

Meta Analysis

Genomic landscape of hepatitis C virus in India: a systematic review and meta-analysis

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ABSTRACT

In India, the prevalence of hepatitis C virus (HCV) infection ranges from 0.5% to 1.5%, with most infected individuals being asymptomatic and without jaundice. HCV is responsible for approximately 25% of liver-related disorders, including hepatocellular carcinoma (HCC) and liver cirrhosis, and is increasingly recognized as a cause of chronic liver diseases. Populations at high risk for HCV transmission include intravenous drug users (IDUs), renal transplant recipients, dialysis patients, and those receiving blood products. HCV is categorized into seven genotypes, each with various sub-genotypes, playing a crucial role in epidemiology and outbreak investigations. An extensive literature search was conducted in Scopus, PubMed, and Web of Science from January 2003 to December 2023, using keywords such as 'hepatitis C' and 'genotype' along with MeSH terms for refinement. Out of 739 retrieved articles, 20 articles fulfilled the inclusion criteria for analysis, with the mean age of participants being 41±4. A statistically significant difference ($p<0.001$) was found between gender and sample size. Notably, genotype 3 was most prevalent (45%), while genotype 1 and 3 showed equal prevalence (26%) in the North-Eastern region. It is essential to implement and monitor government initiatives like NVHCP to enhance understanding and public health regarding HCV.

Keywords: Hepatitis C virus, Indian subcontinent, Viral genotypes, Systematic review, Meta-analysis

INTRODUCTION

Hepatitis C virus (HCV) infection is an emerging cause of chronic liver diseases, which counts approximately 25% of liver-related disorders in India.¹ The chronically infected individuals are at high risk of developing long-term complications such as HCC and liver cirrhosis.^{2,3} The common route of infection is parenteral transmission, which includes individuals who receive blood and blood products, renal transplant and dialysis patients, and IDUs.³⁻⁶ The high circulating viral titer can be transmitted perinatally from mother to their neonates.^{4,7,8} HCV is a positive-sense single-stranded RNA virus with a genome of 9400 nucleotide bases from *Flaviviridae* family.⁹ The incubation period of HCV infection can range from 2 to 26 weeks. HCV-RNA can be detected in plasma/serum by

polymerase chain reaction (PCR) between the initial exposure and a week period.¹⁰ Most of the HCV-infected individuals are anicteric and asymptomatic. After an initial exposure of a week or months, during which a low viral titer and undetectable antibody response, known as "silent period", it takes about 3 months to show positive seroconversion of HCV antibodies in the majority of infected individuals, which may extend up to 6 months in some cases.^{2,10}

A published report shows, the HCV prevalence in India is between 0.5% to 1.5%.¹¹ Based on the genomic variation in the viral genome, the virus is categorized into 7 genotypes and several sub-genotypes.¹² A diverse variation in geography has been observed with HCV genotypes. In European countries and the United States, the

predominance of genotype 1a, 1b, and 2a has been reported, whereas in the Middle East, genotype 4 is predominant. The genotypes 5 and 6 are mostly found in South Africa and Southeast Asia.¹³ The most detected HCV genotype in India is genotype 3, with its sub-genotypes 3a and 3b.^{9,14,15} The region-wise studies conducted in India have also shown that the northern, western and eastern regions of the country are predominated by genotype 3, whereas in the southern region, genotype 1 is most common.^{16,17} The treatment response and relapse are monitored by the HCV viral load, whereas the genotype provides the information regarding the epidemiology, viral biological features, and outbreak investigation of the viral infection.^{3,18,19}

This study aims to draw a genomic landscape for HCV in India by performing a wide-range literature search and qualitative and quantitative meta-analysis of published peer-reviewed articles, to identify the diversity in HCV genotypes among various regions of India.

METHODS

Research question and selection criteria

This systematic review was conducted jointly by the department of microbiology of Sikkim Manipal institute of medical sciences and the department of microbiology of Agartala government medical college, using population, intervention, comparator, outcome (PICO) criteria (Table 1) to find out the prevalence of HCV genotypes in different regions of India.

The inclusion criteria were HCV genotype prevalence studies done in different regions of India, without any restrictions in terms of age and gender. The studies that used standardized methods to detect HCV genotype, such as restriction fragment length polymorphism (RFLP), PCR and sequencing; the studies that were published in English language and peer-reviewed journals were included in this study.

The clinical trials, abstract-only articles were excluded from evaluation in this study.

The study has been registered in the international prospective register of systematic reviews as CRD42024521760.

Included database and search strategy

To identify studies related to the topic, we have searched Scopus, PubMed and Web of Science between January 2003 to December 2023 using the keywords 'Hepatitis C' or 'HCV', 'Genotype' and 'India'. To refine the search strategy, medical subject headings (MeSH) terms with asterisks were used. Additionally, references of each eligible study were assessed to find out additional eligible studies (Table 2). Mendeley desktop V 2.11.1.0 software was used to manage citations, duplicate removal, and to

coordinate the review process. The primary interest was to find out the prevalence of HCV genotype in different regions of India, which was estimated using summary statistics.

Screening and data extraction

Title abstract screening

Two independent authors (AS and LA) have reviewed potentially related titles and abstracts of the obtained studies from the systematic search as per eligibility criteria, and articles for screening of full text were identified. If there was any discrepancy about the inclusion of full text review, the co-authors discussed among themselves to build a consensus and decided on eligibility. If there was still a dispute between co-authors for eligibility of the articles, a third author (TM) was consulted to evaluate the article and to decide whether to include the study for full text review.

Screening of full-text and extraction of data

Two independent authors have reviewed potentially eligible full-text studies for relevance and extracted suitable data from these articles. In case of any disagreement co-authors discussed among themselves to conclude. The third author and fourth author resolved any unsolved disagreements. The extracted data were tabulated in a Microsoft excel spreadsheet for analysis. The extracted data contained the name of the first author, publication year, regions within India, sample size, HCV RNA positivity, mean age, number of participants by gender and method of detection for HCV genotype. The process of literature search, screening, data extraction, systemic review and meta-analysis was performed as per the "preferred reporting items for systemic reviews and meta-analysis" (PRISMA) flowchart.²⁰

Study quality evaluation

Two independent authors assessed the equality of selected articles by using the recommended checklist of Joanna Briggs Institute (JBI) critical appraisal tool for prevalence studies.²¹ This checklist contains 9 questions, which are appropriate to evaluate prevalence and epidemiological studies. The answers were categorized as yes, no, unclear or not applicable.

Statistical analysis

Statistical analysis of the extracted data was done on R Studio 2023.12.1 Build 402 using 'stats', 'meta' and 'metafor' packages.²² To consider statistically significant, data with a $p < 0.05$ at a confidence level of 95% (CI) by the random effect model was used. Descriptive statistics were done for mean age and year of publication. Pearson's correlation was used to define the significance of gender. The I^2 test was used to evaluate study heterogeneity, with

a two-sided p value. Sensitivity testing was performed to address the study bias.

Four subgroup analyses were conducted to identify the cause of heterogeneity: i) gender, ii) region, iii) year of publication (2003-2013 vs 2014-2023) and iv) detection method (RFLP, PCR and sequencing).

A funnel plot was used to evaluate publication bias. The Egger test was done to evaluate small study effects.

RESULTS

The primary search of the literature generated a pool of 739 articles. 651 articles duplicate and thus removed. Titles and abstracts of remaining 88 articles were reviewed, and 60 were excluded. The residual 28 full-text articles were studied, and finally, 20 articles selected as per inclusion and exclusion criteria for both qualitative and quantitative analysis.^{1,3,9,11,15,16,23-39} Process of review and selection is illustrated using PRISMA flow chart (Figure 1).

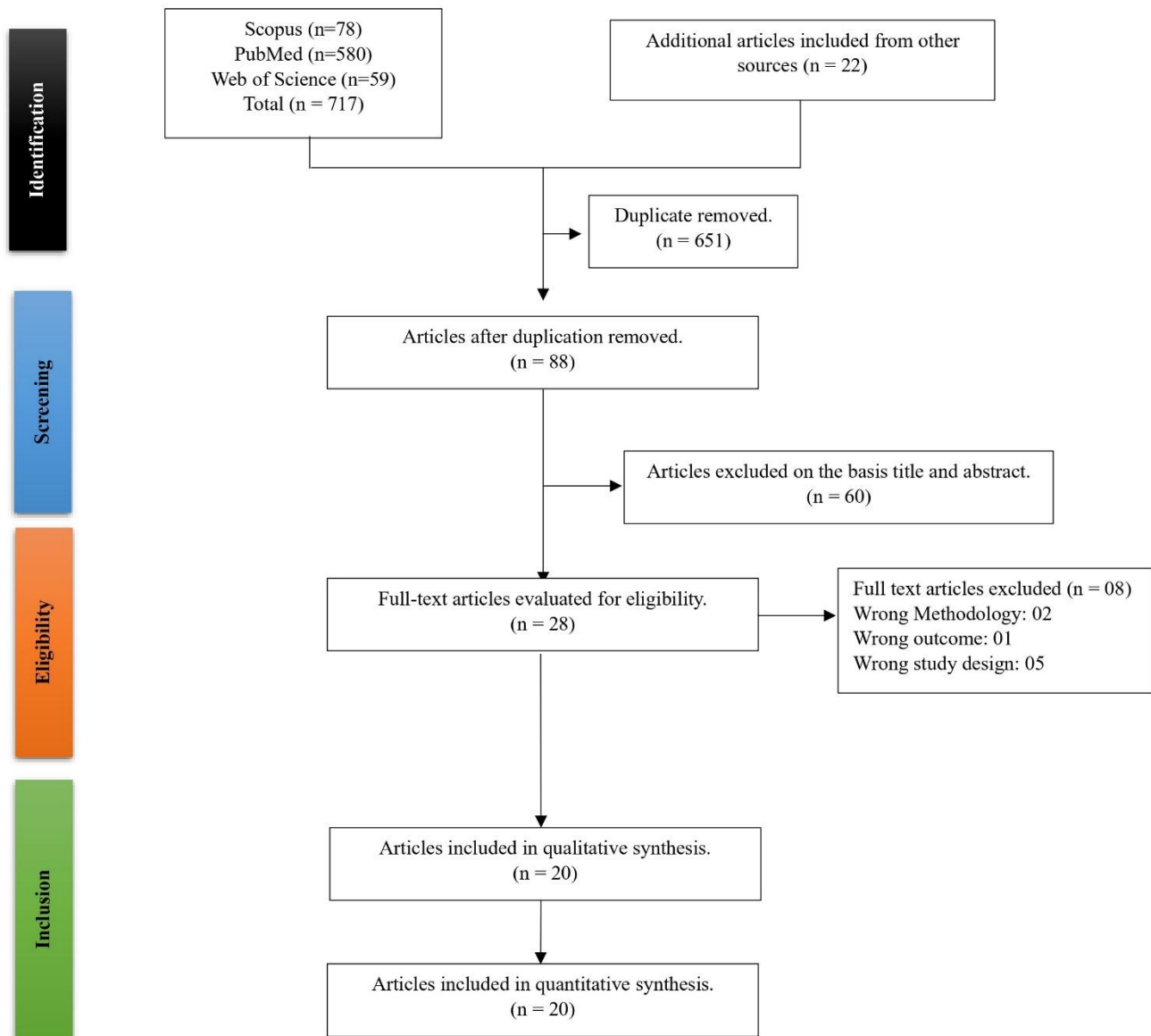


Figure 1: PRISMA flowchart for systemic review and meta-analysis.

The characteristics of the selected 20 articles of this study have been tabulated in Table 3. The total sample size of the included articles was 5050, and the period was between 2003 to 2023. The mean age of the selected studies was 41 ± 4 , ranging from 33 to 51. The Pearson's correlation

showed a statistically significant correlation between gender and sample size, with a $p < 0.001$ (Figure 2 A and B). All the studies have used standard methods to detect HCV genotypes, like PCR, RFLP, and Sequencing.

The critical evaluation of the methodologies for the quality of selected articles is summarized in Table 4, which shows a high methodological strictness of the selected investigators.

A meta-analysis was conducted to find out the prevalence of HCV in India among the 5050 individuals included in this study. Among them, 2121 were positive for HCV. The pooled estimated proportion, based on the random effect model, was 0.58 [0.42; 0.73] at 95% CI. The heterogeneity was high in this meta-analysis [$I^2=100\%$, $p<0.01$] (Figure 3).

Egger's test for publication bias was not significant ($p=0.28$), which shows there is no indication of publication bias between the included studies. A funnel plot was

formed to explain the absence of publication bias (Figure 4).

To find a more accurate estimate regarding the prevalence of HCV in India, a detailed subgroup analysis was done to estimate the differences in estimated prevalence based on gender, different regions of India, year of publication and study location. The pooled proportion of HCV showed a significantly higher value in males [95% CI 0.54- 0.71] than females [95% CI 0.25-0.38], $p\leq 0.01$. The analysis of sub-group based on regions of India shows a significant difference with random effect model a $p<0.01$, where North India [95% CI 0.20-0.82], South India [95% CI 0.09-0.78], East India [95% CI 0.99-1.00], West India [95% CI 0.44-1.00], North-east India [95% CI 0.12-0.93] and Central India [95% CI 0.61-1.00].

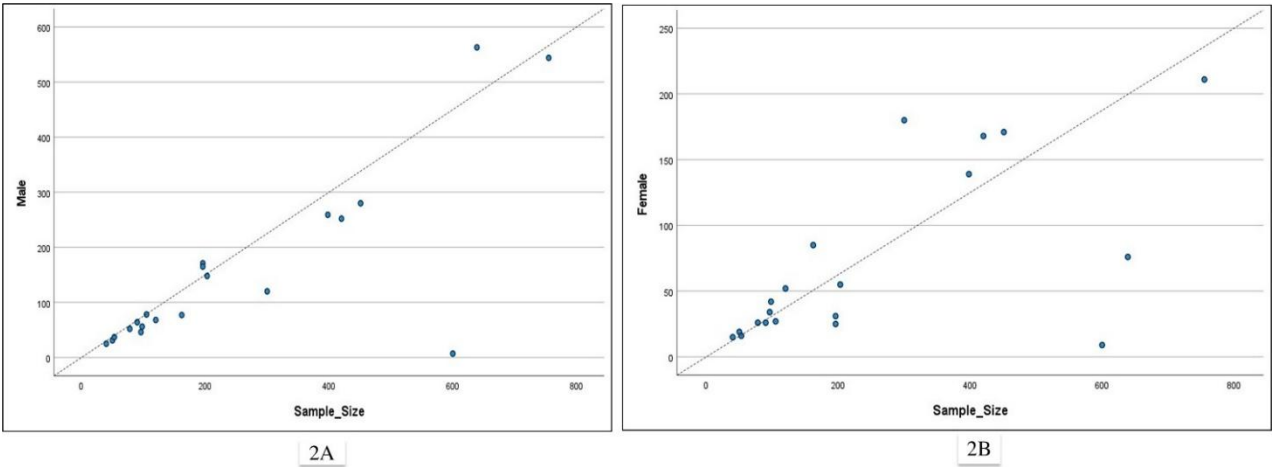


Figure 2 (A and B): Scatter plot of gender wise distribution A-male and B-female.

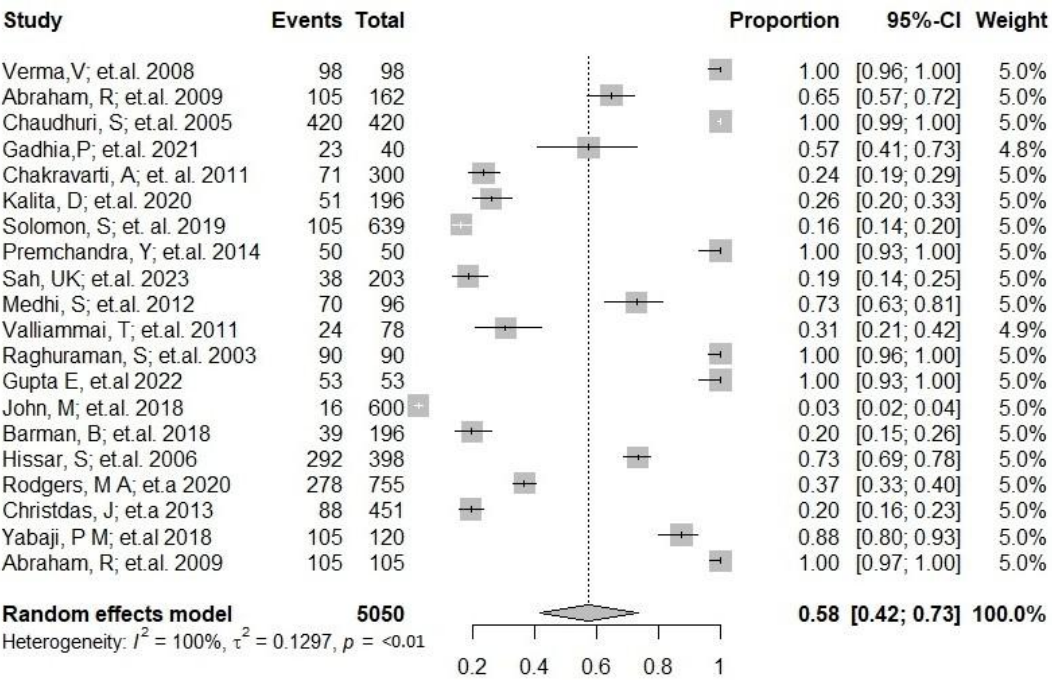


Figure 3: Forest plot of meta-analysis of HCV prevalence in India.

Regarding the trend in HCV prevalence in India, studies between 2003 to 2023 show a prevalence of 0.58 [0.42-0.73] at a 95% CI of the random effect model. However, studies conducted between 2003-2013 showed a higher prevalence of 0.68 [0.49-0.86] compared with studies done between 2014-2023 (0.45 [0.20-0.71] at 95% CI of the random effect model.

Additionally, studies conducted with RFLP, PCR and sequencing as diagnostic methods showed a higher pooled

prevalence with PCR (0.71 [0.47-0.94] over RFLP (0.62 [0.00-1.00] and sequencing (0.46 [0.25-0.68].

The HCV genotypic distribution was calculated from the selected articles, and it shows the majority of genotype 3 (45%) over other reported genotypes.

Table five and Figure five summarize the distribution of the different HCV genotypes in various regions of the India.

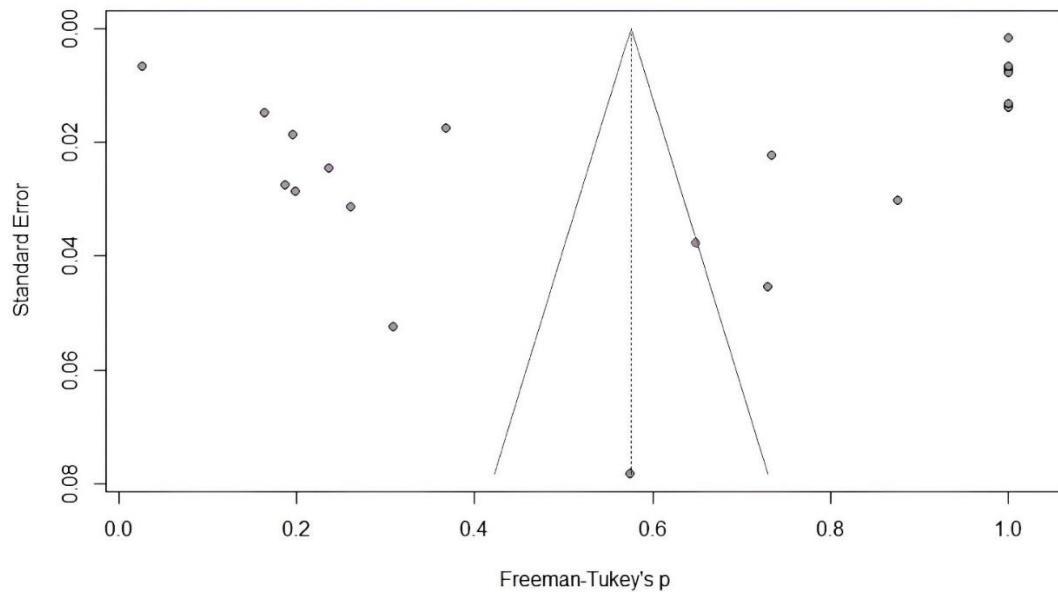


Figure 4: Funnel plot for publication bias for HCV prevalence in India.

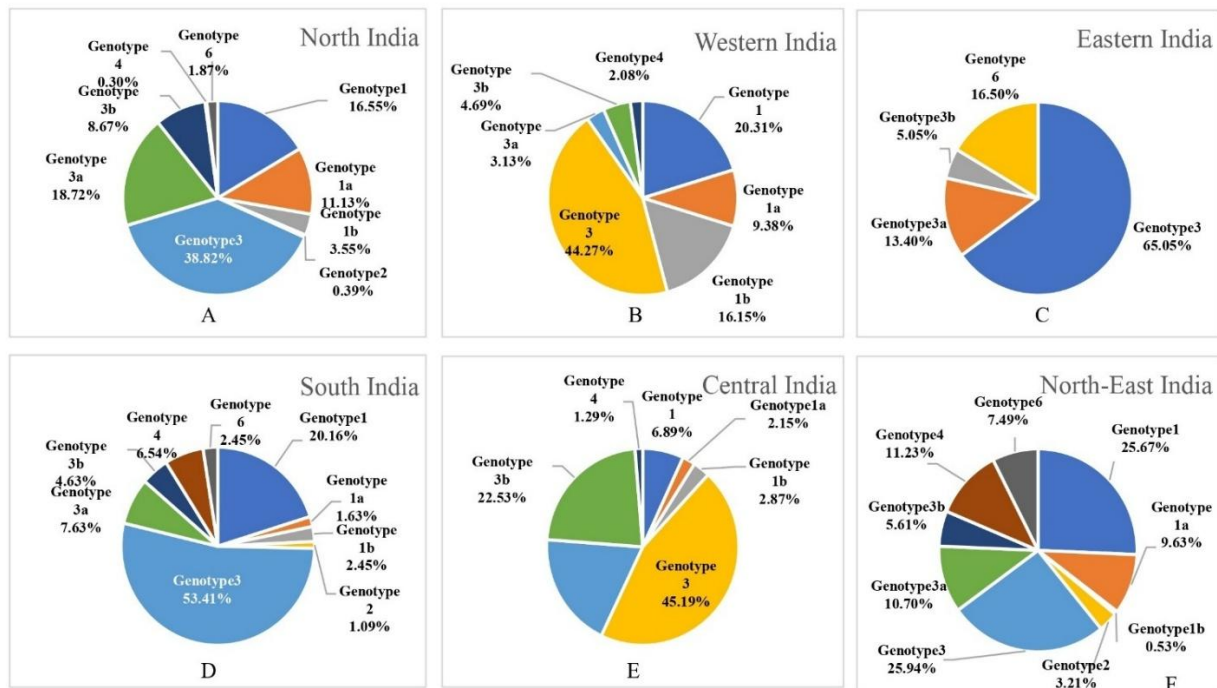


Figure 5 (A-F): Distribution of HCV genotypic variations in India, A-North India, B-Western India, C-Eastern India, D-South India, E-Central India, and F-North-East India.

Table 1: The inclusion and exclusion criteria as per the PICO (Research question: What is the prevalence of hepatitis C viral genotypes in different parts of India?).

Inclusion	Exclusion
Population Indian population diagnosed with confirmed HCV and genotype detected. (Detection methods used were PCR, RFLP and sequencing). All gender. All age groups.	Not done in Indian population.
Intervention NA	
Comparison NA	
Outcome Prevalence of HCV genotype.	
Study design Prevalence studies, cross-sectional studies.	Case-reports, reviews, or systemic reviews

Table 2: Search terms as per searched electronic database (as of 31.12.2023).

Database	No.	Search query	Results
Scopus		((TITLE-ABS (hepatitis C)) OR (TITLE-ABS (India)) OR (TITLE-ABS (Genotype)))	2,688
		((TITLE-ABS (hepatitis C)) OR (TITLE-ABS (HCV)) OR (TITLE-ABS (Genotype)))	388
		#1 AND #2 AND (English [filter]) AND (2003 – 2023 [filter])	78
PubMed	1	"India"[Title/Abstract] OR "Hepatitis C"[Title/Abstract] OR Genotype"[Title/Abstract]	430,583
	2	"Hepatitis C"[Title/Abstract] OR "HCV"[Title/Abstract]	99,531
	3	1 AND 2 AND ((filter[Filter]) AND (excludepreprints[Filter]) AND (observationalstudy[Filter]) AND (humans[Filter]) AND (english[Filter]) AND (2003:2023[pdat]))	580
Web of science	1	((TI=Hepatitis C OR AB= Hepatitis C)) OR ((TI=HCV OR AB=HCV)))	2,350
	2	((((TI=Hepatitis C OR AB= Hepatitis C)) OR ((TI=HCV OR AB=HCV))) OR ((TI=Genotype OR AB=Genotype)))	683
	3	#1 AND #2 NAD (English [filter])	59

Table 3: Descriptions of the selected articles.

Author first name	Year	Region	Sample size	HCV RNA positive	Mean age (in years)	Male	Female	Detection method
Verma et al ¹	2008	North India	98	98	39	56	42	RFLP
Abraham et al ²³	2009	South India	162	105	46	77	85	PCR
Chaudhuri et al ²⁴	2005	East India	420	420	42	252	168	PCR
Gadhia et al ¹⁶	2021	West India	40	23	35	25	15	Sequencing
Chakravarti et al ²⁶	2011	North India	300	71	38	120	180	RFLP
Kalita et al ²⁷	2020	North India	196	51	51	171	25	Sequencing
Solomon et al ²⁸	2019	North-East India	639	105	33	NA	NA	Sequencing
Premchandra et al ²⁹	2014	North-East India	50	50	35	31	19	Sequencing
Sah et al ³⁰	2023	North India	203	38	43	NA	NA	PCR
Medhi et al ³¹	2012	North-East India	96	70	45	46	34	PCR
Valliammai et al ³²	2011	South India	78	24	42	52	26	Sequencing
Raghuraman et al ⁹	2003	South India	90	90	43	64	26	PCR
Gupta et al ³⁴	2022	North India	53	53	44	37	16	Sequencing
John et al ³⁵	2018	South India	600	16	42	NA	NA	Sequencing
Barman et al ³⁶	2018	North-East India	196	39	40	165	31	PCR
Hissar et al ¹⁵	2006	Central India	398	292	38	259	139	Sequencing
Rodgers et al ³⁸	2020	North India	755	278	40	544	211	Sequencing
Christdas et al ³⁹	2013	South India	451	88	41	280	171	Sequencing
Yabaji et al ³	2018	West India	120	105	42	68	52	PCR
Abraham et al ¹¹	2009	Central India	105	105	36	78	27	PCR

Table 4: Methodological evaluation for the quality of selected articles.

Article	1. Was the sample size adequate to address the population?	2. Were the study participants sampled in an appropriate way?	3. Was the sample size adequate?	4. Were the study subjects and setting described in details?	5. Was the data analysis conducted with sufficient coverage of the identified sample?	6. Were appropriate method used to identify the condition?	7. Was the condition measured in a reliable, standard way?	8. Was there appropriate statistical analysis done?	9. Was there appropriate description of the study outcome?	Score
Verma et al ¹	N	Y	N	Y	Y	Y	Y	Y	Y	7
Abraham et al ²³	Y	Y	N	Y	Y	Y	Y	Y	Y	8
Chaudhuri et al ²⁴	Y	Y	Y	Y	Y	Y	Y	Y	Y	9
Gadhia et al ¹⁶	N	Y	N	Y	Y	Y	Y	Y	Y	7
Chakravarti et al ²⁶	Y	Y	Y	Y	Y	Y	Y	Y	Y	9
Kalita et al ²⁷	Y	Y	Y	Y	Y	Y	Y	Y	Y	9
Solomon et al ²⁸	Y	Y	Y	U	Y	Y	Y	Y	Y	8
Premchandra et al ²⁹	N	Y	N	Y	Y	Y	Y	Y	Y	7
Sah et al ³⁰	N	Y	Y	U	Y	Y	Y	Y	Y	7
Medhi et al ³¹	N	Y	N	Y	Y	Y	Y	Y	Y	7
Valliammai et al ³²	N	Y	N	Y	Y	Y	Y	Y	Y	7
Raghuraman et al ⁹	N	Y	N	Y	Y	Y	Y	Y	Y	7
Gupta et al ³⁴	N	Y	N	U	Y	Y	Y	Y	Y	6
John et al ¹⁷	Y	Y	Y	U	Y	Y	Y	Y	Y	8
Barman et al ³⁶	Y	Y	Y	Y	Y	Y	Y	Y	Y	9
Hissar et al ¹⁵	Y	Y	Y	Y	Y	Y	Y	Y	Y	9
Rodgers et al ³⁸	Y	Y	Y	Y	Y	Y	Y	Y	Y	9
Christdas et al ³⁰	Y	Y	Y	Y	Y	Y	Y	Y	Y	9
Yabaji et al ³	Y	Y	Y	Y	Y	Y	Y	Y	Y	9
Abraham et al ¹¹	Y	Y	Y	Y	Y	Y	Y	Y	Y	9

* Y=Yes, N=No, U=Unclear, NA=Not Applicable.

Table 5: Distribution of HCV genotypic variations in India.

Region	Genotype 1	Genotype 1a	Genotype 1b	Genotype 2	Genotype 3	Genotype 3a	Genotype 3b	Genotype 4	Genotype 6
North India	17%	11%	4%	0%	39%	19%	9%	0%	2%
Western India	20%	9%	16%	0%	44%	3%	5%	2%	0%
South India	20%	2%	2%	1%	53%	8%	5%	7%	2%
Eastern India	0%	0%	0%	0%	65%	13%	5%	0%	17%
Central India	7%	2%	3%	0%	45%	19%	23%	1%	0%
North-East India	26%	10%	1%	3%	26%	11%	6%	11%	7%
Total	13%	6%	3%	1%	45%	15%	10%	3%	4%

DISCUSSION

National viral hepatitis program (NVHCP), a government of India initiative for hepatitis surveillance, laboratory diagnosis, treatment, immunization, blood safety, injection safety and infection control, were launched on 28th July 2018.⁴² Implementing such approaches requires the use of efficient scientific facts. This study aimed to draw a geographical landscape towards HCV genotypes across India. This study has highlighted the diverse spread of HCV genotypes throughout the country, remarkably by genotype 3, which predominates with 45%, followed by genotype 1 (13%) and genotype 6 (4%), which is consistent with similar studies.^{9,16,24,29,32,36,39,49} Region-wise all the regions have shown a similar pattern except the Eastern region with genotype 6 (17%) and the North-East region, where genotype 3 and genotype 1 are in equal proportion, i.e., 26%.^{26,27,31,36} Globally, genotype 1 predominates over genotype 3, whereas India's neighboring countries, such as Bangladesh, Myanmar, Bhutan, Nepal, China, and Pakistan, show a similar picture of predominating genotype 3 and its subtypes.⁵³⁻⁴⁹ Recent studies have also stated mixed genotypic HCV infections, which may be a cause of treatment failure in a small number of cases.^{60,61}

In this systemic-review, quality of the studies included was evaluated by using JBI checklist for prevalence studies. After evaluation, it has been observed that the included studies scored high, which indicates a good quality. One study received a score of six, and nine studies received a score of nine on the scale.

In our study, a high level of heterogeneity was observed ($I^2=100\%$). This is clarified by the variability of study design, outcome measurement tools, time frame and study population. For meta-analysis random effect model was used. Analysis of sub-groups was performed to address the heterogeneity across the studies.⁶²

Publication bias occurs when the likelihood of a study being published is influenced by the outcome of the study, leading to an overrepresentation of studies with statistically significant results. This bias can affect the general conclusions drawn from the meta-analysis. Our study has no publication bias, as a symmetrical pattern of the funnel plot has been observed.⁶³

However, the limitations of our study should be readily acknowledged. The lack of literature from various regions of the country can be a limiting factor in getting updated information regarding the disease condition and its progress. Therefore, it is essential to perform observational surveys in all territories of the country to obtain a clear understanding of the disease progression. The other limitations involve the lack of quantitative data from some articles that hampered the possibility of establishing a proper genotypic spectrum, especially the correlation between age group and prevalence of genotypes.

CONCLUSION

The observations of our study revealed that genotype 3 is the utmost prevalent HCV infection. A wide spectrum of genotypic variation has been observed, especially in the North-Eastern region of India. Government initiatives like NVHCP should be properly implemented and monitored to gain more knowledge about the disease and for the betterment of public health.

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