EVALUATION OF LIVER FUNCTION AMONG SUBSTANCE ABUSERS OF GENERAL PUNJABI POPULATION.

Internship Report

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Master of Science in Clinical Biochemistry

Submitted by:

HARUNA SHAMSUDDEEN

(Reg No. 11412766)

SCHOOL OF PHYSIOTHERAPY AND PARAMEDICAL SCIENCE

LOVELY PROFESSIONAL UNIVERSITY, PUNJAB, INDIA

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This is to certify that the present thesis entitled **"Evaluation of liver function among substance abusers in general Punjabi population"** is the outcome of the bonafide work carried out by **Mr. Shamsuddeen Haruna (Registration No: 11412766)** himself under my guidance and the contents of his thesis did not form a basis of the award of any previous degree to him and to the best of my knowledge to anybody else also. The thesis has not been submitted by the candidate for any research degree in any other University.

The dissertation is fit for submission to the partial fulfillment of the conditions for the award of M.Sc. in Clinical Biochemistry. Further certified that the candidate bears a good moral character and nothing adverse has been found against him.

13 May 2016

Internal Guide

Mrs. Anshula Sharma
Lecturer
Department of Paramedical Sciences
Lovely Professional University
Jalandhar

External Guide

Dr. Bhavana Ghosh
MBBS, MD Biochemistry
Associate Professor,
Punjab Institute of Medical Science,
Jalandhar

Dr Ravjit Kaur Sabharwal
Professor and head
Department of Biochemistry
Punjab Institute of Medical Sciences
Jalandhar

DECLARATION

I hereby declare that the work embodied in this Internship report was carried by me under the supervision of Miss. Anshula Sharma (Internal supervisor), Lovely Professional University and Dr Bhavana Ghosh (External supervisor), Punjab institute of medical science. This work has not been submitted in part or in full to any other university for award of any degree or diploma.

Shamsuddeen Haruna
(Registration No: 11412766)
Date :
Place :

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ABBREVIATIONS

ALP: -ALKALINE PHOSPHATASE
ALT: -ALANINE AMINOTRANSFERASE
AST: -ASPARTATE AMINOTRANSFERASE
CLD: -CHRONIC LIVER DISEASE
GGT: -GAMMA GLUTAMYL TRANSFERASE
HA: -HEROIN ADDICTS
HBsAg: -HEPATITIS B SURFACE ANTIGEN
HCV: -HEPATITIS C VIRUS
HIV: -HUMAN IMMUNODEFICIENCY VIRUS
ICD: INTERNATIONAL CLASSIFICATION OF DISEASE
IFCC: INTERNATIONAL FEDERATION OF CLINICAL CHEMISTRY
LFT: -LIVER FUNCTION TEST
LDH: -LACTATE DEHYDROGENASE
OD: -OPTICAL DENSITY
W.H.O: WORLD HEALTH ORGANISATION

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ABSTRACT

Detection of liver disease among substance abusers is an important challenge faced by psychiatrists in the de-addiction centers. In many cases, the disease is unrecognized and treatable in the early stage. In this study the liver function test (Aspartate amino transferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Gamma glutamyl transferase (GGT), Total Protein, Albumin, Total Bilirubin and direct Bilirubin) of substance abusers seeking treatment at de-addiction unit of psychiatric Department of P.I.M.S Jalandhar were carried out. It was then compared with age matched normal control subjects. The result of the study showed an increase in mean value of liver enzymes in substance abusers with AST 45.09 ± 39.69 IU/L, ALT 70.83 ± 78.30 IU/L, ALP 99.43 ± 58.42 IU/L, and GGT 89.27 ± 158.62 IU/L while that of control subjects were AST 21.35 ± 6.59 IU/L, ALT 24.19 ± 10.04I U/L, ALP 69.28 ± 18.34 IU/L and GGT 27.54 ± 7.62 IU/L. An independent sample t-Test was used for the comparison which shows a significant difference between the two groups in liver enzymes.

Serum total protein and albumin were lower in substance abusers. Mean serum total protein was 6. 98 ± 0.69g/dl, albumin was 4.22 ± 0.42g/dl in substance abusers. In the control group, the mean total protein was 7.58 ± 0.62g/dl and albumin was 4.46 ± 0.46g/dl. There was no significant difference in the mean value of total and conjugated bilirubin between the two groups. The prevalence of hepatitis C was 25%, hepatitis B 11% and HIV 1 % among substance abusers.

KEY WORDS: liver function test, AST, ALT, ALP, GGT, Total protein, Albumin, Bilirubin, HCV, HBsAg, HIV, Substance abuse.

INTRODUCTION

1.0 INTRODUCTION

The liver is the largest organ in the body and has a wide variety of functions. It occupies the upper part of the abdominal cavity. (1) Its main function is to filter blood passing through it to the rest of the body. The liver detoxifies chemicals such as organophosphates, arsenide, and nicotine and also metabolizes drugs (therapeutic as well as illicit drugs). The liver secretes bile that ends up in the intestine. It also makes proteins important for transport, clotting, and other important functions. (2)

The function of the liver in health and the diseased state can be assessed in the laboratory by measuring various analytes related to the liver. Tests used to measure liver function include:

I. Enzyme assay (AST, ALT, ALP, and GGT)

II. Total protein and albumin estimation

III Total and direct bilirubin measurement. (3)

1.0.1 LIVER ENZYMES

1.1.1. TRANSAMINASES

Transaminases constitute a group of enzymes that catalyze the interconversion of amino acid to 2-oxo-acids by transfer of amino groups. Aspartate aminotransferase/SGOT (E.C.2.6.1.1) and Alanine aminotransferase SGPT (E.C.2.6.1.2) are the example of transaminases that are of clinical interest. The 2-oxoglutarate/L-glutamate couple serves as one amino group acceptor and donor pair in all amino-transfer reactions. The specificity of individual enzymes derives from the particular amino acid that serves as the donor of the amino group. (4)

Liver disease is the most important cause of increased transaminase activity in serum. (3) These enzymes reside within the cells of the liver (hepatocytes). But when the liver is injured due to any reason, these transaminases are spilled into the bloodstream. (4)

All liver diseases with marked hepatocytes necrosis demonstrate a highly reduced activity of enzymes, specifically hydroxylases, as a result of which inactivation of certain drugs (opiates, sedatives, hypnotics etc.) are difficult and their action on CNS is prolonged. (5)

In most types of liver diseases, ALT activity is higher than that of AST but exception may be seen in alcoholic hepatitis, hepatic cirrhosis, and liver neoplasia. In viral hepatitis, and other forms of liver disease associated with acute hepatic necrosis, serum AST, and ALT concentrations are elevated even before the clinical signs and symptoms of disease appear. (3) Activities of both enzymes may reach values as high as 100 times the upper reference limit.

Persistence of increased ALT for more than 6 months after an episode of acute hepatitis is used to diagnose chronic hepatitis. In acetaminophen-induced hepatic injury the transaminase peak is more than 85 times the upper limit in 90% of cases. (5)

Intravenous use of drugs leads to infection of the liver such as hepatitis B and C viral infection. Hepatocytes are the main site of biotransformation, which through the action of enzymes, enables the removal of the metabolites of drugs and xenobiotics. During these processes, ultrastructural hepatocyte changes and toxic liver damage occur. The morphological changes in the liver tissue are associated with its functional disturbances, which results in the altered metabolism of heroin and other toxins if taken simultaneously (alcohol, drugs) and if these substances are abused it leads to effects that are often surprising. (6)

1.1.2 GAMMA-GLUTAMYL TRANSFERASE

Gamma-glutamyl transferase (E.C.2.3.2.2,) catalyzes the transfer of the gamma-glutamyl group from peptides and compounds that contain it to the same accepters. (7) The gamma-glutamyl accepter is the substrate itself, some amino acid or peptides or even water in which case simple hydrolysis takes place. (4)

Even though renal tissue has the highest concentration of GGT, the enzyme present in serum appears to originate primarily from the hepatobiliary system. GGT is a sensitive indicator of the presence of hepatobiliary disease being elevated in most subjects regardless of the cause. (4) Elevated activities of GGT are found in the sera of patients with alcoholic hepatitis and in the majority of sera from people who are heavy drinkers (8).

Gamma-glutamyl-p-nitroanaline+ glycylglycine → p-nitroaniline +ɤ-Glutamylglycylglycine

1.3 ALKALINE PHOSPHATASE (ALP)

ALP (E.C.3.1.3.1) (orthophosphoric monoester phosphohydrolase) catalyzes the alkaline hydrolysis of a large variety of naturally occurring and synthetic substrates. ALP activity is present in the most organs of the body and is especially associated with membrane and cell surfaces of the small intestine, proximal convoluted tubules of the kidney, in the bone, liver, and placenta. Elevations in serum ALP activity commonly originate from one or both of the sources: the liver and bone. The response of the liver to any form of biliary tree obstruction induces the synthesis of ALP by hepatocytes. Some of the newly formed enzymes enter the circulation to increase the enzyme activity in serum. (9) The elevation tends to be more notable in extra hepatic obstruction than in the intrahepatic obstruction. Liver diseases such as infectious hepatitis that principally affect parenchymal cells typically show only moderate increase or even normal serum ALP activity. (10)

1.2.1 BILIRUBIN

Bilirubin is the orange-yellow pigment derived from red blood cell turnover. It is extracted and bio transformed in the liver and excreted in the bile and urine. Bilirubin is transported from the site of production (mainly spleen), usually bound to albumin, in its native unconjugated form. Measurement of serum bilirubin is helpful in the diagnosis and assessment of severity of liver disease. (8)

1.2.2 PROTEIN

The liver is the primary site of the synthesis of plasma proteins. Synthesis occurs in the rough endoplasmic reticulum of the hepatocytes followed by release into hepatic sinusoids. Although disturbances in protein synthesis occur as a consequence of impaired hepatic function, a variety of other factors also affects plasma protein concentration. These factors include decreased amino acid levels (malnutrition, catabolic state, protein-losing nephropathy, the action of cytokines, actions of hormones, congenital deficiency etc. (8)

1.2.3 ALBUMIM

Albumin is the most commonly measured serum protein and it is synthesized exclusively in the liver. Plasma albumin measurements are useful in assessing the chronicity and severity of liver disease. For example, the plasma albumin concentration is decreased in chronic liver disease. (11)

1.3.0 SUBSTANCE ABUSE

According to W.H.O, substance abuse is a harmful or dangerous use of psychoactive substances, including alcohol and illicit drugs. (12) Substance abuse and drug addiction are two terms that are used interchangeably in many literatures, but the American Psychiatric Association use the term "substance abuse" rather than drug addiction. Drug addiction is considered to be a chronic relapsing disorder characterized by compulsive drug seeking, continued use, despite serious negative socioeconomic and health consequence and by a loss of control over the drug use. (13)

Repeated drug use arises from the drug's neurochemical actions that produce positive reinforcing effects, progressively leading to neurobiological changes in the brain reward circuits and behaviors characteristic of addiction: tolerance, sensitization, dependence, withdrawal and craving. (14)

1.4.0 EPIDEMIOLOGY OF SUBSTANCE ABUSE

Throughout the world, substance abuse is increasing on daily basis. The harmful use of alcohol results in 3.3 million deaths each year. On an average every person in the world aged 15 years or older drinks 6.2 liters of pure alcohol per year. Less than half the population (38.3%) actually drinks alcohol, this means that those who do drink consume on average 17 liters of pure alcohol annually. At least 15.3 million persons have drug abuse disorders. Injecting drug abuse reported in 148 countries, of which 120 reported HIV infection among this population.

Recent estimates are that in 2008, 155 to 250 million people, or 3.5% to 5.7% of the world's population aged 15-64 years, used other psychoactive substances, such as cannabis, amphetamines, cocaine, opioids, and non-prescribed psychoactive prescription medication. Globally, cannabis is the most commonly used drug (129-190 million people), followed by amphetamine-type stimulants, cocaine and opioids. (15) The use of psychoactive substances

causes significant health and social problems for the people who use them and also for others in their families and communities. WHO estimated that 0.7% of the global burden of disease in 2004 was due to cocaine and opioid use and in those countries which have measured the social cost of illicit substance abuse is 2% of the region's GDP. Alcohol abuse disorder caused 107,261 deaths while those for drug abuse accounted for 93,000 deaths globally in the year 2012. (12)

1.5.0 HEROIN

Heroin (diacetylmorphine) is a highly addictive Schedule I drug and a highly abused and extremely potent opiate. It is processed from morphine; a naturally-occurring substance extracted from the opium poppy - *Papaver somniferum* - a plant indigenous to the Middle East and Southeast Asia. Pure heroin (Chitta in Punjabi), which is a bitter-tasting white powder, is rarely sold on the streets. (16)

1.5.1 METHOD OF USE

Heroin is most often injected intravenously for a quick and potent 'high' but there is a rising segment of abusers who sniff, snort and smoke heroin to avoid the dangers of sharing needles. (17)

1.5.2 COCAINE

Cocaine, also known as benzoylmethylecgonine or coke, is a strong stimulant mostly used as a recreational drug. It is commonly snorted, inhaled, or injected into the veins. Mental effects may include losing of contact with reality, an intense feeling of happiness, or agitation. Physical symptoms may include a fast heart rate, sweating, and dilated pupils. (18)

1.5.3 ALCOHOL

An alcoholic beverage is a drink which contains a substantial amount of the psychoactive drug ethanol (informally called alcohol), a depressant which in low doses causes euphoria, reduced anxiety and sociability and in higher doses causes intoxication (drunkenness), stupor and unconsciousness. Long-term use can lead to alcohol abuse, physical dependence and Alcoholism. (19)

1.5.4 NICOTINE

Nicotine is a potent parasympathomimetic alkaloid found in the nightshade family of plants (Solanaceae) and is a stimulant drug. It is made in the roots and accumulates in the leaves of the nightshade family of plants. Nicotine is found in the leaves of *Nicotianarustica* in amounts

of 2–14% (the tobacco plant Nicotianatabacum) (20). It constitutes approximately 0.6–3.0% of the dry weight of tobacco (21) and is present in the range of 2–7 μg/kg of various edible plants. (22)

1.5.5 CANNABIS

Cannabis, also known as marijuana and by numerous other names, is a preparation of the Cannabis plant intended for use as a psychoactive drug or medicine. (20) The main psychoactive part of cannabis is tetrahydrocannabinol (THC); one of the 483 known compounds in the plant, others include at least 84 other cannabinoids, such as cannabidiol (CBD), cannabinol (CBN), and tetrahydrocannabivarin (THCV). Cannabis is often consumed for its mental and physical effects, such as heightened mood, relaxation (23) and an increase in appetite.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

In a study by Rosenblate and colleagues on 50 asymptomatic drug addicts admitted for detoxification 62% increase in serum liver transaminases was reported. Liver biopsies of 45 patients showed a consistent pattern of lymphocytic infiltration in the portal areas and in small foci scattered throughout the lobule. Hyperplasia of the smooth endoplasmic reticulum was also seen in the liver biopsy of addicts. Hence suggesting that these alterations in ultrastructure of hepatocytes could be due to drug or their vehicles (24)

Liver function test, biochemical and immunological parameters were measured in 472 drug addicts seeking treatment from 1994 to 2006 by Roberto and colleagues. They found that drug abuser along with hepatitis B and or hepatitis C viral infections are important risk factors for development of liver fibrosis. (25)

389 intravenous heroin addicts admitted to outpatient treatment clinics throughout California were tested for hepatitis viral infection and liver enzymes, serum proteins and platelet count by Forest and David in 1995.The prevalence of hepatitis A virus was 40.7%, hepatitis B 73.8%, and hepatitis C virus was at 93.6% Those with hepatitis C infections showed greater derangement in liver enzymes and had lower platelet counts. (26)

A study on liver enzymes of heroin addicts was conducted at Ram Ganga hospital and Punjab Institute of mental hospital Pakistan by Farooq, Mubeen and M ltaf 2015, they found 52% of heroin addicts had a high value of liver enzymes. This increase in liver enzymes was highly correlated with duration of abuse and presence of hepatitis B and C viral infection. The study indicated a direct relationship between heroin addiction and liver damage (6).

Empirical “liver function tests” (LFT) were carried out on an unselected group of 89 heroin and cocaine users attending psychiatric out-patient department, or who were admitted as psychiatric hospital in-patients. Some degree of LFT abnormality was seen in 80 percent of the cases. Six of the patients were jaundiced. In them, laboratory findings were compatible with, but not typical of, infective hepatitis. In addition to raised serum bilirubin levels, ALT/SGPT and AST/SGOT levels were very high, but both alkaline phosphatase and serum protein electrophoresis were either normal or minimally abnormal. (27)

In the non-icteric group, serum transaminases were often high or very high in the absence of other evidence of hepatic dysfunction. Serial determinations showed that in some cases, the cessation of intravenous heroin and cocaine injections led to a rapid restoration of liver function to normal. Conversely, resumption of the habit was associated with large and rapid rise in serum transaminase levels. Evidence of liver damage was rare in patients whose abuse was confined to the use of amphetamines, barbiturates or cannabis. It was suggested that while some cases of jaundice in narcotic addicts were viral in origin, transient liver damage was more often the result of either a direct hepatotoxic effect of the large doses of heroin and cocaine used, pyrogens or other contaminants injected unwittingly, or to sub-clinical bacteremia due to the use of septic materials. (27)

Liver function tests performed in 31 Macaca mulatta monkeys addicted to parenteral morphine and compared with 25 nonaddicts in the same colony, do not support the concept of a marked hepatotoxic effect of morphine addiction. Data derived from the control animals may serve as standards of reference for future studies utilizing this species. (28)

In a study conducted by Kenneth Kaplan, thirteen narcotic addicts who were imprisoned for a minimum of 6 months and volunteered for liver function test and liver biopsy. Seven of the thirteen addicts had abnormal liver tissue and ten had abnormal liver function. There is insufficient evidence to ascribe the liver damage to any combination of drugs. While the common occurrence of chronic liver disease in narcotics addicts is confirmed by this study, the etiologic factors are not apparent. (29)

In 2015, 50 male tramadol abusers were studied in a psychiatric clinic in Gaza stripe by Abdelrouf and coworkers. An increased serum AST, ALT, ALP, and LDH was seen in tramadol abusers when compared with non-abusers. Also, direct and total bilirubin, BUN, uric acid and creatinine levels significantly increased in the abusers that were addicted to tramadol. (30)

Liver function tests were performed in500 young servicemen with a history of drug abuse. AST levels were abnormal among 66% of 68 patients with a history of parenteral drug abuse. Forty-one percent of 432 patients with a history of only non-parenteral drug abuse also had elevated AST levels. A high incidence of liver disease in parenteral drug abusers is well established. (31)

Illic et al reported degenerative changes and fatty changes, chronic hepatitis, cirrhosis, dysplastic changes, reduction in the amount of glycogen in hepatocytes, as well as the changes in

the number of kupffer and endothelial cells in the liver autopsy in a group of intravenous heroin addicts. (32)

In another study one hundred and sixteen (116) heroin abusers attending London hospital were studied, 53% of the subjects had raised AST. Also, in the same study, abnormal liver function test was seen more in those with hepatitis B viral infection. (33)

Barry et al reported hepatic dysfunction in 46 heroin users who were admitted to a Methadone Maintenance program over a period of one year with the use of conventional liver function tests. On admission, 21 patients (44%) had significant titers of Hepatitis –Associated Antigen, 14 of the remaining 25% patients with negative hepatitis associated antigen had persistently abnormal result for liver function test. (34)

Lubormirova et al (2011) in a research on subjects with chronic Hepatitis C viral infection and intravenous heroin misuse reported biochemical changes as well as histological changes in a group of patients that were infected with hepatitis C and heroin abusers. The study showed a marked abnormality in liver enzymes i.e. AST ALT, ALP and GGT and total bilirubin. But the role of heroin as in independent damaging factor could not be elicited. (35)

In a study by spencer and collogues, liver function tests of 612 addicts were done before the commencement of methadone therapy. The patients were divided into two categories i.e. alcoholic narcotic addicts and non-alcoholic narcotic addicts. Mean values of (AST, ALT, ALP, and GGT) in the alcoholic cohort was significantly increased compared to those among non-alcoholic ($p<0.01$ to $p<0.001$ for individual test). The mean value of LFT does not change during methadone maintenance in either of the groups. These findings suggested that although elevations in LFT were frequently present in narcotic addicts and were significantly greater among addicts who were also alcoholic. The most elevation is specifically due to alcohol. Conventional LFTs were therefore of limited value assessing alcoholism among addicts. (36)

In a study of fifty asymptomatic drug addicts admitted for detoxification, Rosenblate and colleagues (1975) reported a 62% increase in serum liver transaminases. liver biopsies of 45 patients showed a consistent pattern of lymphocytic infiltration in the portal areas and in small foci scattered throughout the lobule. Hyperplasia of the smooth endoplasmic reticulum was also seen in the liver biopsy of addicts. Rosenblate et all suggests that these alterations in the ultrastructure of hepatocytes could be due to drug or their vehicles (24).

Farid et al analyzed the hepatotoxicity of illicit opium and heroin (confiscated) by government officials. They found a high concentration of arsenic and lead especially in heroin of Indian origin, with 25mg/100g. In this study, 50 mice were divided into ten groups and injected with different concentrations of heroin daily for ten days. The animals were sacrificed and liver tissue was processed for histological analysis. The group of mice injected with carbon tetrachloride (a known hepatotoxic chemical), shows a similar pattern of degenerative changes as with groups injected with confiscated heroin. These changes could also be due to the presence of high levels of impurities (arsenic and lead) in the confiscated heroin. (37)

The liver enzymes (AST and ALT) of 120 opioid dependent patients undergoing treatment of addiction with buprenorphine was evaluated by Nancy et al. Their liver enzymes AST and ALT were measured before the commencement of the treatment, during treatment and after completion of treatment with buprenorphine for 40 days (2, 4 or 8mg/70kg/day). Patients with a history of hepatitis had significant increased levels of AST and ALT ($p<0.05$) even after buprenorphine treatment. The result of their study showed that liver enzymes (AST and ALT) should be monitored closely during treatment of patients with a history of hepatitis. (38)

A study by Weller et al examined the biochemical, histological and ultrastructural features of liver drug abusers. In this study, one hundred and sixteen heroin abusers seeking treatment for addiction at a London de-addiction Centre were recruited. Fifty-three percent of the study subjects had raised serum transaminases. A similar pattern of increased serum transaminases was seen among the study population in patients infected with hepatitis A and hepatitis B and those that had no viral infection. The study suggested that abnormal liver function can be seen in intravenous heroin abusers regardless of infection with viral hepatitis (33).

Barry and colleagues evaluated the hepatic function of 46 heroin abusers who were admitted to a de-addiction treatment program over a period of twelve months. Liver transaminases (AST, ALT, GGT, and ALP) and viral markers (hepatitis- associated antigen) and antibody (HAA-AB) and liver biopsy were evaluated. 44% of the drug abusers have significant titers for HAA or HAA-AB. Abnormal liver function was seen in the majority of the patients. 85% of the addicts had a history of excessive alcohol consumption along with heroin. Also, histologic evidence showed alcoholic liver injury in 10 patients out of twelve biopsies

performed. This showed that alcohol consumption among drug addicts can play a significant role in liver dysfunction. (39)

Apparently healthy adolescent subjects taking heroin, sedative, and airplane glue were evaluated for liver function abnormalities. About 7,272 adolescents were recruited in the study by Iris et al. 37% of the subjects had an abnormal liver function of which increased serum ALT was prominent among the participants. Serum bilirubin elevation was seen in only 10% of the subjects taking heroin and sedatives. There was a large correlation of these abnormalities in subjects taking heroin and sedatives. Airplane glue abusers had moderate or no obvious signs of hepatotoxicity among the subjects. When tested in vitro, that substance appeared to be non-toxic to hepatocytes. (40)

In a study by Allen D et al, 500 young servicemen with a history of drug abuse were tested for liver function. There was 66% increase in serum AST of the subject with a history of parenteral drug abuse while those with non- parenteral drug abuse had 41% increases in serum AST levels. This shows that the magnitude of liver abnormalities is more in subjects with parenteral drug abuse than those with non- parenteral drug abuse (41).

A study on 13 imprisoned narcotic addicts was carried out by Kenneth at the U.S public health hospital. Seven out of the 13 addicts had abnormal liver tissue and 10 out of 13 addicts had abnormal liver function test. Based on this study it could not be confirmed that one particular drug is responsible for abnormal LFT. The etiologic factors responsible for chronic liver disease in narcotic addicts was confirmed by the study (29).

To evaluate the effect of alcoholism on liver function of narcotics, spencer et al recruited 612 narcotic addicts in a randomized control study. 17% of the study population had a history of alcoholism. The mean value of serum AST, ALT, ALP and GGT levels in alcoholic narcotic addict were significantly higher than that of non-alcoholic narcotic addicts. After 6 months to 2 years of methadone maintenance treatment, there was no significant change in mean AST, ALT, ALP and GGT of the both groups. The findings of the study suggest that abnormal LFT is constantly present among narcotic addicts with special emphases on alcoholic narcotic addicts (36).

Rosenblate et al studied 50 asymptomatic heroin abusers who were admitted to detoxification Centre. 62% of the addicts had a mild increase in serum transaminases. One of the

24 patients had hepatitis- associated antigen with acute viral hepatitis. Liver biopsy of 45 addicts shows lymphocytic infiltration and small foci throughout the lobule (24).

To evaluate the prevalence of hepatitis B, hepatitis C, and HIV among drug addicts in Bangladesh, Shirin et al studied 266 drug addicts attending a de-addiction treatment center in Dhaka, the capital of Bangladesh. They found that the prevalence of hepatitis B surface antigen was 6.2% while that of hepatitis C virus was 24.8% among drug addicts. They advocated active education and awareness campaign to drug addicts in order to prevent spread of viral diseases (42)

A study by Rizwana et al showed mild abnormality in the liver enzymes of cannabis abusers. In the study, 51 subjects with established history of substance abuse were recruited. The subjects were divided into two groups, cannabis abusers, and abusers of other substances (e.g. heroin, codeine, methamorphine). 30 apparently healthy subjects were used as a control. The result of the study shows no much difference between the two groups of substance abusers. There was an increase in serum bilirubin in 13.7%, AST in 15.6% of the subjects, ALT in 33.3% and ALP in 74% of the subjects. (43).

In 2009, Stroffolile et al carried out a prevalence survey of hepatitis viral infection among drug addicts seen in Italian treatment centres. The prevalence of hepatitis C infection among intravenous drug users was 63.9% that of hepatitis B2.8% and HIV 3.1 %. (44).

In a study by Illic et al, 50 liver biopsy samples of heroin abusers were analyzed and compared with 10 non- heroin abusers. The microscopic picture of hepatocytes of heroin abusers showed vacuolar degeneration, characterized by the presence of small vacuoles in the hepatocytes-cytoplasm. The percentage of damage to hepatocytes was directly correlated with the duration of heroin abuse (Illic et al 2005). The study concluded that direct hepatotoxic effects of heroin included vascular hepatocytes changes, fatty changes, and alcoholic liver cirrhosis. Also, heroin abuse induced significant reduction in detoxification function of the liver, biotransformation and increased effects on the brain centers to the action of the drug and other toxins (45).

To assess the effect of heroin abuse and chronic hepatitis c infection on the liver, Dessislava et al analyzed the serum of intravenous heroin abusers and that of control who are infected with hepatitis c virus. Also, a liver biopsy of the two groups was taken and analyzed using conventional H&E staining. The data from the study proved liver damage due to

intravenous heroin abuse. Which was proved by the higher serum AST, ALT, ALP, GGT and total bilirubin as well as histological changes in hepatocytes. In the study, biochemical parameters i.e. liver enzymes and total bilirubin of heroin abusers and that of patients with chronic hepatitis C infections showed no significant difference between the two groups. Therefore, it was concluded that heroin abuse could not be seen as an independent risk factor for the development of liver disease (35).

RESEARCH DESIGN

3.0.1 RATIONALE

The harmful effect of chronic use of psychoactive drugs on the liver is not obviously known to drug addicts. Also, the risk of contracting virulent diseases (hepatitis B, hepatitis C, and HIV) due to sharing of injection needles among drug users is either not known or not taken seriously.
In this study, we will correlate the levels of liver damage among drug addicts by conducting liver function test (AST, ALT. ALP, GGT, Total protein and Albumin, Total and direct bilirubin) and the prevalence of hepatitis B and C viral infection compared with normal subjects. Questionnaire will also be given to the participant, from where vital information will be extracted for further discussion.

3.0.2 AIM AND OBJECTIVES

1. To evaluate the harmful effects of drug abuse on the liver function
2. To know the extent of damage to the liver by drug abuse
3. To evaluate the prevalence of hepatitis and viral infection among drug addicts.

MATERIALS AND METHODS

MATERIALS AND METHODS

4.0.LOCATION OF THE STUDY

The study was conducted at the de-addiction unit of psychiatric department of Punjab institute of medical science, Jalandhar Punjab, India.

4.1 SUBJECTS OF THE STUDY

The subjects of the study were patients with established history of substance abuse seeking treatment (detoxification) at the de-addiction unit of psychiatric ward. All patients included in the study were hospitalized. The diagnosis of substance abuse was done by Consultant psychiatrist using ICD criteria (5).

4.2 EXPERIMENTAL DESIGN

At the point of admission, venous blood samples were obtained from the patients for biochemical, hematological and viral markers analysis. In biochemical analysis, liver function test (AST, ALT, ALP, GGT, TOTAL PROTEIN AND ALBUMIN, TOTAL and DIRECT BILIRUBIN) and Viral markers such as HBsAg, Hepatitis C viral antibodies and human immunodeficiency virus antibodies were conducted. The study was performed in accordance with declaration of Helsinki. This is an ethical code on human experimentation drafted by world health organization. Socio-demographic and clinical data were obtained from the patient and reliable attendant through clinical interview. Informed consent was also given by the participants. The study was conducted between January and April of 2016.

4.3 EXCLUSION CRITERIA

Subjects who are not addicted to any of the illicit drugs (opioids, cannabis, and cocaine) were not included in the study. Pregnant women, children below 12 and elderly adults above 70 years were also not included in the study.

4.4 SAMPLE PROCESSING

All biochemical parameters were analyzed using BS-400 chemistry analyzer (fully automated Mindray Analyzer)

4.5 PROCEDURE

Clotted venous blood sample was centrifuged at 500rpm for 5 minutes to separate serum from blood cells. The serum was then place into a labelled sample cups using micropipette (500). Sample cups containing the serum samples are then loaded onto the machine. Various tests were requested using a computer input.

SGPT (ALAT, GPT) FS IFCC METHOD WITH/WITHOUT PYXIDOXAL-5-PHOSPHATE.

METHODOLY: Optimized UV-test according to IFCC (International Federation of Clinical Chemistry and Laboratory Medicine)

PRINCIPLE:

$$\text{L-Alanine} + \text{2-oxoglutarate} \xrightarrow{\text{ALAT}} \text{L-Glutamate} + \text{Pyruvate}$$

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDH}} \text{D Lactate} + \text{NAD}^+$$

Additions of pyridoxal-5- Phosphate (P-5-P) stabilizes the activity of transaminase and avoid falsely low values in sample containing insufficient endogenous P-5-P from patients with myocardial infarction, liver disease and intensive care patient.

Reagents:

Components and concentrations: -

R1: TRIS	pH7.1	5140 mmol/L
L- Alanine		700 mmol/L
LDH (Lactate Dehydrogenase)		>2300 U/L
R2: 2- Oxoglutarate		85 mmol/L
NADH		1 mmol/L
Pyridoxal-5- Phosphate FS		
Good's Buffer	pH 9.6	100 mmol/L
Pyridoxal-5- phosphate		13 mmol/L

Reagent Preparation:

The reagents are ready to use. For the determination with pyridoxal-5- phosphate (P-5-P) mix 1 part of P-5-P with 100 parts of reagent 1.

e.g:- 100 µl P-5P + 10 ml R1

Stability after mixing 6 days at 2-8 °C

24 hours at 15-25 °C

Sample Start:

Without pyridoxal 5 phosphate. Mix 4 parts of R1 + 1 part of R2

(e.g.: - 20 ml R1 + 5 ml R2) = mono reagent.

Stability 4 weeks at 2-8 °C

5 days at 15-25 °C

The mono reagent must be protected from light

ASSAY PROCEDURE:

Wave length: 340 nm, Hg 365 nm, Hg 334 nm

Optical path: 1 cm

Temperature: 37 °C

Measurement: Against air

CALCULATION:

With factor: from absorbance readings calculate Δ/min and multiply by the corresponding factor table below:

	Substrate Start	Sample Start
340nm	2143	1745
334nm	2184	1780
365nm	3971	3235

Normal Range: <40 IU/L

4.5.2 SGOT (ASAT, GOT) FS IFCC METHOD WITH/WITHOUT PYXIDOXAL-5-PHOSPHATE.

METHODOLY: Optimized UV-test according to IFCC (International Federation of Clinical Chemistry and Laboratory Medicine).

PRINCIPLE:

L- Aspartate + 2- Oxoglutarate $\xrightarrow{\text{AST}}$ L- Glutamate + Oxaloacetate.

Oxaloacetate + NADH + H+ $\xrightarrow{\text{MDH}}$ L- Malate + NAD+

Additions of pyridoxal-5- Phosphate (P-5-P) stabilizes the activity of transaminase and avoid falsely low values in sample containing insufficient endogenous P-5-P from patients with myocardial infarction, liver disease and intensive care patient.

Reagents:

Components and concentrations: -

R1: TRIS	pH 7.65	110 mmol/L
L- Aspartate		320 mmol/L
MDH (Malate Dehydrogenase)		800 mmol/L
LDH (Lactate Dehydrogenase)		>1200 U/L
R2: 2- Oxoglutarate		65 mmol/L
NADH		1 mmol/L
Pyridoxal-5- Phosphate FS		
Good's Buffer	pH 9.6	100 mmol/L
Pyridoxal-5-phosphate		13 mmol/L

The reagents are ready to use. For the determination with pyridoxal-5- phosphate (P-5-P) mix 1 part of P-5-P with 100 parts of reagent 1.

e.g.: - 100 µl P-5P + 10 ml R1

Stability after mixing 6 days at 2-8 °C

24 hours at 15-25 °C

Sample Start:

Without pyridoxal 5 phosphate. Mix 4 parts of R1 + 1 part of R2

(e.g.: - 20 ml R1 + 5 ml R2) = mono reagent.

Stability 4 weeks at 2-8 °C

5 days at 15-25 °C

The mono reagent must be protected from light.

ASSAY PROCEDURE:

Wave length: 340 nm, Hg 365 nm, Hg 334 nm

Optical path: 1 cm

Temperature: 37 °C

Measurement: Against air

CALCULATION:

With factor: from absorbance readings calculate Δ/min and multiply by the corresponding factor table below:

	Substrate Start	Sample Start
340nm	2143	1745
334nm	2184	1780
365nm	3971	3235

Normal Range :<40IU/L

4.5.3 ALKALINE PHOSPHATASE (FS), IFCC METHOD 37 °C

METHDOLOGY:

Kinetic photometric test, according to the international federation of clinical chemistry and laboratory medicine (IFCC).

PRINCIPLE:

P- Nitrophenylphosphate + H 0 AP Phosphates + p- Nitrophenol.

Reagents:

Components and concentrations:		
R1: 2 Amino-2- methyl-1-propanol	pH 10.4	1.1 mmol/L
Magnesium acetate		2 mmol/L
Zinc Sulphate		0.5 mmol/L
HEDTA		2.5 mmol/L
R2: P- Nitrophenylphosphate		80 mmol/L

The reagents are ready to use.

Sample Start:

Mix 4 Parts of R1 + 1 Part of R2 (e.g 20 mL R1 + 5mL R2) = mono reagent.

ASSAY PROCEDURE:

Wave length: Hg 405nm, (400-420nm)

Optical path: 1 cm

Temperature: 37 °C

Measurement: Against reagent blank.

CALCULATION:

With factor: from absorbance readings calculate Δ/min and multiply by the corresponding factor table below:

Δ/min factor = AP activity (U/L)

Normal Range:- 25-90 IU/L

4.5.4 GAMMA-GT-IFCC STANDARD

METHDOLOGY:

Kinetic photometric test according to Szasz/Persijn. The test has also been standardized to the method according to IFCC (International Federation of Clinical Chemistry). Result according to IFCC is obtained using a special factor or in case a calibrator (TruCal U) is used, by use of the calibrator value given for the IFCC method.

PRINCIPLE:

GAMMA-GT catalyzes the transfer of glutamic acid to acceptors like glycylglycine in this case. This process release 5-amino-2-nitrobenzoate which can be measured at 405 nm. The increase in absorbance at this wavelength is directly related to the activity of Gamma-GT.

L-Gamma-glutamyl-3-carboxy-4-nitranilide + Glycylglycine

GGT →

Gamma- glutamyl-glycylglycine + 5-Amino-2-nitrobenzoate.

Reagents:

Components and concentrations:

R1: TRIS	pH 8.28	135 mmol/L
Glycylglycine		135 mmol/L
R2: L- Gamma-glutamyl-3- carboxy-4-nitronilide	pH 6.00	22 mmol/L

The reagents are ready to use.

Sample Start:

Mix 4 Parts of R1 + 1 Part of R2 (20 ml R1 + 5ml R2) = mono reagent.

Stability 4 weeks at 2-8 °C

5 days at 15-25 °C

ASSAY PROCEDURE:

Wave length: Hg 405nm, (400-420nm)

Optical path: 1 cm

Temperature: 37 °C

Measurement: Against reagent blank.

CALCULATION:

With factor: from absorbance readings calculate Δ/min and multiply by the corresponding factor table below:

factor = Gamma-GT activity (IU/L)

	Szasz	IFCC
Substrate Start 405nm	1421	1606
Sample start 405nm	1158	1309

Normal Range: 11-50IU/L

4.5.5 TEST FOR HEPATITIS B SURFACE ANTIGEN

To detect Hepatitis B viral infection in addicts' blood, hepacard was used. This is a visual, rapid, sensitive and accurate one step immunoassay.

PRINCIPLE

It is based on antigen capture principle. Antibodies that is conjugated to colloidal gold polyclonal. Antibodies are immobilized on a nitrocellulose strip in the thin line the test serum is introduced and flows through the absorbed pad where it mixes with the signal reagent. Presence of HBsAg in the test sample allows binding of gold-antibody conjugate to the antigen forming an antigen-antibody colloidal gold complex. The complex then migrates by capillary action through the nitrocellulose strip. When the complex meets the line of immobilized antigen the complex is trapped forming an antibody-antigen colloidal complex. This forms a red band indicating the sample is positive for HBsAg. To serve as control, an additional line of anti-mouse antibody c has been immobilized at a distance from the test line on the strip.

TEST PROCEDURE

1. The Required number of heparcard and specimen is brought to room temperature.
2. The card was label with patient's identity with number.
3. 3.70ml of serum is then placed into the sample using a micropipette.
4. The result is then read out after 20 minutes (Paterson d *et.al* 1982)

INTERPRETATION OF RESULT

Appearance of a red colored line on each of the test region T and control C indicates a positive serum for HBsAg. Presence of only one line at the control region c indicates a negative serum for HBsAg. caldwell *et.al* (1977)

4.5.6. TEST FOR HEPATITIS C VIRAL ANTIGEN

4^{th} generation HCV tri dot which utilizes a unique combination of modified HVC antigen from putative core, Ns3, Ns4 and Ns5 regions of the virus to selectively identify all subtypes of Hepatitis C virus with a virus with a high degree of sensitivity and specificity was used to detect Hepatitis C infection among abusers.

PRINCIPLE

HVC antigens are immobilized on a porous immunofiltration membrane. Sample and the reagent pass through the membrane and are absorbed into the underlying absorbent pad. As the patients sample passes through the membrane, HVC antibodies if present in the serum, binds to the immobilized antigen. In the subsequent washing step, unbound serum proteins are removed. In the next step, the protein –A conjugate is added which binds to the fc portion of the HVC antibodies to give distinct pinkish purple dot against a white background at the test region. At the control C a built in quality control dot has been devised to confirmed proper functioning of the device, Reagent and correct procedural application. (Halfan *et.al* 1997).

ASSAY PROCEDURE

1. All reagents and sample are brought to room temperature.
2.100ml of serum is then added to sample well and allow to stand for 2 minutes.
3.100ml of wash buffer is then added to the sample well.
4.2 drops of a protein A conjugate is then added to the sample well.
5.5 drops of buffer solution then added.
6. The result is then readout immediately. (Sayers 1993).

INTERPRETATION OF RESULT

1. Appearance of two dots one at the c control region c and the other at test region t indicates that the sample is positive.
2. Appearance of one dot at the control c region indicates the sample is negative.
3. When no dots appear at the control region c it indicates the test is not valid. (Caypers 1971).

4.5.7. TEST FOR HIV

HIV antigens are immobilized on a porous immunofiltration membrane. Sample and reagents pass through the membrane and are absorbed into the underlying absorbent.as the patients sample passes through the membrane, HIV antibodies if present bind to the immobilized antigens.

ASSAY PROCEDURE

1.Three drops of buffer solution are added at the center of the device.
2. One drop of the serum is then added.
3.Five drops of buffer is then added.
4.Two drops of protein-A conjugate is added.
5.Five drops of buffer solution are then added and result is read.

INTERPRETATION

1. If only one dot (control) appear, the specimen is non-reactive.
2. If two dots appear, the serum is positive for HIV 1.
3. If three dots appear, the serum is positive to HIV 1 and HIV2. (GURTLER 1994).

RESULT

RESULT

The mean AST level of the study group (N=100) was 45.09 ±39.69UI/L while that of the control group was (N=100) was 21±6 IU/L. 35% of the substance abusers had high levels of AST. To test the hypothesis that substance abusers and non-abusers are associated with a statistically significant different liver enzymes level, an independent sample t- test was performed using SPSS statistical software version 24. The independent sample t- test was associated with a statistically significant effect $t=0.000$, $p<0.05$

The mean ALT level of substance abusers (N=100) was 70±78 IU/L while that of the control group was 24±10IU/L. Fifty-nine percent (59%) of the study group had a high value of ALT >40IU/L.

A statistically significant difference was observed $t=0.000$, $p>0.05$. This showed that substance abusers had higher serum ALT than the control group.

The mean ALP level of study group was 99±58 IU/L while that of the control group was 69±18IU/L. 45% of substance abusers have high ALT levels>90 IU/L.

There was a statistically significant difference in the mean ALP value of substance abusers and control group, $t =0.000$, $p<0.05$.

The mean serum GGT of substance abusers was 89± 158IU/L, while that of the control group was 27± 11 IU/L. Forty-seven (47%) of substance abusers had a high value of GGT>50 IU/L. A significant difference in GGT was observed between substance abusers and control groups, $t=0.000$, $p <0.05$.

The mean serum total bilirubin in abusers was 0.51±0.40mg/dl while that of control subjects 0.6±0.51mg/dl. Only 6% of the study group had total bilirubin above the normal reference range. There was no statistically significant difference in serum total bilirubin between abusers and control group, $t=0.051$, $p<0.05$.

The mean value of serum direct bilirubin in abusers was 0.18± 0.19mg/dl and that of the control group was 0.17± 0.67 mg/dl. There was no statistically significant difference between the two groups, $t= 0.591$, $p<0.05$.

	Substance abusers		controls	
Parameters	Mean	SD	Mean	SD
Age (YEARS)	28.54	7.62	27.76	5.44
T Protein(g/d)	6.98	0.69	7.58	0.62
Albumin (g/dl)	4.22	0.42	4.46	0.42
T-Bilirubin (mg/dl)	0.51	0.40	0.51	0.25
D-Bilirubin (mg/dl)	0.18	0.19	0.17	0.06
AST (U/l)	45.39	39.69	21.35	6.59
ALT (U/L)	70.83	78.30	24.19	10.04
ALP U/l)	99.43	58.42	69.28	18.34
GGT (U/l)	89.27	158.62	27.54	7.62

Table 1. Mean Levels of biochemical Parameters in Substance Abusers and controls.

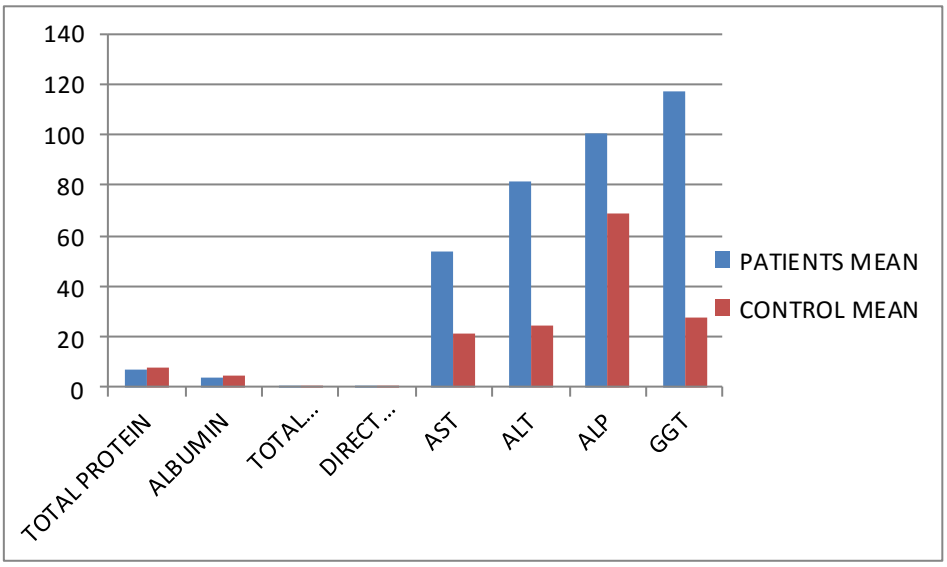

Figure 1: showing biochemical parameters of substance abusers and controls

Independent Samples Test	POPULATION.					
		Levene's Test for Variences Equality		t-test for Means Equality		
	Equal Variance	F	Sig.	T	Df	Sig. (2-tailed)
PROTEIN	Assumed	0.163	0.687	-6.417	198	0.000
	Not assumed			-6.417	195.599	0.000
ALBUMIN	Assumed	1.584	0.210	-4.354	198	0.000
	Not assumed			-4.354	191.485	0.000
TOTAL BILIRUBIN	Assumed	4.133	0.043	-1.967	198	0.051
	Not assumed			-1.967	164.692	0.051
DIRECT BILIRUBIN	Assumed	9.553	0.002	0.538	198	0.591
	Not assumed			0.538	122.768	0.592
AST	Assumed	65.675	0.000	5.974	198	0.000
	Not assumed			5.974	104.464	0.000
ALT	Assumed	44.522	0.000	5.908	198	0.000
	Not assumed			5.908	102.255	0.000
ALP	Assumed	36.527	0.000	4.923	198	0.000
	Not assumed			4.923	118.326	0.000
GGT	Assumed	19.205	0.000	3.886	198	0.000
	Not assumed			3.886	100.092	0.000
AGE	Assumed	0.227	0.634	0.985	198	0.326
	Not assumed			0.985	195.798	0.326

Table 2. Independent sample t- Test. (t test <0.05 is significant)

Description of study population

One hundred substance abusers and an equal number of controls were involved in this study. The average age of the abusers was 28.54± 7.26 years. Out of the one hundred abusers, only two were females (2%) while the rest (98%) were males. The average duration of abuse was 3.4±0.50 years (1.5 -10 years). 55% were taking heroin alone and only 2% were taking only alcohol as substance of abuse whereas rest of 43% were taking different combinations of substances. Leading combination used were: 17% for heroin and alcohol, 10% for heroin and cannabis, 5% for heroin and nicotine the remaining 11% used other combinations of heroin, cannabis, alcohol and psychoactive drugs.

Single substance abusers		Multiple substance abusers	
Heroin	55%	Heroin and alcohol	17%
Alcohol	2%	Heroin and Cannabis	10%
		Heroin and Nicotine	5%
		Others	11%

Table 3. Categories of substance abusers

41% of the subjects administered heroin by injection, 33% are oral alcohol consumers, 19% of the subjects use inhalation method as the route of administering heroin while 7% were smokers.

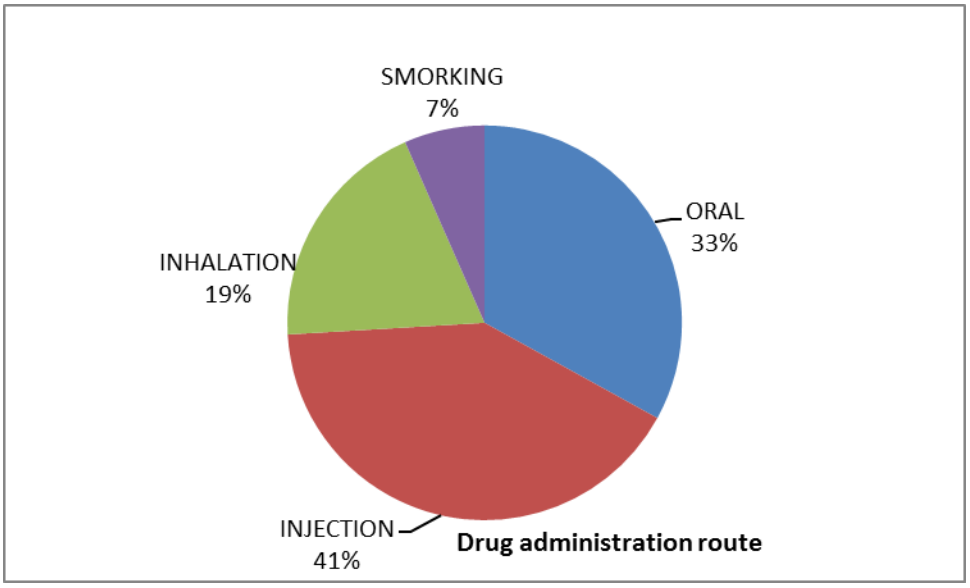

Figure :2 Substance abuse Route of Administration

Most of the subjects obtained the drug either directly or from the dealers or through friends.

The majority of heroin abusers were secondary school dropouts i.e. 43%, primary school dropouts consist of 20%, graduates 23% while those with no formal education were 14% of the study population.

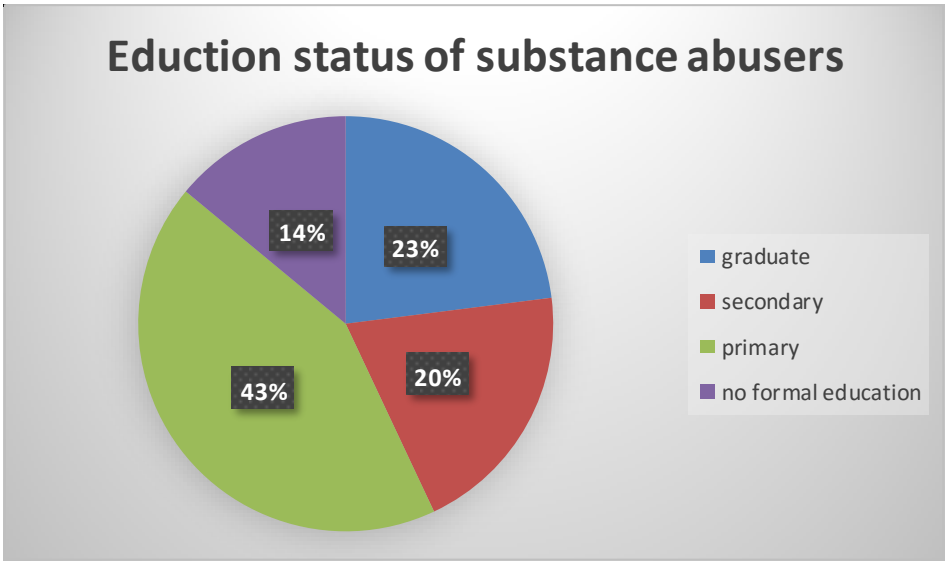

Figure 3: Educational status of substance abusers

In terms of employment 53% of the study group patients have income above 10000 rupees per month, while those with income of 0-10000 are 10% of the population. Unemployed subjects consisted of 37% 0f the study population.

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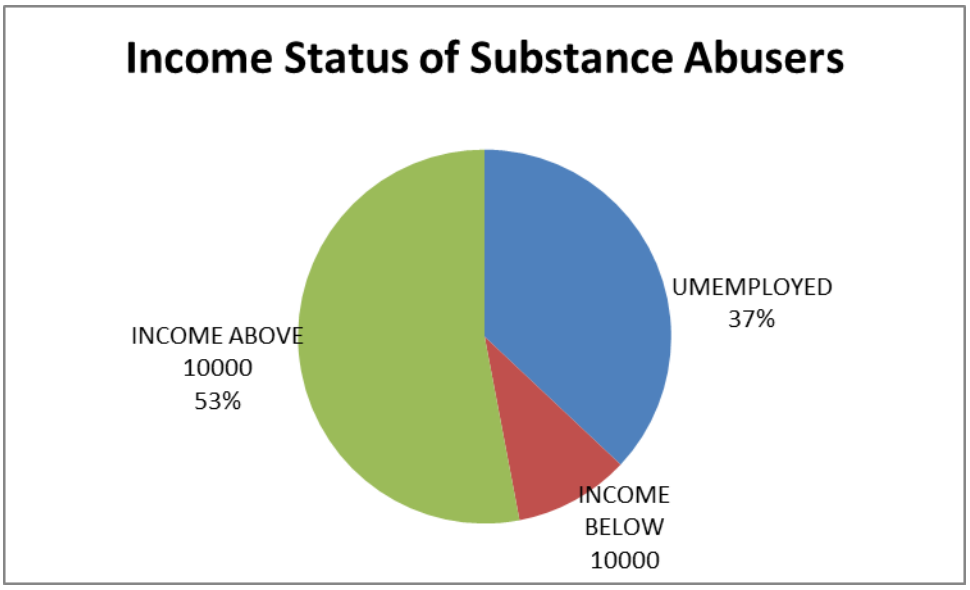

Figure 4: Income Status of Substance Abusers

57% were single, 40% were married while 3% are divorced. 31% of the patients are aware of the risk involved in substance abuse while 69% were not. 84% of the abusers reported their willingness to stop the abuse of drugs while only 16% declined to comment on the willingness to stop. On the issue of availability of family support to addicts, only 33% of the study group had a positive response while 55% reported rejection and negative behavior from their family members.

97% of the study group had serious withdrawal symptoms ranging from depression (31%), headache (22%), sweating (22%), decreased appetite (11%), convulsion (10%) and fatigue (4%).

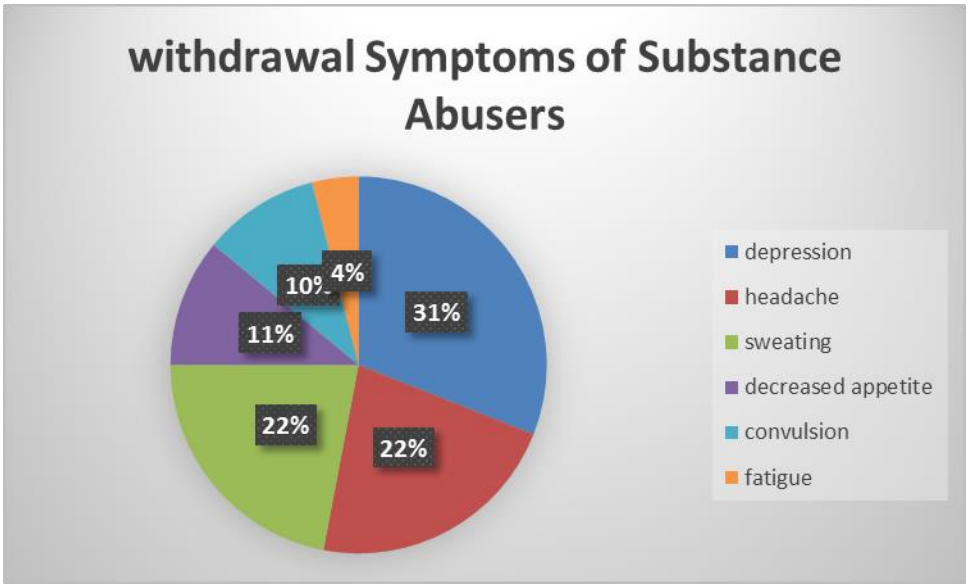

Figure 5: Withdrawal symptoms of Substance Abusers

DISCUSSION

DISCUSSION

The results of this study show that substance abusers have higher values of liver enzymes AST, ALT, ALP, and GGT when compared with non-abusers of the same age group. There is no statistically significant difference between substance abusers and non-abusers in the value of serum bilirubin (total and direct).

The percentage increase in liver enzymes is much higher in addicts that are infected with hepatitis B and or hepatitis C virus. While most studies do not take account of total protein and albumin, in this study, we estimated the level of serum protein and albumin in substance abusers. In comparison with non-abusers, both mean total protein and albumin values were lower in the substance abusers.

A Recent study by Farooqi et al showed that increase in liver enzymes is highly correlated with heroin abuse. In the same study, heroin abusers who were concurrently infected with Hepatitis B and C virus had a higher increase in serum liver transaminases. (6)

Our study was in conformity with that of Allen D et al; in their study they found that serum AST of drug abusers was increased by 66% when compared with non- abusers. Also, liver biopsy of the study group showed either a classic viral hepatitis or a mild non-specific hepatitis. (31)

In earlier studies from New York centre for rehabilitation, liver cirrhosis was significantly associated with alcohol abuse and parenteral drug use and it was more commonly diagnosed in younger patients. (44)

In our study the prevalence of Hepatitis C viral infection among substances abusers was 25%, Hepatitis B viral infection 11% and that of HIV infection 1%. Sharing of injection needles among parenteral substances abusers may be the most likely factor that helped in the spread of the viral infection. Health care providers need to redouble effect in the treatment of substances abusers in order to curb the possible spread of viral infection.

Substance abuse is one the leading disorder among young adults in many societies. Liver damage arising from substance abuse has been proved in many literatures. (20) Heroin is one of the leading substances of abuse in India, especially Punjab. From our study, 55% of the cases of substance abuse are that of heroin. Heroin has high potentials of producing dependency and addiction. (6) Liver enzymes (AST, ALT, ALP and GGT) were very high among substance abusers recruited in this study. The mean age of substance abusers in this study was 28.54±7.26 years. This age group comprises the active labor force of the society. Our data revealed that only 35% of the addicts had no source of income. 53% of the abusers are actively involved in a farming activity with an average income above 10000 rupees ($150) per month. This was in contradiction with the study by Farooqi which concluded that substance abusers are from the poor background with very low income. Pressure from peer group and frustration may lead to this trend of abuse. Lack of family cohesion, affection, and rigidly enmeshed family structure are some of the contributing factors that lead to substance abuse (28) In the present study, 55%of the substance abusers reported negative attitude of family members towards them. This could be one of the contributing factors that lead to substance abuse. From this study, it was observed that majority of substance abusers were males (98%) while only 2% were females. Most common substance of abuse seen in our study was heroin (55%). 41% use injection as a route 33% use oral route, 19% of the subjects use inhalation and 7% used smoking as a route for substance abuse. The daily consumption of heroin was 1-2g/day. The frequency of abuse was daily in the majority of cases.

The presumption that most substance abusers have no any formal education was contradicted in this study. We observed only 14% of the substance abusers have no formal education. College graduates, university graduates, police officers as well as government employees are involved in the practice of substance abuse. Easy accessibility of heroin to gang adults may also be a contributing factor to the rise of abusers in our society.

In this study, the mean AST value of substance abusers was 45±39IU/L, while that of control subjects was 21±6IU/L. There is 35% increase in AST value of substance abusers in this study. Elevation of serum AST in alcoholic was observed by S Patel and O Gorman. They reported 24% increase in AST value of alcoholics and 21.7% increase in drug abusers. (34) Dessislava et al reported that no significant difference in serum AST value of heroin abusers with viral hepatitis C infection and those abusers without viral infection. (35)

Marks v and Chapple also reported an increase in serum AST levels in group of patients with heroin and cocaine abuse. This study was in conformity with that of Abdelrouf et al in which increase in serum AST was reported among tramadol abusers in Gaza strip. (30)

Serum AST elevation was seen in alcoholic narcotics in a study by spencer et al. The study reaffirms the effect of alcohol on liver enzymes with emphases on serum AST levels (36) Increase serum AST was observed in heroin addicts treated with buprenorphine by Nancy et al.

In our study it was also found that the mean serum ALT of substance abusers was 70.83 ± 78.30IU/L while that of the control group was 24.19 ± 10.09IU/L. Statistical analysis showed a significant difference between the two groups. Yih et al reported a similar elevation in the serum ALT of aging narcotic addicts admitted in California civil addict program. (46) Farooqi et al also reported elevation in serum ALT levels of heroin addicts in Punjab. (6) Spencer et al also reported elevation in serum ALT levels of narcotics that are alcohol dependent. In the study, they show that alcoholic narcotics have a higher elevation of serum ALT than non-alcoholics. (36) Elevation in ALT among tramadol abusers was reported by Abdelrouf et al. (46)

CONCLUSION

From this study, we have seen that liver dysfunction is usually present in substance abusers. This is characterized by an elevation in liver enzymes AST, ALT, ALP and GGT. In the past, researchers have shown an increase in liver enzymes as an evidence of substance abuse. (47) Most of the patients recruited in our study were asymptomatic, but with derangement of liver enzymes. This suggested that liver disease can be present in abusers without symptoms, therefore it is highly recommended for medical practitioners to always check and order liver function tests in substance abusers seeking treatment for de-addiction. It was also reported by some researchers that prevalence of viral infection is high among substance abusers this is not unconnected with parenteral drug injection and sharing of needle. (48) In our study the prevalence of hepatitis C infection among substance abusers is also very high, we found that 25% of abusers had positive viral antigen for hepatitis C and 11% with hepatitis B. The presence of hepatitis C infection can also lead to deranged liver function tests which makes it difficult to differentiate from the hepatitis due to substance abuse, hence we need further detailed studies to differentiate the occurrence of hepatitis due to substance abuse or due to presence of hepatitis C infection.

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RESEARCH PERFORMA

NAME SEX/AGE DATE

MRD

TYPE OF DRUG 2 FREQUENCY

CANNABIS	DAILY	
NICOTINE	TWICE WEEKLY	
ALCOHOL	WEEKLY	
OPOIDS	INFREQUENTLY	
OTHER	OTHER	
ADMINISTRATION ROUTE	4	SOURCE OF DRUG
ORAL	MARKET	
INJECTIONS	FRIENDS	
SHARED NEEDLE	STREET	
INHALATION	OTHERS	
SMOKING		
OTHER	6	EMPLOYMENT
UNEMPLOYED		
EDUCATION	INCOME BELOW5000	
NONE	INCOME 5000-10000	
PRIMARY	INCOME ABOVE 10000	
SECONDARY		
GRADUATE	8	NUMBER OF CHILDREN
MALE		
MARTIAL STATUS	FEMALE	
SINGLE		
MARRIED	10	WHAT ARE THE RISK?
DIVORCED	1	
NOT APPLICABLE	2	
3		
ARE YOU AWARE OF THE RISK?	DON'T KNOW	
YES		
NO	12	BEHAVIOUR OF FAMILY TOWARDS ADDICTION
POSITIVE		
WILLINGNESS TO STOP	NEGATIVE	
NO	INDIFFERENT	
YES		
NOT DECIDED	14	DETAIL OF SYMPTOMS
INCREASD APETITE		
ANY WITHDRAWAL SYMPTOMS?	SWEATING	
NO	HEADACHE	
YES	CONVULSIONS	
	DEPRESION	