8. Stop Solution; 2M sulphuric Acid solution
9. Plate Adhesive covers
10. Instruction for Use