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Original Article

Molecular epidemiology of Hepatitis C virus genotypes and clinical relevance in Sindh, Pakistan.

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Abstract:
Introduction: Hepatitis C Virus (HCV) is a common viral infection throughout the world. It is estimated that 3% (around 170 million) of world population is affected by HCV including around 17 million from Pakistan.
Objectives: To obtained definite information on connections between the genotypes and both viral burdens and biochemical markers.
Methodology: This lab-based retrospective study was done from February 2017 to August 2017 at Diagnostic and Research Laboratory; Civil Hospital Hyderabad. Complete data of 261 HCV seropositive patients was collected.
Results: Out of these 261 patients of HCV, 157 (60.1%) were females and 104 (39.8%) were male. The age between 15-50 years bears the biggest number (52.49%) of HCV patients. Genotype PCR investigation of hepatitis C infection showed that 13 (14%) were un-type capable for any genotype. Among the rest tests, the genotype 3a has been observed to be the most transcendent strain in the populace (76%). Different strains recognized were 2a (4.9%), 1a (2.29%) and 1b (2.6%). Genotype 1b was related with the most noteworthy normal viral burden. The largest number of patients were from Hyderabad 151 (57.8%) and least number of patients (1) were found from Bano Aqil. The connection between HCV genotypes and biochemical markers shows prothrombin time was discovered brought up in all genotypes and egg whites' level is low just in genotype 2a.
Conclusion: It is reasoned that the HCV disease is as yet an extraordinary issue so it is prescribed to take further review, need of mindfulness and interferon treatment anticipation and HCV genotyping which is the need of 60 minutes.

Key Words: HCV, Genotype, PCR.

Introduction:

Hepatitis C infection (HCV) is a remarkable wrapped single abandoned RNA infection of flaviviridae family. It was distinguished in 1989 as the causative specialist of most instances of posttransfusion and inconsistent intense and persistent viral hepatitis. HCV is broadly common all through the world and it is assessed that 3% of the populace, around 170 million individuals have constant HCV world wide[1]. HCV disease is among dangerous general medical conditions around the world, with more than 170-200 million contaminated individuals including around 17 million from Pakistan.[2, 3]

The commonness of HCV is 0.5 to 8% in blood givers in various pieces of the world. Studies from Pakistan on blood givers while 4% and 8% for sound family and volunteer blood givers separately. Reports from different investigations show 51% seropositivity of hepatitis C in ongoing liver infection, 57% in cirrhosis and 14% in hepatocellular carcinoma. As per ongoing information hostile to HCV predominance in obstetric cases is 6.6% and 5.31% in everyone. The current pervasiveness in Pakistan shifts from 3 to 7% in various pieces of the country. [1]

The primary driver of HCV transmission in Pakistan is various utilization of needles, minor and significant medical procedure, dental methodology, blood and blood item bonding, utilizing of razors for shaving in the shops of stylists, sharp instruments, nail cutters, tooth brushes and sexual transmission.[4]

Constant HCV contamination as often as possible prompts liver cirrhosis and is related with and raised danger of movement into hepatocellular carcinoma (HCC). Epidemiological reviews have distinguished HCV in 10% to 80% of HCC patients revealed in various populaces. HCV has likewise been accounted for to be the significant reason for HCC in Japan, Italy and Spain, yet is less significant in South Africa and Taiwan. Relationship of HCV contamination with HCC has likewise been very much archived in the United States.[3] HCV can prompt HCC through oxidative pressure, insulin resistance (IR), fibrosis, liver cirrhosis and HCV instigated steatosis. [5]

The HCV genome roughly 10kb long and comprise of a solitary open perusing outline encoding a polypeptide chain of 3000 AA. It comprises of (center, E1, E2 and perhaps P7) proteins and nonstructural (NS2, NS3, NS4A, NS4B, NS5A and NS5B) viral proteins. HCV center is known as the inducer of steatosis, oxidative pressure and HCC. E1 and E2 are associated with infection connection with cells and are considered to the main viral protein comes in counted with the cell. P7 is conceivably worried about particle channel and infection gathering. Distinctive HCV disengages from around the world show generous nucleotide arrangement inconstancy all through the viral genome. In view of the ID of these, genomic contrasts, HCV has been ordered into numerous strains.[5-7] HCV has six genotypes and 52 subtypes predominantly genotypes in the distinctive district of the world are (1a, 1b, 1c, 2a, 2b, 2c, 3a, 3b, 4a, 5a, 6a). Because of nonattendance of editing capacity of the RNA – subordinate RNA – polymerase (NS5B), HCV has a high transformation rate and exists as hereditarily heterogeneous semi species in individual patients. The hereditary variety is over 30% in various genotypes and 20% in subtypes. Varieties in amino corrosive grouping of various HCV genotypes cause contrast in seriousness of pathogenesis. Late investigations have shown variable reactions for interferon (IFN)- ribavirin blend treatment, oxidative pressure/ steatosis and insulin obstruction because of the amino corrosive replacements in the HCV center area of various HCV genotypes.[5]

It is believed that hereditary heterogeneity of HCV might represent a portion of the distinctions in illness result and reaction to treatment noticed the job of genotypes in liver sickness movement or reaction to interferon therapy. [6]

This review was done to record dispersion of HCV genotypes and its subtypes from various urban communities of Sindh (the second most vigorously populated area of Pakistan) and to get more itemized information on connections between the genotypes and both viral burdens and biochemical markers.

Methodology:

Study plan: This lab based observational and graphic review did from February 2017 to August 2017 at Sindh, Pakistan.

Study region and population: In the current review the information was gathered from various urban areas of Sindh.

Sample size: All out 261 patients were gathered by helpful/standard testing strategy.

Inclusion criteria: An incorporation model for patients was to be seropositive for against HCV by third era Enzyme Linked Immunosorbent Assay (ELISA). The data with respect to age, sex and clinical history and birth was acquired from each taking part tolerant.

Exclusion criteria: HCV negative patients were excluded.

Examining and blood screening: After fundamentally assessing the entirety of the moral and examination related issues, the morals board of the exploration Center supported the review. The serum tests were gathered from the patients and an explicitly planned structure was filled to get the patient's assent and the necessary demographical/clinical data. From the get go, all the serum tests were evaluated for HCV antibodies utilizing against HCV ELISA unit of Abbott organization. Just the Anti HCV positive serum tests were remembered for the information alongside the structure containing clinical/clinical history and assent of the patients.

HCV RNA extraction and discovery PCR: Settled opposite record (RT) PCR was accomplished for the subjective recognition of HCV RNA utilizing groundworks that compare to the moderately continuous 5'UTR non coding area of the profoundly changeable HCV. So, HCV viral RNA was extricated from 200 microliter of serum test by utilizing viral RNA extraction unit. Settled PCRs were executed with Taq polymerase and one more arrangement of groundwork. The intensified items for each example were envisioned on 2% agarose gel colored with ethidium bromide over an UV trans illuminator to recognize the particular HCV PCR groups. On discovery of HCV presence, the separate PCR positive examples were additionally continued for HCV genotyping.

HCV genotyping: HCV genotyping was finished by utilizing a particular HCV genotyping convention by utilizing Abbott Real Time HCV Genotype II Assay Kit. This pack gives mechanized example planning utilizing an attractive miniature molecule-based convention to deal with 0.5 ml tests (ACD-A, CPD, Potassium EDTA of sodium EDTA Plasma or Serum). The Abbott m2000 Realtime System (4x24 Preps) utilized attractive molecule innovation to catch nucleic acids. The nucleic acids are then prepared for enhancement. The Abbott Real Time HCV Genotype II Assay utilized four arrangements of PCR preliminaries. One bunch of preliminaries focuses on an arrangement inside the 5' untranslated area (UTR) of the HCV genome. The subsequent preliminary set is intended to intensify the NS5B area of genotype 1a. The third HCV groundwork set enhanced the NS5B area of genotype 1b. During the intensification response, the objective RNA is changed over to cDNA by the converse transcriptase movement of the thermostable rTth DNA Polymerase. Outstanding intensification of the item is accomplished through continued cycling among high and low temperatures, bringing about a billion overlay or more noteworthy enhancement of target successions.

Biochemical markers: Clinical chemistry packs (SGPT Kit) and programmed chemistry analyzer (Cobas - C 501) were utilized to investigate biochemical markers like bilirubin and SGPT. Programmed Analyzer Sysmex Ca 500 was utilized for PT and APTT. Analyzer 501 was utilized for egg whites and Cobas – C 601 was utilized for Alpha Fetoprotein.

Moral concern: The data collected after written permission from Ethical review committee and a composed assent was gotten from every person before cooperation in the review.

Statistical analysis: The information investigated utilizing the Microsoft Excel 2010. All quantitative information presented as mean ± standard deviations.

Results:

During period of study, 261 HCV positive patients having had HCV-RNA PCR done; at Diagnostic & Research Laboratory LUMHS; 157 (60.1%) were females and 104 (39.8%) were males. The patients with respect to age were grouped into four groups. The age between 15-50 years bears the biggest number (79.31%) of patients, The age stratum of 15-30 and 41-50 years showed 26.8% in each stratum as shown in table 1.

Table 1: Age and gender analysis (n=261)

Age groups (years)	No of patients (%)	Males (%)	Females (%)
15-30	70 (26.8)	25 (24.2)	45 (28.4)
31-40	67 (25.6)	28 (27.1)	39 (24.6)
41-50	70 (26.8)	30 (29.1)	40 (25.6)
51-65	54 (20.6)	20 (19.4)	34 (21.5)
total	261	103 (39.4)	158 (60.5)

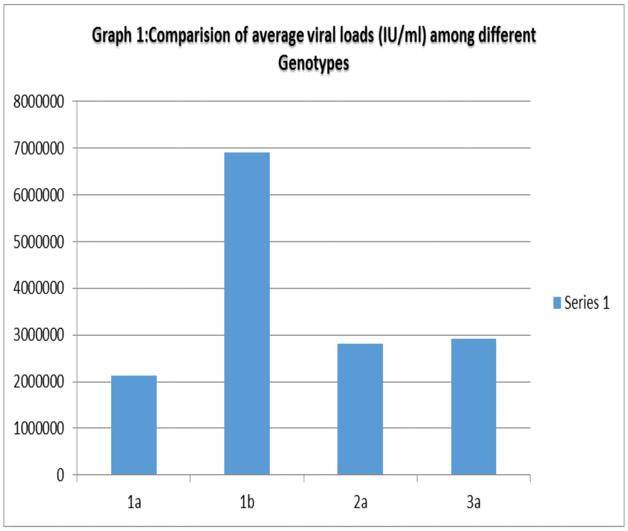
The mean age of patients was 39.5 years ±12.7. Overall female outnumbered male in all age groups. Genotypic PCR examination for hepatitis C infection for 261 HCV positive patients was done. The results show that out of 261 patients, 13 (14%) were un-typeable for any genotype (Table 2).

Table 2: Results of HCV genotypes determination in HCV infected patients (n= 261)

HCV Genotypes	No of patients	Frequency (%)
1a	6	2.29
1b	7	2.6
2a	13	4.9
3a	199	76.2
Un-typeable	26	13.7
Total	261	

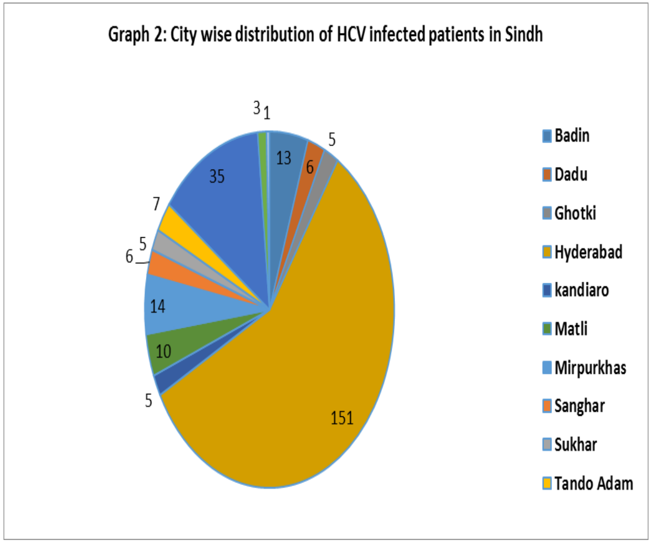
The genotype 3a was identified as the most prevalent strain (76%). Other strains identified were 2a (4.9%), 1a (2.29%)and 1b (2.6%). We classified viral burdens in most minimal (12 IU/ml) and most noteworthy (65,757,000 IU/ml). We identified huge contrasts in viral burdens, as genotype 1b was related with the most elevated normal viral burden (Graph 1).

Graph 1: Average Viral Load.



Graph 2 demonstrates the city wise prevalence of HCV positive patients. It is found that the highest number of patients (151,57.8%) were from Hyderabad 151 (57.8%) and least number of patients (1, 0.38%) found from Pano Aqil.

Graph 2: City wise distribution of HCV infected patients.



The relation between HCV genotypes and biochemical markers such as, bilirubin, serum glutamic pyruvic transaminase (SGPT), prothrombin time (PT), activated partial thromboplastin time (APTT), serum albumin and alpha fetoprotein. None of these biochemical parameters except PT and albumin showed difference between these groups of HCV genotypes (Table 3). The data and the mean with standard deviation of all biomarkers are shown in the table. In this prothrombin time was found raised in all genotypes and albumin level is low only in genotype 2a.

Table 3: Showing the biochemical profile of HCV patients among different genotypes (n=261)

	1a n=6	1b n=7	2a n=13	3a n=199	UT n=36
Bilirubin	0.42 ± 0.049	0.8 ± 0.662	0.7± 0.767	0.68 ± 0.592	0.6 ± 0.614
SGPT	37.5 ± 7.778	41.5 ± 1.732	36.5 ± 5.0479	38.2 ± 4.504	36.5 ± 4.116
PT	15.7 ± 2.217	14.2 ± 2.217	15.4 ± 1.988	15.3 ± 1.771	14.9 ± 1.910
APTT	33.5 ± 0.707	33.0 ± 1.414	33.8 ± 1.345	34.05 ± 1.820	34.0 ± 1.617
Albumin	3.48 ± 0.355	3.5 ± 0.350	3.0 ± 0.508	3.5 ± 0.410	3.7 ± 0.396
Alpha Feto-protein	5.6 ± 6.160	6.8 ± 6.965	2.11 ± 0.262	3.9 ± 3.461	9.4 ± 3.814

Discussion:

The fundamental point of this review was to discover appropriation of HCV genotypes and its subtypes in Sindh and to procure connections between the genotypes and both viral burdens and biochemical markers. The appropriation of HCV genotypes changes as per the geological districts. Genotypes [1-3] are broadly disseminated all through the world[8]. We have examined the relationship between predominance of HCV genotypes in various age gatherings and sex. Results showed that there is larger number of females (60.1%) as compared to males (39.8%), a finding consistent with the published literature.[9] We noticed a high occurrence of HCV among age bunches 15-30 and 41-50 years (26.81%). Then again, the most minimal number of cases were found in 51-65 years

(20.6%). These findings are in concurrence with results of a review in which high predominance was seen in more than 40 years of age[10] and comparable findings were likewise seen by Ali et al[11] and Nabi et al[12]. In any case, results of current study repudiate from that of Riaz et al[9] that high predominance rate in Pakistan was found in advanced age bunch individuals. These findings recommend that early analysis of HCV may be because of the familiarity with public with regards to HCV contamination in this part of the world. So far various investigations have been done to discover the frequencies of different HCV genotypes in various geological districts of Pakistan and the most predominant genotype found is 3a[3, 7,11.] Information Current study also find that genotype 3a (76.2%) has high pervasiveness rate followed by untypeable (14%), 2a (4.9%), 1a (2.29%) and 1b (2.6%). Our example of HCV genotypes dissemination is similar to that from neighboring countries like Iran and India[8, 13] where overwhelming genotype is 3. Hence future counteraction and treatment system ought to be coordinated towards type 3 HCV basically, without ignoring type 1 and 2. However, results of this study are in contrast to that of Kumar J P et al[14] in which genotype 1 was dominating, trailed by 3.

Viral genotype and viral burden are two significant prognostic factors, information on which may be valuable in the treatment preferences. Viral burden is by all accounts is significant as prescient marker for the result of antiviral treatment, since SGPT levels don't really reflect infection severity. Indeed, patients with high popular burden present a helpless reaction to interferon treatment than those with low level viraemia. The connection between HCV genotypes and viral burden stays argumentative; in certain examinations, high titer viremia was associated with cutting edge liver stage.[15,16] In the current review the viral burden in patients with genotype 1 was fundamentally higher that those with genotype 2 and 3. This perception is likewise found in a review by Chakravarti et al from India[8]. This may be because of more proficient viral replication of genotype 1 when contrasted with others.

The biggest number of instances of Hepatitis C were found in Hyderabad city followed by Tando Jam and different urban areas. This enormous number may be the direct result of available Laboratories in these urban communities to analyze the cases. Least number of cases were from Pano Aqil.

Serum biomarkers investigation was done to find any connection of these compound biomarkers to HCV genotypes 1, 2 and 3. The investigation shows low egg whites level in genotype 2a in our review, which is same as in one review conducted by Swaff R et al[17], yet this review finding was negated by Melo I C et al[18]. Prothrombin time was brought up in all HCV genotypes.

Conclusion:

It is concluded that dynamic hepatitis C infection disease are high in Sindh. Patients in age groups between 15-30 and 41-50 years and females are found with high pace of HCV contamination. The most common HCV genotypes in Sindh being 3a trailed by 1a, 1b and 2. Genotype 3 is related with most minimal viral burdens and disease by genotype 1b is related with most elevated viral burdens.

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