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META ANALYSIS OF HUMAN GUT VIROME DIVERSITY OF HEPATOCELLULAR CARCINOMA PATIENTS IN PAKISTAN

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ABSTRACT
Hepatocellular carcinoma (HCC) is the sixth usual form of cancer all over the world. Various types of non-pathogenic viruses like Anelloviruses and Sen Viruses (SENV) are single-stranded, hepatotropic DNA virus. The etiologic role for SEN in the cryptogenic hepatitis could not be recognized, nor was it linked with more severe hepatitis. **Methodology:** Data of four research scholars were collected and meta-analysis were performed for the presence of SEN viruses and Anelloviruses. Statistical analysis was performed and $p<0.005$ were define as statistically significant. **Results:** Among the total 242 normal individuals selected, No one was found infected with SENV and Anelloviruses. Characterization of SENV and *Anelloviruses* among Celiac disease patients showed the presence of SENVH 4/48 (P=0.004) as statistically significant. Leukemic patients were found at a risk for Anelloviruses as 4/30 (P=0.005) were found positive for TTV, 9/30 (P=0.000) for TTMV and TTMDV each. Hepatocellular patients were at greater risk for SENV viruses like 150/150 (P=0.000) SENVH and 72/150 (P=0.000) were found infected with SENVD. A total of 4/10 (P=0.677) for SENVH while 2/10 (P=0.861) for SENVD among HCV-non-HCC patients. **Conclusion:** It is concluded from the current study that the presence of an infection/disease may open a clue for the establishment of non-pathogenic viruses like SENV and Anelloviruses. Furthermore, the presence of non-pathogenic viruses might exaggerate the pathogenesis of present disease in the host.

KEYWORDS: Hepatocellular Carcinoma, Hepatitis C Virus, Hepatitis B Virus, Anelloviruses, Senviruses.

INTRODUCTION
HCC is considering being the cause of death. (Kew, 2010). In advance countries liver cancer is one of the severe health issues. It is reported from sub-Saharan Africa and East Asia, the maximum age accommodated rate is (>20 per 100,000) that accounts about 82% of cancer issues. Specifically, 55% of which are recorded from China (Lawrence, 2001). A half is observed in Southern Europe, lower occurrence (<5 per 100,000) in central and south America and the remaining parts of Europe. The division of HCC in the world differs by gender, etiology and region. Overall, the suffering rate of men is higher than women along the ration of female to man is 1:2 and 1:4. In the Pacific region of Asia (especially North and South Korea, Indonesia, and Vietnam), the percentage in women is four times greater than men. (Bray et al., 2018). The changing age happening of liver cancer is increased between Americans, Indians and Alaskans natives from 1.6 per 100,000 individuals to 4.6 out of 100,000 individuals which is followed by black, White and Hispanics (Altekruse et al., 2009). It is reported from current studies that the involvement of the gut–liver axis is responsible for HCC in the pathophysiological mechanism (Petrosino et al., 2009).

Both of these organs are significant for nutrient absorption and metabolism. The gut and liver have a strong association that suggested from developing evidence. Almost 75 % of blood supply occurs from the hepatic portal vein in the liver that transfers blood from intestine to the liver. The intestinal blood carries nutrients from the gut to stimulate liver functions by increase the operative hepatic processing of the nutrients (Wan & El-Nezami, 2018). A diversified group of microorganisms produced by the human intestine that occur in the host environment. Gut obstruction is serious for maintaining the common physiology of the intestine micro biome. Thus, the intestinal micro biome is caused by a breakdown of this obstruction that can work as the main cause of portal-vein endotoxins, like lipopolysaccharide, in the production of hepatic epidemics (Schroeder & Bäckhed, 2016; Yu & Schwabe, 2017). In the development of hepatic malignancies, the gut microbiota has produced as a dominant causative occurrence. Hence gut microbiota should target to secure eubiosis because of

being the most important organ in human. The gut microbiota is protected by the pathophysiology of viral hepatitis and may lead to progressive HCC levels. The composition of the gut microbiota has a significant effect on the liver's immune response, contributing to the removal or persistence of the virus (Gupta et al., 2019). The composition of the gut microbiota between individuals are differs considerable; recently study shows that between all healthy adults the collection of gut colonizers is shared by composing the healthy humans core microbiota (Tremaroli & Bäckhed, 2012) and is influenced by microenvironment like intestinal motility, pH and nutrient availability (Son et al., 2010). The gut microbiota composition can influence by many factors such as stress, age, diet, medications, lifestyle and illnesses (Conlon & Bird, 2015). Its ninth leading cause of cancer deaths in the United States is HCC (O'Connor et al., 2010). Mainly chronically liver tissues damaged occur due to the HCC because of the inflammatory and the chronic regenerative phenomena that take part to the beginning or development of the HCC (Castven et al., 2017). HCC is a principal fatal cancer acquired from hepatocytes, considered to be 80% of all liver cancers. Some of other liver cancers, comprising hepatoblastoma, angiosarcoma, intrahepatic and cholangiocarcinoma, are somewhat unusually correlated with HCC. For that reason, the HCC occurrence is examined to be the liver cancer morbidness (Zhu et al., 2016; Ahmed et al., 2008). In addition to 21,670 deaths, out of 30,640 novel liver cancers and intrahepatic bile duct were evaluated to arise in 2013 (Crissien & Frenette, 2014). As compared to female HCC occurred more common in male subject than females, with a greater frequency in Eastern Asia and Southern, Middle and Western Africa, Micronesia and Melanesia (Ferlay et al., 2010). It is considered approximately 5.7% circumstances of cancer. Every year, about 1% of mortality rate around the world was due to HCC. In the year 2000 the new cases of HCC had produced by total no of 564300 in which 70% cases found in males (Parkin et al., 2001). 548,600 individuals with HCC died in the same year, showing peoples with 97.2 percent in this condition. Predictably, in Asia there are 77% cases and in Africa 7.4%. In North America the cases of HCC are 12,543 accounted in which the total no of HCC cases is 2.2% registered. The predictable age-adjusted occurrence of liver cancer per 100 000 men in the Asia reached from 18.3 in Southeast Asia, 5.6 in Western Asia, 35.5 in Eastern Asia; in Africa, 13.5 in Western Africa, 24.2 in Middle Africa, 6.2 in Southern Africa, 14.4 in Eastern Africa, to 4.9 in Northern Africa; in Europe, 5.8 in Western and Eastern Europe, 9.8 in Southern Europe, and 5.8 in Western and Eastern Europe. The rates recorded in all regions were two to three times lower in women than in men (Ferlay, 2001).

In Cancer the mortality ranked is fourth of liver cancer after the trachea, bronchus, stomach, Colon and rectum cancer. In males subject the ranked for liver cancer is 3rd, and in females the rank is 5th. Geographically, in Africa the liver cancer deaths rate is 45,000, in America its incidence rate is 37,000, in the Eastern Mediterranean its incidence rate is 15,000, in Europe its prevalence rate is 67,000, in Southeast Asia its prevalence rate is 61,000, and in the Western Pacific its occurrence is 394,000, and most include Japan and China. About 783 000 individuals were confirmed to have died from cirrhosis in the same year, involving 282 000 women and 501000 men.

The HBV is predominating in those countries where the prevalence rate of HCC is high. But in some countries, its incident is beginning to fall (McGlynn et al., 2001). On the other hand, the occurrence of HCC is enhancing in Switzerland, Italy, France, Spain, New Zealand, Australia, and specifically, Japan. In these countries, the HCC is the major cause of chronic liver infection (El-serag et al., 2004).

In adult males the most common cancer is represented and its frequency is high in Pakistan. A known risk factor for HCC is Hepatitis C, which frequency rate is very high in Pakistani population, and through worldwide it has one of the highest incidence rates (>3%) (Nishtar et al., 2013). In the world Pakistan ranked as the sixth most populated state with total individuals of 182142594. When compared with noble states, Pakistan is a low-income State because of lacking of many important factors of healthcares. The incidence of cancer and it mortality rate in the developing world is increasing. In cancer care Pakistan have lack of resources which result a negative impact on patient health (Lyerly et al., 2015). Hepatobiliary cancers have been seen due to the increasing incidence. The most common malignancy in adult males are hepatobiliary cancers according to the data of hospital-based registry in Pakistan, and characterize 10.7% of all cancers (Badar & Mahmood, 2015). In Pakistan the age standardized rate for HCC in male is higher than that of female. It is stated that Hepatitis C is attributable to 60–70% of HCC in Pakistan. The predominant etiology of Hepatitis B in many other Asian pacific countries (Hafeez Bhatti et al., 2016).

HBV belongs to Hepadnaviridae family (Riaz et al., 2016) with a genome of 3.2 kb made of incompletely dsDNA (Pugh & Bassendine, 1990). It is a circular, double-stranded DNA molecule having 8 genotypes from A to H. It is the leading risk factor for HCC all around world. 50% cases of HCC are caused by HBV. It is mostly attained by vertical and perinatal transmission in endemic areas. On the other hand, western countries of low prevalence are generally attained in adulthood by the horizontal transmission (Yang et al., 2011). Numerous influences are described to raise the risk of HCC between HBV carriers, containing viral, demographic, clinical and environmental or life-style factors. The genotypes A and D are common in the Middle East and Europe, whereas genotypes B and C are common in Asian population (Bruix & Sherman, 2011). HBV is conveyed by intravenous injections, sexual contact and contaminated blood transfusions. HBV infection is the principal source vertically transmitted from mother to fetus in the world. The world's 5%

population is infected with HBV (Ott et al., 2012). In Pakistan the spreading of hepatitis B infection is 2.4% in adults and infants (Blumberg et al., 2000). The carriers of HBV having 10%–25% risk of emerging HCC. HBV is unique compare with other sources of hepatitis because it can develop HCC without indication of cirrhosis of the liver (Crissien & Frenette, 2014). HBsAg is not the only hematologic indicator to conveys an important risk for progression of HCC, while subjects who are HBsAg negative with positive HB core antibody (anti-HBc) also remain at risk for progression of HCC (Chen et al., 2006). HCV is a small, single-stranded RNA virus. It shows high heritable variability (Choo et al., 1991). It seems to raise the risk of HCC by persuading hepatic inflammation. Malignant transformation of infected cells is also stimulating by HCV. The HCV risk is maximum between cirrhotic wherever HCC advances at rate of one to four percent per year. However, eight percent rates have been stated in Japan. The progress of HCC in case of HCV happens entirely in the liver with the established cirrhosis (Lok et al., 2009). However, the HALT-C, exhibited that eight percent of patients lacking of cirrhosis of liver, but then again with intermediate to progressive hepatic fibrosis, established HCC (Lok et al., 2011). HCV has six different genotypes. The genotypes I, II, and III are showing predominance in the Western countries and Far East. Whereas, the genotype IV is dominant in the Middle East. The lower rates of CHC infection arise in Europe i-e 0.5%–2.5%, Canada 0.8% and in the United States 1.8% with highest rates occur in Egypt such as 18% (Bostan & Mahmood, 2010). The risk of liver cancer is enhanced by HCV that was 17-times riskier in promoting HCC than CHC; this risk depends on the level of HCV associated with liver fibrosis (Donato et al., 2002).

MATERIAL AND METHODS

The current study was conducted at Molecular Virology Lab, COMSATS University Islamabad. This meta-analysis study was done on the results of different researchers. Different domains were selected for the analysis of viruses by various colleagues. For the meta-analysis study, celiac disease patients, dengue virus infected patients, leukemic patients, hepatocellular carcinoma and HCV non-HCC positive subjects and healthy individuals were considered. Research performed for the detection of SEV (SENVH and SENVD) viruses and Anelloviruses (TTV, TTMV, TTMDV) in various disease patients and normal individuals were included in this study. Research performed for other than SENV and Anelloviruses were excluded from the current study.

Detection of SEV and Anelloviruses in Healthy Population

The detection of SEV viruses and Anelloviruses were performed among various diseases. According to the scientific protocol a positive control/negative protocol is working as a backbone for performing an experiment. A total of 263 normal individuals were studied for the SEV (SENVH and SENVD) viruses and Anelloviruses (TTV, TTMV, TTMDV) which were analyzed meta-analytically in the current study.

Detection of SENV and Anelloviruses in Various Diseases

A total of 273 infected individuals from various diseases including 48 celiac patients, 35 dengue patients, 30 leukemic, 150 Hepatocellular Carcinoma (HCC) patients and 10 HCV non-HCC patients were analyzed for SEV (SENVH and SENVD) viruses and Anelloviruses (TTV, TTMV, TTMDV) meta-analysis.

Statistical Analysis of the Results

The confirmed diseased and healthy controls were arranged by different groups. The numerical values for the incidence of viruses in both healthy and patients systematized in a table and analyzed for multi-variate and univariate regression analysis through Statistical Package Social Sciences Students (SPSS Version 16.00). Various statistical test like Fischer exact test, one way Annova were applied for statistical significance. The statistical change was measured when p value was less than 0.05 ($p< 0.05$).

RESULTS

HCC is reported as the sixth familiar form of cancer worldwide. It shows an increasing frequency and high mortality rate. Its cause is generally related to dietary and environmental aspects. Anellovirus includes four genera of small single-stranded DNA viruses characterized by broad tissue tropism, genetic heterogeneity, and high global distribution. Infection is usually asymptomatic, signifying that it might confer an existence advantage. SEN virus is a circular, single-stranded, hepatotropic DNA virus. The etiologic role for SEN in the cryptogenic hepatitis could not be recognized, nor was it linked with more severe hepatitis.

Evaluation of SENV and Anelloviruses in Normalpopulation

A total of 0/242 normal individuals were found positive SENV and 0/242 were found positive for Anellovirus

Characteristics	Name of the viruses	Infected/ total	P-Value	95% Confidence Interval	
				Lower Bound	Upper Bound
Anelloviruses	TTV	0/242	0.000	-.735	-.567
	TTMV	0/242	0.000	-.809	-.678
	TTMDV	0/242	0.000	-.852	-.717
SEN Viruses	SENVH	0/242	0.000	-.725	-.641
	SENVD	0/242	0.000	-.366	-.260

Identification of SENV and Anelloviruses in CeliacPatients

Among the entire celiac disease patients 9/48 were positive for SENV and 22 /48 were found positive for Anelloviruses.

Characteristics	Name ofthe viruses	Infected/total	P-Value	95% ConfidenceInterval	
				Lower Bound	Upper Bound
Anelloviruses	TTV	4/48	0.327	-.038	.115
	TTMV	9/48	0.116	-.012	.108
	TTMDV	9/48	0.066	-.004	.120
SEN Viruses	SENVH	4/48	0.004	-.096	-.018
	SENVD	5/48	0.944	-.047	.050

Identification of SENV and Anelloviruses inLeukemic Patients

Out of 9/30 leukemic patients showed presence of SENVwhile 22/30 have Anellovirus infections.

Characteristics	Name of the viruses	Infected/total	P-Value	95% ConfidenceInterval	
				Lower Bound	Upper Bound
Anelloviruses	TTV	4/30	0.005	.026	.148
	TTMV	9/30	0.000	.056	.150
	TTMDV	9/30	0.000	.068	.166
SEN Viruses	SENVH	4/30	0.682	-.037	.024
	SENVD	5/30	0.198	-.013	.063

Molecular evaluation of SENV and Anellovirusesin HCV-non-HCC Patients

Characterization of SENV and Anelloviruses showed that a total of 6/10 HCC patients were infected with SENV while 0/10 were infected with Anelloviruses.

Characteristics	Name of the viruses	Infected/ total	P-Value	95% Confidence Interval	
				Lower Bound	Upper Bound
Anelloviruses	TTV	TTV	00/10	0.338	-.054
	TTMV	TTMV	00/10	0.193	-.047
	TTMDV	TTMDV	00/10	0.217	-.048
SEN Viruses	SENVH	SENVH	4/10	0.677	-.022
	SENVD	SENVD	2/10	0.861	-.021

Characterization of SENV and Anelloviruses inHepatocellular Carcinoma Patients

Characterization of SENV and Anelloviruses showed thata total of 150/150 HCC patients were infected with SENVH and 72/150 with SENV D while 0/150 were infected with Anelloviruses

Characteristics	Name of the viruses	Infected/ total	P-Value	95% Confidence Interval	
				Lower Bound	Upper Bound
Anelloviruses	TTV	TTV	00/10	0.338	-.054
	TTMV	TTMV	00/10	0.193	-.047
	TTMDV	TTMDV	00/10	0.217	-.048
SEN Viruses	SENVH	SENVH	4/10	0.677	-.022
	SENVD	SENVD	2/10	0.861	-.021

DISCUSSION

Hepatocellular carcinoma is one of death causing cancer around the globe (Janevska et al., 2015). Mostly causes of the HCC are related with CHB virus and CHC virus (Momosaki et al., 2005). Based on previous data, hepatobiliary cancers represent 10.7% of all cancer. HCC is considered the common cancer in adult males. According to a recent report the age standardized rate for hepatocarcinogenesis in Pakistan is 7.6 out 100,000 persons per year for men and 2.8 for women (Baker et al., 2016). Both pathogenic and non-pathogenic viruses are reported to be the etiological factors for different types of disease like HCC, Celiac and Leukemic. The severity of infection can be influence in patients if they have co-infection with other pathogenic viruses, such as HEV, HSV, VZV, EBV, CMV, HPV and some non-pathogenic viruses, such as the SEN viruses and Anelloviruses (Abbasi et al., 2016).

The current study found that in case of normal population, no one individual were positive for Anello and Sen Viruses. In opposite to the currents study, a highest prevalence rate of Anelloviruses 66.6% of TTMV, 76.3% of TTMDV and 85.2% of TTV were reported by (Al-Qahtani et al., 2016). Some of the reports on single Anellovirus detection showed various prevalence rate

such as 4% prevalence rate of TTV, 92.5%, 23.7%, 66.9% (Shoeib et al., 2011), and 41% (Bouzari et al., 2007) and lowest as 2.9% by (Doosti et al., 2011) In healthy individuals from Iran. While some of the reports detected the presence of SENV in healthy individuals like 10% from China by (Tang et al., 2008), 5.4% and 17.4% Prevalence rate of SENVH and SENVD among healthy blood donors. While 24% SENV-D and SENV-H reported in Greece (Umemura et al., 2003) and 23.08% by (Sharifi et al., 2008).

Celiac disease is complex autoimmune disorder (Nanayakkara et al., 2018) mainly caused due to the intolerance of a specific peptide that is present in plants, including barley, wheat and rye (Bay et al., 2013). According to our study TTV was detected 8.33%, TTMV was 18.75% and TTMDV was 18.75% in celiac patients. The previous studies had shown link of TTV DNA with pro-inflammatory cytokines, especially interferon gamma and TLR9, augment viral replication (Rocchi et al., 2009). Celiac patients have high level of TLR9 and interferon (Moossavi, 2014). According to the above information the incidence of TTV should have been high in celiac disease patients but it is less. This study also found SEN virus H (8.33%) while SEN virus D (10.41%) in celiac patients. Interferon play a vital role in the onset of celiac disease (Ikizler et al., 2017) and according to the previous studies, interferon therapy effect on Sen D and H virus infection in HCV patients.

Leukemia, a malignant disorder, encompasses abnormal production of immature WBCs that dominate the level of normal functional WBCs. It is a multifactorial disorder in which viral, environmental and genetic characteristics are involved (Luo et al., 2015). The detection of AVs revealed 13.33% of TTV and 30% each of TTMV and TTMDV were present in leukemic patients. While the investigation of SEN viruses showed 13.33% SEN-H and 16.66% of SEN-D incidence rate among leukemic patients. Corresponding to our results 30% of incidence rate of TTV in B-cell lymphoma and 50% in non-Hodgkin and Follicular lymphoma were reported by Garbuglia et al., 2003 which indicates that TTV might act as helper virus for lymphoma progression. Likewise, a study reported 15.8 % and 30% TTMV in Hodgkin Lymphoma and non- Hodgkin lymphoma serum samples (Pan et al., 2018) while 55% of prevalence rate of TTMDV in tumor samples by (Salmanizadeh et al., 2012) which is a very high percentage as compared to our studies. SENV is previously associated with various liver pathologies and hepatic carcinomas whereas no data is reported for the prevalence of SENV in leukemic patients till now. In our study, AVs were not found in HCC samples however the entire 100% samples were positive for SENV-H and 48% for SENV-D. The incidence of SENV-H was two to three times higher than SENV-D. Our results are consistent with the findings reported from Japan that showed SEN-V DNA among HCC patients was 42% but greater than the findings reported among Canadian HCC patients which showed the frequency of SEN-V DNA was 32% (Momosaki et al., 2005). These results are contradictory to results from a study in Thai population, which showed the frequency of virus is 19% among HCC patients (Tangkijvanich et al., 2003). There is association that SEN-V infected patients were also co-infected with HCV and HBV. In our results patients infected with SEN-V also had an infection of either HBV or HCV. This is because the prevalence of SENV varies geographically and all these viruses are blood borne; share similar mode of transmission and the transmission of HVC and HBV could also transmit SENV in a risk population. Possible role of SENV as an additional factor in the progression of these HCC cannot be find out. From our results, it is showed that SENV does not have any additional role rise the risk of development of HCC in patients who were already suffering with HCV and HBV. This is because infection of SEN-V is not involved in the etiology of HBV or HVC associated HCC.

Moreover, in our results the incidence of SENV-H and D in HCV patients was 40% and 20% respectively, similar findings of 46% were reported in Iran (Hosseini & Bouzari., 2016). Several studies reported that SENV is not involved in pathogenesis of chronic liver disease. In a recent study in Egypt, it has been reported that presence of SENV might have a protective role in the progression of HCV. On the other hand, a recent Indian study suggested that SENV is capable of causing liver damage (Abu-basha et al., 2012). The finding of various studies suggesting that SENV does not either increase the severity of the disease condition or act as a co-factor in the development of cirrhosis.

CONCLUSION

It is concluded from the current study that the presence of an infection/disease may open a clue for the establishment of non-pathogenic viruses like SENV and Anelloviruses. Furthermore, the presence of non-pathogenic viruses might exaggerate the pathogenesis of present disease in the host.

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CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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