ISSN No:-2456-2165

Comparative Analysis of Human Gyroviruses in Hepatocellular Carcinoma Patients in Pakistan

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Abstract:- Hepatocellular carcinoma (HCC) is the sixth common type of cancer worldwide. Besides the involvement of HCV, HBV and Alcohol intake various non-pathogenic viruses like Anelloviruses and SEN virus are also reported from HCC and various other diseases. The human gyro virus, inducing an immunosuppressive disorder in chickens however investigated in case of various diseased and normal individuals.

Methodology: The current meta-analysis study collected the data of three researchers who analyzed the presence of SEN viruses, Anelloviruses and human gyro viruses. Statistical analysis were performed and p<0.005 were define as statistically significant.

Results: Evaluation of total 42 normal individual showed the absence of SEN viruses, Anelloviruses and human gyro viruses. A total 62.8% (22/35) prevalence rate of SENV among Dengue infected patients however HCV, HBV, Anelloviruses and human gyro viruses were not found. Among Hepatitis B infected individuals 13.8% (9/65) were found co-infected with SENV and 33.8% (22/65) have Anelloviruses infections however infection with other viruses like HCV and human gyro viruses were not observed. Among HCC infected individuals 5% were positive for HBV and 82.5% for HCV however the other non-pathogenic hepatotropic viruses like SEN, Anelloviruses and human gyro viruses were not present.

Conclusion: The current study concluded that the presence of any infection/disease flatten the curve for the co-infection of various non-pathogenic hepatotropic viruses like SENV and AVs however the human gyro viruses is independent of any infection.

Keywords:- *Hepatocellular Carcinoma, Hepatitis C Virus, Hepatitis B Virus, Human Gyro Virus.*

I. INTRODUCTION

The most common primary liver malignancy is Hepatocellular carcinoma (HCC) and it is the prominent reason of death related cancer in the world. In the United States, The leading cause of HCC cancer death is ninth (O'Connor et al., 2010). Mainly chronically liver tissues damaged occur due to hepatocellular carcinoma because of the chronic regenerative and the inflammatory phenomena that take part to the beginning and/or spreading of 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). Intrahepatic bile duct cancer and 30,640 total number of new liver expecting occur in 2013 also in the inclusion of 21,670 deaths (Crissien & Frenette, 2014).

As compare to female HCC occurred more common in male subject (1:2.4), with a greater frequency in Southern Asia and Eastern Asia, Middle and Western Africa, Micronesia/Polynesia 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. Thus, 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,

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 followed by black, white and Hispanics. (Altekruse et al., 2009), the human gyrovirus belongs to the family Circoviridae, which consists of a chicken anemia virus (CAV) that causes chickens to have an immunosuppressive disorder. From the skin swab of healthy volunteers a virus that shows strong CAV homology has recently isolated and has been identified as human Gyrovirus or HGyV. At present, two viruses, such as HGyV and avian gyrovirus 2, have been examined in 4% of normal individuals on the outside of the epidermis and in the serum of infected chickens (Sauvage et al., 2011). The HGV genome organization is identical to CAV, which involves three open reading frames that partly overlap, encoding multiple proteins. A single-stranded DNA virus that is CAV that encoded three structural proteins: VP1 (capsid protein), VP2 (scaffold and phosphatase protein) and VP3 (Noteborn & Koch, 1995). The HGV vp3 (i.e. apoptin) protein with its CAV homologue has 4 an estimated 40 percent identity (Akbari et al., 2019). These two viruses have a close interaction with each other, but the protein sequences vary by just 3-7 percent (VP1-3). Via viral metagenomic study, HGyV was detected in human faeces and explored its association with unedified diarrhea. The cause of HCCC is also thought to be human gyrovirus (Phan et al., 2012), this virus, in particular, apoptosis cancerous cells has specially produced by protein encoding the homolog of CAV apoptin. In lessional Sample of skin 5 in 115 the HGyV was found by screening of PCR. A total of 0 out of 92 fecal specimens and 0 out of 92 bronchoalveolar lavages. The chicken anemia virus Apoptin/VP3 produced a protein from (CAV-Apoptin) that has a significant capacity to produced tumor apoptosis in a different human cancer cells (Guelen et al., 2004). It has been shown that Apoptosis is caused in large diversity of cancer cells by the expression of Apoptin, including hepatoma, osteosarcoma, melanoma, cholangiocarcinoma, carcinoma of the colon, lung cancer, breast cancer, prostate cancer, cancer of the cervix, and gastric cancer, with no impact on normal cell death (Noteborn, 2005). 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 population of 182,142,594. Pakistan is a low-income State, when compared with noble states Pakistan lag behind in several important factors of healthcare. 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). 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).

II. MATERIAL AND METHODS

The current study was performed at Molecular Virology Laboratory (MVL), Department of Biosciences, and COMSATS University Islamabad and Institute of Chemical Sciences University of Peshawar, Pakistan. Molecular characterization of different non-pathogenic viruses including Human Gyro viruses, SENV and Anelloviruses among various diseases like dengue virus infected patients, Hepatitis B virus patients were studied. Research performed for the detection of HBV, HCV, SEN (SENVH and SENVD) viruses, and Anelloviruses (TTV, TTMV, and TTMDV) in various disease patients and normal individuals were included in this study while Research performed for other than HBV, HCV, SENV and Anelloviruses were excluded from the current study.

- *Detection of HBV, HCV, SEV and Anelloviruses in Healthy Population*

According to the study the detection of HBV, HCV, 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 42 normal individuals were studied for the SEN (SENVH and SENVD) viruses and Anelloviruses (TTV, TTMV, TTMDV) which were analyzed Meta analytically in the current study. HBV and HCV viruses were also analyzed Meta analytically in the absence of normal individuals.

- *Detection of HBV, HCV, SENV and Anelloviruses in Various Diseases*

A total of 140 infected individuals from various diseases including 35 dengue virus infected patients, 65 HBV positive patients, and 40 HCC positive patients were analyzed for HBV, HCV, SEN (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 difference was considered when p value was less than 0.05 ($p< 0.05$).

ISSN No:-2456-2165

III. RESULTS

The sixth typical form of cancer is Hepatocellular carcinoma (HCC) throughout the world. Besides the involvement of HCV, HBV and Alcohol intake various non-pathogenic viruses like Anelloviruses and SEN virus are also reported from HCC and various other diseases. The human gyro virus, inducing an immunosuppressive disorder in chickens however investigated in case of various diseased and normal individuals.

- *Evaluation of SENV, Anelloviruses, HCV and HBV in Normal population*

The presence of HBV, HCV, SEV, Anelloviruses and human gyro viruses was not found in normal individuals.

Table 1 Evaluation of SENV, Anelloviruses, HCV and HBV in Normal population

Characteristics	Name of the viruses	Infected/Total	P Value	95% Confidence Interval	
				Lower Bound	Upper Bound
AV	TTV	00/42	0.000	-.400	-.227
	TTMV	00/42	0.000	-.392	-.235
	TTMDV	00/42	0.000	-.390	-.237
SENV	SENVH	00/42	0.002	-.510	-.117
	SENVD	00/42	0.001	-.499	-.128
HB	HBV	00/42	0.109	-.697	.070
	HCV	00/42	0.000	-.418	-.209

- *Identification of SENV, Anelloviruses, HCV and HBV in Dengue Patients.*

Among the entire dengue disease patients 0/35 were positive for SENV 22/35 were found positive for Anelloviruses, 0/35 were found for HBV and 0/35 for HCV.

Table 2 Identification of SENV, Anelloviruses, HCV and HBV in Dengue Patients.

Characteristics	Name of the viruses	Infected/total	P Value	95% Confidence Interval	
				Lower Bound	Upper Bound
AV	TTV	4/35	0.618	-.113	.068
	TTMV	9/35	0.503	-.054	.110
	TTMDV	9/35	0.701	-.064	.095
SENV	SENVH	0/35	0.351	-.301	.107
	SENVD	0/35	0.324	-.290	.096
HB	HBV	0/35	0.634	-.497	.303
	HCV	0/35	0.081	-.206	.012

- *Identification of SENV, Anelloviruses, HCV and HBV in HB Patients.*

Out of 9/65 HB patients showed presence of SENV, 22/65 have Anelloviruses infections, 0/65 were found for HBV and 0/65 were found for HCV.

Table 3 Identification of SENV, Anelloviruses, HCV and HBV in HB Patients.

Characteristics	Name of the viruses	Infected/ total	P Value	95% Confidence Interval	
				Lower Bound	Upper Bound
AV	TTV	4/65	0.002	-.293	-.066
	TTMV	9/65	0.014	-.232	-.026
	TTMDV	9/65	0.005	-.241	-.042
SENV	SENVH	4/65	0.059	-.010	.502
	SENVD	5/65	0.015	.060	.544
HB	HBV	0/65	0.320	-.755	.247
	HCV	0/65	0.000	-.390	-.117

- *Characterization of SENV, Anelloviruses, HCV and HBV in HCC Patients*

Characterization of SENV, Anelloviruses, HBV and HCV showed that a total of 0 /40 HCC patients were infected with SEN, 0/40 were infected with Anelloviruses, 2/40 were infected with HBV and 33/40 were infected with HCV.

ISSN No:-2456-2165

Table 4 Characterization of SENV, Anelloviruses, HCV and HBV in HCC Patients

Characteristics	Name of the viruses	Infected/Total	P Value	95% Confidence Interval	
				Lower Bound	Upper Bound
AV	TTV	0/40	0.039	-.073	-.002
	TTMV	0/40	0.023	-.069	-.005
	TTMDV	0/40	0.019	-.068	-.006
SENV	SENVH	0/40	0.360	-.117	.043
	SENVD	0/40	0.334	-.113	.038
HB	HBV	2/40	0.000	.806	1.119
	HCV	33/40	0.000	.920	1.005

IV. DISCUSSION

The infection with SENV and AV among different diseased patients is reported throughout the World, however there is contradiction in the involvement of SENV, AV and human gyro viruses in disease progression. (Reyes, A. 2013). The prevalence of these viruses is found high in various other diseases like HCV, HBV and HIV infected individuals however there is still lack of data about the involvement of these non-pathogenic viruses in the progression of disease and its involvement in the increasing pathogenic effect in other diseases. 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. The current meta-analysis study of various researcher is an attempt to investigate the relationship of various non-pathogenic viruses with normal and various diseased patients.

In the current study of meta-analysis, the occurrence of the Anelloviruses, SENV and human gyroviruses among normal individuals was not detected anywhere in-contrast to the study of a highest prevalence rate of aneloviruses 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 anelovirus 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 arte 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). Dengue is a public health hazard and a viral infection that is caused by a vector *(Aedesagypti)* (Chaturvedi et al., 2018). Dengue shock syndrome (DSS) and the Dengue hemorrhagic fever (DHF) are the most severe form of this disorder (Syenina et al., 2015). The currents study observed AV's among dengue subjects as, 11.42% prevalence of TTV, 25.71% prevalence of TTMV and 25.71% prevalence of TTMDV. The existence of SEN viruses and human gyroviruses was not detected in the current study. The higher percentage of Aneloviruses like TTV in dengue patients suggested that it might be involved in the increased pathogenic effect of dengue so far, having high prevalence rate. According to a study conducted on dengue individuals showed that the percentage and frequency of TTV rises in plasma of the patient's increases, as the temperature of the fbrile patients increased (McElvania TeKippe et al., 2012). As in dengue, temperature also rises and it should raise the percentage of TTV however the higher percentage of TTMV in the current study was in coincide with the previous study conducted on the prevalence of TTMV in HIV and HCV positive groups previously it was also found that there is a low percentage of TTMV in plasma and NP specimens (McElvania TeKippe et al., 2012). Comparing our current results with previous studies we can assumed that TTMV can only be prevalent in diseases which have viral etiology. The presence of TTMDV in dengue patients of the current study suggested that TTMDV was prevalent in diseases with viral as well as non-viral etiologies (McElvania TeKippe et al., 2012). In present study less prevalence rate of TTV in comparison with previous studies may suggest that previously TTV might not be involved in any pathogenesis or causing inflammation and hence this genotype would be easily escaping immune responses. But now, in our study its prevalence is indicating that TTV may be causing any inflammation in the body and then caught by immune system which ultimately blocks it replication.

All over the world the one of the death causing cancer is Hepatocellular carcinoma (HCC) (Janevska et al., 2015). Mostly causes of the HCC are associated with the chronic HBV and HCV (Momosaki et al., 2005). This study observed that majority of the hepatocellulatr carcinoma patients was HCV infected (82.5%), followed by 5% of HBV however no incidence of Anelloviruses, SEN viruses and human gyroviruses which validates the observation of (Bhatti et al., 2016) reported that HCV infection and not HBV is known to be the major cause of HCC especially in Pakistan. However, in other Asian countries for HCC one of the common risk factor is HBV. Our study also confirmed HCV positive infection in the majority of HCC patients. According to Farhat et al., 2014 no vaccine are yet been available in Pakistan for HCV due to its error prone replication and generation of multiple genomic types as it is an RNA virus. However, HBV vaccines are available in Pakistan.

One of the most common cause of cause of chronic liver infections among humans due to HBV, has become main global health anxiety (Cheung et al., 2019). Asia is reported to be endemic for HBV infection (Wait et al., 2016). The current study found that 6.15% of the HB patients were also infected by TTV, 13.84% by TTMV and TTMDV however among SEN Viruses, SENVH (6.15%) and SENVD (7.69%) was found while absence of human gyroviruses found among Hepatitis B Virus infected patients. In the previous studies, association between TTV Hepatic disorders like non A-E viral hepatitis, transfusion associated hepatitis, cryptogenic chronic liver disease, liver cirrhosis; hepatocellular carcinoma was observed (Kao et al., 2002). Persistent TTV infections in patients can lead to hepatic failure in the patients. The results suggest that replication of TTV occurs mainly inside the Hepatocytes and has major role in HBV pathology.

V. CONCLUSION

The current study concluded that the presence of any infection/disease flatten the curve for the co-infection of various non-pathogenic hepatotropic viruses like SENV and AVs however the human gyro viruses is independent of any infection.

❖ *Author Contributions*

All the authors have accepted responsibility for the entire content of this submitted manuscript and have approved its submission.

❖ *Conflict of Interest*

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

❖ *Funding*

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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