**MATERIALS AND METHODS:**

Study will be carried out in the Microbiology Department of Rama Medical College & Hospital, Kanpur (U.P.).

1. **Selection of Patients and controls:**

   Patients will be selected from the OPD and IPD of Rama Medical College & Hospital, Kanpur (U.P.).

Sample size:

\[ n = 194 \approx 200 \]

Written informed consent will be taken from all patients.

**Inclusion criteria:**

1. **Chronic liver disease** cases included disease of the liver which had history, sign and symptoms over a period of 6 months suggestive of liver pathology. These were confirmed by blood tests, ultrasound and liver biopsy.

2. Patients who are reactive for anti-HCV antibodies

**Exclusion criteria**

Studies in non-representative populations, (e.g., people who inject drugs (PWID’s), haemophiliacs, minority ethnic groups, refugees, etc.),

1. Epilepsy
2. Other malignancy
3. Pregnancy and lactation period
4. Therapy involving cytotoxic and bone marrow depressant.
5. HIV positive patients

2. **Collection and storage of sample:**

10 ml blood will be collected by venipuncture from suspected HCV patients of both sex and age. All sampling and testing will be is accordance with ethical standards. The serum will be separated immediately by a single centrifugation step method at 3000 rpm for 10 min.\[27-28\] Separated serum will be stored at 4°C for upto 4 days. \[27-28\]

3. **Detection of Hepatitis C Antibody (HCV Ab)**

   5-7 ml sample will be used for antibody detection by using SD Proline kit (Rapid card and ELISA) as per manufacturer’s Instructions.

4. **Nucleic acid separation:** 5-7ml sample will be used for RNA extraction and RT-PCR as per manufacturer's instructions (SD Proline).

5. **Detection of nucleic acid (RNA).** Nucleic acid will be analyzed for Qualitative Testing & Quantitative Testing by using (SD Proline) as per manufacturer's instructions.

6. **Liver enzyme Tests** 10 ml sample will be used for analysis of Alanine amino transferase (ALT) \[29\], Aspartate aminotransferase (AST) \[30\] by UV(international Federation of Clinical Chemistry recommended) Kinetic Method by using kit (SD Proline).
7. **Statistical Analysis:**

Data will be analyzed & statistical correlation will be established by using Z test.

**Sample size:**
The incidence of HCV in India is reported1 to be 1.9% [37].

We suppose to study symptomatic acute chronic cases of HCV genotype to determine the difference of 1% in the incidence of HCV burden with 80% power (p = .02) and confidence of 95% i.e. (Gama = .05)

The sample size was determined by the following formula:

\[
Z_{\text{power}} = \frac{p - q}{\sqrt{\frac{pq}{n}}} \\
p = .02 \quad q = 1 - .02
\]

\[
0.84 = \frac{0.2}{\sqrt{\frac{0.196}{n}}} - 1.96
\]

or

\[
0.2 \left[ \frac{0.196}{n} \right] = 0.84 + 1.96
\]

or

\[
0.2 \left[ \frac{\sqrt{n}}{0.196} \right] = (0.84 + 1.96)
\]

or

\[
\sqrt{n} = \frac{(0.84 + 1.96) \times \sqrt{0.196}}{.02}
\]

or

\[
n = \frac{(0.84 + 1.96)^2 \times 1.96}{.02^2}
\]

or

\[
n = \frac{7.84 \times 0.196}{.0004}
\]

or

\[
n = 194 \cong 200
\]

**WORK PLAN**

Sera from HCV suspected Patients

HCV antibody (+)
RIBA

(Confirmation n=50)

↓

RNA Extraction & Amplification

↓

c DNA Synthesis & Amplification

↓

PCR

SEQUENCER (For genotyping) or Line probe assay (For genotyping)