UNIVERSITY OF GHANA

COLLEGE OF HEALTH SCIENCES

HEPATITIS C VIRUS (HCV) INFECTION AMONG SICKLE CELL DISEASE PATIENTS AT THE KORLE-BU TEACHING HOSPITAL

BY

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DECLARATION

I, Gifty Mawuli, hereby declare that this project, aside other cited articles and references, is the result of a research I duly conducted which was supervised by Dr. Bartholomew Dzudzor, Department of Medical Biochemistry and Dr. J H Kofi Bonney, Department of Virology- Noguchi Memorial Institute for Medical Research, University of Ghana.

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ABSTRACT

Background: Hepatitis C virus (HCV) infection is a leading cause of chronic liver disease among sickle cell disease (SCD) patients. Disease progression has been implicated with circulating genotypes of HCV. Determination of RNA and genotypes of HCV in sickle cell disease patients may give an indication of their contribution to the observed clinical manifestations and disease progression which will inform appropriate clinical management.

Aim: This study sought to identify and characterize HCV in sickle cell disease patients.

Methods: This was a cross-sectional study which enrolled 142 sickle-cell patients from the Ghana Institute for Clinical Genetics, Korle-Bu Teaching Hospital. Patient information was obtained through a questionnaire and 3/mls of whole blood was collected. The plasma obtained was screened by serology and the viral nucleic acid extracted was amplified by reverse transcriptase- polymerase chain reaction (RT-PCR), with primers targeting the HCV core gene. Amplified DNA were purified and sequenced. Sequenced products were purified and put into the genetic analyzer for analysis. HCV genotypes were obtained by phylogenetic analysis.

Results: A total number of 142 SCD patients were recruited in this study with majority of them being females (64%). The median age was 25 years. Seventy-two (51%) had been transfused. Out of the 142 patients' samples collected 12 (9%) were sero-positive for anti-HCV total antibodies. HCV RNA was amplified from 8 (6%) out of the 142 patients' samples. One of the 12 seropositives was HCV RNA positive. Five (63%) out of the HCV RNA positive samples were successfully sequenced. The phylogenetic tree constructed

with GenBank reference sequences, clustered all five sequences from our study into genotype 1, specifically 1b.

Conclusion: This study established the seroprevalence of 9% of total antibodies to HCV among sickle cell patients whilst circulating HCV among the study population were found to be genotype 1 strain.

DEDICATION

I humbly dedicate this thesis to my dearest husband, Nana Akosah Sarpong and children; Nana Yaw Sarpong and Nana Kwabena Mawuli Sarpong for their relentless help, love, patience and prayers throughout my studies.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

CC:	Haemoglobin C
CHS:	College of Health Science
CD81:	Cluster of Differentiation 81
CLDN:	Claudin
DAA:	Direct acting anti-virals
ELISA:	Enzyme Linked Immunosorbent Assay
ER:	Endoplasmic Reticulum
GICG:	Ghana Institute of Clinical Genetics
HbS:	Sickle Haemoglobin
HCV:	Hepatitis C Virus
HIV:	Human Immunodeficiency Virus
IRB:	Institutional Review Board
IRES:	Internal Ribosomal Entry Site
ISGs:	Interferon stimulated genes
KBTH:	Korle- Bu Teaching Hospital
LDL:	Low Density Lipoprotein
MAFFT:	Multiple Alignment using Fast Fourier Transform

MEGA:	Molecular Evolutionary Genetics Analysis
mRNA:	Messenger RNA
NMIMR:	Noguchi Memorial Institute for Medical Research
NS:	Nonstructural
PBMC:	Peripheral Blood Mononuclear Cell
PCR:	Polymerase Chain Reaction
PIAS:	Protein inhibitor of activated STAT 1
PKR:	Protein Kinase RNA
RBCs:	Red blood cells
RNA:	Ribonucleic Acid
RT-PCR:	Reverse Transcription Polymerase Chain Reaction
SC:	Haemoglobin SC
SCD:	Sickle Cell Disease
SD:	Haemoglobin D-Punjab
SF:	Fetal haemoglobin
SOCS:	Suppressors of cytokine signaling
SRB1:	Scavenger Receptor class B type 1
SS:	Haemoglobin SS
STC:	Scientific and Technical Committee

SVR: Sustained Virological Response

TNFα: Tumor Necrosis Factor alpha

UTR: Untranslated Region

VHFs: Viral Haemorrhagic Fevers

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

One of the greatest hereditary diseases in the world is sickle cell disease (SCD) and this affects mainly people of African ancestry (Cordero et al., 2009; Centre for Disease Control and Prevention, 2017). Sickle cell disease is an autosomal disorder of the blood and it is caused by inherited abnormal haemoglobin, which is the main transporter of gases such as oxygen and carbon dioxide within the red blood cells. SCD is a point mutation disease of the beta globin chain and this leads to the replacement of valine with glutamic acid (Pauling et al., 1949). The resulting abnormal haemoglobin (HbS) is responsible for the changes in the red blood cells which ends up in the blocking of the microcirculation causing inadequate blood supply and organic dysfunction (Bunn, H. F., 1997; Gladwin, M. T. & G. J. Kato, 2005).

Transfusion of normal red blood cells is given as prophylaxis and therapy for major complications of SCD such as stroke and acute chest syndrome. Also, other different health problems such as leg ulcers, priapism, pulmonary hypertension, vaso-occlusive crises and complications during pregnancies may require blood transfusion (Raghupathy et al., 2010). Only a single transfusion may be needed for some problems, however, other patients require multiple transfusions, and this could be receiving blood once in a month for a long period. However, there are some possible risks of chronic transfusion, which may include iron overload, transfusion reactions and transmission of infections. Currently, blood products are screened for infectious disease such as hepatitis and HIV before patients are transfused, though, the risk of getting infection is still present (Jude, 2008).

Hepatitis C virus was first detected by Choo and colleagues in 1989 (Farci, 2002) and it is primarily acquired through contact with contaminated blood. This infection is one of the leading causes of chronic liver disease. Globally, about 71 million of the total population who are chronically infected with this disease are unaware of their condition (EASL, 2018). The virus has the ability to cause both acute and chronic hepatitis. The acute phase is self-limiting, hardly leads to hepatic failure, and mostly ends with a chronic infection. About 50-80% of infected individuals have chronic infection which could last for many years and often results in cirrhosis, liver cancer or require liver transplantation (Sanjiv & Lai, 2019).

About 15% of individuals infected with the HCV show spontaneous viral clearance (Bobek et al., 2010) . Such patients are positive by serology but HCV RNA negative without antiviral therapy (Renda et al., 2011). The current HCV drug regimen are effective and the main aim is to obtain sustained virologic response (SVR). This is the absence of HCV RNA 12 weeks or 24 weeks after completion of treatment. Factors that may affect viral clearance are age, sex, race, frequent alcohol intake, viremia level and HCV genotypes (Freeman et al., 2001; Chen, S. L. & Morgan, 2006).

The most broadly studied mode of HCV transmission has been blood or blood derivatives (Freeman et al., 2001). People whose blood were transfused before serologic screening for anti-HCV antibodies at blood centers have HCV prevalence ranging from 2 to 30% (Schreiber et al., 1996). Although transmission by sexual intercourse is rare, the risk may be high among people with multiple partners (MacDonald, M. A. et al., 2000). A study by MacDonald and Ohto established that the rate of vertical transmission is low (Freeman et al., 2001). Intravenous drug users could get HCV infection by repetitive exposure to contaminated injection equipment (Paintsil et al., 2014).

At present, HCV vaccine is unavailable (Shoukry, 2018), however, clinical care has improved enormously for patients with HCV related liver diseases. There is now a greater understanding of the viral genome and proteins and this has given a better insight into the pathophysiology of HCV infection, therapy and diagnostic procedures (Pockros et al., 2016). A cure for HCV infection has been the primary goal of therapy. Treatment regimens prevent the complications associated with HCV-related liver diseases. They improve the standard of life of patients and prevents onward transmission (EASL, 2018). The current drugs available are the multiple direct-acting antivirals (DAAs) which target specific steps within the life cycle of HCV. These drugs, defined by their modes of action and therapeutic targets, disrupt the viral replication cycle by targeting specific nonstructural proteins which make their role in viral replication inactive (Pockros et al., 2016).

The virus has a greater degree of genetic diversity and this helps it present as a group of heterogenous but closely related species *in-vivo* (Jardim et al., 2013). Hepatitis C virus currently has seven genotypes, namely, genotype 1 to 7 and also includes about 67 closely related subtypes (Smith et al., 2014). Genotype 1 is found in the Americas, Japan and Europe, with 2 and 3 being the most prevalent globally. Genotypes 4 and 5 are mostly found in Africa and Asia, and genotypes 6 and 7 are distributed in Southeastern Asia (Owusu et al., 2018). From literature, genotype 1 has a lower SVR which is about 30% as compared to genotypes 2 and 3, whose sustained virologic response is 65%. Globally, the predominant subtypes are 1a and 1b and these accounts for about 60% of HCV infections. Patients who develop active chronic HCV normally have genotype 1b and have higher plasma concentration of HCV RNA (Le Pogam et al., 1998). It has become increasingly evident that genotypes of hepatic viruses are a major factor contributing to differences

observed in clinical manifestations, antiviral treatment and vaccination. However, poor and non- documented data on HCV infection and genotypes in SCD patients in Ghana are available in literature and it emphasizes the importance of genotyping studies.

1.2 Problem Statement

Presently, studies have shown that infections through blood transfusion has reduced significantly (Dean et al., 2018) This is attributed to better screening methods currently available; however, the risk is still present. Sickle cell disease patients who have frequent blood transfusions are at risk of HCV infection (Ancel et al., 2009; Shah et al., 2017). Hepatitis C virus causes chronic liver diseases among these patients. At least 10% of adult sickle cell patients are HCV positive and often have liver dysfunction (Silva et al., 2006). Disease progression has been associated with circulating genotypes of HCV. Even though HCV is endemic in sub-Saharan Africa, there is a dearth on information on the occurrence of HCV and the different genotypes among SCD patients in Ghana. In addition, the immunocompromised status of most SCD patients makes them prone to severer liver damage when infected with HCV. It is therefore relevant to determine the occurrence of HCV, the genotypes circulating among sickle cell disease patients within the study area and analyze the genomic diversity among these genotypes.

1.3 Justification

Determination of anti-HCV antibody, RNA and genotypes of HCV in sickle cell disease patients may give an indication of their contribution to the observed clinical manifestations

and disease progression, which will inform appropriate clinical management. It is therefore necessary to determine the occurrence of HCV, detect HCV RNA and identify the genotypes circulating in the Ghanaian population with sickle cell disease.

1.4 Aim

I. To identify and characterize HCV in sickle cell disease patients at the GICG-KBTH.

1.5 Specific Objectives

II. To determine the prevalence of anti-HCV antibodies among SCD patients.

III. To detect HCV RNA among the SCD patients.

IV. To genetically characterize the genotypes of HCV among the SCD patients.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Structure of Hepatitis C Virus

Hepatitis C virus (HCV) was first isolated in 1989. This is an RNA virus which is enveloped, positive-sense and single stranded. This virus is about 55-65 nm in size and spherical in shape. It belongs to the viral family, Flaviviridae. This family has three genera which are the flaviviruses, pestiviruses and hepaciviruses. Hepatitis C virus presently, is the only member of the genus, hepacivirus (Park et al., 2014). The viral genome is about 9.6 kbp and has one large open reading frame which is flanked by conserved untranslated regions (UTR) at the 5' and 3' ends . The untranslated regions contain control elements required for initiation of replication and translation (Sharma, 2010). The RNA encodes structural and nonstructural proteins which are the core and enveloped glycoproteins E1 and E2, NS2, NS3, NS4A, NS4B, NS5A and NS5B (Figure 2.1). The p7 protein is an additional protein found in the middle of structural and nonstructural proteins.

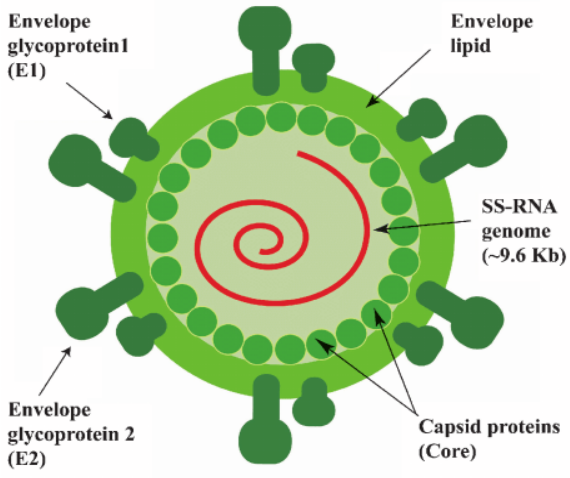

Image source: (Sharma, 2010)

Figure 2.1: Structure of the Hepatitis C Virus

2.2 **Hepatitis C Viral Genome**

2.2.1 The 5' Untranslated Region

The 5' untranslated region of the hepatitis C viral genome consists of 341 nucleotides located upstream of the open reading frame (ORF) translation initiation codon (Figure 2.2). The 5' UTR is the most conserved region of the genome (Chevaliez & Pawlotsky, 2006). The 5' untranslated region has four well defined domains (I- IV) and contains many stem loops (Brown et al., 1992) . The internal ribosomal entry site (IRES), which forms part of the 5' UTR, consists of domains II, III, IV and the first 12 to 30 nucleotides of the core region (Honda et al., 1996). The IRES is capable of forming a firm pre-initiation complex. The 5' UTR contains an IRES that directs translation, binds to elongation factor 3 and ribosomal protein S9 during translation (Chen, Z. & Weck, 2002). Also a microRNA with binding domains in the 5' UTR facilitates HCV replication.

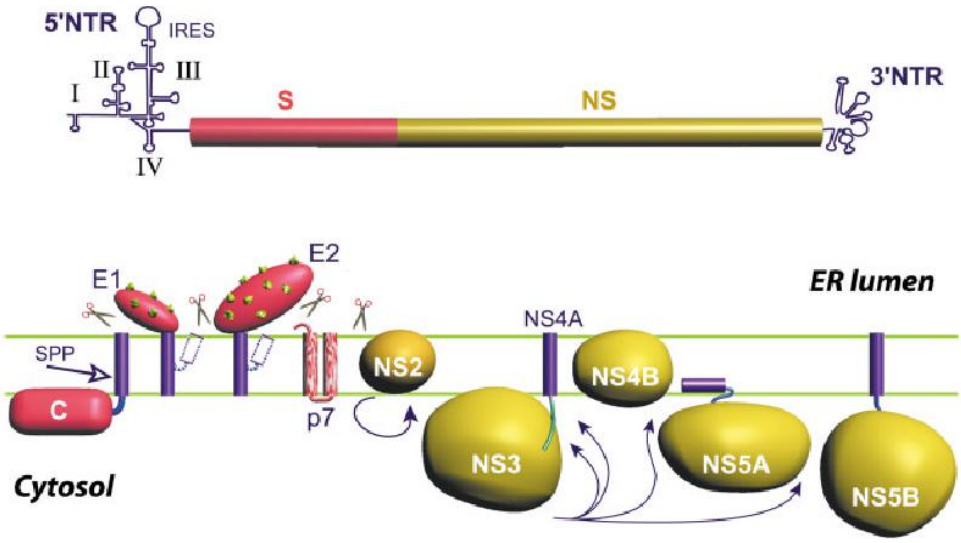

Image Source:(Penin, 2003)

Figure 1.2: HCV Genome Organization (up) and Polyprotein processing (down).

Hepatitis C virus has a viral enveloped, a nucleocapsid with a positive-sense RNA of 9.6 kilobytes. The 5'UTR has the four highly structured domain and also the IRES. There are three unique

domains at the 3' UTR which are the poly U/UC tract, the variable region and the X- tail region with 3 stem loops. During translation, the 5' and 3' do not undergo translation but are essential for translation and replication of the genomic RNA.

2.2.2 The 3' Untranslated Region

The 3' UTR is about 225 nucleotide bases long. It has three distinct regions which are the variable region, a poly U/UC tract and a highly conserved 3'-terminal stretch (3' X region). The 3'X regions also comprise of 3 stem-loop structures SL1, SL2 and SL3 (Kolykhalov et al., 1996; Sharma, 2010). There exists long- range RNA/RNA interactions between the 3' and 5' UTR which are necessary during replication and speeds up translation from the HCV IRES (Park et al., 2014).

2.2.3 The HCV Core Protein

The core protein of the viral genome is an RNA-binding protein which is extremely basic in nature and is capable of self-assembly in HCV-like particles in the endoplasmic reticulum (ER) (Raney et al., 2010). It has 3 distinct predicted regions which are the N-terminus hydrophilic domain (domain D1), the C-terminus hydrophobic domain (domain D2) and the last domain (D3) serves as a signal peptide for the downstream envelop protein E1(Chevaliez & Pawlotsky, 2006) . One of the regulatory functions of the core protein is the assembling of the seven nonstructural proteins to the lipid droplet-associated membranes. A portion of the core protein which is made up of 112 to 152 amino acids (Chevaliez & Pawlotsky, 2006) is essential for the link with the ER and also the outer mitochondria membrane (Wang et al., 2010). This association with the outer mitochondria

membrane allows it to regulate calcium ion concentrations and apoptotic signals. The core protein associates with chaperons of mitochondrial proteins and results in oxidative stress. Detection of the core antigen in plasma of a patients is an indication of active infection (EASL, 2018).

2.2.4 Envelope glycoproteins

These consist of two structural proteins E1 and E2 which make up the fine spike-like projections on the viral outer coat. The envelope proteins are highly glycosylated and both proteins are required for viral entry by binding to host receptors (Hu et al., 2005). The E1 is the subunit for fusion whiles E2 acts as a receptor binding protein containing a binding site for the human CD81. It is also able to utilize glycosaminoglycans and low-density receptors on hepatocytes as initial attachment factors (Idrees et al., 2013) .

2.2.5 The p7 Protein

The p7 is a membrane protein consisting of 63 amino acids found in the ER. The p7 protein is a viroporin, which forms ion channels that translocate protons across cellular compartments (Atoom et al., 2013; Aweya et al., 2013). Though, the viroporin does not aid in replication, it is important for the packaging and budding of the infective virions. It is reported that p7 is able to associate with at least four HCV proteins (core protein, NS2, NS4, NS5B) during morphogenesis (Popescu et al., 2014).

2.2.6 Nonstructural Proteins

2.2.6.1 Nonstructural protein 2 (NS2)

The nonstructural protein 2 (NS2) is transmembrane protein needed to complete the replication of the virus (Ashfaq et al., 2011). This protein is non-glycosylated with a molecular weight of 21-23 kDa (Chevaliez & Pawlotsky, 2006). Two internal signal sequences belonging to NS2 at amino acid positions 839-883 and 928-960 are responsible for endoplasmic reticulum association. The N- terminal part of NS2 is highly hydrophobic and it forms about four transmembrane helices that insert into the ER membrane. The C-terminal which resides in the cytoplasm is important in NS2/NS3 auto protease activity together with the N-terminal domain of NS3 . The protease activity of NS2 is lost after self-cleavage from NS3 (Chevaliez & Pawlotsky, 2006) .

2.2.6.2 The Nonstructural protein 3-4A (NS3-4A)

The NS3 is a multifarious viral protein which is about 67 kDa. The N-terminus contain a serine protease whiles the C-terminus contain a helicase/NTPase domain (Chevaliez & Pawlotsky, 2006) . The NS4A acts as a cofactor of NS3 protease activity. The protease activity of the NS3 protease is influenced through the NS4 protein acting as a co-factor. The interaction between the NS3 and the NS4 proteins initiates an attachment with the ER membrane (Wolk et al., 2000). The NS3-4A properties is essential in the lifecycle and disease pathogenesis, this is through interaction with host cellular processes. For this reason, NS3-NS4A protease is a viral target for anti-HCV therapeutics. This protease is important for HCV replication through the cleavage of HCV polyproteins at the following

junctions, NS3/NS4A, NS4A/NS4B, NS4B/NS5A and NS5A/NS5B (Chevaliez & Pawlotsky, 2006).

2.2.6.3 The Nonstructural protein 4B (NS4B)

The NS4B is a small hydrophobic protein of 261 amino acids with an endoplasmic reticulum or endoplasmic reticulum- derived membrane localization (Lundin et al., 2003; Ashfaq et al., 2011). Topological studies have predicted NS4B to contain 4 transmembrane domains and an N-terminal amphipathic helix which are responsible for membrane association (Elazar et al., 2004). Studies have shown that NS4B- induced morphological changes to ER and it forms the membranous web. Viral proteins are found in this area and this is where the replication complex is formed (Egger et al., 2002).

2.2.6.4 The Nonstructural protein 5A (NS5A)

The NS5A is a phosphorylated zinc-metalloprotein of 56-58 kDa. This protein plays an essential role in viral replication, regulation of cellular pathways and interferon response (MacDonald, A. et al., 2004). NS5A has no transmembrane domains. Studies have confirmed the association of NS5A with viral proteins which confirms its presence in replication. Its numerous interaction with other proteins affect cell signaling. NS5A regulates cell signaling by pro and anti- apoptotic mechanisms. Mutations in the NS5A sequences inhibits HCV replicon RNA replication (Elazar et al., 2003)

2.2.6.5 The Nonstructural protein 5B (NS5B)

The NS5B is a member of membrane proteins which are called the tail-anchored proteins (Chevaliez & Pawlotsky, 2006). As an RNA polymerase, it synthesizes a new RNA genome from an RNA template. Replication continuous through the generation of a negative strand RNA complementary to the genomic RNA. Subsequently uses the negative strand RNA as an intermediate to synthesize positive strand RNA (Ashfaq et al., 2011). The RNA dependent RNA polymeraseis a therapeutic target, which are the Direct Acting Anti-virals (DAAs) (Pawlotsky, 2006).

2.3 The Hepatitis C Virus (HCV) lifecycle

The virus replicates in the liver cells (hepatocytes) and these cells are its main targets. Research have shown that the virus could replicate in peripheral blood mononuclear cells (PBMCs). This could be the reason for the high level of immunological disorder in chronically infected patients. The virus RNA-dependent RNA polymerase lacks a proofreading mechanism and this could account for the wide variety of HCV genotypes (Le Guillou-Guillemette et al., 2007).

The initial stage of viral replication is the attachment (Figure 2.3). This occurs through a complex interaction which occurs between the viral envelope glycoproteins and cell surface receptors such as CD81, SR-BI, DC-SIGN, members of the Claudin family (CLDN1, 6 and 9) (Zeisel et al., 2009; Popescu et al., 2014). The virus bound to the cell surface receptors is endocytosed and the nucleocapsid is freed into the cytoplasm. Just like most RNA viruses, viral replication and translation occurs in the cytoplasm. Hepatitis C Virus uses most of host cell's protein and molecules for replication (Lindenbach & Rice, 2005). The viral genome is translated into a single protein which is around 3011 amino acids long. The

polyprotein is post translated into three structural and seven nonstructural proteins. This occurs with the help of viral and cellular proteases, which are the NS2 cysteine autoprotease, and the NS3-4A serine protease. One of the nonstructural proteins, NS5B is an RNA- dependent RNA polymerase and it produces a negative strand RNA intermediate from the RNA template. The negative RNA is the synthesized into new positive strand viral genomes. The mature viral particle is exocytosed and infects other hepatocytes (Lindenbach & Rice, 2005).

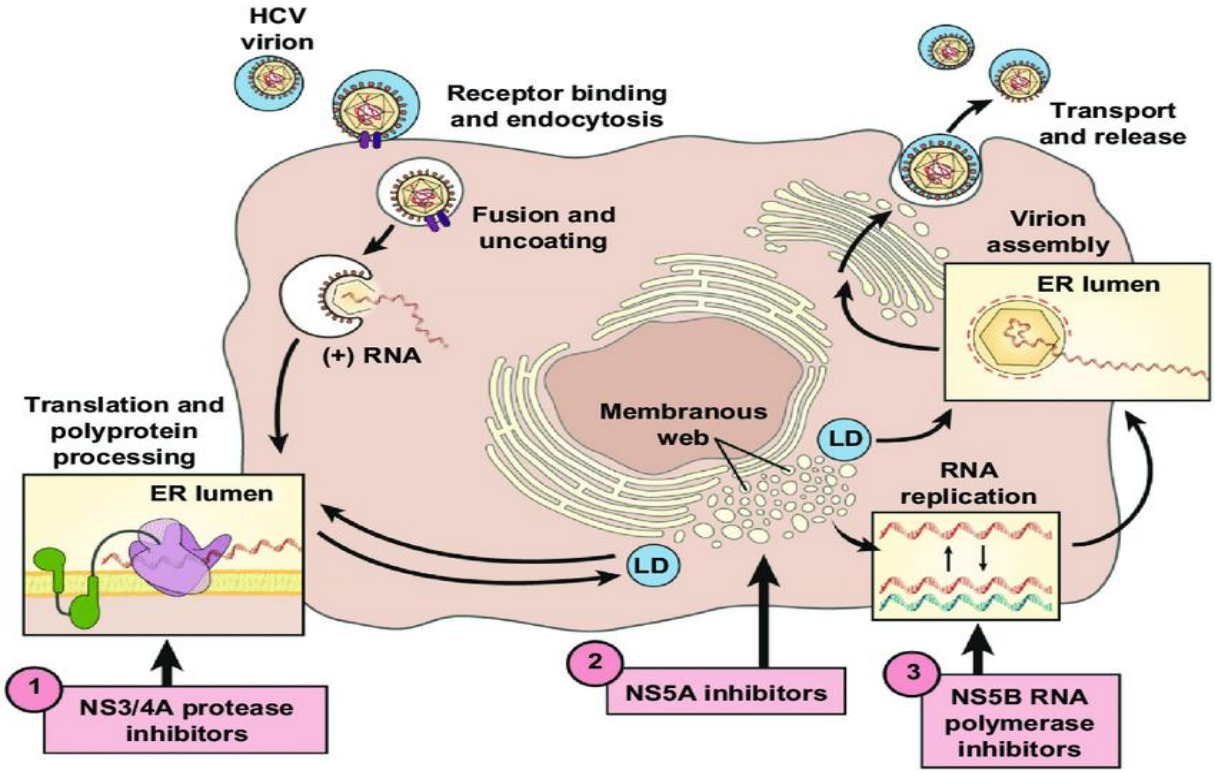

Image source: (Kayali & Schmidt, 2014)

Figure 2.2: HCV life cycle and Therapeutic targets

Hepatitis C virus replicates in the liver cells (hepatocytes) and circulates throughout the body. The initial stage is an attachment between the viral envelope glycoproteins (E1 and E2) and the cell surface receptors which are the CD81, Scavenger Receptor class B1 and members of the Claudin family (CLDN 1, 6 and 9). The virus linked to the cell surface

receptors is endocytosed. The nucleocapsid is released into the cytoplasm. The genomic RNA is removed from the nucleocapsid ant it is used for polyprotein translation and replication. The viral RNA acts as a messenger RNA (mRNA) since it is a positive sense RNA. Translation of the mRNA occurs in the rough endoplasmic reticulum (ER). This is followed by a post translation of the ploy protein into three structural proteins (core, E1 and E2) and seven nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B). The virus matures in the golgi apparatus, exocytosed to infect other hepatocytes.

2.4 Global Distribution of the Seven Genotypes of Hepatitis C Virus

The nonstructural protein 5B, lacks a proof reading mechanism and this accounts for the high genomic diversity of the hepatitis C virus (Le Guillou-Guillemette et al., 2007).

The current therapeutics are genotype specific and therefore it is important to know the circulating genotypes in a region, which would have important clinical implications. The virus has seven genotypes. Presently, it has 67 subtypes and 20 yet to be subtyped (Messina et al., 2015). Borgio et al (2018) have discovered a novel HCV genotype 8 in Punjab, India (Borgia et al., 2018). Phylogenetic analysis have shown that HCV strains which belong to the same subtype differ with a maximum of 15 percent of nucleotide sites (Smith et al., 2014). It has been reported that subtypes 1a, 1b, 2a and 3a are globally predominant and a high percentage of HCV infection in developed countries have these subtypes (Messina et al., 2015). Endemic strains of HCV subtypes are comparatively rare and circulates in more restricted region. In West Africa, the endemic strains are from genotypes 1 and 2, genotype 3 in South Africa, Central Africa and Middle East with genotype 4, genotypes 5 and 6 are found in Southern Africa and South Asia respectively (Figure 2.4). Genotype 7 which was reported in Canada was isolated from a central African immigrant (Taylor, 2006; Sandmann & Ploss, 2013; Messina et al., 2015). These finding suggests that, the genetic

variations of HCV strains across the globe are likely to be subject to human migration (Culasso et al., 2012) .

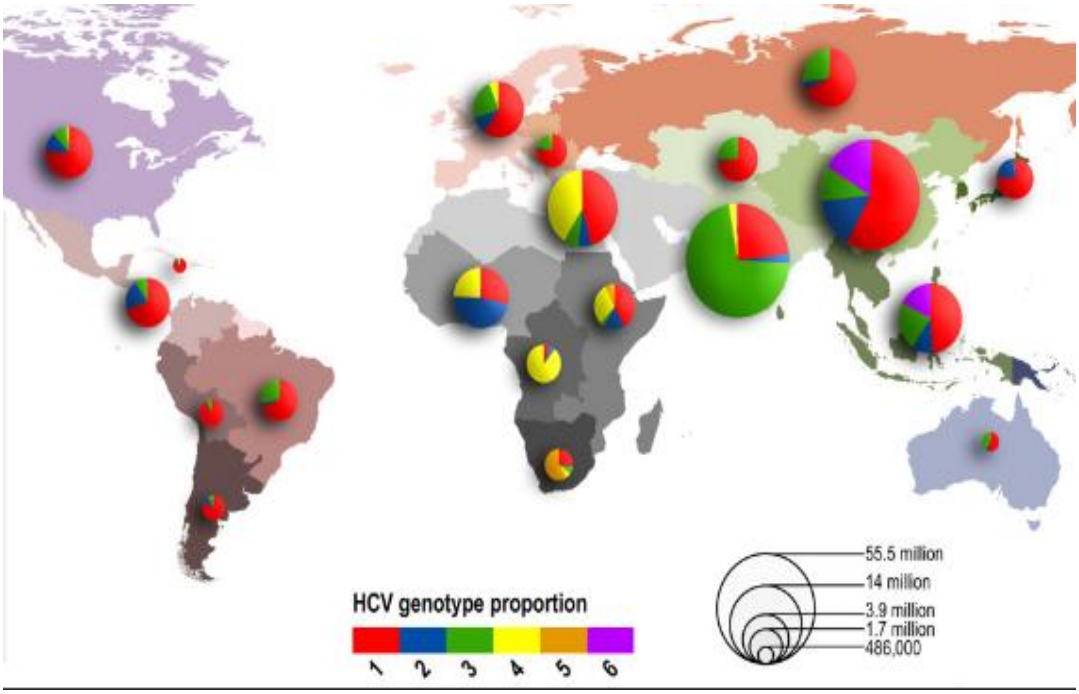

Image source: (Messina et al., 2015)

Figure 2.4: Map showing the global distribution of HCV genotypes

2.5 HCV Evasion Mechanisms and Host Immune Response

A strong type 1 interferon response is induced in the liver of infected persons during an HCV infection (Sheahan et al., 2014). The host's innate immune response puts the liver cells in an antiviral state in two phases, the initiation and the effector phases. The host immune factors recognize the HCV by using pattern recognition receptors which are found on cell surfaces and the endosomes (Ashfaq et al., 2011). Once the virus has been identified, interferon stimulated genes (ISGs) in the host are induced. Pattern recognition receptors used by the host in identifying HCV genome include the TLR-2, 3, 7, and RIG-I

which signal through adaptor proteins TRIF, MyD88, and IPS-1(Hu et al., 2005). The initiation phase of the host response includes the identification of the virus, phosphorylation of adaptor proteins and movement of transcription factors into the nucleus to initiate transcription of the appropriate genes (Interferon type 1 genes). The effector phase begins when the genes have been translated, thereby leading to the secretion of the interferons into the cytoplasm. The interferons then bind to cytokine receptors which signal through the JAK-STAT pathway. Binding of interferon to the receptor causes a conformational change in the juxtaposition of JAK associated with the receptor making it possible for cross–phosphorylation of neighboring kinases (Jatiani et al., 2010). Apart from interferon 1 stimulation, natural killer cells and natural killer T cells cause cytotoxic effects to the virus by releasing granules which contain perforins and granzymes.

To successfully thrive in the host, the virus uses several mechanisms to evade the host's immune response mounted against it. HCV NS3/4A proteases inhibits RIG-1 which signal through the IPS-1 pathway and TLR3 which also signals by the TRIF pathway (Wieland & Chisari, 2005). The core protein is reported to bind to a number of proteins in the JAK-STAT pathway; the core protein is able to bind to the SH2 domain of STAT1 to prevent it from binding to the JAK kinase, thus inhibiting phosphorylation of STAT 1 and nuclear translocation of other STAT proteins (Kwon et al., 2014). The core protein inhibits phosphorylation of the JAK kinase itself and induces proteins which suppress signaling such as the suppressors of cytokine signaling (SOCS 1 and 3), and protein inhibitor of activated STAT 1 (PIAS 1). Other viral proteins such as the E2 and NS5A down-regulate the expression of interferon sensitive genes by stimulating the expression of Interleukin 8

(IL-8) and inhibiting the antiviral activity of Protein Kinase RNA (PKR) (Okwuraiwe et al., 2012).

2.6 Treatment of HCV

Hepatitis C Virus infection is a global health importance and this calls for effective treatment. Currently, vaccines for the prevention of HCV infection are unavailable due to the extraordinarily high degree of genetic diversity (Shoukry, 2018). Hepatitis C virus can down-regulate the expression of its proteins. This mechanism allows it to stay very long in the host cells without being noticed by the immune cells. Due to this, the acute phase of HCV infection is rarely diagnosed, and treatment of acute infections is limited. Chronic HCV infections (infection persisting over more than six months with positive HCV-antibody and HCV-RNA detectable in the serum) are however treated with the direct acting anti-virals (DAAs) which targets HCV at various stages in the life cycle. Historically, interferon alpha and ribavirin were the main HCV drug therapy. However, these drugs had severe side effects on patients. Currently, the DAAs which are used for the treatment of HCV comes with minimal side effects and short course of therapy. They have high cure rates of more than 90 percent (Messina et al., 2015).

The main aim of HCV therapy is to achieve a sustained virologic response (SVR) (Shah et al., 2017). This is defined as undetectable HCV RNA 12 weeks or 24 weeks after treatment completion (EASL, 2018). From literature, the risk of liver- related mortality has significantly reduced, however, not eliminated in patients with cirrhosis who clear HCV as compared to untreated patients and non-sustained virologic responders (Perz et al., 2006; Arase et al., 2013). The four classes of the DAAs are the NS3/4A serine protease inhibitors,

nucleotide analog inhibitors of NS5B polymerase, NS5A protein inhibitors and the non-nucleoside NS5B polymerase inhibitors. These drugs targets HCV at various stages in its replication cycle (Li & Lo, 2015).

2.7 Sickle Cell Disease

Sickle cell disease (SCD) is a class of hereditary haemoglobin disorder which affects mainly people of African ancestry (Centre for Disease Control and Prevention, 2017)). SCD is a point mutation disease of the beta globin chain and this leads to the replacement of valine with glutamic acid (Pauling et al., 1949; Browning et al., 2007). This change in amino acids helps deoxygenated HbS to become "sticky" and polymerize to form stiffness and sickle forming fibers (Christoph et al., 2005). The high, sickle cells can sometimes get stuck in small blood vessels and pile up to block blood flow. This blockage prevents the flow of gases (oxygen and carbon dioxide) to tissue and organs and this results in an extremely painful condition known as ischemia (Bunn, F. H., 1997; Gladwin, Mark T. & Gregory J. Kato, 2005). Sickled RBC's have a lifespan of 10-20 days compared to normal RBC's 120-day lifetime leading to chronic anemia in patients with SCD (Ohnishi et al., 2000). Sickle cell disease signs include hand-foot syndrome, anemia, priapism, weakness and paleness, pain spells, chronic inflammation, eye problems, jaundice, and delayed development(HET, 2017).

2.7.1 Genotypes of Sickle Cell Disease

There are four main SCD genotypes: SS, SC, β+, and β 0. The most common and extreme variant is homozygosity of sickle mutation, SS (John, 2010; Bennett, 2011). Hemoglobin

SD, hemoglobin CC and fetal hemoglobin SF are uncommon and mainly not associated with severe symptoms. In Africa, S frequency, and thus SS, is the highest in equatorial low-altitude areas. Compound heterozygosity for S and C (SC) is the second common subtype of SCD in Africa. The C allele is found almost exclusively in people of descent from West Africa, being the most prominent among Burkina Faso and northern Ghana. Thalassemia (S-thalassemia) compound heterozygosity is a type of SCD that is thought to be uncommon in most sub-Saharan Africa (Fleming, 1989). In Central, East, and Southern Africa, SCD is generally assumed to be synonymous with SS disease. The prevalence of SCDs in Ghana remained at 2% and in the population at 25% (Ohene-Frempong et al., 2008).

2.8 HCV infection in Sickle Cell Disease

Recent studies have shown that the risk of having an infection through blood transfusion has reduced significantly (Bihl et al., 2007). This is mainly due to better screening assays; however, the risk still exists. Sickle cell patients are at high risk for acquiring HCV infection because of the large number of blood transfusions received. A substantial proportion of patients with sickle cell disease acquired HCV infection through blood transfusions, before it became possible to screen donated blood for HCV in 1990-1992 (Hasan et al., 1996). At least 10% of adult sickle cell patients are hepatitis C-virus positive and often have liver dysfunction (Hassan & Najim, 2018). In sickle cell patients, blood transfusions also result in high iron overload which is additive to the liver damage caused by HCV infection. In addition, the immunocompromised status of most SCD patients predisposes them to severer form of the infection.

CHAPTER THREE

3.0 METHODS

3.1 Research Design

This was a cross-sectional and hospital-based study involving SCD patients

3.1.1 Study site

Study participants were recruited from the Ghana Institute for Clinical Genetics, Korle-Bu Teaching Hospital in the Greater Accra Region, Ghana. The KBTH serves as a tertiary referral health facility and ranked as the third largest hospital in Africa. This hospital provides Level III facility-based clinical services with over 1500-bed capacity (KBTH, 2019). Korle-Bu Teaching Hospital attends to averagely, 1500 patients daily and 250 patient admissions. There are 17 diagnostic departments at this facility which includes the Sickle Cell Disease Unit at the GICG. The GICG was established in 1974 and sees an average 40 patients daily with one third attending the center's daycare for management of crises. It is a day clinic that runs from 8:00-14:00 hours GMT on Mondays through to Fridays. Since its inception, more than 25000 patients have been registered.

3.1.2 Study population and sample size calculation

Study participants were sickle cell disease patients who were within the inclusion criteria.

Calculation of sample size was done using the formula:

$$N = z^2 * p\ (1-p)/d^2$$

Where N is the sample size, Z is the statistic corresponding to level of confidence, p is the expected prevalence, d is the precision (Mora et al., 2016)

With a 95% confidence interval, Z= 1.96. Taking a proposed prevalence rate of 10.1% (Hasan et al., 1996) and precision set at 5%, the minimum number of samples based on the above calculate calculation is 141.

3.1.3 Inclusion Criteria

Any sickle cell disease patient above the age of five years who has consented to take part in the study.

3.1.4 Exclusion Criteria

I. Patients who are sickle cell disease negative.

II. Sickle cell disease patients who were below the age of five (5) years.

III. Sickle cell disease patients who did not consent to partake in the study.

3.2 Ethical approval

Ethical approvals were obtained from the Ethical and Protocol Review Boards at Korle-Bu Teaching Hospital, College of Health Sciences and NMIMR after approval of the scientific content through the Scientific and Technical Committee (STC) (Appendices 3, 4, 5).

3.3 Data and Sample Collection

Eligible patients who consented to partake in the study were recruited by convenient sampling (Appendix 7). Demographic and clinical data such as age and sex, nationality,

residence and number of times of blood transfusion were obtained from the patients with a structured questionnaire (Appendix 2). A one-time point sampling was obtained from participants with the help of a trained phlebotomist from the GICG unit. Three milliliters (3 ml) of venous blood from enrolled patients were collected using a 5 ml syringe into a BD Vacutainer K2E (EDTA, purple-topped) tubes. Blood specimen collected from participants were labeled with a unique lab ID for easy identification. The blood samples were sealed with parafilm, kept in a zip lock bag and put in a cool box with ice packs. Transportation of samples was done under cold chain condition from KBTH to the Virology-Department, NMIMR where the serological and molecular testing were done. Plasma was separated from the blood samples using a refrigerated centrifuge at 2500 rpm for 10 minutes. Two 2 ml vials of polypropylene tubes were labeled for each sample with their respective lab IDs. About 1 ml each of plasma was transferred into the labelled vials using pasteur pipettes and stored at 2-8^{o}C for use within 2-3 days or stored at -80^{o}C for a longer period.

3.4 Laboratory procedures and analyses

3.4.1 Serologic Detection of Anti-HCV Antibodies Using Anti-HCV Rapid Diagnostic Test Kit

Plasma from all 142 blood samples were screened for anti- HCV antibodies using the advanced Quality Rapid Anti-HCV Test Strip (InTec Products, INC). This kit uses the principle of immunochromatography and qualitatively detects antibodies to HCV in plasma, serum and blood. One (1) drop or 10 µL of patient plasma was pipetted onto the sample pad of the test strip and two (2) drops of sample diluent were added to the sample pad after addition of the specimen. The results of the test were observed after 15 minutes.

The test was said to be positive if both the control and test bands appeared. If a band was only seen on the control line (C), the test was valid and negative. However, if a band was observed on the test line (T) and not on the C line, the test was invalid and was repeated.

3.4.2 Genomic detection of HCV RNA

Viral ribonucleic acid (RNA) was extracted using the QIAamp® Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Viral RNA was reverse transcribed and amplified by a conventional PCR with primers targeting HCV core gene. The amplicons were visualized I n a 2% agarose gel with gel red under ultraviolet trans-illumination using the BioDoc-It 220 Imaging System (Upland, CA, USA). Amplified DNA were purified and sequenced. Purified sequenced products were loaded into the genetic analyzer (ABI 3130xl, Applied Biosystems, USA).

3.4.2.1 Extraction of HCV RNA

Extraction of genomic RNA was done using the QIAmp Viral RNA mini kit following the manufacturer's instructions. Each patient's plasma was thawed to room temperature (23ºC to 26ºC). In a class II biosafety cabinet (Airtech ClassIIA/B3, AirTech, Japan), 560 ml of buffer AVL and carrier RNA (56 µL) were aliquoted into appropriately labelled Eppendorf tubes. The patient's plasma was vortexed and 140 µL of each sample added to the lysis buffer, pulse vortexed for about 15 seconds and incubated at room temperature (25^0C) for 10 mins. Five hundred and sixty microliters of cold absolute ethanol (molecular grade) was dispensed into the tubes containing the lysates and pulse vortexed for about 15 seconds. Six

hundred and thirty microliters (630 µL) of the lysate/ethanol mixture was aliquoted onto a labelled spin column and spun for a minute at 8000 rpm. The tube containing the filtrate was thrown away, and the spin column placed into a new filtrate collection tube. This process was repeated for the remaining 630 µL of the lysate/ethanol mixtures in the Eppendorf tubes. After the centrifugation of all the lysate/ethanol mixture, 500 µL of wash buffer AW1 was dispensed into each of the spin columns and centrifuged for a minute at 8000 rpm. The collection tubes were thrown away, and the spin columns put into new collection tubes. For the second wash, 500 µL of buffer AW2 was added to each the spin columns and centrifuged for 3 minutes at 14000 rpm. Discarding the collection tubes and placing the spin columns into new collection tubes, the centrifugation at 14,000 rpm was repeated for 1 minute. The collection tubes and the filtrates were discarded, and the spin columns placed into new appropriately labelled Eppendorf tubes after which 60 µL of buffer AVE, an elution buffer, was added directly to membrane of the spin columns and allowed to sit on the bench at room temperature (25^0C) for 1 minute. The tubes were centrifuged at 8000 rpm for a minute to elute RNA. The eluted RNA extracts were kept at 2ºC to 8ºC for use within 48 hours or at -40ºC for longer storage.

3.4.3 Conventional RT-PCR for Detection and amplification of Hepatitis C Virus Ribonucleic Acid (Core gene)

3.4.3.1 First Round PCR

The viral RNA was detected by amplifying the core gene (C gene) of HCV using a QIAGEN OneStep RT-PCR (QIAGEN, USA) commercial kit. The PCR final reaction volume of 25 µL constituted of 5 µL RNA extract, 5 µL of Reaction Buffer (5 X), 5 µL of

water, 1 µL dNTP mix (10 mM), 5 µL Q solution (5x), 1 µL of enzyme mix and 1.5 µL (10 µM) each, sense (5'-CTT CAC GCA GAA AGC GTC TA-3') and anti-sense (5'-CAA GCA CCC TAT CAG GCA GT-3') primers. Polymerase chain reaction was carried out using the ESCO Thermal Cycler (ESCO Microplate Ltd, Singapore). The reaction was subjected to the following cycling conditions: reverse transcription stage at 50^0C for 30 minutes, followed by an initial denaturing at 95^0C for 15 minutes, then 45 cycles of denaturation for 15 seconds at 95 ^{0} C, annealing at 60^0C for 15 seconds, extension for 15 seconds at 72^0C and a final extension for 30 seconds at 72^0C. The amplicons were stored at -4^0C for further analyses.

3.4.3.2 Second round PCR (Re-amplification)

The protocol used was a re-amplification method where the primers for the first run were repeated for the same targets in the second run using the Platinum Taq DNA Polymerase kit (Invitrogen, Thermo Fisher Scientific). The master mix for the re-amplification included 33.5 µL of nuclease free water, 5µL of platinum buffer (10 X), 3 µL of $MgCl_2$ (50 mM), 1 µL of dNTP mix (10 mM), 0.6 µL of each of sense and anti-sense primers (10 µM) and 0.5 µL Platinum Taq. Polymerase chain reaction was performed with 1 µL of the first round PCR amplicons as templates. The reaction was subjected to the following cycling and thermal conditions: an initial denaturing at 3 minutes at 95^0C, followed by 35 cycles of denaturation at 95^0C for 20 seconds, annealing at 55^0C for 20 seconds, extension for 60 seconds at 72^0C and extended finally for 20 minutes at 72^0C. The amplicons obtained were stored at 4^0C for further analysis.

3.4.4 Agarose gel Electrophoresis

A 2% agarose gel with a gel red DNA staining dye was prepared using 1X Tris-acetate EDTA (TAE) buffer. Five microliters of the PCR amplified products were added to a 1 µL of 6X Blue/orange loading dye. One kilo base pair (1kb) DNA ladder (GeneRuler, ThermoFisher Scientific, USA) was used to aid in the verification of the expected band size of 243 base pairs. The gel was run at 100 volts (V), 500 milliamps (mA), for 45 minutes. To visualize the presence or absence of the amplified products on the gel, a UV-Transilluminator (BioDoc-it^{TM} 220 imaging system; Upland, CA, USA) was used.

3.4.5 Post PCR purification

Amplicons positive for HCV were cleaned using the QIAquick gel extraction kit following manufacturer's instructions (Qiagen GmbH, Hilden, Germany). With the aid of a clean surgical blade and the gel on a UV plate, DNA fragments within the expected band size were excised from the gel. Each cut gel was weighed in a colorless 2 ml Eppendorf tube. To one volume of the gel, 3 volumes of binding buffer QG was added. The mixture was incubated on a heat block at 56^{o} C for 10 mins, vortexing to mix every 2 minutes till all the gel was dissolved. After complete dissolution of the gel slice, one gel volume of isopropanol was dispensed into the sample tubes and mixed. The QIAquick spin columns were labelled and attached to their respective collection tubes. To bind DNA, the mixture was pipetted on to the membrane of the QIAquick spin columns and centrifuged for a minute at 13000 rpm in a microcentrifuge (Eppendorf AG 22331 Hamburg). Discarding the flow through and reusing the collection tubes, 500 µL of binding Buffer QG was dispensed onto the QIAquick column and centrifuged for a minute at 13000 rpm. The filtrate was

discarded and collection tubes were reused. A volume of 750 µL wash buffer PE was dispensed onto the QIAquick column and then centrifuged for a minute at 13000 rpm. The flow through was discarded and the column placed back and centrifuged for an extra minute at 13000 rpm. The QIAquick columns were placed in appropriately labelled 1.5 mL microcentrifuge tubes and 30 µL of elution buffer EB dispensed directly onto the center of the membrane in the QIAquick column and incubated at room temperature (25^0C) for a minute. To elute the purified DNA, the columns were centrifuged for a minute at 13000 rpm. The purified DNA was then kept at 4^oC and quantified using the Nanodrop 2000C spectrophotometer (Thermo Scientific) prior to cycle sequencing.

3.4.6 Sequencing of the HCV core gene

Cycle sequencing of the HCV core gene was done using the BigDye® Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, USA). The protocol used was adapted from the HIV drug resistance study at the Virology Department at NMIMR. Two separate master mixes for forward and reverse primers were prepared. A total volume of 10 µL reaction mixture was prepared which consisted of 2.0 µL of DNase/RNase free water, 2.0 µL of 5 x Sequencing Buffer, 2.0 µL of BigDye terminator v3.1, 2.0 µL of sense (2 µM) or anti-sense (2 µM) primer and 2 µL of the purified DNA. The mixture was then sequenced using the following cycling and thermal conditions: 94^0C 2 minutes / (94^0C/ 30 seconds; 50^0C/ 15 seconds; 60^0C/ 4minutes) x 25 cycles/ 4^0C hold on the ESCO Thermal Cycler (ESCO Microplate Ltd, Singapore).

3.4.7 Post cycle sequencing purification

The sequenced products were purified to remove excess dNTPs, primers, and other unwanted products using the Agencourt CleanSeq Dye Terminator Removal Kit (Beckman Coulter, Inc., U.S.A.). To each of the sequenced products, 10 µL of magnetic beads and 42 µL of 85% molecular grade ethanol were added and placed on the magnetic field to stand for 3 minutes. After the 3 minutes incubation and with the microtubes still on the magnetic field, the fluid mixture in the tubes was pipetted out leaving the DNA bound magnetic beads in the tubes. A 100 µL of 85% molecular grade ethanol was then added to each tube and allowed to further sit on the magnetic field for 3 minutes. After the 3-minute incubation wash the ethanol was aspirated from the tubes and the tubes left to air-dry for 10 minutes on the magnetic field. To elute the purified products, 50 µL of nuclease-free water was added to each tube. The tubes were removed from the magnetic field and placed on a plastic rack for at least 5 minutes. This was done to make sure the magnetic beads and the sequenced DNA separated with the magnetic beads falling of the PCR tube walls and sedimenting at the bottom leaving the DNA in solution. The tubes were then placed onto the magnetic field again for 2-3 minutes after which 40 µL of the clear solution containing DNA was then pipetted into a 96 well standard plate. The 3130xl Genetic analyzer (ABI 3130xl, Applied Biosystems, USA) was used to base call the sequenced products.

3.4.8 Nucleotide Sequence Analyses

Nucleotide sequences generated by the ABI3130XL Genetic analyzer were viewed in the Chromas software (version 2.6.2) to detect background noise in the base calls. Sequences were then imported into the BioEdit software, version 7.2.5, trimmed, assembled to form a

contig and manually edited. The reference strains for the different genotypes of HCV gene from the GenBank were compared to the consensus sequence obtained from the genetic analysis with the help of ClustalW multiple alignment accessory tool in the Bioedit software. The phylogenetic analysis was done using the Molecular Evolutionary Genetics Analysis (MEGA X) software. A bootstrap test and reconstruct ion were done 500 times to confirm the reliability of the phylogenetic tree.

3.5 Statistical Analysis

Data was analyzed statistically using Microsoft Excel (2012). The association between each variable (age, sex, blood transfusion, sickling genotype) and the dependent variable (HCV RNA and anti HCV antibodies) were tested. The Fisher Exact test was used in determining the significance and strength of association. Bivariate analysis was performed to determine statistical associations at 95% confidence interval setting the significance at p-value of <0.05.

3.6 Data handling and Dissemination of Results

All data collected was handled with strict confidentiality. The patients were given unique laboratory IDs. Results for both serological and molecular tests were given within 10 working days to physicians on duty at GICG. Data obtained from this study would be made available by formal request to the investigator or the supervisors.

CHAPTER FOUR

4.0 RESULTS

4.1 Demographic Characteristics of study population

A total number of 142 blood samples were collected from SCD patients who visited the day care clinic for their routine check-up at the Ghana Institute of Clinical Genetics - Korle-Bu. Study participants were patients diagnosed of any of the sickle cell disease traits which includes; SS, SC, SD, CC and SF (Table 1).

An average number of 10 patients were recruited in a day with majority of them being females representing 64% of the study population with the remaining 36% being males. Majority of the patients (35%) were within the age range of 20 to 29 years. The median age was 25 years (12-79 years). Majority (62%) of the study population were homozygous haemoglobin SS, with the lowest number of 1 being haemoglobin C. A little above half of the total population (51%) had received blood transfusion and the remaining 49% had never received blood transfusion (Table 1).

An overall total of 64 patients out of the 72 had received blood transfusion once, 6 patients had had transfusions two or three times and the remaining 2 had received blood more than 3 times (Figure 4.1). None of the study participants new their HCV status. The number of times of blood transfusion in SCD patients against sex, age groups and sickling status have been summarized in Figure 6 (A, B and C).

Table 1: Demographic distribution of SCD patients by age group, sex, sickling and blood transfusion status at GICG, KBTH.

Characteristics	Number of Cases	Percentage
Overall Total	142	100
Gender		
Males	51	36
Females	91	64
Age group (years)		
≤ 19	41	29
20 to 29	49	34
30 to 39	22	15
40 to 49	13	9
≥ 50	17	12
Median age	25 (12-79)	
Sickling status		
SS	88	62
SC	47	33
SD	4	3
SF	2	1
CC	1	1
Blood transfusion		
Yes	72	51
No	70	49

Key: SS: Haemoglobin SS, SC: Haemoglobin SC, SD: Haemoglobin D-Punjab, SF: Fetal haemoglobin

CC: Haemoglobin C.

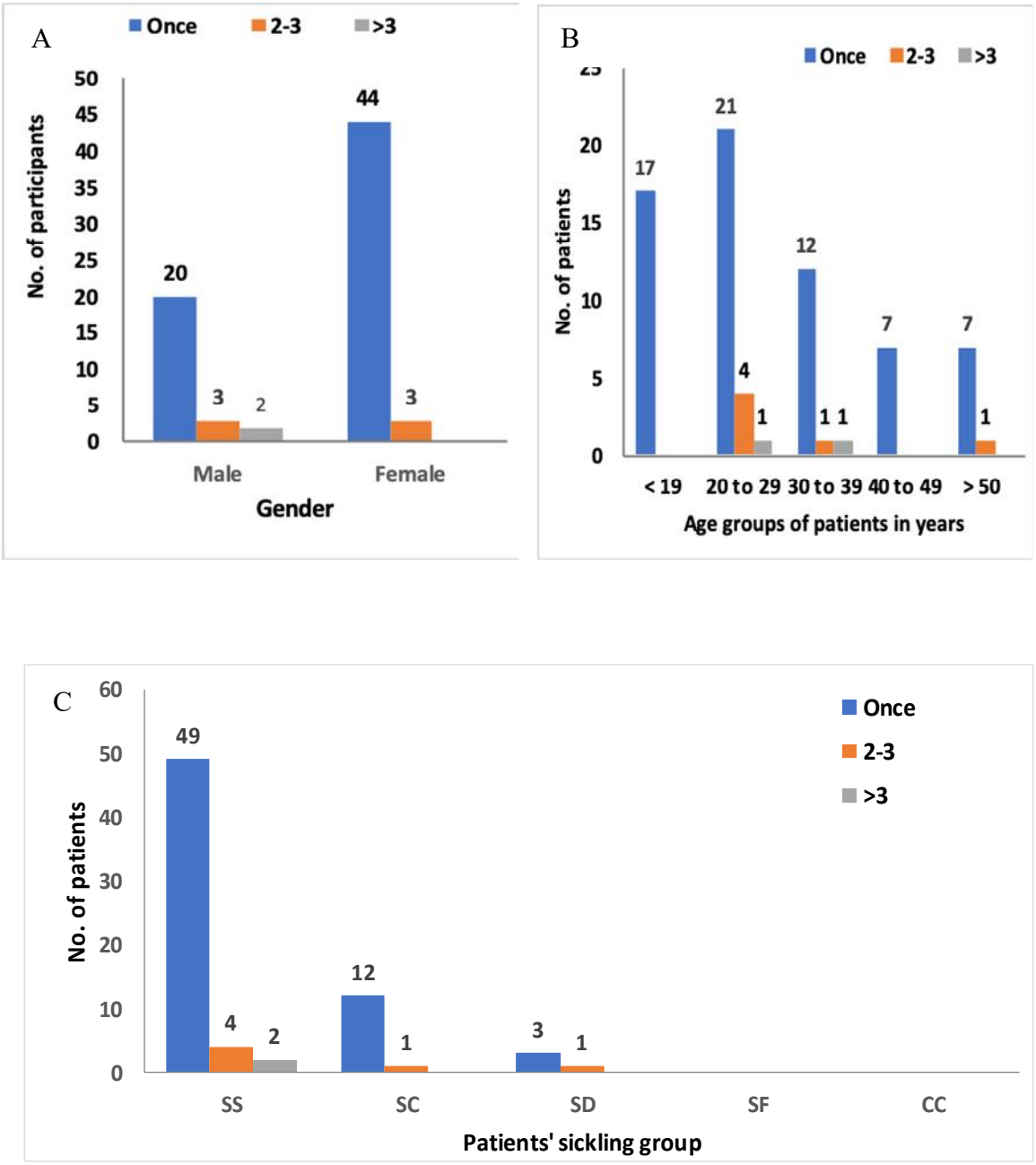

Figure 4.1: Distribution of number of times of blood transfusion in SCD patients by:

A. Gender of distribution **B**. Age group distribution and **C**. Sickling status

Table 2: Distribution of SCD patients by age group, sex, sickling genotype, and blood transfusion status against HCV detections at GICG, KBTH.

Characteristics	No of patients N (%)	HCV (Serology) Number of positives n (%)	Odds ratio (95% confidence interval)	P-value	HCV (RT-PCR) Number of positives n (%)	Odds ratio (95% confidence interval)	P-value
Overall Total	142 (100)	12 (9)			8 (6)		
Gender							
Males	51 (36)	8 (16)	4.04 (1.1540-14.1893)	0.0281[α]	3 (6)	1.075 (0.2461-4.6956)	0.9999
Females	91 (64)	4 (4)	1		5 (6)	1	
Age group (years)							
≤ 19	41 (29)	4 (10)	1.0811 (0.1819-6.4263)	0.9999	3 (7)	0.7895 (0.1218-5.1191)	0.9999
20 to 29	49 (35)	5 (10)	1.1364 (0.0203-6.3467)	0.9999	1 (2)	0.2083 (0.0179-2.4300)	0.2249
30 to 39	22 (15)	2 (9)	1	1	1 (5)	1	
40 to 49	13 (9)	1 (8)	0.833 (0.0681-10.2021)	0.9999	1 (8)	0.833 (0.0681-10.2021)	0.9999
≥ 50	17 (12)	0 (0)	*	*	2 (12)	*	*
Sickling status							
SS	88 (62)	7 (8)	0.8469 (0.2548-2.8153)	0.7664	5 (6)	1.0241 (0.2347-4.4685)	0.9999
SC	47 (33)	5 (11)	1.4966 (0.4485-4.9942)	0.5319	3 (6)	1.2273 (0.2805-5.3703)	0.9999
SD	4 (3)	0 (0)	*		0 (0)	*	
SF	2 (1)	0 (0)	*		0 (0)	*	
CC	1 (1)	0 (0)	*		0 (0)	*	
Blood transfusion							
Yes	72 (51)	4 (6)	0.4459 (0.1308-1.5890)	0.2408	2 (3)	0.3048 (0.0594-1.5646)	0.1545
No	70 (49)	8 (11)	1		6 (9)	1	

p-value set at <0.05, [α] = significant finding, * indicating calculation not applicable due to no positive detected, and 1 indicates reference variable. *SS: Haemoglobin SS, SC: Haemoglobin SC, SD: Haemoglobin D-Punjab, SF: Fetal haemoglobin, CC: Haemoglobin C*

4.2 Detection of anti-HCV by Rapid Anti-HCV Test

Out of 142 samples collected, 12 (9%) were reactive by the Advanced Quality Rapid Anti-HCV Test Strip (Table 2, Table S1). A representative picture of the reactive samples is shown in Figure 4.2.

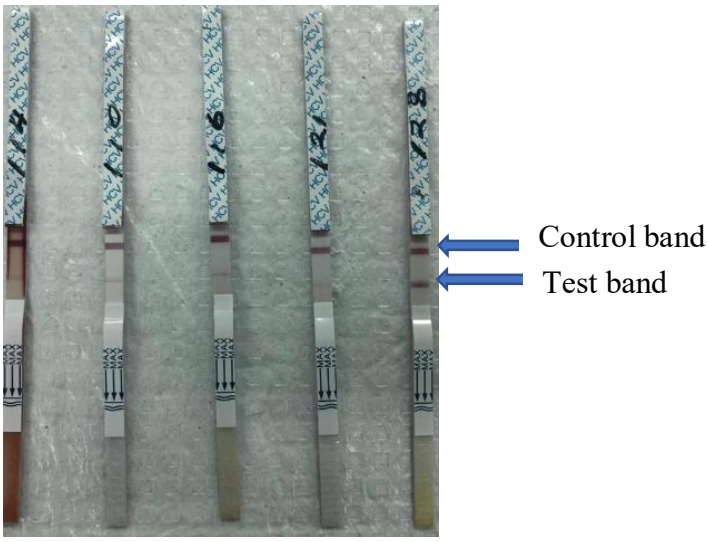

Figure 4.2: Reactive samples by Advanced Quality Rapid Anti-HCV Test Strip.

4.3 Genomic Detection of HCV RNA

The presence of the hepatitis C virus was investigated in all 142 Sickle-cell disease patients by amplifying the core gene (C-gene) segment of the virus using polymerase chain reaction. A band size of 243 base pairs indicated successful amplification of the C-gene. Eight (8) out of the 142 SCD patients representing 6% were HCV-RNA positive (Table S2). A representative gel is shown in figure 8.

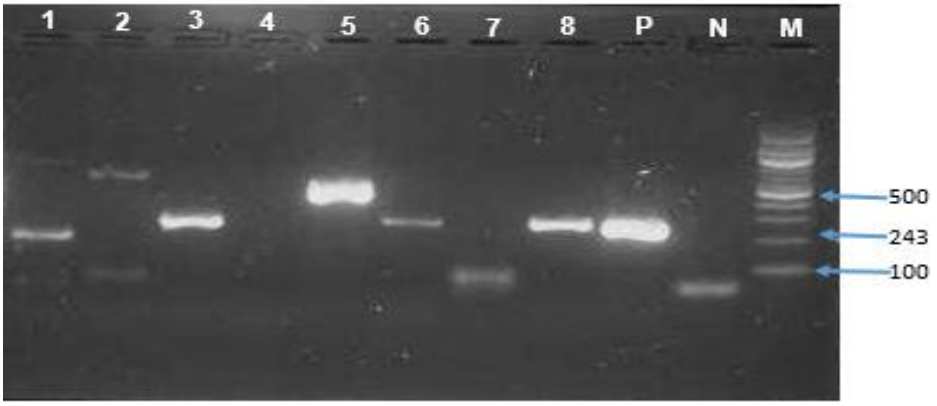

Figure 4.3: PCR Amplification of the C-gene of HCV in Sickle-cell disease patients.

M is 1kb Molecular weight marker (Thermo-scientific, U.S.A). S1 to S8 represent study samples; P is HCV positive control and N contained a negative control. The expected amplicon size was 243 bp. Samples, S1, S3 S6 and S8 were successfully amplified for HCV core gene. Failed amplification was observed in S2, S4 and S7.

4.3.1 Comparison between Serological and Molecular HCV test results

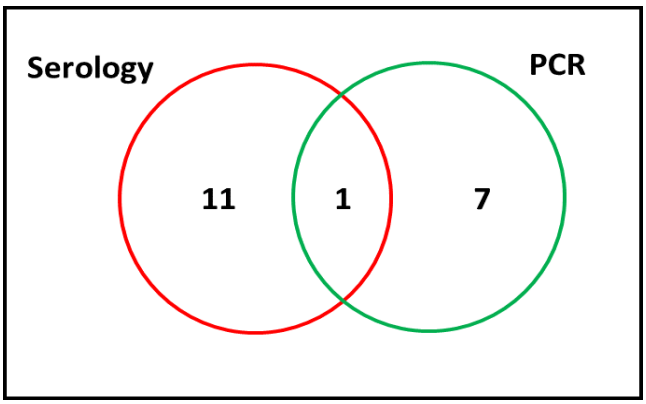

Figure 4.4: Relationship between the serologic and molecular testing.

Only one patient tested positive both for the Advanced Quality Rapid Anti-HCV Test Strip and PCR. Red circle indicates the 12 patients who were HCV seropositive. The green circle indicates the total number of patients who were HCV RNA positive. One patient was positive by serology and PCR (Figure 4.3.1).

4.4 Nucleotide Sequence Analyses

Five (63%) out of the 8 HCV-RNA positives were successfully sequenced for the C gene of hepatitis C virus. Thirty eight reference strains, representative for the different genotypes and sub-genotypes of HCV gene were obtained from the GenBank and used for phylogenetic analysis (Appendix 1). The Ghanaian HCV strains were closely related to each other and form within genotype 1, related to sub-genotype 1b (Figure 4.5). Phylogenetic analysis was carried out to determine the relatedness of the identified genotypes in this study with reference strains. Nucleotide sequence for genotype 1 isolates clustered with reference sequences from different parts of the world (Figure 4.5).

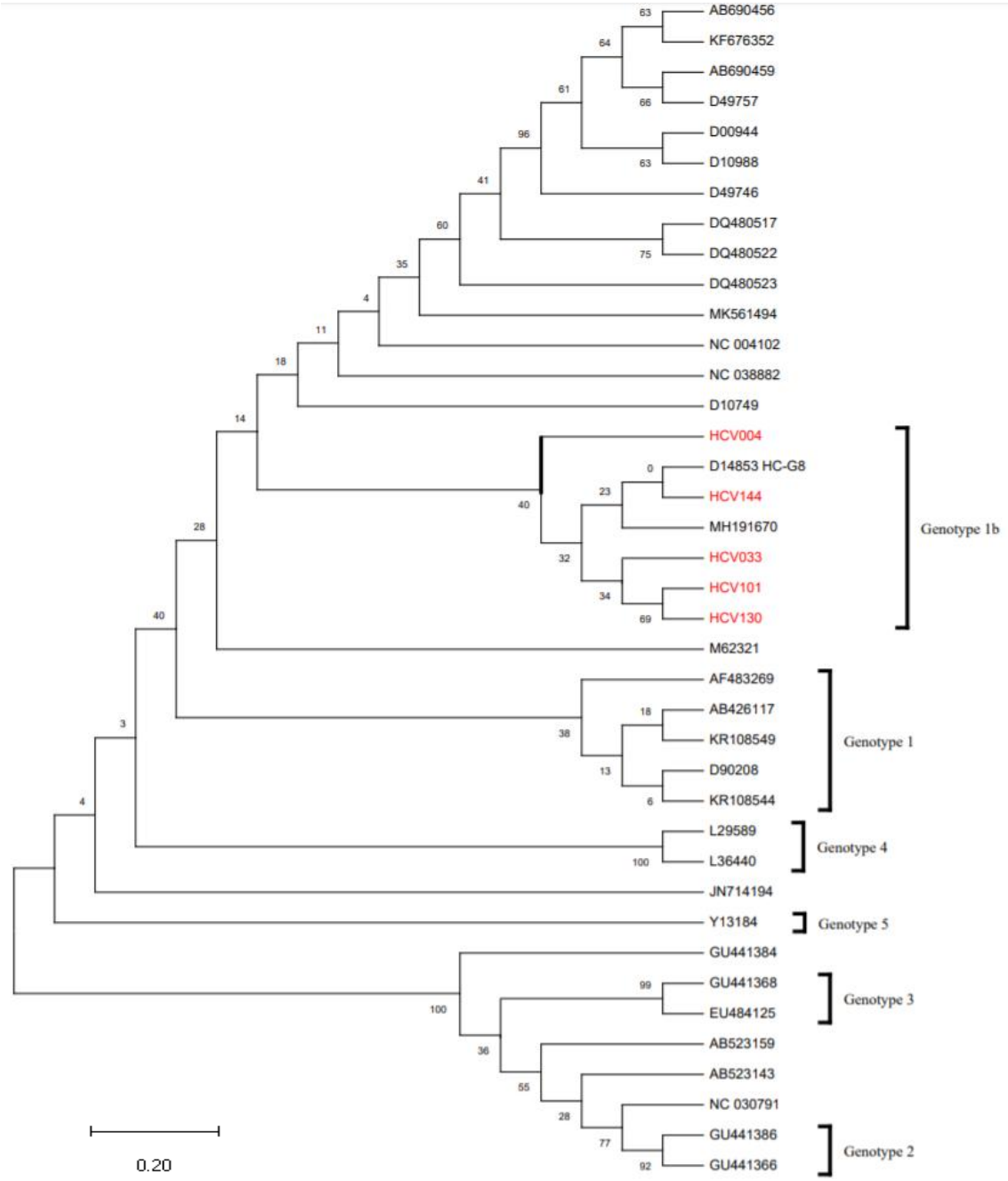

Figure 4.5: Phylogenetic relationship between HCV core sequences.

Key: Samples from this study are in red font.

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 Discussion

Hepatitis C virus is known to cause a significant proportion of liver diseases with an estimated 70% (55-85%) resulting in chronic infection which may progress to liver cirrhosis or cancer (WHO, 2019). Patients with sickle-cell disease have a high risk of chronic liver diseases due to high iron overload, sickle cell hepatopathy and chronic viral hepatitis (Ancel et al., 2009; Moon et al., 2017). Studies done in different countries have confirmed that the rate of HCV infection among SCD patients has significantly reduced after screening of blood for HCV before transfusion (Hassan & Najim, 2018). However, patients who are subjected to frequent blood transfusion, exposed to contaminated needles and objects are still at risk of having the infection (Jeannel et al., 1998). This study, therefore, sought to identify and characterize HCV infection in sickle cell disease patients.

Approximately 9% of anti- HCV total antibodies and 6% HCV-RNA positivity was established among SCD patients who visited the GICG, Korle-Bu between the period of November 2018 to March, 2019. This result is comparable to similar studies done in Basra-Iraq and Washington DC, with anti-HCV sero-positivity rate among SCD patients being 9.2% and 10%, respectively (Hassan et al., 2003; Hassan & Najim, 2018). A greater prevalence of HCV infection (9%) was observed in the current study as compared to the varied proportions on HCV sero prevalence in different population such as HIV patients, pregnant women, children, blood donors and the general population in Ghana and neighboring West African countries. The population variability could account for the differences in percentages (Acquaye & Tettey-Donkor, 2000; Candotti et al., 2003). Another factor could have been the testing algorithm, which was one or more enzyme immune assay (EIA) screenings with or without a

confirmatory assay. The highest sero prevalence of 11.6% report in Ghana was among jaundiced patients (Owusu et al, 2018). Currently, no study in Ghana has reported HCV prevalence in the high-risk groups such as injectable drug users (IDUs) and sickle cell disease patients. This study is the first to report on the sero-positivity of HCV among SCD patients in Ghana.

About 15% to 25% of patients infected with HCV show spontaneous viral clearance, which is undetectable HCV-RNA in patient's plasma, but positive for anti-HCV antibodies in the absence of antiviral therapy (Bobek, 2010). Approximately 80% of infected individuals progress to chronic infection which normally comes with no symptoms but can lead to end- stage disease (Candotti et al., 2003). In this study, 11 (92%) out the 12 anti-HCV antibodies positive patients were HCV-RNA negative by PCR. From this result, we speculate that these 11 patients might have gone through a spontaneous viral clearance. In addition, plasma HCV RNA levels vary significantly overtime and this may result in a false-negative result mainly when the rate of viral replication is low. Another factor for a false negative result by PCR could be due to viral persistence limited to the liver. These factors could account for the low positivity rate of HCV-RNA for patients who were anti HCV antibodies positive. Results from this study is not different from similar studies conducted in sub-Saharan Africa which reported undetectable HCV-RNA between 41 and 89% of individuals who tested positive by serology (Agbodjan et al., 1995; Tucker et al., 1997). However, the different assays used and lack of a confirmatory assay in the present study makes the comparison difficult. Although this group have supposedly cleared the virus, anti- HCV antibodies usually persist for life and these neutralizing antibodies are not protective (Freeman et al., 2001) . Reinfection with any genotype of HCV can occur in individuals who spontaneously cleared the virus or from treatment (CDC, 2016).

As early as 7 to 21 days after HCV infection, the viral RNA can be detected by molecular testing (PCR) (Haydon et al., 1998). A percentage of individuals who are exposed to HCV have detectable RNA but are anti-HCV antibodies negative (Schneeberger et al., 1998). This group of people are known to have late seroconversion which could last over a year (Beld et al., 1999) . This study recorded a number of 7 out of 8 patients who had detectable HCV RNA by PCR but serologically negative. The core protein of HCV is said to appear in the early stage of the illness and since the PCR targeted the core region, we could conclude that these patients could have been in the early or acute phase of the infection.

Presumed chronic Hepatitis C with active virus replication was reported in a patient who tested positive by serology and PCR. Hepatitis C virus RNA detection by PCR confirms chronic HCV infection only when a serological test is reactive for anti-HCV antibodies (Haydon et al., 1998; Bhutta et al., 2019) . This patient (HCV 114) had received transfusions in 2013, a year when screening for anti HCV RNA in blood donors was already in place. Torres et al., 2003, who conducted a similar research reported on a HCV chronically infected patient who had received blood transfusion during the screening era (Torres et al., 2003). Whether the infection was through the blood transfusion received is difficult to prove. Several studies have reported on the risk of HCV transmission through blood transfusion among SCD patients, however, we cannot conclusively associate HCV infection to blood transfusion. This is because a higher number of HCV positives were recorded among study participants who had not been transfused (Table 2).

The proportion of males' positive (16%) for HCV was significantly higher than females (4%) with a *p-value* of 0.0281. The ratio of anti-HCV antibody infection of males to females was about 4 times with an odd ratio of 4.04 (1.1540-14.1893). This results is comparable to a study among SCD patients in Brazil which equally recorded

predominance of HCV infection among males (Torres et al., 2003). Other host factors in chronic HCV infection, which may accelerate the disease progression to cirrhosis include age at time of HCV infection (> 40 years old), male gender, frequent consumption of alcohol (>2-3 drinks per day) and infection with other diseases such as hepatitis B and HIV (Freeman et al., 2001; CDC, 2016). Male participants who were HCV RNA positive and the only chronically infected male are likely to progress faster to cirrhosis as compared to the HCV RNA positive females although this speculation could not be supported with additional data.

Another parameter which could have accounted for the spontaneous viral clearance is age as reported by Dustin et al.(2016). From this data, all the 11 patients who supposedly showed SVC were below the age of 40. We could therefore suggest that these patients had cleared the virus naturally.

Since the inception of a global screening for HCV in 1992, the possibility of getting the virus through blood transfusion is quite low (Chen, S. L. & Morgan, 2006). From literature, the risk of having HCV infection from blood transfusion is higher in patients who received more than 10 units of red blood cells than those who received less than 10 units (Moon et al., 2017). In addition, HCV positivity rate is directly proportional to the number of times of receiving blood transfusion. Almost all transfused participants in this study had received less than 10 units of blood and this confirms the low prevalence rate of 6% anti-HCV and 3% HCV RNA among these study participants (Table 2). A number of studies have confirmed the greater reduction of HCV infection among blood transfused patients after the routine serological screening for anti HCV antibodies at blood banks was implemented (Donahue et al., 1992; CDC, 2016).

Majority of patients who have chronic HCV infection are not aware of their condition. This creates a big gap for the elimination of the infection. Hepatitis C virus infection

normally presents with no symptoms during the acute phase although 15-30% may experience non-specific symptoms (Hajarizadeh et al., 2013; Dustin et al., 2016). In the present study, none of the patients who tested positive either by serology or PCR was aware of their condition. This is not different from a similar study by Dustin et al (2016) who observed that patients who were HCV positives were unaware of the infection due to the absence of clinical symptoms at the early stages. For this reason the disease is reported when the infection is quite advanced and has compromised the liver and other organs of the body. There is therefore a need for a routine screening for HCV in high-risk populations.

Most of the patients (62%) were haemoglobin SS (HbSS). This is consistent with the known epidemiology of the SCD, which records HbSS as the most common type the disease. Patients with HbSS experience the worst form of the disease and often immunocompromised (HET, 2017). Patients who are on long-term immune suppressants or those with agammaglobulinemia (lack of gamma globulin in the plasma) may not be able to mount a normal antibody response to HCV (CDC, 2016). This study recorded 6% of HCV RNA infection in HbSS population. However, recorded minimal clinical information of study participants, which admittedly is a limitation though the primary aim was on diagnostics. Therefore, conclusive statement could not be made regarding patient's sickling genotype and HCV infection.

The knowledge of HCV genotypes in sickle cell patients gives an indication of their contribution to clinical manifestations and disease progression which will inform appropriate clinical management. Globally, the distribution of the 7 HCV genotypes are highly characteristics of certain regions. Although little is known on the extremely diverse strains of HCV in Ghana, this study showed clearly a 100% predominance of genotype 1 specifically 1b in the study population. All the Ghanaian strains were

closely related. This finding contradicts studies conducted in Ghana which reported genotype 2 as the highest circulating type in the country (Candotti et al., 2003; Bonney et al., 2013). Moreover, the different routes of transmission have been associated with the circulating genotypes. Subtypes 1a, 1b, 2a, 2b, 2c and 3a have been related to parenteral routes. It is assumed that all the Ghanaian patients might have had the infection through a parenteral pathway. Some studies have documented that females with genotype 1 have higher clearance rate as compared to the opposite sex (Grebely et al., 2014; Dustin et al., 2016). We speculate that females who were positive for HCV RNA have a greater chance of clearing the virus.

Most importantly, all the 12 serologically positives and 8 HCV RNA positives subjects were referred from the GICG to the Gastroenterology Unit, KBTH to engage them in care and treatment.

LIMITATIONS

Anti-HCV confirmatory assay reveals a better rate of spontaneous viral clearance (Candotti et al., 2003) and hence this could have affected the outcome of our results since no anti-HCV confirmatory was available.

This study failed to record clinical symptoms of patients, so HCV results could not be linked with disease severity.

5.2 CONCLUSION

This study established the seroprevalence of 9% of total antibodies to HCV among sickle cell patients whilst circulating HCV genotype among the study population was found to be genotype 1 strain, specifically 1b.

5.3 RECOMMENDATIONS

Further genotypic studies in a larger population of SCD patients would be important to clarify the molecular epidemiology and selection of HCV genotypes, and analyze the degree of infections among different regions for public health interventions.

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Appendices

Appendix 1: Multiple Sequence Alignment of the HCV core genome

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D00944          cttgtggtactgcctgatagggtgcttg
AB690459        cttgtggtactgcctgatagggcgcttg
D49757          cctgtggtactgcctgatagggtgcttg
D10988_         cttgtggtactgcctgatagggtgcttg
JN714194        cttgtggtactgcctgatagggtgcttg
L29589          atggtggtgctgccaccatg--tgttcc
L36440          atggtggtgcagccaccctt--tgctct
L39281          ttggcggaggggctgccctc--tgctcc
AB523143_       gccgtcctgggctcagcccgggtaccct
AB523159        gcaggactgggctcagcccggataccct
GU441384        gccggcctgggcccagcccgggtaccct
GU441368        gccggcctgggctcagcccgggtacccc
EU484125        gccgacctgggctcagcccgggtaccct
LC271217        gcaagcctggggtcgcccaggatаccca
GU441386        gcaagcctgggggaaaccaggatacccc
GU441366        gcaagcctggggaagtccaggatatccc
U33436          gaaggcctgggcacagccaggatаccca
NC_030791       gtaaacctggggcaagcccgggtaccct
EU484153        -------ttctatcttccttcttgctct
```

The online MAFFT software was used to perform multiple sequence alignment for the core genomes of HCV positive samples in this study. Reference sequences were downloaded from the GenBank: ncbi.nlm.nih.gov/

Table S1: An excel sheet showing demographic data of study participants reactive by serology.

1	Sample	Age	Sex	Blood Transfusi	Month and Year	Number of times	HCV (Serolog	HCV RT-PCR	Sicklin
14	HC-014	28	M	NO			Reactive	Negative	SC
20	HC-020	37	F	NO			Reactive	Negative	SC
61	HC-062	22	F	NO			Reactive	Negative	SS
76	HC-095	12	F	NO	2008	1	Reactive	Negative	SS
104	HC-091	26	M	YES	2008	1	Reactive	Negative	SS
112	HC-110	19	F	NO			Reactive	Negative	SC
116	HC-114	44	M	YES	2013	1	Reactive	Positive	SS
118	HC-116	24	M	NO			Reactive	Negative	SS
120	HC-121	23	M	YES	2010	1	Reactive	Negative	SS
125	HC-128	15	M	YES	2015	1	Reactive	Negative	SS
132	HC-134	18	M	NO			Reactive	Negative	SC
138	HC-139	36	M	NO			Reactive	Negative	SC

Table S2:An excel sheet showing demographic data of study participants positive by PCR

1	Sample	Age	Sex	Blood Transfusi	Month and Year	Number of times	HCV (Serolog	HCV RT-PCR	Sicklin
5	HC-004	52	F	NO			Non-Reactive	Positive	SC
31	HC-033	39	F	YES	2008	1	Non-Reactive	Positive	SC
44	HC-035	17	M	NO			Non-Reactive	Positive	SS
79	HC-101	19	M	NO			Non-Reactive	Positive	SS
97	HC-008	59	F	NO			Non-Reactive	Positive	SC
116	HC-114	44	M	YES	2013	1	Reactive	Positive	SS
127	HC-130	26	F	NO			Non-Reactive	Positive	SS
143	HC-144	17	F	NO			Non-Reactive	Positive	SS

Appendix 2: HCV Survey Questionaire Form

HCV SURVEY QUESTIONAIRE FORM

Title: Hepatitis C virus infection among sickle cell disease patients at the Korle Bu Teaching Hospital in Accra, Ghana.

Sample ID:..... Age:.....yrs

Sex: M ☐ F ☐ Hospital Folder Number:.....

Nationality:..... Place of Residence: .....

Date blood sample taken: ..... Sickling Genotype: .....

Blood Transfusion:

Date of first blood transfusion: .....

Number of times of blood transfusion taken: .....

Units of blood received: .....

HCV Status: Pos ☐ Neg ☐ If positive, on Treatment? ☐ ☐

Contact of Interviewer

Mobile No. ..... E-mail:.....

Appendix 3: Approval of Protocol, STC-NMIMR

NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH
Established 1979
A Constituent of the College of Health Sciences
University of Ghana

Phone: +233-320-501180/513202 (Direct)
+233-320-501178/9 (S/board)
Fax: +233-320-502182/513202
E-mail: Director@noguchi..ug.edu.gh

Post Office Box LG 581
Legon, Accra
GHANA

My Reference:

Your Reference:

3rd April, 2019

Mrs. Gifty Mawuli
Department of Virology
NMIMR
Legon

Dear Madam,

APPROVAL OF PROTOCOL

The Scientific and Technical Committee of the Noguchi Memorial Institute at its meeting on 10th December, 2018, reviewed the protocol entitled: "Laboratory investigations for Hepatitis C Virus (HCV) infection among Sickle-Cell patients at the Korle-Bu Teaching Hospital in Accra, Ghana", STC Paper 1(3) 2018-19, which was submitted by Ms. Gifty Mawuli.

The Committee after the review approved the protocol on Tuesday, 12th March 2019, and recommended that the study should be carried out.

The Scientific and Technical Committee avails to you the assurances of its highest consideration.

Thank you.

Yours faithfully,

Professor Abraham Kwabena Anang, PhD
DIRECTOR

Appendix 4: Ethical Clearance- CHS

UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES
ETHICAL AND PROTOCOL REVIEW COMMITTEE

Ref. No.: EPRC/FEB/2019 February 08, 2019

Gifty Mawuli
Department of Medical Biochemistry
School of Biomedical and Allied Health Sciences
Korle-Bu

ETHICAL CLEARANCE

Protocol Identification Number: ***CHS-Et/M.6 – 5.16/2018-2019***

FWA: 000185779 **IORG: 0005170** **IRB: 00006220**

The College of Health Sciences Ethical and Protocol Review Committee (EPRC) at its February 08, 2019 full board meeting reviewed and approved your re-submitted research protocol.

Title of Protocol: **"Hepatitis C Virus (HCV) Infection among sickle -cell Patients at the Korle Bu Teaching Hospital"**

Principal Investigator: **Ms. Gifty Mawuli**

This approval requires that you submit six-monthly review report(s) of the study to the Committee and a final full review report to the EPRC at the completion of the study. The Committee may observe, or cause to be observed, procedures and records of the study before, during and after implementation.

Please note that any significant modification(s) to this project/study must be submitted to the Committee for review and approval before its implementation.

You are required to report all serious adverse events related to this study to the EPRC within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee's duty to review the ethical aspects of any manuscript that may be produced from this study. You will therefore be required to furnish the Committee with any manuscript for publication.

This ethical clearance is valid till February 10, 2020.

Please always quote the protocol identification number in all future correspondence in relation to this protocol.

Signed: .....
Professor Andrew Anthony Adjei
Chair, Ethical and Protocol Review Committee

cc: Provost, CHS
Dean, SBAHS
Head, Department of Medical Biochemistry

• P. O. Box KB 52, Korle Bu, Accra, Ghana. • Telephone: +233 (0) 306 665103/4 • Fax: +233 (0) 302 660762
• Email: admin.chs@ug.edu.gh / provost.chs@ug.edu.gh • Website: www.chs.ug.edu.gh

Appendix 5: Approval of Protocol-STC, KBTH

In case of reply the number
And the date of this
Letter should be quoted

My Ref. No. KBTH/md/Ec.3/19

Your Ref. No......

P. O. BOX KB 77,
KORLE BU, ACCRA.

Tel: +233 302 667759/673034-6
Fax: +233 302 667759
Email: Info@kbth.gov.gh
pr@kbth.gov.gh
Website: www.kbth.gov.gh

22nd January, 2019

GIFTY MAWULI
MEDICAL BIOCHEMISTRY
SCHOOL OF BIOMEDICAL AND ALLIED HEALTH SCIENCES
UNIVERSITY OF GHANA
LEGON

INSTITUTIONAL APPROVAL: KORLE BU TEACHING HOSPITAL-SCIENTIFIC AND TECHNICAL COMMITTEE/INSTITUTIONAL REVIEW BOARD (KBTH-STC/IRB/000126/2018

Following approval of your study entitled "Laboratory investigation for Hepatitis C virus (HCV) infection among sickle cell patients at Korle Bu Teaching Hospital, Accra, Ghana" by the Korle Bu Teaching Hospital-Scientific and Technical Committee/Institutional Review Board.

I am pleased to inform you that institutional approval has been granted for the conduct of your study in Korle Bu Teaching Hospital.

Please contact the Head of Department to discuss the commencement date of the study.

Please note that, this institutional approval is rendered invalid if the terms of the Institutional Reviewed Board/Scientific and Technical Committee approval are violated.

Sincere regards,

Dr. Samuel Asiamah
Director of Medical Affairs
For: Chief Executive Officer

Cc: The Chief Executive
Korle Bu

Appendix 6: Ethical Approval-KBTH

And the date of this
Letter should be quoted

My Ref. No. KBTH/MI/CS/19

Your Ref. No......

KORLE BU, ACCRA.

Tel: +233 302 667759/673034-6
Fax: +233 302 667759
Email: Info@kbth.gov.gh
pr@kbth.gov.gh
Website: www.kbth.gov.gh

21st January, 2019

GIFTY MAWULI
MEDICAL BIOCHEMISTRY
SCHOOL OF BIOMEDICAL AND ALLIED HEALTH SCIENCES
UNIVERSITY OF GHANA
LEGON

LABORATORY INVESTIGATION FOR HEPATITIS C VIRUS (HCV) INFECTION AMONG SICKLE CELL DISEASE PATIENTS AT THE KORLE BU TEACHING HOSPITAL IN ACCRA, GHANA

KBTH-IRB /000126/2018

Investigator: Gifty Mawuli

The Korle Bu Teaching Hospital Institutional Review Board (KBTH IRB) reviewed and granted approval to the study entitled "Laboratory investigation for Hepatitis C virus (HCV) infection among sickle cell patients at KBTH, Accra Ghana"

Please note that the Board requires you to submit a final review report on completion of this study to the KBTH-IRB.

Kindly, note that, any modification/amendment to the approved study protocol without approval from KBTH-IRB renders this certificate invalid.

Please report all serious adverse events related to this study to KBTH-IRB within seven days verbally and fourteen days in writing.

This IRB approval is valid till 30th December, 2019. You are to submit annual report for continuing review.

Sincere regards,

MR OKYERE-BOATENG
CHAIR (KBTH-IRB)

Cc: The Chief Executive Officer
Korle Bu Teaching Hospital

Appendix 7: Consent Forms

Consent Form

Research Topic: Hepatitis C virus (HCV) infection among sickle cell disease patients at the Korle Bu Teaching Hospital.

Principal Investigator: Gifty Mawuli
Address: NMIMR, P. O. Box LG 581 Legon Accra

Purpose of The Study/ General Information

The purpose of this study is to check for the presence of hepatitis C virus (HCV) disease in the blood of sickle-cell patients at the Korle-Bu Teaching Hospital in the Greater Accra Region of Ghana. Hepatitis C virus (HCV) infection is one of the major causes of chronic liver disease among sickle-cell patients. Since most sickle-cell patients get frequent blood transfusions, they are at risk of contamination with HCV. Information from this study will help doctors know the kind of treatment to give to sickle-cell patients who are positive for HCV. The information will also be used to develop future programs and interventions to address any effects the presence of HCV in sickle-cell patients have on public health in Ghana.

Study Procedures

If you agree to be in this study, you will participate in a survey with a staff member where your age, sex, your background, health, history of HCV and history of blood transfusion taken. You will be given identification number (ID) to hide and protect your identity and privacy. Information about your diagnosis will be taken as well as the type of drugs you are taking if on treatment. It will take up to 10 minutes to complete the survey form and any information you provide will be kept confidential. A trained laboratory person will take about 3mls of blood from you at the time of filling the survey form. The blood samples will be sent to Noguchi Memorial Institute for Medical Research, Legon, for analysis and storage.

Why have I been asked to participate? You have been chosen because we are looking for hepatitis C virus in sickle cell disease patients. We would like to take 3mls of blood from you at the time of filling the survey form because you had received blood transfusion.

Do I have to take part? You are free to choose to take part of this study or not. If you decide not to take part in this study, it will not affect the care you will receive in anyway. If you agree to participate you are free to end your participation at any time you want to.

Will I be paid any money? There will be no money paid to you if you take part in this study.

What will happen to me if I take part? If you take part, a trained staff member will collect information from your medical records. Your name will not be written down and you will be asked a few questions. You will be asked to provide 5ml (1 teaspoon) of blood at the time of filling the survey form for research purposes.

I would also like to store some of your blood to be used for later testing after obtaining permission from an ethics board. You may take part in the study without having your blood stored for later testing. We will store your samples for 5 years

Will my taking part in this research study be kept private? All information and blood samples will be kept private. Names will not be written on the forms or the blood tubes. All information will be kept well and taken to Noguchi Memorial Institute for Medical Research where they will be stored in a locked cabinet that can only be opened by an assigned research staff and the people in charge of this study. When the study is over and the results are submitted to the Department of Medical Biochemistry of the University of Ghana and also published, your name will not appear in any of such documents.

What will I get by participating in this research study?

By participating in the study, you will know your HCV status. You are also helping me to identify hepatitis C virus in sickle-cell patients at the Korle-Bu Teaching Hospital, Ghana. The information will help generate future studies and improve hepatitis programs in Ghana.

What is the risk of being in this research study?

Should you take part in this study, the risk associated is small. All the things that will be used to take the 3mls of blood at the time of filling the form are clean and safe. They have never been used before and will be thrown away after each use. Some people may feel dizzy when blood is drawn and there may be some pain or discomfort from the needle prick. You may develop a bruise or swelling where the needle goes into your arm. If you have any pain, bleeding, or swelling from taking blood, please let us know so we can take care of it.

What will happen to the results of this research study?

The results will be written into my thesis which will not have any individual names. This thesis will be used by the Ministry of Health/ Ghana Health Service and other donors to improve the management of liver diseases caused by hepatitis C virus in Ghana.

Confidentiality

Your personal information and laboratory results will be held in the strictest confidence, and no identifying information of any kind will be released to any other person or agency without your specific permission in writing. Your samples collected for research purposes will be labelled with a code number and will be taken to Noguchi Memorial Institute for Medical Research for processing. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.

Your rights as a Participant

This research has been reviewed and approved by the Ethical and Protocol Review Committees of College of Health Sciences, Noguchi Memorial Institute for Medical Research and Korle-Bu Teaching Hospital. If you have any questions about your rights as a research participant you can contact the office between the hours of 8am-5pm or call on +233 (0) 306 665103/4

Volunteer Agreement

The document detailing the risks, discomfort, benefits and procedures involved in the research work titled Hepatitis C virus (HCV) infection among sickle-cell patients at the Korle Bu

Teaching Hospital, has been read and adequately explained to me. I have been given full opportunity to have any questions I may have answered to my satisfaction. Therefore, I agree to participate as a volunteer.

Do you agree to be in the study? _____Yes ☐ No ☐

Do you agree to have your blood stored for future studies? Yes ☐ No ☐

_____ _____

Signature (or thumb print) of consenting individual Date

If a volunteer cannot read the document, then a witness is needed; I was present during the reading and explanation of the consent document to the volunteer; all questions from the volunteer were duly answered and the volunteer agreed to participate in this study.

Signature of witness…… Date……

I certify that the purpose and the nature of the research, the potential benefit and possible discomfort associated with this research has been explained to the volunteer who has agreed to voluntarily participate

Signature of person who obtained the consent…… Date……

If You Have a Problem or Have Other Questions

Please call Gifty Mawuli (0249083765) if you have further questions about the research.