PREVALENCE OF HEPATITIS C VIRUS INFECTION IN DIABETIC SUBJECTS AT ENUGU STATE UNIVERSITY OF SCIENCE AND TECHNOLOGY TEACHING HOSPITAL, PARKLANE, ENUGU.

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PG/M.Sc/10/55042

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SUPERVISOR: DR T.K.C.UDEANI

DECEMBER, 2014.

DECLARATION

I hereby declare that this research work was carried out by me under the supervision of DR.T.K.C. Udeani and has not been submitted elsewhere for the award of Master of Science degree. All references were quoted appropriately.

…… **……**
AGBO I. U. **DATE**

UN/CM/MLS/ 15th December, 2014

CERTIFICATION

Mr./Mrs./Miss AGBO IFEOMA UZOMA PG/M.Sc./10/55042 a postgraduate student of the Department of Medical Laboratory Sciences, Faculty of Health Sciences & Technology, College of Medicine, University of Nigeria, Enugu Campus majoring in MEDICAL MICROBIOLOGY completed the requirements for the research work. The results embodied in the work have not been submitted in partial or full to any Diploma or Degree of this or any other University.

Supervisorôs Name: DR. T.K.C. UDEANI

Signature:----------

DEDICATION

This work is dedicated to the Almighty God who saw me through this work.

ACKNOWLEDGEMENT

My deep gratitude goes to the Almighty God who initiated this programme and also gave me the enabling grace and succour that ensured the successful completion of this work.

During the pursuit of this research work a lot of people played prominent roles worthy of mention. My sincere gratitude goes to my darling husband, Mr. Ndubuisi Agbo for the moral, financial and spiritual support he gave to me during this work.

My innermost appreciation goes to my project supervisor Dr. T.K. C. Udeani for the quality time, guidance and support he offered me throughout this undertaking. He brought his wealth of experience and knowledge to bear on this work.

I appreciate all the lecturers in the department. Many thanks also to the following professional colleagues for their co-operation during the stages of sample collection and test analysis. They are Mrs. Ofordile, a Chief Matron with the ESUT Teaching Hospital, Parklane, Enugu, Mr Chinedu Chukwubuike and Mrs, Chioma Pujah both of Virology Unit, Microbiology Department, UNTH, Ituku-Ozalla. Ijeoma Nwagbara, Nnenna Okafor, and Juliet Osaretin are not left out.

May the Most High God bless you all.

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ABSTRACT

The objective of this study was to assess the prevalence of anti-HCV antibodies amongst diabetics in Enugu. This is important as it has been indicated that presence of HCV in diabetics could lead to insulin resistance and thus increases diabetic complications. A total of 138 diabetics were recruited for this study, with a population control of 102 apparently healthy subjects, within the same age. A structured questionnaire was used to obtain information on the demographic, co-morbid and diabetic complications. Blood samples were collected through venepuncture and serum extracted from them. The serum was analysed for anti-HCV antibodies using anti-HCV antibody EIA, 4th generation kit. The lipid profile and alanine amino transaminase levels of the infected diabetics and few uninfected diabetics were also estimated using a commercially prepared kit respectively. The results were analysed statistically with significant levels taken at $p<0.05$. Of the 138 diabetics, 7(5.1%) were seropositive for anti-HCV antibodies while in the control subjects, 11(10.8%) were seropositive for anti-HCV antibodies. Among the diabetics and the control subjects, the females had higher seropositivity of anti-HCV antibodies than the males. In the diabetics, the females were 4(2.9%) and the males 3(2.2%) while among the control subjects, the females were 9(8.8%) and males 2(2.0%). In the diabetics, the age group 46-60 and 61 + recorded a HCV seropositive of 5(3.6%) and 2(1.4%) respectively while the seropositive was prevalent in all the age groups in the control subjects with statistical significance of $p=0.030$. The ALT of 4 out of the 7 seropositive samples tested were elevated; (mean value 17.13 ± 5.28 ul). The lipid profile of the seropositive samples analysed, all of the 7 samples had elevated triglyceride (TG) (1.84 ± 0.24mmol/l) and 1 had low density lipoprotein (LDL) (3.37 ± 1.2lmmol/l) elevated while the total cholesterol (TC), high density lipoproteins (HDL) and very low density lipoproteins (VLDL) were all normal. Among the risk factors analysed for HCV infection in diabetics, those that have a family history of diabetes were more infected with HCV than those without family history 4(2.9%) and 3(2.2%) with a p-value 0.092. Other risk factors were not statistically significant. The complications due to diabetes showed that only hypertension was statistically significant with $p= 0.008$. There is an effect due to HCV infection in diabetic patients, as HCV can induce hypertensive complications in diabetics.

CHAPTER ONE

1.0 INTRODUCTION

Hepatitis C virus (HCV) was first known as a distinct disease entity in 1975 when most of transfusion associated hepatitis were found not to be caused by the only two hepatitis viruses recognized at that time viz Hepatitis A virus and Hepatitis B virus. The disease at that time was called ñnon ï A non ï B hepatitisò (NIH, 2002). The discovery of hepatitis C genome in 1989 has led to the understanding that this virus is a cardinal health problem worldwide (NIH, 2002). It is marked by a chronic development with mild to severe liver disease, including liver fibrosis, cirrhosis and in lesser proportion, hepatocarcinoma (Sene *et al*., 2004).

Hepatitis C virus infection is one of the most common causes of chronic liver disease and Hepatitis C virus linked disease is a leading indication for liver transplantation today (Mason, 2001). According to World Health organization (WHO) press release in April 1998, prevalence of 0.5% to greater than 10% has been found in population samples around the world. Approximately 170 million people are affected with HCV worldwide. The problem keeps on deteriorating, especially in developing countries. New infections continue to occur because of intravenous drug use, unscreened transfusion, failure to sterilize hospital and injection equipment, occupational exposure, tattooing and high risk sexual activity. Frequent hepatitis C mutations and numerous subtypes have made the search for an HCV vaccine challenging, although research continues and preliminary clinical trials of an experimental vaccine are in progress (WHO, 2000; WHO, 1998).

A number of infectious agents have been associated with chronic diseases, human immunodeficiency virus (HIV) has been linked with acquired

immunodeficiency syndrome (AIDs), human papilloma virus (HPV) with cervical cancer, Epstein-Barr virus with Burkittôs lymphoma and hepatitis B virus (HBV) with hepatocellular Carcinoma (Blattner *et al.*, 1988; de-The *et al*., 1978; Szmuness, 1978 and Walboomers *et al*., 1999). Biologic plausibility and strong epidemiologic evidence were used to demonstrate that these infectious agents caused the associated chronic disease. Epidemiologic associations can be made by detecting either an increased prevalence of the infection among persons with chronic disease or vice versa.

Infection with hepatitis C virus is chiefly a disease of the liver. Notwithstanding, HCV has also been linked to a variety of chronic conditions involving several other organ and tissue systems, including the kidneys and skin. For some of these diseases, there is strong epidemiologic evidence and biologic plausibility, while for others there is only epidemiologic evidence. *Diabetes mellitus* (DM) has recently been associated with HCV infection as one of the extra hepatic manifestations (Sene *et al*., 2004; Mason, 2001). However, it remains to be determined whether hepatitis C infection leads to diabetes mellitus or vice versa, but it is argued that patients with diabetes have an increased risk of exposure to Hepatitis C infection. While patients with liver disease are known to have a higher prevalence of glucose intolerance, preliminary studies suggest that Hepatitis C infection may be an additional risk factor for development of *Diabetes mellitus* (Mason, 2001).

Simo brought forth the idea for the first time in 1993 followed by Allison *et al* in 1994 but the history of appreciation of this association goes back to as far as 1977 when Russian endocrinologists found out abnormalities of glucose tolerance in patients with chronic liver disease (Simo *et al*., 1996; Chen *et al*., 2003; Shlimovich,

1977). Fraser *et al* confirmed in their study in July 1996, that there is an association between *diabetes mellitus* and chronic Hepatitis C but not chronic hepatitis B (Fraser *et al*., 1996). Korean study in March 2001 has also confirmed the fact that diabetes is more prevalent in patients with HCV than HBV (Ryu *et al*., 2001). Mason *et al*., (1999) found an increased prevalence of HCV infection among clinic patients with diabetes (4.2%).

1.1 Justification

A high prevalence of Hepatitis C virus infection in patients with diabetes has been consistently reported, and there is growing evidence to support the concept that HCV infection is a risk factor for developing diabetes. The cause of higher prevalence of HCV infection in diabetics remains unclear. The specific mechanisms by which HCV leads to diabetes are not fully understood, this and other possible mechanisms also need clarification. At present, there is paucity of data on the association of HCV and diabetes in patients attending clinic at Enugu State University of Science and Technology Teaching Hospital (ESUTTH), Parklane, Enugu.

1.2 Aim and Objectives of the Study

1.2.1 Aim

To determine the prevalence of HCV infection in diabetic patients.

1.2.2 Objectives

1. To estimate the level of alanine aminotransferase(ALT) as a marker for liver damage.
2. Evaluate the lipid profile of the infected subjects.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Hepatitis C Virus

The discovery of hepatitis C virus (HCV) in 1989 and the development of a diagnostic assay for circulating antibodies uncovered the aetiology for 90% post transfusion ñnon-A, non-B hepatitisò. The hepatitis C virus is a small, enveloped RNA virus. Although humans are the only known reservoir of HCV, the virus has been successfully transmitted to Chimpanzees in experimental settings. It is unrelated to the other common hepatitis virus (for example, hepatitis A or Hepatitis B). HCV is a member of the Flaviviridae family of viruses. Other members of this family of viruses include those that cause yellow fever and dengue. Hepatitis C virus enters a susceptible host either directly, through needle inoculation or transfusion of contaminated blood products, or inadvertently through breakage of a percutaneous barrier. HCV primarily targets hepatocytes, but other organs and cell types such as lymphoid cells, may also be involved. The cardinal disease manifestation is cirrhosis, more severe and late stage complications include hepatocellular carcinoma and end-stage liver disease (Hu and Tong, 1999). The HCV genome and different viral proteins have been characterized in detail despite the lack of efficient culture systems and small animal models (Gale and Beard, 2001).

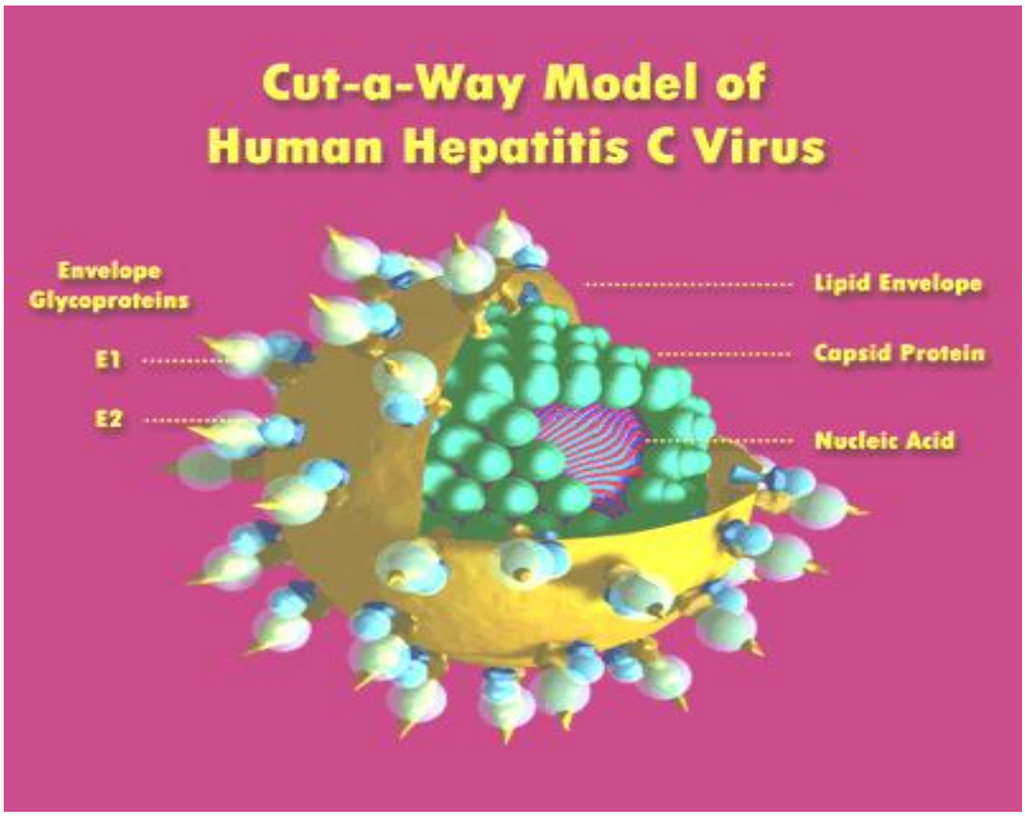

FIG 1: Three dimensional model of HCV.

Created by: Louis E, Henderson.

2.2 Classification of HCV

HCV is classified as a hepacivirus of the flaviviridae family, HCV has a 9.6-kb single-stranded positive ï sense RNA genome, which consists of a 5^1 non translated region (NTR), a single open reading frame (ORF), and a short 3^1 NTR (fig 2)

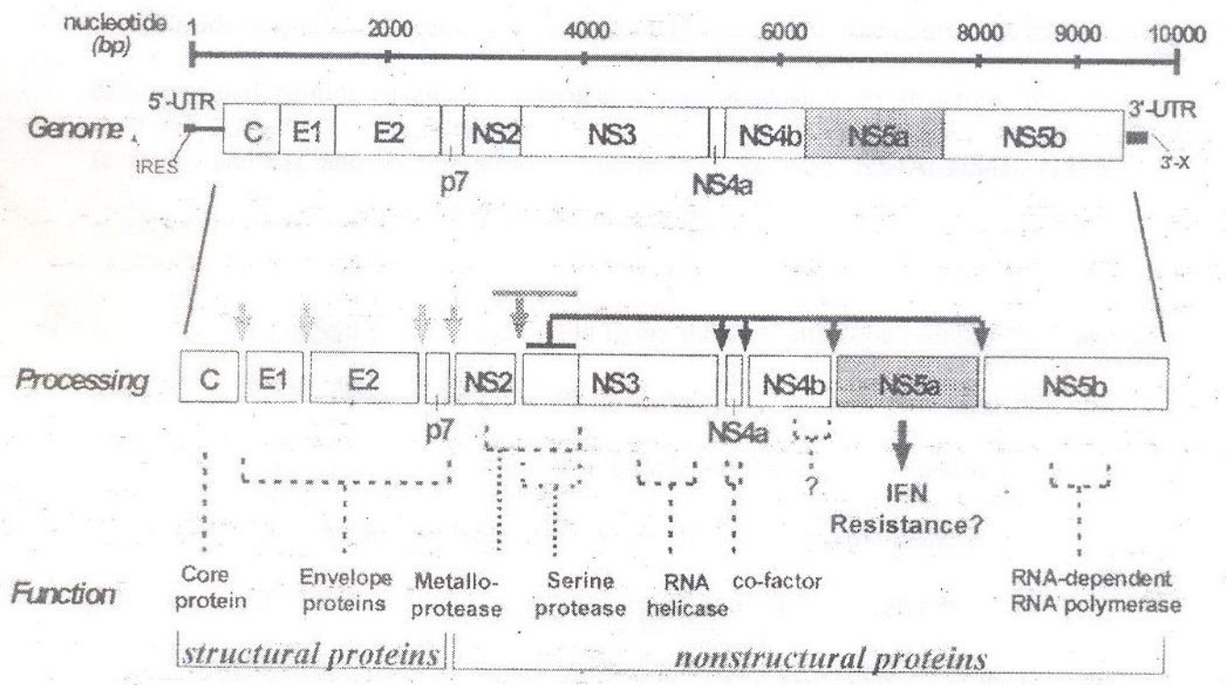

FIG. 2. The hepatitis C virus (HCV) genome and viral proteins. HCV has a single-stranded, positive-sense RNA genome that encodes a single polyprotein, which is processed cotranslationally and posttranslationally by host and viral proteases into structural and nonstructural proteins.

The 5^1 NTR of the viral genome is the most highly conserved region and contains an internal ribosome entry site (IRES) that initiates viral protein translation in a cap-independent manner. The ORF encodes a large polyprotein of approximately 3,000 amino acids, which is processed co-translationally and post translationally by both cellular signal peptidases and viral proteases into at least three structural proteins (core, E_1, and E_2), and six non structural proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B).

Core is the viral capsid protein, and E_1 and E_2 are the viral envelope proteins. NS2 and NS3 are the viral proteases required for processing of the non structural proteins. NS4A is a cofactor for NS3 protease. The function of NS4B still remains unknown. Even though the function of NS5A is not completely understood, there are strong indications that the protein mediates viral interferon (IFN) ï resistance and may regulate viral replication. NS5B, which is the viral RNA ï dependent RNA polymerase (RDRP), mediates replication of the viral genome (De Francesco, 1999; Major and Feinstone, 1997).

Because of the error-prone nature of the viral RDRP, the HCV genome displays a high level of sequence variation. Based on this variation, HCV is classified into 6 major genotypes, with 11-12 subtypes, which display a distinct geographical distribution (Bukh *et al*., 1995 and Simmonds, 1995). HCV genotype 1, including subtypes 1a and 1b, is the predominant genotype in America, Europe and Asia. Genotype 4 in north and central Africa, Genotype 1 in West Africa and genotype 5 in South Africa (Pearlman, 2006).

HCV exists in individual patients as a population of slightly different and closely related viral genomes, also known as quasispecies (Bukh *et al*., 1995), which differ mostly in the hypervariable regions (HVRS) of the envelope genes (Enomoto and Sato, 1995). Partially because of the quasispecies nature of HCV, a vaccine for HCV is not available, which reminds us of our experience with human immunodeficiency virus (HIV).

2.3 Epidemiology

Since its identification in 1989, HCV has been recognized as a serious global medical problem with considerable burden on the health care system (Leigh *et al*.,

2001). HCV infects 2% of the world population, including approximately 4 million Americans and causes an estimated 230,000 new infections annually worldwide (Alter, 1997; Alter and Seeff, 2000). HCV infection is characterized by a high frequency of persistence and the virus sets up a chronic infection in about 80% of infected individuals. Chronic HCV infection causes recurring hepatitis and frequently leads to cirrhosis, hepatic failure and hepatocellular carcinoma (HCC) (Di Bisceglie, 1997). HCV ï associated liver failure is the current leading indication for liver transplantation and causes estimated 8,000 ï 10,000 deaths annually in that country. The data in Africa is not well established but recent report suggest a prevalence of about 10.9% in normal Africans. It is less than 0.5% in the Scandinavia whereas it is up to 20% in Egypt (Bojuwoye, 1997). HCV has also been recognized as a major risk factor for the development of autoimmune disease. HCV primarily targets hepatocytes, but other organs and cell types such as lymphoid cells, may also be involved (Manns, 1999).

2.4 Pathology and Immune Response

The study of HCV pathology and of the anatomic branch in particular has been complicated by the lack of an effective animal or cell culture model. Also, because HCV preferentially targets the liver. It is difficult to draw conclusions about how HCV behaves based on how it grows in non-liver cells.

The variation in reaction profiles observed in HCV patients further complicates the elucidation of HCV pathology. This diversity of response is believed to be related to differences in Human Leucocyte Antigen (HLA) haptotypes, immune competence and viral genotypes. Lastly, pathological studies have been hindered by

the lack of a dependable test for disease progression short of invasive liver biopsy. Because of these complications, little is known with certainty about HCV pathology (Lau and Nelson, 1997). What is understood has mostly been gleaned from indirect lines of evidence and sometimes from liver tissue biopsy.

2.4.1 Direct HCV Cytotoxicity

The most important controversy over HCV pathology concerns its mode of tissue destruction, which is often manifested as total liver failure. Experiments have revealed the existence of both direct and indirect HCV cytotoxicity (Farci e*t al.,* 1992*).* There remains, however, much debate over which of these mechanisms is the primary mechanism of necrosis, even greater uncertainty exists over the pathological mechanisms behind HCVôs high incidence of hepatocellular carcinoma-a rare form of cancer as well as assortment autoimmune reactions (Farci e*t al.,* 1992*)*. Researchers reason that if HCV causes direct cell death, then patients with elevated levels of HCV RNA or HCV proteins should present with high incidence of necrosis and, therefore, chronic disease. So far, no such relationship has been demonstrated (Lau and Nelson, 1997). PCR amplification to determine serum concentration of HCV RNA has shown no direct correspondence to nephron necrosis.

Furthermore, transgenic animal models, with high levels of HCV antigens expressed in hepatocytes, demonstrate no associated cell death (Fang *et al.,* 1994). Other studies have shown that hepatocyte cell death does occur when HCV concentration reaches certain critical levels, but within the majority of patients, such high levels of viremia are rare. Some severely immuno compromised HCV patients classified as ñultra quick progressorsò have demonstrated extremely rapid liver

failure. It is thought that this is the result of abnormally high levels of HCV viremia, resulting in direct cytotoxicity (Farci e*t al.,* 1992*)*.

In conclusion, while it is likely that some degree of cell death results from direct HCV damage, current evidence points away from direct cytotoxicity and towards the effects of an immune response, as the leading cause of HCV pathology.

2.4.2 Indirect Cytotoxicity

The immune response: HCV has been shown to stimulate all normal pathways of the immune response. Early in the immune response, HCV stimulates the effector cells of innate immunity including natural killer (NK) cells. NK cells are thought to contribute, at least in part, to the elevated levels of interferon (IFN) common in HCV patients. Soon after, the acquired immune response is stimulated and both HCV specific B and T cells are recruited (Farci e*t al.,* 1992*)*.

Humoral Immunity:

Antibody (AB) production to both structural and non structural HCV proteins has been demonstrated in nearly all HCV patients. In particular, a large gamut of Abs reactive to HCV envelope proteins E_1 and E_2 develop. The clinical significance to these Abs has not been clearly demonstrated but it is believed that hypervariable regions in the E_1 and E_2 proteins prevent these Abs from clearing the disease and instead drive the antigenic drift of HCV (Farci e*t al.,* 1992*)*.

Further evidence supporting the ineffectiveness of Abs to clear infection has come from a set of experiments conducted by Farci *et al*. These researchers demonstrated that chimpanzees that recovered from HCV infection could later be re-infected by both heterologous (different genotype) and autologous (same genotype)

challenge. The criteria for órecoveryô in these studies involved an HCV RNA ï negative status and normal Alanine amino-transferase levels.

There is speculation that auto reactive Abs may opsonize hepatocytes and stimulate both antibody dependent cytotoxicity (ADCC), as well as the complement (C^1) cascade. Either route of cell death presents possible explanations of hepatocyte necrosis observed in HCV biopsy. However researchers have yet to find anti ï HCV antibody that is reactive to cell membranes (Farci e*t al.,* 1992*)*. Consequently, ADCC and C^1 pathways are considered unlikely, or at least largely insignificant. It is improbable that the humoral immune response results in the widespread liver necrosis observed in HCV patients. Lines of evidence do, however, support that some of the auto-immunity observed in correlation with HCV infection may result from humoral immunity. A subset of B-cells characterized by the CD_5 surface molecule has been shown to undergo clonal expansion during HCV infection. CD_5+B-cells have been implemented by such autoimmune response as rheumatoid arthritis, and may represent a possible cause of the observed auto-immune reaction (Fang *et al.,* 1994). Furthermore, certain HCV antigens have been found to bear sequence homology to self antigens. It is postulated that chronic exposure and activation of the humoral immune system to HCV, as well as its failure to clear the infection, could result in clonal expansion of self reactive antibodies (Fang *et al.,* 1994). Specifically, auto-antibodies to the liver and the kidneys have been found in HCV patients (Lau and Nelson, 1997).

Cell Mediated Immunity (CMI)

The study of cell ï mediated ï immunity (CMI) in HCV infection has proven somewhat difficult due to lack of a good animal model, and because peripheral blood T-cell levels are not reflective of liver T-cell concentrations.

The hepatitis C virus typically induces a strong T-cell reaction, generating both Th_1 and Th_2 responses. It is believed that the relative intensities of these two distinct responses is related to significant cell death such as that observed in the liver of HCV-infected patients. In particular, unusually high amounts of Th_1 cells have been linked to nephron necrosis. Patients with chronic HCV show high titers of the Th_1 cytokines including 1L-2, Tumor Necrosis Factor (TNF) ï alpha, and IFN ï gamma ï all of which implicate Th_1 T-cells in cytotoxicity (Farci e*t al.,* 1992*)*.

Furthermore, it has been found that elevated levels of IFN ï gamma mRNA and 1L-2 mRNA in the liver correlated with fibrosis and inflammation yet another indication that Th_1 cytokines play some role in hepatocellular damage. Researchers have also been able to expand HCV relative T-cells for liver biopsies.

Nearly all of these lymphocytes have turned out to be activated cytotoxic T-lymphocytes (CTL_s). It has been hypothesized that insufficient clearance of the hepatitis C virus, in the presence of large scale CTL activation, can lead to tissue necrosis (Fang *et al.,* 1994).

In addition to evidence identifying an aggressive Th_1 response as an important cause of cell death, further evidence supports the Th_2 response as being preventative against HCV progression (Lau and Nelson, 1997). Studies have shown that 73% of all patients who contract HCV (measured by circulating HCV RNA), but present with no clinical signs of infection (indicated by normal liver biopsy and ALT levels), have

high CD_4^+ responses to immunogenic core proteins such as NS4. In contrast, only 10% of those who test positive for both HCV and clinical progression (indicated by necrosis in biopsy and elevated ALT levels) have strong CD_4^+ proliferative responses.

This correlation of CD_4^+ clonal expansion with non-progressive HCV suggests that Th_2 CD_4^+T cells offer protection against cell damage (Lau and Nelson, 1994). This relationship is consistent with classical dogma; it is understood that Th_2 cytokines have a suppressive effect on the Th_1 response. Future medical therapies for HCV may be able to make use of this observation by trying to induce a stronger Th_2 response and hence repressing the damaging effects of CTL activation.

2.5 Transmission

HCV is spread (transmitted) most efficiently through exposure to infected blood (Conry-contelena, 1996). The most common route of transmission is needles shared among users of illicit drugs. Accidental needle ï sticks in health care workers also have transmitted the virus. The average risk of getting HCV infection from a stick with a contaminated needle is 1.8% (Kiyosawa *et al*., 1991).

Prior to 1992, some people acquired the HCV infection from transfusions of blood or blood products. Since 1992, all blood products have been screened for HCV and cases of HCV due to blood transfusion now are extremely rare. HCV infection also can be passed from mother to unborn child. Approximately 4 of every 100 infants born to HCV infected mothers become infected with the virus (Ohto *et al*., 1994). A smaller number of cases are transmitted through sexual intercourse (Bernstein, 2008). The risk of transmission of HCV from an infected individual to a non-infected spouse or sexual partner without the use of condoms over a life time has been estimated to be between 1% and 4%.

Finally, there have been some outbreaks of HCV when instruments exposed to blood have been re-used without appropriate cleaning between patients.

2.6 Replication

Hepatics C virus enters a susceptible host either directly, through needle inoculation or transfusion of contaminated blood products, or inadvertently, through breakage of a percutaneous barrier (as exemplified by sexual or perinatal transmission) (Alter, 1999). The virus then enters hepatocytes or other susceptible cells, probably through a unique surface molecule or molecules, as the viral receptor (Pileri and Matsu, 1998). After uptake, the virus uncoats and releases the genome to begin replication. The viral genome first serves as the template for translation of the polyprotein. The processed non-structural proteins then form a complex with the genome and initiate synthesis of the negative strand, which in turn function as the template for positive strand synthesis. The replication complex probably resides in a membranous compartment in the cytoplasm, presumably derived from the endoplasmic reticulum. The RNA replicative intermediate matures and interacts with the core and envelope proteins to assemble into a virion. Although most of the replicative processes have not been defined, some non structural proteins clearly play critical roles in viral replication and productive infection; these proteins are therefore the focus of antiviral development.

2.7 Diseases and Symptoms

HCV is the main cause of hepatitis C infection. The virus is responsible for causing hepatitis C and many other forms of liver disease (hepatic fibrosis, complicated cirrhosis, hepatocellular carcinoma). Although there are many risk

factors in the occurrence of liver disease, infection with HCV is considered to be the major cause of hepatitis C. Hepatitis C virus is the main cause of hepatitis C and other infectious liver diseases in humans and chimpanzees, generating similar symptoms in both species.

2.7.1 Hepatitis C infection Symptoms

About 75% of people have no symptoms when they first acquire hepatitis C viral infection. The remaining 25% may complain of fatigue, loss of appetite, muscle aches or fever. Yellowing of the skin or eyes (Jaundice) is rare at this early stage of infection. Over time, the liver in people with chronic infection may begin to experience the effects of the persistent inflammation of the liver caused by the immune reaction to the virus. Blood tests may show elevated levels of liver enzymes, a sign of liver damage, it is often the first suggestion that the infection may be present. Patients may become easily fatigued or complain of non-specific symptoms. As cirrhosis develops, symptoms increase and may include weakness, loss of appetite, weight loss, breast enlargement in men, a rash on the palms, difficulty with the clotting of blood and spider-like blood vessels on the skin. In patients with advanced cirrhosis, the liver begins to fail. This is a life threatening problem. Confusion and coma (encephalopathy) may result from the inability of the liver to process certain toxic substances. Increased pressure in the blood vessels of the liver (portal hypertension) may cause fluid to build up in the abdominal cavity (ascites) and result in engorged veins in the swallowing tube (esophageal varices) that tear easily and can bleed suddenly and massively. Portal hypertension also can cause kidney failure or an enlarged spleen resulting in a decrease of blood cells and the development of low platelets (thrombocytopenia) which can promote bleeding. In advanced cirrhosis, liver

failure causes decreased production of clotting factors. Patients with advanced cirrhosis often develop jaundice because the damaged liver is unable to eliminate bilirubin formed from the haemoglobin of old red blood cells.

2.8 Diagnosis

The diagnosis of HCV infection can be made by detecting either anti-HCV or HCV RNA. Detection of anti-HCV is recommended for routine testing of asymptomatic persons and should include use of both enzyme immuno assay (EIA) and supplemental antibody testing (ie RIBA) for all positive anti-HCV results of EIA is preferred, particularly in settings where clinical services are not provided directly (De Medina and Schiff, 1995).

Relatively few patients seek medical care for acute hepatitis C, since most patients are asymptomatic or have only mild, flu-like symptoms. Of those who do present with acute hepatitis C, 70 to 80 percent have detectable anti-HCV at clinical presentation and 90% have detectable anti-HCV by 12 weeks after onset (Vallari *et al.,* 1992). Therefore, anti-HCV testing should be repeated if acute hepatitis C is suspected and the initial test result is negative. Most patients with acute hepatitis C remain chronically infected and approximately two thirds or more of patients with chronic infection have abnormal ALT activity. Based on serial serum samples with normal values for ALT and negative results for HCV RNA, it is estimated that in 15% of HCV infected patients, the infection resolves and the patients recover from hepatitis C. In most instances evidence of chronic HCV infection is discovered by chance through screening tests at the time of blood donation or routine physical examination. Most persons who are found to be positive for anti-HCV in these

situations are chronically infected (HoofNagle, 1997). No tests are available to differentiate acute, chronic and resolved infection and the diagnosis of chronic hepatitis C is usually based on the presence of elevated ALT value in patients who are positive for anti-HCV. For anti-HCV positive patients with a normal ALT value, the presence of ongoing liver inflammation should be assessed by monitoring serum ALT values several times over six to 12 months, because abnormalities may be present only intermittently in patients with chronic hepatitis C.

Table 1: Tests for Hepatitis C Virus Infection

Test/type	Application	Comments
Anti-HCV. EIA, RIBA	Indicates past or present infection but does not differentiate between acute, chronic or resolved infection. All positive EIA results should be verified with a supplemental assay (RIBA)	Sensitivity: > = 97% EIA alone has low positive predictive value in low prevalence populations.
HCV RNA. Qualitative tests RT-PCR amplification of HCV RNA by in-house or commercial assays (eg amplicor HCV)	Detects presence of circulating HCV RNA. Monitors patient on antiviral therapy	Detects virus as early as 1 to 2 weeks after exposure. Detection of HCV RNA during course of infection may be intermittent, a single negative RT-PCR test is not conclusive. False positive and false negative results are Possible.
Quantitative tests. RT-PCR amplification of HCV RNA by in-house or commercial assays.	Determines concentration of HCV RNA. May be useful for assessing the likelihood of response to antiviral therapy.	Less sensitive than qualitative RT-PCR. Should not be used to exclude the diagnosis of HCV infection or to determine treatment end point.
Genotype. Several methodologies available (eg hybridization sequencing).	Gps isolates of HCV based on genetic differences into six genotypes and > 90 subtypes. With new therapies, length of treatment may vary based on genotype.	Genotype I (subtypes 1a and 1b) is the most common in the US and is associated with lower response to antiviral therapy.
Serotype EIA based on immune reactivity to synthetic peptides (eg murex HCV serotyping 1-6 assay)	No clinical utility	Cannot distinguish between subtypes. Dual infections often observed

EIA = Enzyme Immune assay

RIBA = Recombinant Immunoblot Assay

Anti-HCV = Antibody to Hepatitis C Virus

RT-PCR = Reverse Transcriptase Polymerase Chain Reaction

bDNA = branched chain DNA

2.9 Prevention and Control

Prevention programs have been aimed at avoiding needle sharing among drug addicts. Needle exchange programs and educational interventions have reduced transmission of HCV infection. However, the population of drug addicts is a difficult population to reach, and rates of HCV remain high among addicts. Among health care workers safe needle usage techniques have been developed to reduce accidental needle sticks. Newer syringes have self-capping needle systems that avoid the need to manually replace a cap after drawing blood and reduce the risk of needle sticks. There is no clear way to prevent transmission of the HCV from mother to child.

Screening tests for blood products have almost eliminated the risk of transmission of HCV infection through transfusion, estimated by the CDC to be less than one in two million transfused blood products. People with HCV infection should not share razors or toothbrushes with others. It is critical that health workers follow manufacturerôs directions for sterilizing/cleaning instruments and that disposable instrument be discarded properly.

It is important to realize that HCV is not spread by casual contact. Thus, shaking hands, and hugging are not behaviours that increase the risk of transmission. There is no need to use special isolation procedures when dealing with infected patients.

Finally, immunization against HCV is not available, therefore, identifying persons at risk but not infected with HCV provides opportunity for counseling on how to reduce their risk for becoming infected.

2.10 Treatment of HCV Infection

Interferon (IFN) ï Ŭ monotheraphy was first approved by the Food and Drug Administration (FDA) in 1991 and became the mainstay for treatment of HCV infection until recently, when IFN ï Ŭribavirin combination therapy became available. Interferon based therapies, currently the only ones available for HCV infection, have been unable to eliminate viral infection in the majority of patients. Many studies suggest that HCV possesses mechanisms to antagonize the IFN-induced antiviral response. Overcoming IFN resistance of HCV still poses a majority challenge for effective IFN-based therapy and future management of the HCV pandemic.

2.11 Definition of *Diabetes mellitus*

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both (Diabetes care, 1997). The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels (Diabetes care, 1997). Thus diabetes covers a wide range of heterogeneous diseases.

2.12 Classification of *Diabetes mellitus*

The majority of diabetes cases fall into two categories: type 1 and type 2. Type 2 diabetes can be distinguished from type I diabetes by its epidemiology, etiology and pathogenesis.

2.12.1 Type I (beta-cell destruction, usually leading to absolute insulin deficiency).

This form of diabetes, previously encompassed by the terms insulin-dependent diabetes, Type I diabetes, or juvenile-onset diabetes, results from autoimmune

mediated destruction of the beta cells of the pancreas. The rate of destruction is quite variable, being rapid in some individuals and slow in others (Zimmet *et al*., 1994). The rapidly progressive form is commonly observed in children, but also may occur in adults (Humphrey *et al*., 1998). The slowly progressive form generally occurs in adults and sometimes referred to as latent auto immune diabetes in adults (LADA). Children and adolescents, may present with ketoacidosis as the first manifestation of the disease (Diabetes, 1985). Others have moderate fasting hyperglycaemia that can quickly change to severe hyperglycaemia and/or ketoacidosis in the presence of infection or other stress. Adults in particular, may retain residual beta-cell function, sufficient to prevent ketoacidosis, for many years (Zimmet, 1995). Individuals with this form of type I diabetes often become dependent on insulin for survival eventually and are at risk for ketoacidosis (Willis *et al*., 1996). At this stage of the disease, there is little or no insulin secretion as manifested by low or undetectable levels of plasma C ï peptide (Hother-Nielsen, 1988). Markers of immune destruction, including islet cell autoantibodies, and/or autoantibodies to insulin, and autoantibodies to glutamic acid decarboxylase (GAD) are present in 85 ï 90% of individuals with type I diabetes mellitus when fasting hyperglycaemia is initially detected (verge *et al*., 1996). The peak incidence of this form of diabetes occurs at any age, ranging from childhood to the ninth decade of life (Molbak *et al*., 1994).

These patients may also have other autoimmune disorders such as Gravesô disease, Hashimotoôs thyroiditis, and Addisonôs disease (Betterle *et al*., 1983). Idiopathic form of type I diabetes is a term used to refer to some forms of type I diabetes which have no known aetiology. Individuals in this category have permanent insulinopenia and are prone to ketoacidosis, but have no evidence of autoimmunity

(Mclarty *et al*.,1990)**.** This form of diabetes is more common among individuals of African and Asian origin. In another form found in Africans an absolute requirement for insulin replacement therapy in affected patients may come and go, and patients periodically develop ketoacidosis (Ahren and Corrigan, 1984).

2.12.2 Type 2 (predominantly insulin resistance with relative insulin deficiency or predominantly an insulin secretory defect with/without insulin resistance).

Diabetes mellitus of this type previously cover non-insulin-dependent diabetes, or adult-onset diabetes. It is a term used for individuals who have relative (rather than absolute) insulin deficiency. People with this type of diabetes frequently are resistant to the action of insulin (Defronzo *et al*., 1997). At least initially, and often throughout their lifetime, these individuals do not need insulin treatment to survive. This form of diabetes is frequently undiagnosed for many years because the hyperglycaemia is often not severe enough to provoke noticeable symptoms of diabetes (Harris, 1993).

Nevertheless, such patients are at increased risk of developing macrovascular and microvascular complications (Harris, 1993). There are probably several different mechanisms which result in this form of diabetes, and it is likely that the number of people in this category will decrease in the future as identification of specific pathogenetic processes and genetic defects permits better differentiation and a more definitive classification with movement into ñother typesò. Although the specific aetiologies of this form of diabetes are not known, by definition autoimmune destruction of the pancreas does not occur and patients do not have other known specific causes of diabetes.

The majority of patients with this form of diabetes are obese, and obesity itself causes or aggravates insulin resistance (Campbell and Carlson, 1993). Many of those who are not obese by traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region (Kissebah *et al*., 1982).

The risk of developing type 2 diabetes increases with age, obesity, and lack of physical activity (Zimmet, 1991). It occurs more frequently in women with prior Gestational diabetes mellitus (GDM) ï and in individuals with hypertension or dylipidaemia. Its frequency varies in different racial/ethnic sub-groups (Zimmet, 1991). It is often associated with strong-familial, likely genetic, predisposition (Valle *et al*., 1997).

However, the genetics of this form of diabetes are complex and not clearly defined. Some patients who present with a clinical picture consistent with type 2 diabetes have autoantibodies similar to those found in type I diabetes, and may masquerade as type 2 diabetes if antibody determinations are not made. Patients who are non-obese or who have relatives with type I diabetes and who are of Northern European origin may be suspected of having late onset type I diabetes.

2.12.3 Gestational Diabetes mellitus

Gestational diabetes is carbohydrate intolerance resulting in hyperglycaemia of variable severity with onset or first recognition during pregnancy. It does not exclude the possibility that the glucose intolerance may antedate pregnancy but has been previously unrecognized. The definition applies irrespective of whether or not insulin is used for treatment or the condition persists after pregnancy.

Individuals at high risk of gestational diabetes include older women, those with previous history of glucose intolerance, those with a history of large gestational age babies, women from certain high risk ethnic groups, and any pregnant woman who has elevated fasting, or casual, blood glucose levels. It may be appropriate to screen pregnant women belonging to high ï risk populations during the first trimester of pregnancy in order to detect previously undiagnosed diabetes mellitus. Formal systematic testing for gestational diabetes is usually done between 24 and 28 weeks of gestation (WHO, 1999).

2.12.4 Other specific types

Other specific types are currently less common causes of diabetes mellitus, but are those in which the underlying defect or disease process can be identified in a relatively specific manner. They include, for example, diabetes as a result of genetic defects in ß-cell function, genetic defects in insulin action, disease of the exocrine pancreas, endocrinopathies, Drug or chemical ï induced diabetes, infections, uncommon forms of immune ï mediated diabetes and other genetic syndromes sometimes associated with diabetes (Diabetes care, 2005).

2.13 Association of Diabetes Mellitus and Chronic Hepatitis C Infection

The association between diabetes mellitus and cirrhosis has been speculated since decades. The hypotheses that have been put forward for this association are summarized by Hadzyiyannis (1999) as follows: (a) Diabetes could be the cause of associated liver disease because the nuclear and cytoplasmic glycogen deposit, fat deposit in the hepatocytes and perisinusoidal fibrosis seen in diabetes are also seen in liver cirrhosis. It has been proven that cirrhosis is a rare consequence of diabetes (Cruzelfeldt *et al*., 1970), but recently diabetes was implicated in the pathogenesis of

cirrhosis through lesions of non-alcoholic steatohepatitis (NASH). About 25-75% patients with NASH have diabetes and similarly 90% of NASH patients have been found to be obese. In many diabetics, progression of NASH to cirrhosis has been documented (Katamna, 1997). The known causes of chronic liver disease like HCV infection may also coexist in diabetics and thus result in cirrhosis. (b) The other possibility is implication of chronic liver disease in the etiology of diabetes. Liver plays a pivotal role in the carbohydrate metabolism. This metabolism is deranged in terms of impaired glucose tolerance in about 70% of the cases suffering from liver cirrhosis while frank diabetes occurs in few (Bacon *et al*., 1994; Kruszynska *et al.*,1992).

Hyperinsulinemia, insulin resistance and hyperglucagonemia characterize this abnormal glucose metabolism. These patients with chronic liver disease develop hyperglycemia with therapeutic doses of steroids or interferon (Farbis *et al.,* 1992; Fattovich *et al*., 1997). (c.) Thirdly the association of chronic liver disease with diabetes may be due to common causes like alcohol, hemochromatosis and autoimmune conditions. Hepatitis B and C are common liver diseases and they tend to occur more in diabetics than in general population. Frequent parenteral exposures in diabetics may be the cause of this high association of HBV and HCV infections. Extra hepatic sites of viral replication i.e. kidneys, pancreas, spleen etc. may also produce many co-morbid conditions especially in relation to organ of extra hepatic manifestation (Allison *et al.*, 1997; Hadziyannis *et al.,* 1997). There are several studies pointing to possible link between HCV infection and Type 2 Diabetes Mellitus (Caronia *et al*., 1999). In one of the retrospective studies on 100 orthotoptic liver transplantations for end stage liver disease, Type 2 *Diabetes Mellitus* was found in

50% of the cases with HCV related liver cirrhosis versus 9% in patients having liver disease unrelated to HCV(Allison *et al*.,1994). In another study there was an increased incidence of HCV infection in 200 Type 2 *Diabetes Mellitus* patients recruited from UK for a prospective study (Gray *et al.,* 1995). Reports from Europe, Middle East and North America also show an increased prevalence of diabetes in patients having chronic liver disease when compared with other chronic liver diseases like primary biliary cirrhosis, primary sclerosing cholangitis, alcoholic liver disease or for that matter chronic HBV related disease (El-Zayadi *et al.,* 1997; Mason *et al.,* 1999). Mason *et al* reported a case controlled study on the association of chronic hepatitis C with diabetes mellitus (Mason *et al.,* 1999). Though many studies have been done in the past to see this association but the study by Mason *et al* (1999) deals systematically with all known causal possibilities in relation to diabetes and chronic liver disease. In this study all possible factors related to abnormal glucose handling were excluded. The prevalence of diabetes was found to be higher in HCV related chronic liver disease (21%) than in chronic HBV (12%). Moreover prevalence of chronic HCV infection was much higher in diabetics (4.2%) than in controls (1.6%). Similar association was reported in advanced liver disease cases when transplant was not available.

The food for thought is - should diabetes be included in the list of extrahepatic manifestations of HCV infection? The reasons for debating this association is the chances of introduction of HCV infection in diabetics as a result of frequent admissions and venepunctures that occur in these patients. Moreover, chronic HCV infection in adults has a very high transition rate to convert to chronicity when compared to HBV, therefore if exposure to the two viruses is similar the prevalence of

chronic HCV infection is expected to be much higher than that for chronic HBV. The mechanism involved in this extrahepatic disease is not yet clear but (a) cytopathic damage due to the viral replication at these extrahepatic sites (b) immunological tissue damage (c) virus associated autoimmunity are the possibilities (Strassburg *et al*., 1996). In this context, the HCV infection may either trigger latent autoimmunity or induce de novo an autoimmune disease through molecular mimicry and immunological dysregulation. The reasons to support the latter are thyroiditis, thrombocytopenia and lichen planus and aggravation of these disorders by interferon therapy (Hadziyannis *et al*.,1997). Studies have shown some association of HCV genotype 2a with extrahepatic disease especially diabetes (Zignego *et al*., 1996; Andreone *et al*., 1996) .In Pakistan, the HBsAg carrier rate varies between 4- 10% (Andreone *et al*., 1996; Zuberi *et al.,* 1977). The Meta analysis of various HBV studies gives a figure of 4% HBV carrier rate (unpublished data). The anti HBsAg figures in various population groups are around 32% indicating a natural seroconversion, as these figures are of pre vaccination era (Zuberi *et al.,* 1977). The HCV positivity rate in blood donors and healthy population varies from 1.4-4% (Luby *et al*., 1997; Kakepota *et al.,* 1996). Keeping these figures in mind it appears that the two viruses are hitting the Pakistani population at almost the same rate of 4%.(Qureshi *et al.,* 2002) in this issue of the journal report a rather unusual finding of equal occurrence of diabetes in both HBV and HCV. Their plea is that as two viruses have the same exposure rate therefore their extrahepatic involvement is also similar. The National Health survey of Pakistan reports a 5% prevalence of *Diabetes mellitus* (PMRCNHS, 1998), which again appears to confirm the above hypothesis. Most of the above figures for HBV are of pre or early HBV vaccination era. It would be

interesting to study these figures 2-3 decades after the HBV vaccination when the HBV figures are likely to fall, and see if this equal predisposition of diabetes mellitus persists in chronic HCV and HBV cases.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Population

Patients that were diagnosed of diabetes in a tertiary hospital, ESUT Teaching Hospital Enugu, were enrolled for the study. The diabetics were recruited while reporting for routine medical check-up; or those that were hospitalized, for diabetic complications. The personal and clinical information were obtained and recorded on a structured questionnaire. The diabetics of all ages were enrolled, while those with HIV infection were not enlisted in the study. Participation in the study was voluntary and informed consent was obtained from those that agreed to participate. The study was reviewed and approved by the ethical committee of the hospital. The study was matched with a population control of individuals that are apparently healthy.

3.2 Collection of Sample

Blood samples were collected using sterile syringes and needles. Five millilitres of blood was collected, allowed to clot and centrifuged at cold temperature at 2000rpm for 5 minutes, the sera samples were separated and stored in cryovials at ï 95^0C until assayed.

3.3 Detection of Anti-HCV Antibodies

Anti-HCV antibodies were detected using fourth generation enzyme-linked immunosorbent assay. Anti-HCV antibodies kit (INTECO Diagnostics, UK) which consisted of recombinant antigens corresponding to core (C) and nonstructural (NS2,NS3,NS4A,NS4B,NS5A and NS5B) regions of HCV was used.

3.4.1 Principles of the Assay

This kit employs solid phase, indirect ELISA method for detection of antibodies to HCV in two-step incubation procedure. Polystyrene microwell strips are pre-coated with recombinant, highly immunoreactive antigens corresponding to the core and the non-structural regions of HCV (Fourth Generation HCV ELISA). During the first incubation step, anti-HCV specific antibodies, if present, will be bound to the solid phase pre-coated HCV antigens.

The wells are washed to remove unbound serum proteins, and rabbit anti-human lgG antibodies (anti-lgG) conjugated to horse radish peroxidase (HRP-conjugate) is added. During the second incubation step, these HRP ï conjugated antibodies will be bound to any antigen-antibody (lgG) complexes previously formed and the unbound HRP-conjugate is then removed by washing. Chromogen solutions containing tetramethylbenzidine (TMB) and urea peroxide are added to the wells and in presence of the antigen-antibody ï anti-lgG (HRP) immunocomplex; the colourless chromogens are hydrolyzed by the bound HRP conjugate to a blue colour product. The blue colour turns yellow after stopping the reaction with sulphuric acid. The amount of colour intensity can be measured and is proportional to the amount of antibody captured in the wells, and to the sample respectively. Wells containing samples negative for anti-HCV remain colourless.

3.3.2 Test Procedures for HCV antibodies

The reagents and samples were allowed to reach room temperature for 30 minutes. The stock wash buffer was diluted 1 to 20 with distilled water using a clean vessel. Using manufacturerôs manual, 100µl specimen diluent was added into each well

except the blank. 10µl of positive control, negative control and sample were added into their respective wells. Separate disposable pipette tip was used for each sample. The plate was mixed by tapping gently. The plate was covered and incubated for 30 minutes at 37^0C.

At the end of incubation, each of the microwells was washed 5 times with diluted wash buffer using Optic Ivymen System model 2600C. 100µl of HRP ï conjugate was added to each well except the blank. The plate was covered and incubated for 30 minutes at 37^0C. Washing was repeated as stated above. 50µl of chromogen A and 50µl chromogen B solution were added into each well including the blank. It was mixed by tapping the wells gently. The plates were incubated for 15 minutes at 37^0C avoiding light. 50µl stop solution was added into each of the wells and mixed by tapping gently. Intensive yellow colour developed in positive control and anti-HCV positive sample wells. Microlitre plate reader: thermo scientific multi skan EX was used to read the absorbance at 450nm. The cut-off value was calculated and the results were evaluated.

3.4.2 Interpretation of the Results

S = individual absorbance (OD) of each specimen

C.O = cut-off.

Negative results: (S/C.O.<1): samples giving absorbance less than the cut-off value are negative for this assay; which indicates that no antibodies to hepatitis C virus have been detected with the HCV ELISA Kit. Positive results (S/C.O.≥1): samples giving an absorbance greater than or equal to the cut-off value are considered reactive, which

indicates that antibodies to hepatitis C Virus have been detected using this anti-HCV ELISA kit.

3.5 Lipid Profile Test

3.4.1 Principle for triglyceride test.

Sample triglycerides incubated with lipoproteinlipase (LPL), liberate glycerol and free fatty acids. Glycerol is converted to glycerol-3-phosphate (G3P) and adenosine -5-diphosphate (ADP) by glycerol kinase and ATP. Glycerol-3-phosphate (G3P) is then converted by glycerol phosphate dehydrogenase (GPO) to dihydroxyacetone phosphate (DAP) and hydrogen peroxide (H_2O_2).

In the last reaction, hydrogen peroxide (H_2O_2) reacts with 4- aminophenazone (4-AP) and P ï chlorophenol in the presence of peroxidase (POD) to give a red coloured dye:

Triglycerides + $H_2$0 LPL → Glycerol + free fatty acids

Glycerol + ATP Glycerol kinase → G 3P + ADP.

G3P + O_2 GPO → DAP + H_2O_2

H_2O_2 + 4 ï AP + P ï chlorophenol POD → Quinone + H_2O

Reagent used was Bio Trust.

References values:

Men Ÿ 40 ï 160mg/dl (0.452 ï 1.808 mmol/L)

Women Ÿ 35 ï 135mg/dl (0.3955 ï 1.5255 mmol/L)

Conversion factor: mg/dl x 0.0113mmol/L

3.4.2 Principles for cholesterol test

Cholesterol esters + H_2O cholesterol Esterase → cholesterol + fatty acids.

Cholesterol + H_2O + O_2 cholestrol Oxidase cholestenone + H_2O_2.

H_2O_2 + 4ï Aminoantipyrine + 3,5-Dichlorophenol POD coloured quinonic derivative + 4H_2O

QCA Reagent was used and ChoD ï POD METHOD.

Normal range

< 5.17 mmol/L (200mg/dl) desirable blood cholesterol.

5.17 ï 6.18 mmol/L (200- 239mg/dl) bordeline high blood cholesterol.

> or = 6.20mmol/L (240mg/dl) high blood cholesterol.

3.5.3 Principles for HDL cholesterol

Low density lipoproteins (LDL) and very low density lipoproteins (VLDL) are precipitated from serum by the action of a polysaccharide, in the presence of divalent cations. Then high density lipoproteins cholesterol (HDL ï cholesterol) present in the supernatant is determined.

Clinical interpretations

Low HDL ï Cholesterol increases the risk for heart disease.

<40mg/dl Ÿ < 1.04 low

High HDL ï Cholesterol reduces the risk for heart disease.

≥ 60mg/dl Ÿ ≥ 1.55 high

3.4.4 Principle for ALT (alanine aminotransferase)

∝ - oxoglutarate + L ï alanine GPT L ï glutamate + Pyruvate.

Alanine aminotransferase is measured by monitoring the concentration of pyruvate hydrazine.

Reagent used; Randox method by Reitman, S.

Normal value up to 12ul.

3.6 Precautions

The kits were used by qualified medical laboratory scientists. All personnel involved in performing the assay wore personal protective equipment. Cross-contamination between kit reagents was avoided by using disposable tips. The waste materials were disposed appropriately and the work bench was cleansed with an antiseptic solution.

3.7 Statistical Analysis

Basic descriptive statistics (Mean, SD, percentages) were calculated. Prevalence estimates of HCV were generated using HCV positive cases.

Prevalence of risk factors was generated using data from all consented diabetic subjects.

The variables were compared using Chi-square and studentôs t- test at 95%C.I and P value less than or equal to 0.05 were used to access differences in risk factors. All statistical analysis was done with Statistical Package for Social Science (SPSS) version 17.

CHAPTER FOUR
RESULTS

A total of 138 diabetics were recruited for this study. They comprised of 53 males and 85 females with mean age of 57.63 ± 11.18 (age range of between 21 and 85 year olds). The study was matched with a population control, of individuals that are apparently healthy, which comprised of 40 males and 62 females, with a mean age of 37.8 ± 11.8. Of the 138 diabetics, 7(5.1%) were seropositive for anti-HCV antibodies while in the control subjects, 11(10.8%) were seropositive for anti-HCV antibodies. Among the diabetics and the control subjects, the females had higher seropositivity of anti-HCV antibodies than the males. In the diabetics, the females were 4(2.9%) and the males 3(2.2%) while among the control subjects, the females were 9(8.8%) and males 2(2.0%). The age group of 46-60 and Ó61 had a HCV seropositive of 5(3.6%) and 2(1.4%) respectively in the diabetics. In the control subjects, the seropositive was prevalent in all the age groups (Table 2a and 2b). The age group was statistically significant among the control subjects (p=0.030).

In the analysis of risk factors for HCV infection among diabetics, the family history indicates that those that have a family history of diabetes were more infected with HCV than those without family history 4(2.9%) and 3(2.2%) with a p-value 0.092. Among the other risk factors analyzed, such as previous blood transfusion, surgery, sharing of sharp objects, none was statistically significant (Table 2a).

Alcohol consumption and cigarette smoking are known factors that enhance the progression of HCV and diabetes, none was statistically significant in this study.

The complications due to diabetes were assessed (Table 3), of the 71 diabetics with hypertensive complications, 7(5.1%) were seropositive for HCV and this was

statistically significant, (p=0.008). The multivariant analysis showed an OR of 1.100 (95% CI: 1.027-1.198) (Table 5).

The other complications assessed were bad eye sight and diabetic foot ulcer, and the seropositivity of HCV was not statistically significant (Table 3). Co-morbid factors that were assessed included previous kidney disease, liver disease, reaction to insulin dependency and all those that responded to having any of these problems were all seronegative for HCV. The biochemical changes indicated that 34(24.6%) of the diabetics, have persistent elevated glucose level, of which 4 (2.9%) were seropositive for anti- HCV antibodies. The alanine aminotransferase (ALT) indicated of 4 (17.13 $\pm$ 5.28u/l) with elevated ALT, though not statistically significant. Of the eleven (11) diabetics with normal ALT values, 3(12.0 $\pm$ 0.00) had HCV infection. The lipid profile indicated that 8 had elevated Triglycerides (TG) and 8 had elevated low density lipoproteins (LDL),7 and 1 were seropositive for HCV respectively, each of the TC, HDL cholesterol, VLDL lipid profile, markers with normal values had anti-HCV antibodies.

Table 2A: Demographic and risk factors associated with Hepatitis C Virus infection in diabetics.

Variable	Number	HCV +ve(%)	P-Value
Sex			
Male	53	3(2.2)	0.804
Female	85	4(2.9)	
Age (years)			
< 30	4	0	0.583.
31-45	11	0	
46-60	66	5(3.6)	
61 above	57	2(1.4)	
Family History of Diabetes.			
Yes			
No	40	4(2.9)	0.092
	98	3(2.2)	
Previous			
Blood			
Transfusion			
Yes	15	2(1.4)	0.122
No	123	5(3.6)	
Sharing of sharp			
Objects			
Yes	7	0	0.530
No	131	7(5.1)	
Alcohol in take			
Yes	58	2(1.4)	0.459
No	80	5(3.6)	
Smoking cigarette			
Yes	7	0	0.530
No	124	7(5.1)	

Table 2B: Demographic and risk factors associated with Hepatitis C Virus infection in apparently healthy control subjects.

Variable	Number	HCV +ve (%)	P-value
Age			
<=30	82	6(5.9)	0.030
31-45	13	2(2.0)	
46-60	5	2(2.0)	
61 above	2	1(1.0)	
Sex			
Male	40	2(22.0)	0.130
Female	62	9(8.8)	
Previous blood transfusion			
Yes	8	0(0)	0.306
No	94	11(10.8)	
Surgery			
Yes	18	2(2.0)	0.961
No	84	9 (8.8)	
Sharing of sharp objects			
Yes	2	0(0)	0.619
No	100	11(10.8)	
Alcohol intake			
Yes	56	5(4.9)	0.505
No	46	6(5.9)	
Smoking cigarette			
Yes	4	0	0.478
No	98	11(10.8)	

Table 3: Association of HCV with diabetic complications

Variable	Test (n)	HCV +ve (%)	p-value
Bad eye Sight			
Yes	56	3(2.2)	0.900
No	82	4(2.9)	
Hypertension			
Yes	71	7(5.1)	0.008
No	67	-	
Foot ulcer			
Yes	7	0	0.531
No	131	7(5.1)	
Previous kidney disease			
Yes	2	0	0.742
No	136	7(5.1)	
Liver disease			
Yes	0	0	NA
No	138	7(5.1)	
On Insulin therapy			
Yes	5	0	0.559
No	133	7(5.1)	
Insulin therapy Reaction			
Yes	4	0	0.639
No	134	7(5.1)	

Table 4: Biochemical markers of HCV and diabetes

Parameters	Number	HCV +ve (mean value ±SE)	P-value
Glucose (FBS)			
normal	75	3	0.126
elevated	34	4	
ALT(u/I)			
Normal	11	3(12.0± 0.00)	0.270
Elevated	5	4(17.13+-5.28)	0.858
TC (mmol/L)			
Normal	15	7(5.09 ± 0.26)	0.563
Elevated	1	0	
TG(mmol/L)			
Normal	8	0	0.614
Elevated	8	7 (1.84± 0.24)	
HDL (mmol/L)			
Normal	15	7(1.05± 0.08)	0.447
Elevated	1	0	
LDL (mmol/L)			
Normal	8	6(2.65± 0.33)	
Elevated	8	1(3.37+_1.21)	
VLDL (mmol/L)			
Normal	9	7(0.80± 0.37)	0.447
Elevated	8	0	

Table 5: Multivariate Analysis

Variable	OR	95% CI
Hypertension	1.109	1.027-1.198
Family history	0.284	0.061-1.333

CHAPTER FIVE

DISCUSSION AND CONCLUSION

Discussion

Diabetic patients could be at risk of infection by hepatitis C Virus because of the frequent iatrogenic exposure (Ratziu *et al*., 2004). The goal of this study was to assess the prevalence of anti-HCV antibodies amongst diabetics in Enugu. The prevalence rate of 5.1% and 10.8% were obtained from diabetics and non-diabetics (control) respectively. Comparatively, fewer cross-sectional studies assessing the prevalence of HCV infection among people with diabetes have been performed, and few have included a comparison group of non-diabetic persons. The prevalence rate obtained in this study is high when compared with the prevalence rate of 4.2% and 1.6% obtained from diabetics and non-diabetics respectively by Mason *et al. (*1999). In a similar study, Ndako *et al.* (2009), in Jos, Nigeria, reported a prevalence rate of 11% although no control. In Addis Ababa Ethiopia, a higher prevalence rate of 9.9% was reported amongst patients with diabetes and 3.3% amongst apparently healthy subjects (control) (Ali *et al.,* 2012). This study shows that the occurrence of HCV among the general population (control) is higher than that obtained in Ethiopia while the prevalence of HCV in diabetics was higher in Ethiopian subjects than in this study. The cause of this increased prevalence of HCV in diabetic patients could not be easily explained. The differences in these findings may be attributed to the fact that HCV was acquired before the on-set of diabetes or that HCV was induced due to iatrogenic exposure (Ratziu *et al*., 2004). In this study, the females had higher prevalence of 2.7% which is similar to the findings of Cacoub *et al* .(1999) who found female gender as a frequent factor in the extrahepatic manifestation of HCV infection

but in a similar study by Ali and co-workers (2007), there was no gender difference in the seropositive cases. The problem associated with the females having higher prevalence is the possibility of congenital transmission of HCV or mother to child transfer in some that are of child bearing age. In addition, this study detected HCV infection in people aged between 46 years and above and not in the younger ones, whereas, in the control subjects, HCV was detected in all the age groups and it was statistically significant. This agrees with previous studies by El-Hawaly *et al*. (2011) that reported that HCV infection was more in the elderly diabetic patients than the younger ones, the assumptions in this study may be that; the diabetic patients probably acquired HCV during the onset of type 2 *Diabetes mellitus* or that few of them maybe as a result of extra-hepatic infection due to earlier infection with HCV at a tender age as observed in the control subjects. It has been severally reported that higher prevalence of HCV infection in diabetic subjects may not be related to the main risk factors associated with HCV seropositivity (Rudoni *et al.,* 1999; Okan *et al.,* 2002). Furthermore, the risk factors assessed in this study, showed that 1.4% each of those that received blood transfusion and those that had had surgery were seropositive for HCV although not statistically significant. These two risk factors are associated with HCV infection, where as family history of diabetes which is a known risk for acquisition of diabetes (Butt *et al.,* 2004) with 2.9% HCV seropositivity was not statistically significant. Therefore, the risk factor for presence of HCV infection in diabetic patients may not really be ascertained. This assumption is being supported by the report of Strassburg *et al.* (1999) who suggested that viral factors such as cytopathic damage due to the viral replication at these extrahepatic sites or virus associated autoimmunity may play a stronger role in diabetes rather than the well

known documented risk factors. Alcohol intake has differential influence in both HCV and diabetes. For instance, alcohol induced liver disease itself has a strong association with diabetes, perhaps because of altered carbohydrate metabolism, hyperglycemia and glucose intolerance or because of direct pancreatic toxicity (Bunout, 1999). In non-diabetic subjects with HCV, it has been suggested that higher levels of alcohol consumption contribute to the development of progressive liver disease (Peters and Terrault 2002; Corraco and Arico, 1998). In this study, both the diabetic patients who consume alcohol and those who do not consume alcohol share the HCV seropositivity. The effect of the alcohol may lead to metabolic disorder in the subjects affected because HCV replication will be enhanced by the alcohol metabolism. Diabetic patients are characterized by various forms of complications which may involve bad eye sight, hypertension, diabetic foot ulcer. Amongst these complications, hypertension was statistically significant. This indicates that the presence of HCV in these subjects may provoke and sustain the onset of hypertension as a complication in those diabetic subjects. In a similar discovery, Younossi *et al.* (2013) stated that there is an association between HCV and hypertension. They postulated that HCV patients are at increased risk for congestive heart failure. The diabetics with HCV seropositivity, may likely develop serious cardiac problem as a result of extra-hepatic complications of HCV infections. All the diabetic subjects in this study, never had previous liver disease, which is suggestive that either they are not aware of harbouring HCV which remained undiagnosed or they were infected while undergoing therapy for diabetes.

The infection due to HCV in the liver is monitored by the biochemical parameters such as liver enzyme transaminases while the lipid profile equally measures the

internal body fats in diabetic patients and they are also relevant in HCV. In this study, ALT, as a marker of liver damage is elevated in 2.2% of diabetic patients with HCV. In a Taiwanese study, age and serum Glutamic Pyruvic Transaminase (GPT) or ALT levels were regarded as predictive factors for the presence of HCV infection (Lin *et al.,* 1995). The implication of elevated ALT is increased liver cirrhosis and probably poor anti-diabetic drug metabolism. This may result to poor response to insulin therapy. Lipid abnormalities are commonly found in persons with *Diabetes mellitus* and this increase in lipids affects the replication of HCV (Agnello *et al.,* 1997). Thus, the lipid profile was assessed in the diabetics with HCV, and diabetics without HCV, 7 and 1 of the seropositive cases had elevated TG and LDL respectively. HDL and VLDL were normal in all the cases. The implication of this is that the diabetics with HCV infection may develop metabolic syndrome, this association has been suggested to be related both to the host and viral factors (Younosi *et al.,* 2009). Previous studies have shown that HCV affects glucose insulin homeostatis (Petrides, 1994) and as well as lipid synthesis. HCV core protein inhibits microsomal triglyceride transfer protein (MTTP) activity and thereby inhibits assembly of very-low density lipoprotein (VLDL) particles from apolipoprotein B (Apo B) and triglycerides (TG). This process results in intracellular storage of lipids and eventually leads to steatosis (Younossi, 2009). Therefore, the association between diabetes and HCV in relation to lipid may enhance liver damage because VLDL affects the replication of HCV in the liver. This increased replicative ability may influence the spread of the virus thereby inducing multiple heightened complications among the HCV infected diabetics. Another problem with this is the faster production of auto antibodies and probably non-response to HCV therapy.

5.2 Conclusion

Conclusively, it was observed in this study, that in this locality, that HCV increases the risk of hypertension in diabetic patients. Thus, suggesting that HCV/diabetic patients are at increased risk for cardiovascular disease and or congestive heart failure. In the Nigeria environment, with an estimated occurrence of 10-15% hypertension (Akinkugbe, 2000), there is a need to check the impact of HCV among the hypertensive patients. Moreso, screening for and prevention of diabetes in persons with HCV infection could be started earlier than the suggested age of $\geq$ 45 years (ADA, 2008) for the general population considering that the HCV seropositivity was prevalent in all the age groups used for the control.

References

ADA American Diabetes Association (2008). Diagnosis and classification of *Diabetes mellitus. Diabetes Care.* **31** S1,.S55-S60

Agnello, V., Abel, G. and Elfahel, M. (1999). Hepatitis C virus and other Flaviviridae Viruses enter Cells Via Low Density Lipoprotein. *Proc. National Academy of Science. USA* **96**: 12766-12771.

Ahren, B. and Corrigan, C.B. (1984). Intermittent need for insulin in a subgroup of diabetic patients in Tanzania. *Diabetes Medicine*. **2**:262-264.

Akinkugbe, O.O. (2000). Non Communicable Disease in Nigeria: the Next Epidemic Abayomi Memorial Lecture. *Niger Journal of Medical Practice.* **3**: 904-907.

Ali, S., Abora, S., Mihret, A. and Abebe, T. (2012). Association of Hepatitis C Virus Infection with type II diabetes in Ethiopia: A Hospitalï Based Case- Control Study. Interdisciplinary Perspectives on Infectious Diseases Volume.

Allison, M.E., Wreghitt, T., Palmer, C.R. and Alexander, G.J. (1994). Evidence for a link between hepatitis C virus infection and *Diabetes mellitus* in a cirrhotic population. *Journal of Hepatology* **21**(6): 1135 ï 1139.

Allison, M.E.D., Wreghitt, T. and Palmer, C.R. (1994). Evidence for a link between hepatitis C virus infection and *Diabetes melltius* in a cirrhotic population. *Journal of Hepatology.* **21**:1135-9.

Alter, H.J. and Seeff, L.B. (2000). Recovery, Persistence and sequelae in Hepatitis C Virus infection: a perspective on long-term outcome. *Seminar Liver Disease* **20**:17-35.

Alter, M.J. (1997). Epidemiology of hepatitis C. *Hepatology* **26**: 625-655.

Alter, M.J. (1999). Hepatitis C Virus infection in the United States. *Journal of Hepatology* **31**:88-91

Andreone, P., Gramenzi, A., Cursaro, C. (1996). Monoclonal gammopathy in patients with chronic hepatitis C virus infection. *Blood* **88**:1122.

Anonymous (1999). Global Surveillance and control of hepatitis C Report of a WHO Consultation organized in Collaboration with the viral Hepatitis prevention Board, antiwerp, Belgium. *Journal of Viral Hepaitis* **6**: 35-47.

Bacon, B.R, Farahvash, M.J, Janney, C.G. and Neuschwander-Tetri, B.A. (1994). Non alcoholic steatohepatitis: an expanded clinical entity. *Gastroenterology*. **107**:1103-1109.

Bernstein, D. (2008). Diagnosis and Management of Hepatitis C available at http://www.medscape.com/medscape/gastro/clinicalmgmt/cm.vol/public/index-cm.vol.html.

Betterle, C., Zanette, F., Pedini, B., Presotto, F., Rapp, L.B., Monsciotti, C.M. and Rigon, F. (1984). Clinical and subclinical organ-specific autoimmune manifestations in type I (insulin-dependent) diabetic patients and their first-degree relatives. *Diabetologia* **26**(6): 431-436.

Blattner, W., Gallo, R.C. and Temin, H.M. (1988). HIV causes AIDs. *Science* **241**: 515-516.

Bojuwoye, B.J. (1997). The burden of viral hepatitis in Africa. *West African Journal of Medicine*. **16**(4): 198-203.

Bukh, J., Miller, R. H. and Purcell, R. H. (1995). Genetic heterogenecity of hepatitis C Virus: quasispecies and genotypes. *Seminar Liver Disease* **15**:41-63.

Bunout, D. (1999). Nutrition and Metabolic Effects of Alcoholism: their Relationship with Alcoholic Liver Disease. *Nutrition* **15**: 583-589.

Butt, A.A., Fultz, S.L., Kwoh, C.K., Kelley, D., Skanderson, M. and Justice, A.C. (2004). Risk of Diabetes in HIV Infected Veterans Pre and Post HAART and the role of HCV Co-Infection. *Hepatology.* **40**: 115-119.

Cacoub, P., Poynard, T., Ghillani, P., Charlotte, F., Olivi, M., Piette, J.C. (1999). Extrahepatic Manifestations of Chronic Hepatitis C. *Arthritis Rheumatology.* **42** (10): 2204-2212.

Campell, P. J. and Carlson, M.G. (1993). Impact of obesity on insulin action in NIDDM. *Diabetes*. **42**:405-410.

Caronia, S., Tayl, R. and Pagliaro, L. (1999). Further evidence for an association between Non insulin dependent *Diabetes mellitus* and chronic hepatitis C virus infection. *Hepatology*. **30**: 1059-1064.

Chen, I.K., Hwang, S.J., Tsai, S.T., Luo, J.C., Lee, S.D. and Chang, F.Y. (2003). Glucose intolerance in Chinese patients with chronic hepatitis C. *World Journal of Gastroenterology* **9**(3): 505-508

Conry-contilena, C., Vanraden, M., Gibble, J., Melpolder, J., Shakil, A.O., Viladominu, L., Cheung, L., DiBisceglie, A., Hoofnagle, J., Shih, J.W., Kaslow, R., Ness, P. and Alter, H.J. (1996). Routes of infection, Viraemia and liver disease in blood donors found to have hepatitis C virus infection. *New England Journal of Medicine*. **334**(26): 1691-1696.

Corrao, G. and Arico, S. (1998). Independent and Combined Action of Hepatitis C. Virus Infection and Alcohol Consumption on the risk of Symptomatic Liver Cirrhosis. *Hepatology.* **27** (4): 914-919.

Cruzelfeldt, W., Frerichs, H. and Sickinger, K. (1970). Liver disease and *Diabetes mellitus*. In Popper, H. and Schaffner, F, (eds.). Progress in liver disease. Vol 3. New York: Grune and Stratton, pp. 371-407.

De Francesco, R. (1999). Molecular virology of the hepatitis C virus. *Journal of Hepatology* **31**:47-53.

De Medina, M. and Schiff, E.R. (1995). Hepatitis C diagnostic assays. *Seminar Liver Disease*. **15**: 33-40.

Defronze, R.A., Bonadonna, R.C. and Ferrannini, E. (1997). Pathogenesis of NIDDM: In: ALberti, K.G.M.M., Zimmet, P., Defronzo, R.A. eds. International Textbook of *Diabetes mellitus*. 2nd edition. Chichester; John Wiley, pp 635-712.

deï The, G., Geser, A., Day, N.E., Tukei, P.M., Williams, E.H., Beri, D.P., Smith, P.G., Dean, A.G., Bronkamm, G.W., Foorino, P. and Henle, W. (1978). Epidemiological evidence for causal relationship between Epstein-Barr virus and Burkittôs lymphoma from Ugandan prospective study. *Nature* **274** (5673): 756-761.

Di Bisceglie, A.M. (1997). Hepatitis C and hepatocellular carcinoma. *Hepatology* **26**:345-385.

El-hawary, E. I., Mahmoud, G.F., El-Daly, M.A., Mekky,M. F.A., Esmat, G.G. and Abdel-Hamid, M. (2011). Association of HCV with *Diabetes mellitus*: an Egyptian Case Control Study. *Virology Journal.* **8**: 367.

El-Zayadi, A., Selim, O. and Daddous, H (1994). Prevalence of *Diabetes mellitus* among HCV positive chronic liver disease Egyptian patients and its control with alpha interferon in some cases. *Hepatology*. **19**:43.

Enooto, N. and Sato, C. (1995). Hepatitis C virus quasispecies populations during chronic hepatitis C infection. *Trends in Microbiology* **3**:445-447.

Expert Committee on the Diagnosis and classification of *Diabetes mellitus*. Report of the expert committee on the diagnosis and classification of *Diabetes mellitus*. *Diabetes Care* 1997:**20**:1183-1197.

Farbis, P., Betterle, C. and Floreani, A. (1992). Development of type I *Diabetes mellitus* during interferon alfa therapy for chronic HCV hepatitis. *Lancet* **340**:548.

Farci, P., Alter, H.J., Govindarajan, S., Wong, D.C., Engle, R., Lesniewski, R.R., Mushahwar, I.K., Desai, S.M., Mille, R.H., Ogata, N. against re-infection with Hepatitis C virus. *Science* **258**:135-140 258(5079): 135-140.

Fattovich, G., Giustina, O., Favarato, S. and Roul, A. (1997). Investigators of the Italian Association for the Study of the Liver. A survey of adverse events in 241 patients with chronic viral hepatitis treated with alfa interferon. *Journal of Hepatology. Viral Hepatology*. **4**:9-28.

Feld, J.J. and Hoofnagle, J.H. (2005). Mechanism of action of Interferon and Ribavirin in treatment of Hepatitis C. *Nature* **436**: 967-972.

Frang, J.W.S., Lau, G.K.K., Marousus Gonzales Peralta, R.P., Davis, G.L., Gish, R., Moyer, R.W., Kao, K.J. and Lesniewki, R. Failure to detect cell surface expression of hepatitis C structural proteins using a vaccinia expression system.

Fraser, G.M., Harman, I., Meller, N., Niv, Y. and Porath, A. (1996). *Diabetes mellitus* is associated with chronic hepatitis C but not chronic hepatitis B infection. *Israel Journal of Medical Science* **32** (7): 526-530.

Gale, M., Jr and Beard, M.R. (2001). Molecular clones of hepatitis C virus: applications to animal models. *ilar Journal* **42**: 139-157.

Genovese, D., Dettori, S., Argentini, C., Villano, U., Chionne, P., Angelico, M. and Rapicetta, M. (2005). Molecular epidemiology of Hepatitis C Virus genotype 4 isolates in Egypt and analysis of the variability of envelope proteins E_1 and E_2 in patients with chronic hepatitis. *Journal of Clinical Microbiology*. **43**(4): 1902-1909

Gray, H., Wreghitt, T. and Stratton, I.M, (1995).High prevalence of hepatitis C infection in Afro-Carribbean patients with type 2 diabetes and normal liver function tests. *Diabetes Medicine* **12**:244-249.

Hadziyannis, S.J. (1997). The spectrum of extrahepatic manifestations in hepatitis C virus infection. *Journal of Viral Hepatology*. **4**:9-28.

Hadzyiyannis, S. and Karamanos, B. (1999). *Diabetes mellitus* and chronic hepatitis C virus infection. (editorial). *Hepatology*. **29**:604-605.

Harris, M.I. (1993). Undiagnosed NIDDM; Clinical and public health issues. *Diabetes Care.* **16**: 642-652.

Hoofnagle, J.H. (1997). Hepatitis C: The Clinical Spectrum of diseases. *Hepatology*. **26**:155-205.

Hother-Nielsen, O., Faber, O., Sorensen, N.S. and Beck-Nielsen, H. (1988). Classification of newly diagnosed diabetic patients as insulin-requiring or non-insulin-requiring based on clinic and biochemical variables. *Diabetes care.* **11**:531-537.

Hu, K.Q. and Tong, M.J. (1999). The long-term outcomes of patients with compensated hepatitis C virus-related cirrhosis and history of parenteral exposure in the United States. *Hepatology* **29**:1311-1316.

Humphrey, A.R., McCarty, D.J., Mackay, I.R., Rowley, M.J., Dwyer, T. and Zimmet, P. (1998). Autoantibodies to glutamic acid decarboxylase and phenotypic features associated with early insulin treatment in individuals with adult-onset *Diabetes mellitus. Diabetic Medicine*. **15**(2):113-119.

Japan and Pittsburgh childhood Diabetes Research Groups, (1985). Coma at onset of young insulin-dependent diabetes in Japan: the result of a nationwide survey. *Diabetes* **34**(12): 1241-1246.

Kakepota, G.N., Bhally, H.S., Khaliq, G. (1996). Epidemiology of blood borne viruses, A study of healthy blood donors in southern Pakistan Southeast. *Asian Journal of Tropical Medicine Public Hygiene.* **27**:703-706.

Katamna, B.H., Petrelli, M., and Mc Cullough, A.J. (1997). The liver in *Diabetes mellitus* and hyperlipidemia. In Gitlin N,ed. *The Liver and systemic disease*. New York, Churchill Livingstone, pp.73-113.

Kissebah, A.H., Vydelingum, N., Murray, R., Evans, D.J., Hartz, A. J., Kalkhoff, R. K. and Adams, P.W. (1982). Relationship of body fat distribution to metabolic complications of obesity. *Journal of Clinical Endocrinology Metabolism*. **54**:254-260.

Kiyosawa, K., Sodeyama, T., Tanaka, E., Nakano, Y., Furuta, S. and Nishioka, K. (1991). Hepatitis C in hospital employee with needle sticks injuries. *Annals of Internal Medicine.* **115**: 367-369.

Kruszynska, Y.T. and McIntyre, N. (1991). Carbohydrate metabolism. In McIntyre, N., Benhamou, J.P., Bircher, J., Rizzetto, M. and Rodes, J. (eds.). Oxford Text book of clinical Hepatology Oxford: University Press, pp.129-143.

Lau, J. Y. and Nelson, D.R. (1997). Pathogenesis of hepatocellular damage in chronic hepatitis C virus infection. *Clinics in Liver Disease*. **1**(3): 515-528.

Leigh, J.P., Bowlus, C. L., Leistikow, B.N. and Schenker, M. (2001). Cost of hepatitis C *Archives of Internal Medicine* **161**:2231-2237.

Lin, S.C., Shih, S.C., Kao, C.R.and Chou, S.Y. (1995). Prevalence of Antibodies to Hepatitis C Virus in Patients with non-alcoholic fatty liver. *Zhonghua Yi Xue Za Zhi.* (Taipei) **56**(2): 80-85.

Luby, S.P., Qamaruddin, K.and Shah, A.A. (1997). The relationship between therapeutic infection and high prevalence of hepatitis C infection in Hafizabad, Pakistan. *Epidemiology of Infection.* **119**:349-356.

Major, M.E. and Finstone, S.M. (1997). The molecular virology of hepatitis C *Hepatology* **25**: 1527-1538.

Manns, M.P. and Rambusch, E.G. (1999). Autoimmunity and extrahepatic manifestations in hepatitis C virus infection. *Journal of Hepatology* **31**(1): 39-42.

Mason, A. (2001). Viral induction of type 2 diabetes and auto immune liver disease. *Journal of Nutrition* **131**(10):2805S-2808S

Mason, A.L., Lau, J.Y.and Hoang, N. (1999). Association of *Diabetes Mellitus* and Chronic Hepatitis C virus infection. *Hepatology*, **29**:328-333.

Mason, A.L., Lau, J.Y., Hoang, N., Qian, K., Alexander, G. J., Xu, L., Guo, L., Jacob, S., Regenstein, F.G., Zimmerman, R., Everhart, J.E., Wasserfall, C., Maclaren, N.K. and Perrillo, R.P. (1999). Association of *Diabetes mellitus* and chronic hepatitis C virus infection. *Hepatology* **29** (2): 328-333.

Mclarty, D.G., Athaide, I., Bottazzo, G.F., Swai, A.B.M and Alberti, K.G.M.M. (1990). Islet cell antibodies are not specifically associated with insulin-dependent diabetes in rural Tanzanian Africans. *Diabetes Research Clinical Practice*. **9**(3):219-224.

Molbak, A.G., Christay, B., Marner, B., Borch-Johnsen, K. and Nerup, J. (1994). Incidence of insulin-dependent *Diabetes mellitus* in age groups over 30 years in Denmark. *Diabetes Medicine*. **11**:650-655.

National Institute of Health: NIH Consensus Development Conference on Management of Hepatitis C. *consensus.nih.gov/2002/2002hepatitis2002116html.htm*

Ndako, J.A.,Echeonwu, G.O.,Shidali, N.N.,Bichi,I. A.,Paul,G.A.,Onovoh, E. and Okeke, L.A.(2009).Occurrence of Hepatitis C Virus infection in type 2 diabetic patients attending Plateau state specialist hospital Jos, Nigeria.*Virology Journal*.**6**:98.

Ohto, H., Terazawa, S., Sasaki, N., Hino, K., Ishiwata, C., Kako, M., Ujiie, N., Endo, C., Matsui, A., Okamoto, H. and Mishiro, S. (1994). Transmission of hepatitis

C virus from mothers to infants. *New England Journal of Medicine*. **330**(11): 744-750.

Okan, V., Araz, M., Aktaran, S., Karsligil, T., Meram, I., Bayraktaroglu, Z.and Demirci, F. (2002). Increased Frequency of HCV but not HBV Infection in type 2 Diabetic Patients in Turkey. *International Journal of Clinical Practice.* **56**: 175-177.

Omayra De Jesus, Phylogenetic analysis of hepatitis C virus infection in Nigeria, http://aids.psm.edu/publications/posters/poster2.pdf.

Pakistan Medical Research Council National Health Survey of Pakistan 1990-94. Islamabad, Pakistan Medical Research Council, 1998, pp. 56-77.

Pearlman, B.L. (2006). Hepatitis C Virus infection in African Americans. *Clinical infectious Disease* **42** (1): 82-91 pubmed.

Peters, M.G. and Terrault, N.A., (2002). Alcohol use and Hepatitis C. *Hepatology.* **36**: S220-255.

Petrides, A.S., Vogt, C. and Schulzeï Berge, D. (1994). Pathogenesis of Glucose Intolerance and *Diabetes Mellitus* in Cirrhosis. *Hepatology* **19**: 616-627.

Pileri, P., Matsu, U.E.Y., Campagnoli, S., Galli, G., Falugi, F. and Petracca, R., (1998). Binding of hepatitis C virus to CD 8. *Science* **282**: 938-41

Qureshi, H., Ahsan, T.and Mujeeb, S.A. (2002). Diabetes is equally frequent in chronic HCV and HBV infection. *Journal of Pakistan Medical Association*. **52**: 280-283.

Ratziu, V., Trabut, J.B., Poynard, T. (2004). Fat, Diabetes and Liver Injury in Chronic Hepatitis. *Curriculum Gastroenterology Rep*. **6**: 22-29.

Rudoni, S, Petit, J.M., Bour, J.B., Aho, L.S., Castaneda, A., Vallant, G., Verges , B. and Brun, J.M. (1999). HCV Infection and *Diabetes mellitus*: Influence of the use of Finger Stick Devices on Nosocomial Transmission. *Diabetes Metabolism.* **25**; 502-505.

Ryu, J.k., Lee, S.B., Hong, S.J. and Lee, S. (2001). Association of chronic hepatitis C. virus infection and *Diabetes mellitus* in Korean patients. *Korean Journal of Internal Medicine* **16**(1): 18-23.

Sene, D. Limal, N. and Cacoub, P. (2004). Hepatitis C virus associated extrahepatic manifestations. A review. *Metabolic Brain Disease* **19** (3-4): 357 ï 381

Shlimovich, P.B., Zus, B.A., Evdokimov, A.R. (1977). Clinical picture and pathogenesis of *Diabetes mellitus* in chronic Hepatitis and Cirrhosis of the liver. *Problems of Endocrinology* (Mosk) **23**(4): 7-14.

Simmonds, P. (1995). Variability of hepatitis C virus. *Hepatology* **21**:570-583.

Simo, R., Hernandez, C., Genesca, J., Jardi, R. and Mesa, J. (1996). High prevalence of hepatitis C virus infection in diabetic patients. *Diabetes Care* **19**(9): 998-1000.

Strassburg, C.P., Obermayer-Straub, P., Manns, M.P. (1996). Autoimmunity in hepatitis C and D virus infection. *Journal of Viral Infection*. **3**:49-59.

Svensson, M., Eriksson, J.W. and Dahlquist, G. (2004). Early glycemic control, age at onset, and development of microvascular complications in childhood-onset type I diabetes: a population based study in northern Sweden. *Diabetes care*. **27**:955-962.

Szmuness, W. (1978). Hepatocellular Carcinoma and the hepatitis B virus: evidence for a causal association. *Progress in Medical Virology* **24**:40-69.

Three dimensional model of HCV created by Lous E, Henderson, PhD, Federick cancer research center.

Vallari, D.S., Jett, B. W., Alter, H. J., Mimms, L.T., Holzman, R. and Shih, J.W. (1992). Serology markers of post transfusion hepatitis C. viral infection. *Journal Clinical Microbiology*. **30**:552-556.

Valle, T., Tuomilehto, J. and Eriksson, J. (1997). Epidemiology of NIDDM in Europids. In Alberti, K.G.M.M, Zimmet, P., Defronzo, R.A. eds: *International Textbook of Diabetes mellitus* 2nd Edition. Chichester: John Wiley. pp 125-142.

Verge, C.F., Gianani, R., Kawasaki, E., Yu, L., Pietropaolo, M., Chase, H.P., Eisenbarth, G.S. and Jackson, R.A. (1996). Predicting type I diabetes in first-degree relatives using a combination of insulin, GAD, and ICA 512bdc/1A-2 autoantibodies. *Diabetes*. **45**:926-933.

Walboomers, J.M., Jacobs, M.V., Manos, M.M, Bosch, F.X., Kummer, J.A. Shah, K.V., Snijders, P.J., Peto, J., Meijer, C. J. and MuŔoz, N. (1999). Human papillomavirus is a necessary cause of invasive cervical cancer world wide. *Journal of Pathology* **189**(1): 12-19

WHO, (1999). Definition, Diagnosis and Classification of *Diabetes mellitus* and its complication. Report of a WHO consultation. WHO/NCD/NCS/99.2

Willis, J.A., Scott, R.S., Brown, L.J., Forbes, L.V., Schmidli, R.S., Zimmet, P.Z., Mackay, I.R. and Rowley, M.J. (1996). Islet cell antibodies and antibodies against glutamic acid decarboxylase in newly diagnosed adult ï onset *Diabetes mellitus*. *Diabetes Research Clinical Practice.* **33**(2):89-97.

www.who/int (fact sheet No. 164, revised October 2000)

www.who/int (press release WHO/36; 1st May 1998)

Younossi, Z. M.and Stepanova, M., Nader, F., Younossi, Z. and Elsheikh, E. (2013). Associations of Chronic Hepatitis C with Metabolic and Cardiac Outcomes. *Alimentary Pharmacology and Therapeutics.* **37**: 647-652.

Younossi, Z.M., McCullough, A.J. (2009). Metabolic Syndrome, Non-Alcoholic Fatty Liver Disease and Hepatitis C Virus: Impact on disease Progression and Treatment. *Liver International.* **29** (2): 3-12.

Zignego, A.l., Ferr, C.and Giannini, C. (1996). Hepatitis C virus genotype analysis in patients with type II mixed cryoglobulinemia. *Annalsof Internal Medicine* **124**:31-4.

Zimmet, P., Tuomi, T., Mackay, R. I., Rowley, M.J., Knowles, W., Cohen, M. and Lang, D.A. (1994). Latent autoimmune *Diabetes mellitus* in adults (LADA): the role of antibodies to glutamic acid decarboxylase in diagnosis and prediction of insulin dependency. *Diabetic Med.* **11**(3): 299-303.

Zimmet, P.Z. (1992). Kelly West Lecture 1991: Challenges in diabetes epidemiology: from west to the rest. *Diabetes Care*. **15**:232-252.

Zimmet, P.Z. (1995). The pathogenesis and prevention of diabetes in adults. *Diabetes Care* **18**(7): 1050-1064.

Zuberi, S.J., Samad, F.and Lodi, T.Z. (1977). Hepatitis B surface antigen in health care personnel. *Journal of Pakistan Medical Association*. **27**:373.