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PREVALENCE OF HEPATITIS C AMONGST HIGH-RISK GROUPS AND PATIENTS IN A TERTIARY CARE CENTER IN CENTRAL KERALA AND THEIR CLINICO-HEMATOLOGICAL PROFILE.

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KEYWORDS :

INTRODUCTION

Hepatitis C is a positive stranded RNA virus, which belongs to the family Flaviviridae.It was first identified in the late 1970's as a separate disease causing post-transfusion hepatitis and was initially termed as non-A non-B hepatitis.

Acute phase of Hepatitis C is often undiagnosed due to the absence of clinical symptoms. Hence, it has been rightly named as the silent killer due to late hepatic manifestations like hepatocellular cancers (HCC) and liver cirrhosis. Chronic Hepatitis C causes 25% of hepatocellular carcinoma and 27% of cirrhosis worldwide[1]. Hepatitis C is also one of the most common chronic blood borne infections.

Global prevalence of Hepatitis C is 180 million worldwide i.e. nearly three percent of the entire world is infected with Hepatitis C[2].The prevalence of HCV is variable in different geographical regions. Overall,the rate varies from < 1% in Northern Europe to > 2.9% in Northern Africa. Chronic hepatitis C causing liver cirrhosis and chronic liver disease, is the most common cause of liver transplantation in the United States, Australia and most of the European countries[3].In India it is estimated that around 12.5 million people are suffering from Chronic Hepatitis[4].Hepatitis C is an emerging infection in India. The risk factors being, poor screening of blood donors, aseptic therapeutic practices, reuse of needles and professional blood donations. Prospective studies have shown that 80% of cases of acute Hepatitis C, progresses to chronic infection;10–20% of these will develop complications of chronic liver disease, such as liver cirrhosis, within two to three decades of onset, and 1–5% will develop hepatocellular carcinoma[5].However, the epidemiological data of the prevalence of HCV from Kerala is sparse.Few studies have been conducted in India to evaluate the epidemiological prevalence of Hepatitis C, these studies were conducted in blood banks, as a surrogate marker for the population at large. With the then presence of professional blood donors, these results have been considered as fallacy.

Thus, this is a retrospective study conducted to evaluate the epidemiological prevalence of Hepatitis C amongst the population attending atertiarycare centre in Central Kerala.

MATERIALS & METHODS

This study was conducted at tertiary care hospital and medical college, located in Central Kerala in the Department of Microbiology from January 2014 to June 2015. Human ethical clearance for this study was obtained from Institution Ethics Committee prior to collection of samples and a detailed proforma was collected from patients included in the study. The sample size was calculated to be 600, by using the confidence level of 99% and tolerance error of 1% after considering the prevalence of HCV from previous studies.All patients (outpatients and inpatients) whose samples that were received in the microbiology department as a part of screening and diagnosis of Hepatitis C infection were classified into low risk and high-risk group.High risk groupincluded current or former injecting drug users; people on long-term haemodialysis; health care workers (especially those working in dialysis units, critical care and emergency medicine); patients who have received multiple blood transfusions; people living with HIV (human immunodeficiency virus) and/or HBV (hepatitis B virus); people with abnormal liver tests and alcoholics..Low risk group included the patients enrolled for pre-surgical screening. Risk factors, clinico-hematological features were collected in a detailed profoma. Ante-natal cases and children less than 16 years have been excluded from the study. All the samples collected were subjected to enzyme immunoassay for detection of HCV antibodies, by third generation ELISA(HCV Microlisa,J.Mithra& Co Pvt Ltd(with a sensitivity 100 % and specificity 97.4%).Samples that were positive for HCV antibodies were subjected to RT-PCR for the detection of HCV RNA.

RESULTS

Out of the total 600 samples screened 398 (66.3%) were males and 202 (33.6%) patients were females.The proportion of age and gender distribution included in this study is listed in table no:1.

TABLE 1 : Age and gender Distribution (n= 600)

Age group (years)	Males	Females
16 – 39	106(17.6%)	97 (16.1%)
40-60	171 (28.5%)	47 (7.8%)
61-100	121 (20.16%)	58 (9.6%)
Total	398 (66.3%)	202 (33.6%)

The representation of various categories of patients included is listed in table no: 2.

TABLE 2: Various Categories Of Patients Included In The Study

Population at risk		Number of patients screened (Percentage)
High risk	Dialysis	123 (20.9%)
	Alcoholics	100 (16.7%)
	Health care workers (HCW)	100 (16.6%)
	Patients with liver diseases (Others)	233 (38.3%)
Low-risk	Pre-surgical Screening	46 (7.6%)
	Total	**600**

Out of the 600 samples screened, 27 samples were reactive for HCV antibodies by ELISA. These 27 samples were subjected to PCR, out of which 16 were positive for HCV RNA by PCR.The common risk factors commonly associated with HCV transmission among these 27 patients with HCV antibody reactiveswere hemodialysis 13/27(48.1%),blood transfusions 9/27(33.3%),past surgical history 5/27 (18.5%) and multiple sexual partners 1/27(3.7%).

Of the total 27 reactive cases; 23 patients were symptomatic and four patients were asymptomatic. None among these 27 patients had co-infections with HCV,Hepatitis B or HIV. None of them were drug abusers.The common symptoms in these patients were jaundice(60.8%), fatigue (69.5%), abdominal pain (47.8%),pruritis(39.1%), dark colored

urine (26%) and clay colored stools (26%).The common laboratory parameters like SGPT(Serum glutamic pyruvic transaminase), SGOT (serum glutamic oxaloacetic transaminase), ALP (Alkaline phosphatase) and total bilirubin were analyzed for the 23 symptomatic patients.

Table 3: Laboratory Parameters Among The Hcv Positive Symptomatic Cases

Laboratory Parameters	Reference range	Number of cases with values more than the upper limit (percentage) (n=23)	Number of cases that were PCR positive with values more than the upper limit (percentage) (n=12)
SGOT	13 -52.8IU/L	13 (56.5%)	6 (50%)
SGPT	10-68 IU/L	9 (39.1%)	6 (50%)
SGOT/SGPT ratio	0.5-0.7	11(47.8%)	7(58.33%)
ALP	44 -147 IU /ml	23(100%)	12(100%)
Total Bilirubin	0.3 – 1.3 mg/dl	5 (21.7%)	5 (41.66%)

TABLE 4 :SGOT/SGPT ratio among HCV antibody positive cases (n=27)versus HCV RNA PCR positive cases(n=16)

SGOT/SGPT ratio	HCV antibody positive(27)	HCV RNA positive(16)
0.5 – 0.7	**7 (26%)**	**4 (25%)**
0.7 -1.1	**3 (11 %)**	**5 (31.25%)**
>0.16	**14 (51.8%)**	**7(43.75%)**

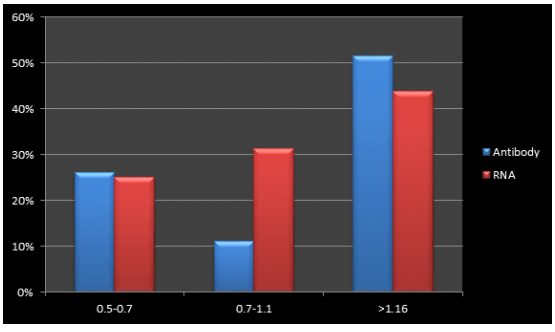

FIGURE 1: SGOT/SGPT ratio among HCV antibody positive cases (n=27)versus HCV RNA PCR positive cases(n=16)

DISCUSSION

The global burden of Hepatitis C infection is nearly130-170 million, of the world population with 3 to 4 million people being newly infected every year globally [6]. Hepatitis C infection is a serious public health problem, 80% of the infected cases proceeds to chronic HCV, 15 to 20 % develops liver cirrhosis and 5% develops HCC[7]. HCV accounts for 26% of all liver cirrhosis and 28% of all cases of HCC globally[8]. In India HCV accounts for 15 – 20 % of all chronic liver diseases and 5 to 10% of all HCC [9]. This study includes most of the high-risk groups for HCV, attending the hospital during the study periodincluding23 dialysis patients (20.9%), 100 patients attending the de-addiction alcoholic clinics (16.7%), 100 health care workers from high-risk areas includingdialysis units, criticalcare units and the emergency department, which contributed to 16.6%(Table no:2). The largest group (38%) screened in this study were patients presenting with symptomatic causes of liver disease.

The diagnosis of chronic HCV is based on detection of anti-HCV antibody and HCV RNA. Anti-HCV antibodies are the first line diagnostic test for HCV infections. But PCR (Polymerase Chain Reaction) is the gold standard for diagnosing, acute and chronic HCV. If the patient is reactive for anti-HCV antibody, the sample must be tested for HCV RNA; if HCV RNA is negative, the patient must be re-tested for PCR after three months [11]. In case of an acute HCV infection and in case of immune-suppressed patients, EIA can be non-reactive. Following the onset of treatment or spontaneous viral clearance, HCV antibodies can persist, even in the absence of HCV RNA. The specificity of third generation ELISA is 97% and of RT-PCR is 97–99% [12]. A total of 600 samples were screened in our study, of these there were 27 samples which screened reactive for HCV antibody (4.4%).

All these 27 samples were subjected to a qualitative RT-PCR, to detect HCV RNA. Out of the 27 samples tested, 16 samples (59.2%) came positive for HCV PCR, which suggests the presence of detectable HCV RNA in peripheral blood. Not all the 27 specimens reactive for anti HCV antibodies had a detectable RNA. This is probably because in most cases of acute HCV infections viremia persist in only 50 to 85% reflecting a good immune response [13].The HCV RNA was undetectable in 11 of our cases, most probably due to a viral load below the detectable threshold by the qualitative RT-PCR. The detectability of HCV RNA can also decrease in routine laboratory settings, as the samples are usually kept at room temperature for periods involving processing and due to repeated freezing and thawing. This drawback was considered early on in our study and was minimized by direct collection of the specimens from reactive cases and transported to the lab in ice packs and the separated serum was stored immediately at -200C. Thus, the degeneration of HCV RNA was limited and false negatives in this study were attempted to be minimized.Repeat testing of these negative specimens were advised after three months, but patients were lost to follow-up.

Majority of the antibodyreactive cases were from Aleppey district (62.5%) followed by Pathanamthitta district (18.5%), Kottayam district (12.5%) and lastly Idukki (6.25%) district of Kerala. The gender distribution seen in our study was higher among males at 66.3% with the predominant age group being 40-60 years. The reason for a higher percentage of males being affected was studied by E.ElWahabet al., from Egypt [10]. He was able to isolate behavioral and community acquired exposures in males like unsafe sexual practices, sharing drug paraphernalia, smoking, alcohol abuse, imprisonment, military services and traveling. These risk factors were less elicited from the women population who were confined to their households. The above-mentioned risks in males holds good for our community as well, weremalesespecially from Kerala, usually travel abroad for work especially to the Middle Eastern countrieswhile women are usually left behind. The higher number of cases in 40-60 age group correlates well with the global trends. These groups of people borne in between 1945-1965 are called as baby boomers. CDC mentions that the baby boomers were infected between 1960 -1980, when the transmission of HCV was at its peak. This age group has been exposed to medical equipment's and procedures before standard precautions were introduced. The lack of screening of blood and blood products before 1992, also has resulted in higher risk of HCV infections in the age group 40-60 years.

Risk factors commonly associated with HCV transmission include transfusion of blood and blood product, transplantation of organs from infected donors, injectable drug use, unsafe therapeutic injections, occupational exposure to blood, birth to an infected mother. The common risk factors which we could identify in our study were haemodialysis (48%) and history of blood transfusions seen in 33% of cases. Among the HCV antibodyreactive cases in our study, 3.7% of the cases were on multiple hemodialysis for less than 5 years, 37% were hemodialysis for period more than 10 years. Of the 16 HCV RNA positive patients 31.25%. were on hemodialysis. The prevalence of HCV in patients on haemodialysis is quite high compared to the general population and increases with the duration of ongoing dialysis. According to WHO the percentage of HCV positives with dialysis as a risk factor is 33% globally[2]. In India, in a recent study conducted In Hyderabad in 2005, the prevalence of HCV among dialysis patients was found to be 13.2% [14].Dialysis specific infection control practices have been advised by CDC. Transmission of the virus to hemodialysis patients is generally nosocomial with possible risk factors being failure to disinfect devices between patients, sharing of single-use vials for infusions, poor sterile technique, poor cleaning of dialysis machines, and poor distance between chairs[15].To add on most of these patients are anemic and often require multiple blood transfusions.Blood transfusion was a risk factor in nine of the antibody positive cases (33.3%) and in 31.25% of the HCV RNA PCR positive cases. In a study done in Mexico among HCV positive cases the risk of blood transfusion was 38% [16]. In India, in a study conducted in Haryana by Malhotra et al, blood transfusion was a risk factor in 12% of the sample[17]. Hepatitis C is a blood borne disease, unscreened blood transfusion being one of the main modes of its spread. Until 1992, screening of blood and blood products were not mandatory, which would have been a major route of its spread.

History of past surgeries was seen in 18.5% of our antibody reactive and in 25% of the HCV PCR positive cases.Unsafe therapeutic injections, both by professional and non-professionals, accounts for the spread of HCV worldwide. Nearly two million HCV infections are acquired annually from contaminated health care injections, and accounts for 40% of infections acquired worldwide[18]. In developing countries, the nosocomial spread of HCV is on the rise due to the reuse of needles, syringes, scalpels. In Egypt, the mass treatment of endemic schistosomiasis made use of unsterilized needles and syringes, which has caused the spread of HCV among all age groups. This suggests that unsafe therapeutic practices in the past with reuse of needles and syringes may have accounted for HCV infection in our locality.

A history of multiple sexual partners could be elicited in 3.7% of our antibody reactive and 6.25% of HCV RNA PCR positivecases. The presence of multiple sexual partners as a separate risk factor for HCV is controversial. According to studies conducted in United States of America by Alter MJ in 1980, multiple sexual partners were considered as separate risk factor for HCV[19]. In few other studies conducted in the 1990, there was no correlation between HCV and multiple sexual partners and among male homosexuals, which was previously considered as a risk factor[20]. In few other studies which were reviewed, the history of sexual activity was not elicited as it remains a social taboo. Hence the significance of sexual mode of transmission as a risk factor for HCV remains controversial.

None of the cases had history of intravenous drug abuse, occupational exposure or needle stick injuries as compared to other studies. It might be due to the lower prevalence of IV drug abuse among younger generation in our locality. HCV transmission by occupational exposures and needle stick injuries were also nil in this study, as standard precautions are followed routinely, and safer phlebotomy practices are followed in our hospital set up.

Symptoms developed during HCV infections are vague. In our study out of the 27 cases with antibody reactive; 23 cases (85%) were symptomatic and 4 cases (14.8%) were asymptomatic. Of the 16 HCV RNA PCR positive cases 12 (75%) were symptomatic while 4(25%) were asymptomatic.These asymptomatic cases were picked up as a part of surgical screening. Jaundice was seen in 14 symptomatic antibody reactivecases (60.8%)and in 68.5% of the symptomatic HCV RNA PCR positive cases.In the acute stage of HCV infection, jaundice is found only in 25% of cases[21]. The rate of chronic HCV infection is lower in patients who develop jaundice or symptoms during the acute onset of HCV infection as compared to those who are anicteric. The presence of jaundice in the acute phase is associated with good immune response and rapid clearance of the virus. None of our 27 patients belonged to the acute stage of HCV infection. They were either long term carriers or patients suffering from chronic disease.

In our study SGOT is elevated in thirteen cases (54%), while SGPT is elevated in nine cases (37.5%) of the antibody reactive cases, while 50% of our HCV RNA PCR positive cases had elevated SGPT and elevated SGOT. During chronic HCV infection, the principal abnormality is an elevation of serum amino transferase activity, usually SGPT exceeding SGOT. The release of SGOT and SGPT from liver cells to blood cells represents hepatocellular damage or death. These enzymes are usually released at a constant rate, which represents the turnover of apoptosis and clearance of the enzyme from plasma. But once cirrhosis sets in SGOT exceeds SGPT. In cases of chronic hepatitis C, elevated serum SGOT correlates to fibrosis rather than serum SGPT.When compared to other chronic liver disease, in chronic HCV infections there is episodic fluctuation in aminotransferase activity.This is due to the burst of hepatic necro-inflammatory activity accompanying the emergence of new quasispecies,which overcomes host immunological containment of HCV.

As the elevation of SGOT is more than SGPT in our study it correlates with most of our patients who were cirrhotic or had chronic HCV infection. The normal SGOT/SGPT ratio is in the range 0.5 to 0.7. Figure 1 explains the variation of SGOT/SGPT ratio in antibody reactiveversus HCV RNA PCR positive cases our study. Seven cases of antibody reactive and four cases of HCV RNA PCR positive caseshadSGOT/SGPT ratio within the normal range of 0.5-0.7 (25.9%), three antibody reactive cases (11%) versus five cases (31.25%) of HCV RNA PCR positive caseshad an SGOT/SGPT ratio between 0.7-1.1,which is suggestive of poor prognosis. Fourteencases(51.8%)of the antibody reactive cases versus seven cases, 43.75% of HCV RNA PCR positive cases had SGOT/SGPT more than 1.16, which suggest bad prognosis. In chronic viral illness the ratio of SGOT/SGPT is predictive of long term complications like cirrhosis and fibrosis. Typically, inchronic viral hepatitis SGOT/SGPT is below 1, but as the disease progresses and as cirrhosis andfibrosis sets in this ratio starts rising above 1. Hence in chronic hepatitis C infection raised SGOT/SGPT is suggestive of fibrosis, rather than necro-inflammatory changes[22].A raised SGOT/SGPT over 1.09 is predictive of the progression of chronic viral hepatitis to cirrhosis.Anincreased SGOT/SGPT of over 1.16 is predictive of poor survival. It also correlates well withother prognostic scores like Child Pugh test and Model for end stage liver disease score(MELD).Hence, in our study 51.8% of HCV antibodyreactivecases and 43.75% of HCV RNA PCR positive cases had poor prognosis with cirrhosis.

Thus, to conclude HCV has a prevalence of 4.4% among the high-risk groups and patients attending our tertiary care centre in central Kerala. The safety measures to prevent transmission and spread of HCV in hospital settings must be in place.

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