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HEPATITIS C INFECTION: A RETROSPECTIVE ANALYSIS OF PATIENTS AT CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL, SOUTH AFRICA

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A research report submitted to the University of Witwatersrand, Johannesburg, in fulfilment for the requirement of the degree of Master of Medicine

DECLARATION

I, Wamda Abuelhassan, declare that this research report is my own work which is being submitted for the degree of Master of Medicine (in the submissible format with my protocol and an extended literature review) in the branch of Internal Medicine at the University of the Witwatersrand, Johannesburg.

It has not been submitted before for any degree or examination at this or any other university

……

28th September 2018

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Professor Menezes, my second supervisor for always being there. Your sound advice, research knowledge and enthusiasm played an important role in my completion of this project.

Professor Gasim, my husband, for your continuous mentoring and support and your assistance with the statistical analysis and for your patience and understanding during this time.

Last, but not least, to my children, Mohammed, Moein and Muram for your patience, unwavering support and love.

ABSTRACT

Background: Hepatitis C is a viral infection that leads to chronic liver disease resulting in significant morbidity and mortality. This study aimed to describe the demographics and clinical presentation of patients with chronic hepatitis C infection. Aspartate transaminase-to-platelet ratio index (APRI) and fibrosis index based-on-4-factors (FIB4) were assessed for prediction of liver fibrosis.

Methods: We retrospectively reviewed 87 patients who presented to the Liver Clinic, Division of Gastroenterology, Chris Hani Baragwanath Academic Hospital from January 2007 to December 2016. Patients' records were reviewed and analysed. Convenience sampling was used**.**

Results: The patients' age was 52.6(12.3) years, mean(SD). Fifty-four percent were females. Genotype 5 was exclusively found in blacks (P=<0.001) constituting 53.5% of infections in this ethnic group and 44% of the cohort, followed by genotype 1 (18.4%), genotype 3 (13.8%), genotype 4 (9.2%) and mixed-genotype infections which was 3.4%. Genotype 5 patients were older (mean age 56.7(9.8) years) than genotype 1 (46.3(11.4)) years and genotype 3 (42 (9.8)) years (P=0.002 and <0.001 respectively). The Receiver Operating Characteristic curve (ROC curve) for Metavir F0 and (APRI) (cut off <0.7) showed a moderate correlation with an AUC of 0.349 (P=0.002), sensitivity of 78.8%, specificity of 70.6% and a negative predictive value (NPV) of 63.2%. Metavir F4 vs APRI (cut off ≥1.5) showed an AUC = 0.881 (P=0.001) with sensitivity of 85.7%, specificity of 93% and a positive predictive value (PPV) of 67 %. Metavir F0 vs (FIB 4) (cut off <1.45) showed moderate correlation with AUC = 0.332 (P=0.021), sensitivity of 78.3%, specificity of 53.8% and a negative predictive value (NPV) of 73.7 %. Metavir F4 vs FIB 4 (cut off >3.25) had a strong correlation with AUC= 0.952 (P=<0.001), sensitivity of 63.6%, specificity of 100% and PPV of 100 %. Twelve-week EVR was found to predict sustained virological response to therapy (OR=27.8, 95% CI=2.8-274.3, P= 0.004).

Conclusion: Genotype 5 is predominant in this cohort particularly in older age groups compared to other genotypes. Moreover, APRI and FIB4 scores were powered enough to diagnose advanced fibrosis among HCV patients. Finally, early virological response during therapy was found to determine sustained virological response.

ABBREVIATIONS

ALP	Alpha feto protein
APRI	Aspartate transaminase-to-platelet ratio index
AUC	Area under the curve
BMI	Body mass index
CHBAH	Chris Hani Baragwanath Hospital
DAAs	Directly-acting antivirals
EVR	Early virological response
FIB 4	Fibrosis index based-on-four factors
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
IQR	Interquartile range
LFT	Liver function test
NPV	Negative predictive value
PPV	Positive predictive value
RNA	Ribonucleic acid
ROC	Receiver operating characteristics
RVR	Rapid virological response
SD	Standard deviation
SVR	Sustained virological response
WHO	World Health Organization

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CHAPTER 1 – PROTOCOL WITH EXTENDED LITERATURE REVIEW

1. BACKGROUND

Hepatitis C is a viral infection that leads to chronic liver disease resulting in significant morbidity and mortality with many patients progressing to liver cirrhosis and hepatocellular carcinoma. It tops the list of indications for liver transplantation in Japan, Europe and the United States (1).

Estimates made by the WHO in 2015 reflect that 71 million individuals are living with chronic hepatitis C infection. That constitutes about one percent of the world's population. It is also estimated that 1.75 million individuals developed new HCV infections worldwide in 2015 (2). There are no official figures reflecting the prevalence of hepatitis C in South Africa however it is estimated to have ranged between 0.1-1.7% (3). Asymptomatic patients are detected during routine screening or blood donation. The rest progress until they present with some form of chronic liver disease (4).

1.1 The virus

Hepatitis C virus (HCV) is a positive stranded RNA virus that is a member of the Flaviviridae family. Its 9.6-kb genome is characterised by one large open reading frame (ORF) encoding for at least ten proteins; three structural (core, E1, and E2) which form the virions and seven non-structural proteins including p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B that are crucial for viral replication and maturation (5). These proteins are essential for regulating the activity of cellular genes thus potentially triggering malignant changes in the hepatocyte (6,7).

1.2 Hepatitis C genotypes

About seven major HCV genotypes have been recognised since the identification of HCV in 1989. The genotypes are further subdivided into a series of subtypes. HCV genotypes follow a characteristic epidemiological distribution worldwide. These genotypes are one of the most critical factors determining response to therapy (8). Genotype 5 is reported to be the predominant HCV genotype in South Africa constituting about 40% of HCV infections followed by genotype 1 (33%)(9).

1.3 Acquisition of infection and progression factors

The risk factors for hepatitis C infection include blood and blood products transfusion and organ transplantation prior to 1992, intravenous drug use, long term haemodialysis, multiple sexual partners, tattooing and scarification, unsafe medical practices, patients infected with human immunodeficiency virus (HIV), and children born to HCV-infected mothers (10–13). Interestingly, the ethnic background of patients has also been reported to determine the prevalence of HCV-infection(14).

HCV infection factors known to determine progression include ethnicity, male gender, long duration of HCV infection, history of alcohol consumption, smoking, and comorbidities such as obesity, diabetes, co-infection with HIV and hepatitis B (15–17).

1.3.1. HCV co-infection with HIV and Hepatitis B

HIV coinfection results in increased replication of hepatitis c virus leading to high viral loads. Individuals with HIV coinfection progress to cirrhosis three times faster than mono-infected individuals (18,19). In areas endemic with hepatitis B, an appreciable number of individuals are coinfected with hepatitis C due to the shared modes of transmission. Those individuals are at increased risk of liver cirrhosis and hepatocellular carcinoma (20). Hoffmann C *et al* found a very low prevalence of HCV amongst HIV infected individuals. They attributed this to the

possibility of very low circulating HCV infection among South Africans, low rates of intravenous drug use and of healthcare related transmission (21).

1.4 Natural history

The progression from acute to chronic hepatitis C usually passes unnoticed. Detailed studies are easier said than done owing to the silent nature of the disease. The possibility of spontaneous remission of the disease is predetermined by various host genetic factors, such as IL28B polymorphisms and DQB1*0301 of the human leucocyte antigens type II (22). Current evidence shows that eradication of the virus in the acute phase without evolution to chronic disease is not associated with florid disease, but rather trivial histological alterations. This has been observed in subjects becoming anti-HCV seropositive and HCV RNA seronegative. Individuals succeeding in eradicating the virus are still at risk of acquiring a new infection. Highly susceptible groups include men who have sex with men and HIV-infected patients. Only about 15-20% of infected individuals manage to clear the virus with the remaining 80% progressing to chronic infection. Untreated chronic infection progresses to cirrhosis in 10-20% of patients over two to three decades. Once patients develop cirrhosis, there is an annual risk of progression to hepatocellular carcinoma (HCC) that approaches 5% and an annual risk of liver decompensation of 6% per year. Treatment with anti-HCV therapy does not totally abolish the risk of developing HCC (8).

1.5 Pathogenesis

Being a non-cytopathic virus, the pathogenesis of HCV liver disease results from viral interaction with the host's immune system and other factors such as insulin resistance and fatty liver. Both natural and acquired immunity contribute to HCV pathogenesis. Cytotoxic T lymphocytes are essential for viral elimination or viral endurance and are controlled by HCV proteins and variants and various metabolic factors (23).

Hepatitis C is an example of a pathogen succeeding in establishing a chronic infection by escaping the host's immune response. The majority of infected subjects develop a chronic infection while only a small proportion clear the virus spontaneously (24). As a response to viral infection, the body senses such invasion via pathogen recognition receptors and tends to release pro-inflammatory cytokines such as tumour necrosis factor (TNF), Interleukin-6 and type 1 interferons (25,26). In chronic HCV infection recruited mononuclear cells infiltrate the liver. These cells include natural killer cells, natural killer T cells , cluster of differentiation (CD) 4+ and 8+ T lymphocytes and B lymphocytes (27).

1.6 Clinical presentation

The possibility of progression from acute to chronic HCV infection is very high and estimated to be between 80 and 100 percent (24). Acute hepatitis C infection is usually asymptomatic. Most of chronic HCV-infected patients present with trivial vague symptoms if any (11).They commonly present with fatigue. Other infrequent symptoms include nausea, loss of appetite, muscle pains, joint pains, general body weakness, right upper quadrant pain, itching and possibly weight loss and dark urine (28). Extra-hepatic manifestations described with HCV include dermatological manifestations such as porphyria cutanea tarda, leukocytoclastic vasculitis and lichen planus; renal such as membranoproliferative glomerulonephritis; and haematological disorders such as thrombocytopenia, anaemia, lymphomas and essential mixed cryoglobulinemia (29,30).

1.7 Diagnosis

Anti-HCV antibodies are used for screening for hepatitis C. Thereafter qualitative and quantitative essays are used to detect the HCV virus RNA and to determine the number of existing viral copies. Further tests are done to determine the genotypes and their subtypes.

Patients diagnosed with hepatitis C usually require a liver biopsy before starting treatment unless contraindicated e.g. in patients with thrombocytopenia and in haemophiliacs. This is to assess for the degree of fibrosis and to assess for other liver disorders such as fatty liver and iron overload. The Metavir score is usually used to report on the liver histology (31). (Table 1) Recently, non-invasive scores have been introduced as predictors of liver fibrosis. These include the aspartate aminotransferase to platelet ratio index (APRI) and fibrosis index based-on-four factors (FIB4) (32,33). APRI and FIB4 scores have been reported to identify hepatitis C-related fibrosis in many studies thus alleviating the need for the invasive liver biopsy (34–36). Search of the literature did not show results regarding the utility of those scores in HCV infected individuals in South Africa.

Table 1 - Metavir Score

Necro-inflammatory activity score		Fibrosis score	
A0	no evidence inflammation	F0	no fibrosis
A1	evidence of mild inflammation found	F1	portal fibrosis without septa
A2	evidence of moderate inflammation	F2	portal fibrosis with few septa
A3	evidence of severe inflammation	F3	bridging fibrosis
		F4	Cirrhosis

1.8 Treatment of HCV

The standard treatment used for chronically infected patients with hepatitis C in South Africa is the combination of pegylated interferon (PegIFN) and ribavirin for twenty four or forty eight weeks (3), with sustained viral response (SVR) achieved in up to 80% of those treated (37).

(Table 2). However, achievement of an SVR is governed by the HCV genotype along with other factors such as genetic factors e.g. IL 28B (8,22). Hadziyannis *et al* showed that individuals infected with genotype 1 need standard doses of ribavirin while those infected with genotypes 2 and 3 are adequately treated with low doses (38). Given the serious side effects of such a combination (39), and hurdles of use among large sections of HCV patients, the use of the combination is not always an easy decision (40).

Since the FDAs approval of the use of directly acting antiviral (DAAs) agents in May 2011 in the US, therapy of hepatitis C has evolved markedly in recent years. The cure rates have increased with shorter duration of therapy and less toxic regimens. A hindrance to the wide use of DAAs remains the unaffordable costs to most developing countries.

Table 2 - Response to therapy definitions

Virological Response	Definition
Rapid virological response (RVR)	defined as undetectable HCV RNA levels at week 4
Complete early viral response (cEVR)	undetectable HCV RNA at treatment week 12
Partial early viral response (pEVR)	a decline ≥ 2 logs at 12 weeks, but no complete early viral response
Sustained viral response (SVR)	undetectable HCV RNA levels 12- 24 weeks after the end of therapy
Non-responder	non-clearing of HCV RNA after week 24 of treatment
Relapse	detectable HCV RNA after 24 weeks of the end of therapy

1.8.1. Predictors of response

Several factors are known to predict response to HCV therapy including (i) the patient's age, where age less than 40 years is associated with a better response. (ii) The patient's race whites do better than blacks). (iii) BMI (BMI less than 25 kg/m^2 do better than higher BMI's). (iv) HCV genotype. (v) Viral load. (vi) Rapid viral response. (vii) The degree of liver fibrosis, alpha fetoprotein (AFP), and IL28B (3).

Despite the impact of the HCV infection on the South African population especially on considering its frequent coexistence with the highly prevalent HIV infection, there is lack of published data. Therefore, this study aims to describe the demographics of patients with chronic hepatitis C, disease presentation, clinical parameters, coinfection with hepatitis B and HIV and response to therapy. (Definitions of response to therapy: Table 2). It is expected that the findings of the current study will help to provide data on the spectrum of hepatitis C infection in a tertiary hospital setting in South Africa.

2. STUDY OBJECTIVES

i. To determine the demographics along with the risk factors of patients presenting with HCV-infection.
ii. To describe the clinical parameters of patients presenting with HCV infection.
iii. To describe the presentation of HCV coinfection in patients presenting with hepatitis B and HIV infection.
iv. To assess response to therapy including predictors of response in patients presenting with HCV-infection who received treatment.

3. METHODOLOGY

3.1 Study design and Study population

This is a retrospective study of HCV- infected subjects attending the liver clinic, the Division of Gastroenterology at Chris Hani Baragwanath Academic Hospital (CHBAH), between 2007 and 2016. CHBAH which is based in Soweto, Johannesburg is one of the largest teaching hospitals worldwide with a capacity of more than 3200 beds (41).

Convenience sampling will be used whereby all the patients who attended the clinic will be included. The sample size is estimated to be 60 patients.

3.1.1 Inclusion Criteria:

i. Patients older than 18 years of age

ii. Hepatitis C PCR positive or detectable hepatitis C viral load.

3.1.2 Exclusion criteria

i. Age less than 18

ii. Lack of clinical records

3.2 Data collection and analysis

Data will be extracted from patients' clinical records and laboratory results and recorded on excel format. Information will be entered anonymously. Data extracted will include patients' demographics such as age, gender and ethnic group and clinical presentation including history and examination. Laboratory data extracted will include parameters such as full blood count (FBC), liver function tests (LFT), renal function, hepatitis C viral load and genotype, HIV and hepatitis B serology and liver biopsy results. Treatment received as well as viral load results during therapy and LFT result at the end of therapy will also be recorded.

3.3 Statistical Methods

All data will be analysed using SPSS statistical software (version 24.0 for Windows; SPSS Inc., Chicago, IL, USA). Categorical data such as gender, ethnicity and symptoms will be analyzed using Pearson's chi-square test or Fisher's exact tests when more than 20% cells have an expected frequency less than 5. Continuous data such as age and viral load will be analysed using parametric methods and presented as means and standard deviations for normally distributed data, or median and interquartile ranges (IQRs), or percentiles, for data that is not normally distributed. The T-test will be used in the analysis of normally distributed continuous data; otherwise the Mann Witney U test will be used. The binary logistic regression will be used

to determine the risk factors for HCV-infection among the study cohort after calculating their univariate logistic regression. Wilcoxon signed-rank test will be used for analysis of treatment effect on viral load and liver function. A P value of <0.05 will be considered statistically significant.

4. ETHICS

Written permission will be obtained from the Clinical Head of the Department of Internal Medicine and the Chief Executive Officer (CEO) of the hospital. Thereafter approval will be obtained from the Human Research Ethics committee of the University of the Witwatersrand. The study will be conducted in adherence with the declaration of Helsinki.

5. TIMING

Dec 2016–Sep 2018

	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Òct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
Literature review																						
Preparing protocol																						
Protocol assessment																						
Ethics application																						
Collecting data																						
Data Analysis																						
Writing up thesis																						
Writing up paper																						

6. FUNDING

Costs of stationery and printing will be self-funded.

7. LIMITATIONS

1. It is a single centre study and thus doesn't reflect the national data.
2. Incomplete records or missing results.

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CHAPTER 2: SUBMISSIBLE ARTICLE

Title:

Hepatitis C Infection: A Retrospective Analysis of Patients at Chris Hani Baragwanath Academic Hospital, South Africa

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Abstract

Background: Hepatitis C is a viral infection that leads to chronic liver disease resulting in significant morbidity and mortality. This study aimed to describe the demographics and clinical presentation of patients with chronic hepatitis C infection. Aspartate transaminase-to-platelet ratio index (APRI) and fibrosis index based-on-4-factors (FIB4) were assessed for prediction of liver fibrosis.

Methods: We retrospectively reviewed 87 patients who presented to the liver Clinic, Division of Gastroenterology, Chris Hani Baragwanath Academic Hospital from January 2007 to December 2016. Patients' records were reviewed and analysed. Convenience sampling was used**. Results:** The patients' age was 52.6(12.3) years mean(SD). Fifty-four percent were females. Genotype 5 was exclusively found in blacks (P=<0.001) constituting 53.5% of infections in this ethnic group and 44% of the cohort, followed by genotype 1 (18.4%), genotype 3 (13.8%), genotype 4 (9.2%) and mixed-genotype infections which was 3.4%. Genotype 5 patients were older (mean age 56.7(9.8) years) than genotype 1 (46.3(11.4)) years and genotype 3 (42 (9.8)) years (P=0.002 and <0.001 respectively). The Receiver Operating Characteristic curve (ROC curve) for Metavir F0 and (APRI) (cut off <0.7) showed a moderate correlation with an AUC of 0.349 (P=0.002), sensitivity of 78.8%, specificity of 70.6% and a negative predictive value (NPV) of 63.2%. Metavir F4 vs APRI (cut off ≥1.5) showed an AUC = 0.881 (P=0.001) with sensitivity of 85.7%, specificity of 93% and a positive predictive value (PPV) of 67 %. Metavir F0 vs (FIB 4) (cut off <1.45) showed moderate correlation with AUC = 0.332 (P=0.021), sensitivity of 78.3%, specificity of 53.8% and a negative predictive value (NPV) of 73.7%. Metavir F4 vs FIB 4 (cut off >3.25) had a strong correlation with AUC= 0.952 (P=<0.001), sensitivity of 63.6%, specificity of 100% and PPV = 100%. Twelve-week EVR was found to predict sustained virological response to therapy (OR=27.8, 95% CI=2.8-274.3, P= 0.004).

Conclusion: Genotype 5 is predominant in this cohort particularly in older age groups compared to other genotypes. Moreover, APRI and FIB4 scores were powered enough to diagnose advanced fibrosis among HCV patients. Finally, early virological response during therapy was found to determine sustained virological response.

Keywords:

Hepatitis C, HCV, genotype 5, South Africa, FIB 4 score, APRI score.

Background

Hepatitis C is a viral infection that leads to chronic liver disease resulting in substantial morbidity and mortality with many patients progressing to liver cirrhosis and hepatocellular carcinoma. It tops the list of indications for liver transplantation in Japan, Europe and the United States (1).

About seven major HCV genotypes have been recognised since the identification of HCV in 1989. These genotypes are one of the most critical factors determining response to therapy (8). Genotype 5 is reported to be the predominant HCV genotype in South Africa constituting about 40% of HCV infections followed by genotype 1 (33%) (9).

The risk factors for hepatitis C infection include blood and blood products transfusion and organ transplantation prior to 1992, intravenous drug use, long term haemodialysis, multiple sexual partners, tattooing and scarification, unsafe medical practices, patients infected with human immunodeficiency virus (HIV), and children born to HCV-infected mothers (4-7). Interestingly, the ethnic background of patients has also been reported to determine the prevalence of HCV-infection (8).

Anti-HCV antibodies are used for screening for hepatitis C. Thereafter qualitative and quantitative essays are used to detect the HCV virus RNA and to determine the number of existing viral copies. Further tests are done to determine the genotypes and their subtypes.

Patients diagnosed with hepatitis C usually require a liver biopsy before starting treatment unless contraindicated e.g. patients with thrombocytopenia and in haemophiliacs. This is to assess for the degree of fibrosis and to assess for other liver disorders such as fatty liver and iron overload. The Metavir score is usually used to report on the liver histology(9). Recently, non-invasive scores have been introduced as predictors of liver fibrosis. These include the aspartate aminotransferase to platelet ratio index (APRI) and Fibrosis index based-on-four factors (FIB4) (32,33). APRI and FIB4 scores have been reported to identify hepatitis C-related fibrosis in

many studies thus alleviating the need for the invasive liver biopsy(34–36). This has not been reported on in HCV infected individuals in South Africa.

The standard treatment used for chronically infected patients with hepatitis C in South Africa is the combination of pegylated interferon (PegIFN) and ribavirin for twenty four or forty eight weeks (42), with sustained viral response (SVR) achieved in up to 80% of those treated (37). However, achievement of an SVR is governed by the HCV genotype along with other factors such as genetic factors e.g. IL 28B (8,22). Hadziyannis *et al* showed that patients infected with genotype 1 need standard doses of ribavirin while those infected with genotypes 2 and 3 are adequately treated with low doses (38).Given the serious side effects of such a combination (39), and hurdles of use among large sections of HCV patients, the use of the combination is not always an easy decision (40).

Several factors are known to predict response to HCV therapy including (I) the patient's age, where age less than 40 years is associated with a better response. (II) The patient's race (whites do better than blacks). (III) BMI (BMI less than 25 kg/m^2 do better than higher BMI's). (IV) HCV genotype. (V) Viral load (VI) Rapid viral response, (VII) The degree of liver fibrosis, alpha fetoprotein, and IL28B (42).

Despite the impact of HCV infection on the South African population, especially on considering its frequent coexistence with the highly prevalent HIV infection, there is lack of published data. Therefore, this study aims to describe the demographics of patients with chronic hepatitis C, disease presentation, clinical parameters, coinfection with hepatitis B and HIV and response to therapy. It is expected that the findings of the current study will help to provide data on the spectrum of hepatitis C infection in a referral hospital setting in South Africa.

Methods

Study population

We retrospectively reviewed 87 patients who presented to the liver clinic, Division of Gastroenterology at Chris Hani Baragwanath Hospital (CHBAH) between January 2007 and

December 2016. Patients diagnosed with hepatitis C infection were referred from local clinics, South African National Blood Services (SANBS), CHBAH and from private clinics. Their records were reviewed and analysed. Convenience sampling was used.

Data collection

All patients attending the clinic underwent an initial assessment including history taking and examination. Baseline investigations including a full blood count (FBC), liver function tests (LFT), urea and electrolytes (U&E), hepatitis C viral load and genotype, hepatitis B serology and HIV serology were done. A liver biopsy was performed in all consenting patients except where it was contraindicated or when it was not part of the treatment protocol (not done in patients with genotype 3). Patients eligible for treatment were treated with peginterferon and ribavirin. Those on treatment were followed up for the duration of therapy. Hepatitis C viral load was done during treatment and at 24 weeks after treatment completion to determine sustained virological response (SVR).

Definitions

Treated patients were deemed to have achieved a rapid virological response (RVR) if they had an undetectable HCV viral load at week 4 of therapy. Early virological response (EVR) was defined as complete when viral load was undetectable at 24 weeks of treatment or partial EVR when there was a 2 log drop in viral load at 24 weeks of treatment. Sustained virological response (SVR) was defined as an undetectable HCV viral load 24 week after completion of treatment.

Statistical analysis

All data were analysed using SPSS statistical software (version 24.0 for Windows; SPSS Inc., Chicago, IL, USA). Categorical data such as gender, ethnicity and symptoms were analysed using Pearson's chi-square test or Fisher's exact tests when more than 20% of cells have an

expected frequency less than 5. Continuous data such as age and viral load were analysed using parametric methods and presented as means and standard deviations or median and percentiles for non-normally distributed data. The geometrical mean was used to analyse body mass index (BMI) and viral load. T-test was used in the analysis of normally distributed continuous data; one-way ANOVA with Dunett-t correction was used to compare between the ages of patients with the different genotypes; otherwise the Mann Whitney U test was used. Multinomial logistic regression was used to determine the risk factors for HCV-infection among the study cohort after calculating their univariate logistic regression. Wilcoxon signed-rank test was used for analysis of treatment effect on viral load and liver enzymes. Eta correlation was used to assess the correlation between the Metavir, APRI and FIB4 scores, while Spearman correlation was used for the correlation between APRI and FIB4 scores. Receiver operating characteristics curve (ROC) was also used to compare APRI and FIB4 to the gold standard Metavir. A P value of <0.05 was considered to be statistically significant.

Results

Demographics

Eighty-seven patients' records were identified (Figure 1). The mean age of the cohort at presentation was 52.6 (SD12.3) years. Female patients constituted 54% of the group. There were 71(81.7%) black individuals, 9 (10.3%) Asians and 7 (8%) whites. Black HCV infected individuals tended to be older with a mean (SD) age of 55.7(10.60) which was statistically significant (P=0.001) (Table 3).

Risk factors

Twenty-three of black individuals had history of blood transfusion but it was not statistically significant. Five individuals were haemophiliacs. There was no statistically significant difference in the prevalence of haemophilia between the different ethnic groups. White individuals had more tattooing and history of intravenous drug use when compared to blacks

and Asians which was statistically significant (P= <0.001) (Table 1). History of previous surgery and scarification were not significantly different between the ethnic groups. Data for men who have sex with men (MSM) was not available.

Clinical parameters of patients

Thirty-one (35.6%) individuals were incidentally diagnosed. Forty-seven (60.3%) patients were symptomatic. In 9 patients, presentation was not documented. The most frequently symptoms and signs were joint pain in 12.8%. This was followed in frequency by ascites in 6 patients, lower limb oedema in 5 patients and jaundice in 5 patients.

Comorbidities

Comorbidities in this cohort included diabetes mellitus (10 individuals), chronic kidney disease (8 black individuals) and cardiovascular disease (7 individuals). There was no statistically significant difference in the prevalence of co-morbidities between the different ethnic groups. Chronic hepatitis B was encountered in 3 (2 blacks and 1 Asian) of the 87 individuals while human immunodeficiency virus (HIV) infection was encountered in 17 subjects {16(22.5%) black and 1(14.3%) white patients}. The median (Interquartile range - IQR) CD4 for HIV co-infected individuals was 448 (283-900) cells/µL. Of the 78 patients in which the information was available, 9 were smokers. Thirteen of 81 patients consumed alcohol. The geometric mean and SD of BMI was 25.12 (1.2).

Genotypes

Genotype 5 was exclusively found in blacks. It constituted 53.5% of infections in this ethnic group and 44% of the whole cohort, followed by genotype 1 which formed 18.4%; genotype 3 (13.8%); genotype 4 (9.2%) and mixed genotype infection (3.4%). When genotype 5 was compared to HCV genotypes in other ethnic groups, a statistically significant difference was seen (P= <0.001). Genotype 5 patients were found to be older (mean (SD) age 56.7(9.8) years)

than genotype 1 (46.3(11.4) years and genotype 3 (42 (9.8) years (P=0.002 and <0.001 respectively).

Viral loads

The mean viral load for the cohort was log 5.7(0.9). There was no statistical difference between the different ethnic groups or different genotypes.

Metavir and FIB4 scores

Metavir and APRI scores and FIB4 and APRI score where found to be strongly correlated. r=1, 0.997 and 0.817 respectively. The Receiver Operating Characteristics curve (ROC curve) for Metavir F0 and APRI (cut off <0.7) showed a moderate correlation with an AUC of 0.349 (P=0.002), sensitivity of 78.8%, specificity of 70.6% and a negative predictive value (NPV) of 63.2% and for Metavir F4 vs APRI (cut off ≥1.5) showed an AUC = 0.881 (P value 0.001) with Sensitivity 85.7%, Specificity 93% and a positive predictive value (PPV) of 67% (figure2). Metavir F0 Vs FIB 4 (cut off <1.45) has a moderate correlation with AUC = 0.332 (P=0.021), sensitivity of 78.3%, specificity of 53.8% and NPV 73.7 %. Metavir F4 Vs FIB 4 (cut off >3.25) has a strong correlation with AUC= 0.952 (P=<0.001), sensitivity of 63.6%, specificity of 100% and PPV of 100% (figure 3).

Treatment

All treated patients received pegylated interferon α 2a (Pegasys) and ribavirin. Of 48 patients who were treated, 44 completed treatment. 34 individuals achieved SVR while 9 did not. In one patient results were not available (figure 1). All patients but one developed side effects secondary to treatment. Anaemia was the most common side effect (33 (68.8%) individuals) followed by leucopenia (31(65%)) and fatigue (27(56.2%)). Growth factors (EPO and G-CSF) were used in 17(35.4%) and 6(12.5%) individuals respectively during treatment.

Both ALT and AST showed a statistically significant decline post treatment (P= <0.001). Twelve-week EVR was found to predict sustained virological response to therapy (OR=27.8, 95% CI=2.8-274.3, P=0.004) (Table 4).

Discussion

Chronic HCV infection causes substantial morbidity and mortality as it remains asymptomatic for prolonged periods of time until the individual develops chronic liver disease and/or HCC, yet it is potentially curable.

Having the same mode of transmission as HIV and the high prevalence of HIV in South Africa; it was thought that South Africa will have a large burden of patients with HCV infection. There is paucity of data on HCV infection in South Africa. In the current cohort of 87 patients we describe the clinical characteristics of patients with specific emphasis on genotype 5 which is endemic in South Africa with scarcity of literature worldwide on this genotype (43). Genotype 5 was seen in 44% of the cohort subjects; this is more or less similar to what Smuts *et al* has described from a sample of 79 patients (9). On the other hand, Prabdial-Sing *et al* described a lower percentage of genotype 5 in a larger cohort having done the study at the National Health Labrotary Service (43). Genotype 5 was found exclusively in South African blacks. This could possibly be atributed to previous apartheid policies segregating groups of people together particularly that patients infected with genotype 5 were found to be significantly older than genotype 1 and 3. The observation that it occurs more frequently in older age has been made by french researchers a couple of years ago (44). Genotypes 1, 3, 4 and mixed-genotype infections followed genotype 5 in frequency with a percentage of 18.4%, 13.8%, 9.2%, and 3.4% respectively; with similar distribution to Prabdial-Sing et al report apart from genotype 1 which was far less encountered than what they have reported(43). On the other hand, these figures are much higher than Smuts et al's figures, though sample sizes were not that different.

Both HIV and hepatitis B occurred in low rates in this cohort despite the shared modes of transmission of those infections with HCV.

In this cohort, scores for liver fibrosis such as the APRI score and the FIB4 score correlated significantly with advanced Metavir score of fibrosis (Metavir F3 and F4). Such a conclusion has been reached by a number of researchers (34,35). Implementing these scores in clinical practice can reduce the need for invasive liver biopsies.

Multiple factors were found to affect clearance of the virus, however in this cohort week-twelve early virological response (EVR) was the only predictor of sustained virological response among the study cohort. Rao *et al* had a similar conclusion on their multicentre study that included 125 study recruits of chronic hepatitis C patients (36).

The major strength of this study is that it adds significant information on the clinical characteristics of patients with HCV in South Africa even though it is a retrospective single centre study and does not include patients who have been treated with directly acting antivirals (DAAs), however, this has since changed and DAAs are going to be the standard of care of treatment of HCV patients in SA.

Conclusion

The current study concludes that genotype 5 is the predominant genotype in a single South Africans centre. It is more common among older South African blacks compared to other genotypes. Moreover, APRI and FIB4 scores were powered enough to diagnose advanced fibrosis among HCV patients alleviating the need for liver biopsy in selected individuals. Despite their shared modes of transmission, hepatitis B and HIV infections were not encountered commonly among HCV patients in this cohort. Finally, early virological response was found to determine who is likely to have a sustained virological response.

List of abbreviations

AFP	Alpha feto protein
APRI	Aspartate transaminase-to-platelet ratio index
AUC	Area under the curve
BMI	Body mass index
CHBAH	Chris Hani Baragwanath Academic Hospital
DAAs	Directly acting antivirals
EVR	Early virological response
FIB 4	Fibrosis index based on four factors
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
IQR	Interquartile range
LFT	Liver function test
NPV	Negative predictive value
PPV	Positive predictive value
RNA	Ribonucleic acid
ROC	Receiver operating characteristics
RVR	Rapid virological response
SD	Standard deviation
SVR	Sustained virological response
WHO	World Health Organization

Declarations

1. Ethics approval and consent to participate

Ethical approval was obtained from University of Witwatersrand Human Research Ethics committee (medical), clearance certificate No. M170538.

2. Consent for publication

   Not applicable

3. Availability of data and material

   The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

4. Competing interests

   The authors declare that they have no competing interests

5. Funding

   The study was privately funded

6. Authors' contribution

   WA wrote the manuscript. GIG analyzed the patients' data and assisted in writing the manuscript. RA and CM assisted in writing the manuscript. All authors read and approved the final manuscript.

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Not applicable

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Tables and Figures

Table 3: Hepatitis C infection: Cohort characteristics according to ethnicity

Characteristics		Ethnicity		
		Black (71)	**White (7)**	**Asian (9)**
Age		55.7(10.6)	33.1(3.9)	43.6(11.6)
Gender	Male	33(46.5)	3(43)	4(44.4)
	Female	38(53.5)	4(57)	5(55.6)
Comorbidities	DM	8(11.3)	0(0)	2(22.2)
	CKD	8(11.3)	0(0)	0(0)
	Hemophilia	4(5.65)	1(14.3)	0(0)
	Chronic Hepatitis B	2(2.83)	0(0)	1(11.1)
	HIV	16(22.6)	1(14.3)	0(0)
BMI		25.1(1.2)	24(1.2)	26.3(1.15)
Risk Factors	Blood transfusion	23(32.4)	1(14.3)	2(22.2)
	Tattoo	0(0)	3(43)	0(0)
	Scarification	1(1.4)	0(0)	0(0)
	IV drug use	0(0)	3(43)	0(0)
	Surgery	11(15.5)	0(0)	2(22.2)
Genotypes	GT 1	11(15.5)	2(28.6)	4(44.4)
	GT 3	3(4.2)	4(57.2)	5(55.6)
	GT 4	8(11.3)	0(0)	0(0)
	GT 5	38(53.5)	0(0)	0(0)
	>1 GT	3(4.2)	0(0)	0(0)
Viral load		Log 5.8(0.8)	Log 5.6 (1.2)	Log 5.5 (1.2)
Total treated (48)		37(52.1)	5(71.4)	6(66.7)
Response to treatment (44)	SVR No (34)	27(73)	5(100)	2(33.3)
	Non-responders	5(13.5)	0(0)	2(33.3)
	Relapse	2(5)	0(0)	1(16.7)

All data are expressed as number and percentile n(%) except for age, BMI and VL which are expressed using mean and standard deviation n(SD)

BMI: body mass index, VL: viral load, SVR: sustained virological response, DM: diabetes mellitus, CKD: chronic Kidney disease, HIV: human immunodeficiency virus, GT: genotype

Table 4: Predictors of SVR

Variables		SVR	NO SVR	P
Age mean (SD)		47.2(11.8)	54.7(9.9)	0.103
Gender	Male (n:19)	15	4	0.889
	Female (n:24)	18	6	
Ethnicity	White (n:5)	5	0	
	Black (n:34)	27	7	0.090
	Asian (n:4)	2	2	
HIV (n:4)		4	0	0.999
Genotypes	GT 1	5	3	
(GT)	GT3	5	1	
	GT4	1	3	0.079
	GT5	20	2	
	>1 GT	3	0	
RVR (n:27)		23	4	0.211
EVR 12 (n:42)		34	9	0.006
FIB4 (n:40)		33	7	0.036
APRI (n:40)		33	7	0.079

On multivariate analysis EVR12 was found to be the only significant factor determining response with a p value of 0.004

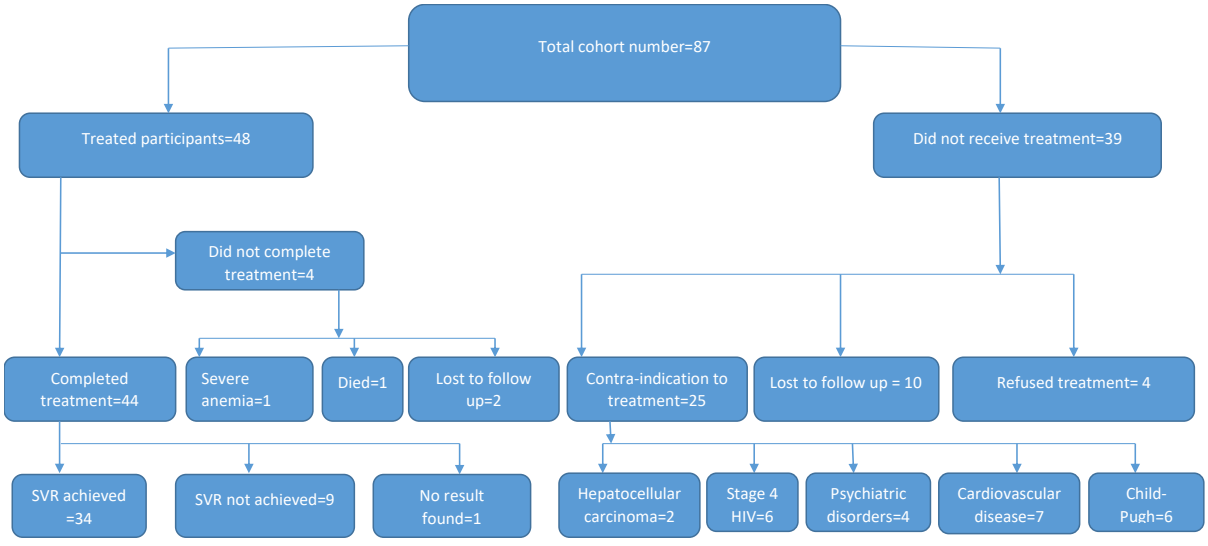
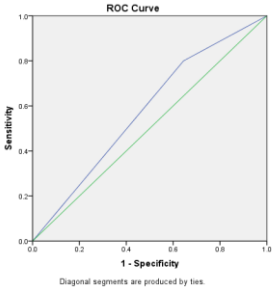


Figure 1. Flow chart of the study cohort



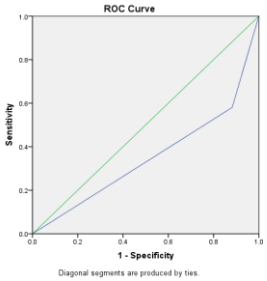
APRI (cut of <0.7) vs Metavir F0

AUC=0.349, P=0.002

Sensitivity= 78.8%

Specificity=70.6%

NPV = 63.2%



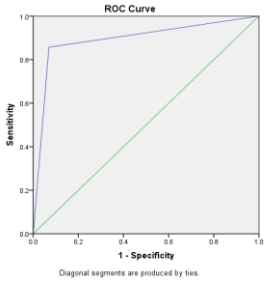
APRI (cut of 0.5-2) vs Metavir F3

AUC=0.578, P=0.571

Sensitivity= 20%

Specificity= 6%

PPV =79%



APRI (cut of ≥1.5) vs Metavir F4

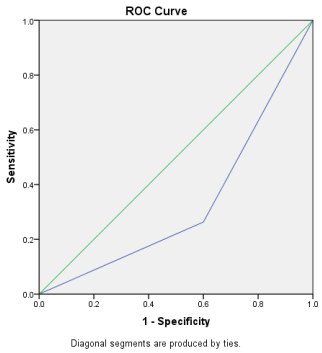
AUC = 0.881, P value 0.001

Sensitivity=85.7%

Specificity=93%

PPV = 67 %

Figure 2. Correlation between APRI and Metavir Scores



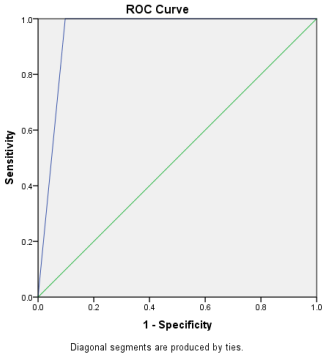
a. FIB 4 cut off <1.45 vs Metavir F0

AUC = 0.332, P=0.021

Sensitivity =78.3

Specificity= 53.8,

NPV = 73.7%



b. **FIB 4 (cut off >3.25) vs Metavir F4**

AUC= 0.952, P=<0.001

Sensitivity= 63.6%,

Specificity =100%,

PPV = 100%

Figure 3. Correlation between FIB4 and Metavir

CHAPTER 3: SUPPLEMENTARY TABLES AND FIGURES

Table 5: Liver enzymes response to treatment

Variable	Pre-treatment Median	Post-Treatment Median	P value
AST	40	28	<0.001
ALT	44.5	20	<0.001

Table 6: The correlation between Metavir, FIB4 and APRI scores (Eta correlation)

Variables	Metavir	FIB4	APRI
METAVIR	1	1	0.997
FIB4	1	1	0.817
APRI	0.997	0.817	1

Table 7: Characteristics of Hepatitis C genotype 5 infection in comparison to other genotypes (Univariate analysis)

Genotype / **Variable**		GT5	GT 1	P	GT 3	P	GT 4	P	>1 GT	P
Age mean (SD)		56.(9.8)	46.3(11.4)	0.002	42 (9.8)	<0.001	60.6(6.5)	0.296	46(20.5)	0.089
Gender	Male	16	8	0.732	7	0.329	3	0.810	3	NA
	Female	22	9	NA	5	NA	5	NA	0	NA
Ethnicity	White	1	2	0.992	4	0.992	0	0.997	0	NA
	African	37	11	0.991	3	0.990	8	NA	3	NA
	Asian	0	4	NA	5	NA	0	NA	0	NA
IV drug use		0	0	0.999	3	0.995	0	0.999	0	NA
Surgery		7	4	0.662	1	0.419	0	0.690	0	NA
Tattooing		0	0	0.999	3	0.995	0	0.999	0	NA

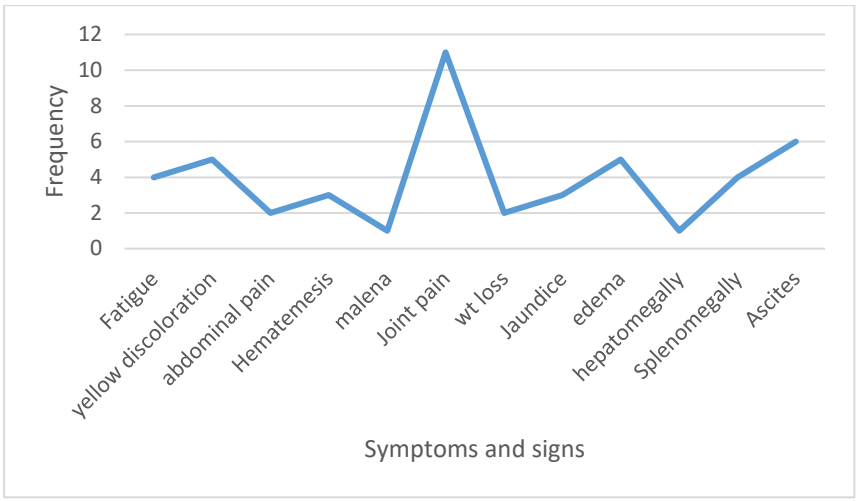


Figure 4. HCV infection: symptoms and signs

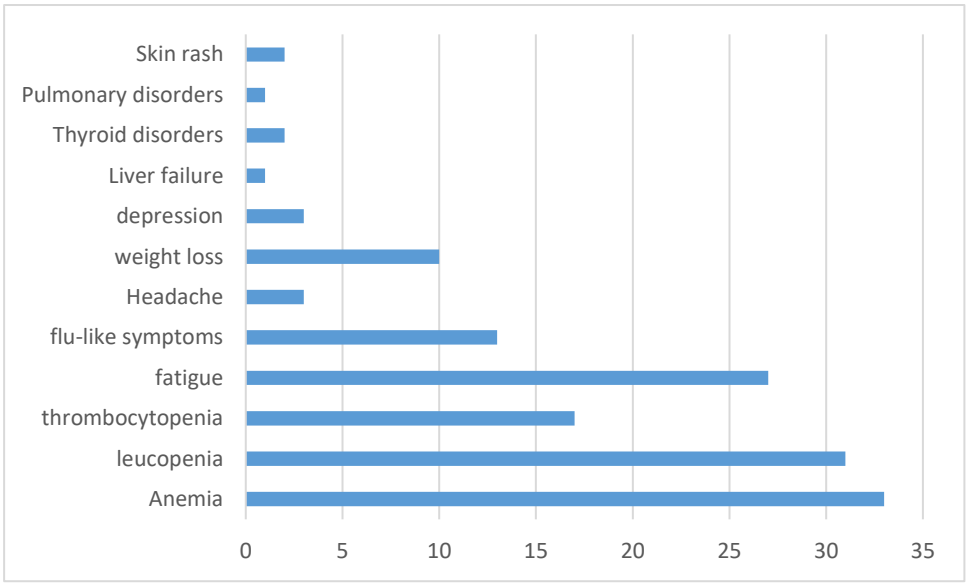


Figure 5. Side Effects of Treatment

CHAPTER 4: APPENDICES

Appendix 1 - Data Collection Sheet

Data Collection Sheet

Participant Identification Number_____

1. Age (at time of presentation) _____ (years)
2. Gender: Male ☐ Female ☐
3. Ethnic group
   White ☐ Black African ☐ Mixed Ancestry ☐ Asian ☐
   Other ☐ (specify_____)
4. Presentation: Incidentally Diagnosed ☐ Fatigue ☐
   Abdominal pain ☐ Jaundice ☐ Ascites ☐ Hematemesis ☐
   Body Swelling ☐ Melena ☐ Joint Pains ☐ Weight loss ☐
   Hepatocellular carcinoma ☐ Other ☐ (specify_____)
5. Comorbidities:
   Diabetes Mellitus ☐ Hypertension ☐
   Cardiovascular disease ☐ Haemophilia ☐
   Chronic kidney disease ☐ Rheumatological conditions ☐
   Chronic hep B ☐ Psychiatric illness ☐
   HIV: known ☐ unknown ☐ (if known CD4_____ treatment_____)
   Other ☐ (specify_____)
6. Risk factors: Previous blood transfusion ☐ Tattoo ☐
   Scarification ☐ IV drug use ☐
   Other ☐ (specify_____)
7. Alcohol use: Yes ☐ No ☐ Smoking: Yes ☐ No ☐
8. Examination: Weight (at time of treatment):_____kg Height ____m
   Jaundice ☐ Edema ☐ Hepatomegaly ☐
   Splenomegaly ☐ Ascites ☐ Other ☐ (specify_____)
9. Investigations: Genotype _____ Hep C Viral Load _____Log value_____
10. HIV status: Positive ☐ Negative ☐
11. Hepatitis B surface antigen: Positive ☐ Negative ☐
12. Pre-treatment blood tests: Hemoglobin _____gm/dl Platelets _____x10^9/L
    WCC_____ x10^9/L Total Bilirubin: _____umol/L Albumin: _____ g/L
    ALT _____U/L AST_____U/L ALP_____U/L GGT_____U/L
    Creatinine_____umol/L INR: _____ Ferritin: _____ug/L % sats_____
    Transferrin: _____g/L AFP_____

13. Liver Biopsy performed pre-treatment: Yes ☐ No ☐

14. If yes: Metavir score: A1 ☐ A2 ☐ A3 ☐
F1 ☐ F2 ☐ F3 ☐ F4 ☐

15. Treated: Yes ☐ No ☐

16. If no, treatment exclusion reason:
Refused ☐ contraindicated ☐ (specify_____)
Other ☐ (specify_____)

17. Pegylated-Interferon initiating dose: 180ug/wk ☐ 135ug/wk ☐ 90ug/wk ☐
18. Ribavirin initiating dose_____ mg/day

19. Week 4 PCR (Rapid viralogical response: RVR) Positive ☐ Negative ☐
20. Week 12 PCR (Early viralogical response: EVR) Positive ☐ Negative ☐

21. Total duration of treatment: _____ (weeks)
22. End of treatment (EOT) PCR: Positive ☐ Negative ☐

23. Sustained viralogical response(SVR) achieved? Yes ☐ No ☐
24. Was any G-CSF (e.g. Neupogen) required? Yes ☐ No ☐

25. Was any EPO (e.g. Recormon) required? Yes ☐ No ☐

26. Side Effects of treatment: Yes ☐ No ☐ if yes specify below:

Anaemia ☐	Leukopenia ☐	Thrombocytopenia ☐
Flu-like symptoms ☐	Fatigue ☐	Headache ☐
Weight loss ☐	Depression ☐	Hypersensitivity reaction ☐
Liver failure ☐	Thyroid disorder ☐	(specify_____)

Pulmonary disorder ☐ (specify_____) Other ☐ specify_____

27. EOT ALT_____U/L_ AST_____U/L

RVR: Rapid viralogical response, EVR: early viralogical response, EOT: End of Treatment;

SVR : Sustained Viralogical Response (Hep C PCR negative 24 weeks after the completion of treatment)

Appendix 2 – Ethical clearance certificate

R14/49 «Tit Init name»

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170538

NAME: (Principal Investigator)	Dr Wamda Abuelhassan
DEPARTMENT:	Internal Medicine Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	Hepatitis C Infection: A Retrospective Analysis of Patients at Chris Hani Baragwanath Academic Hospital, South Africa
DATE CONSIDERED:	26/05/2017
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Reidwaan Ally and Prof Colin Menezes
APPROVED BY:	Professor P. Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	29/05/2017

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary 3rd floor, Phillip Tobias Building, Parktown University of the Witwatersrand. I/We fully understand the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially review in May and will therefore be due in the month of May each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES