Sudan University of Science and Technology

College of Graduate Studies

Assessment of High Sensitive C-reactive Protien of Hepatitis B in Sudanese Patients in Khartoum State

تقويم بروتين سي التفاعلي عالي الحساسية لدى مرضى الكبد الوبائي (ب) للمرضى السودانين في ولاية الخرطوم

A dissertation Submitted in Partial Fulfillment for MS.c Degree in Medical Laboratory Science (Clinical Chemistry)

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بسم الله الرحمن الرحيم

قال تعالى :-

((رَبِّ أَوْزِعْنِي أَنْ أَشْكُرَ نِعْمَتَكَ الَّتِي أَنْعَمْتَ عَلَيَّ وَعَلَى وَالِدَيَّ وَأَنْ أَعْمَلَ صَالِحاً تَرْضَاهُ

وَأَدْخِلْنِي بِرَحْمَتِكَ فِي عِبَادِكَ الصَّالِحِينَ))

صدق الله العظيم

النمل: الآية (19)

Dedication

To my mother and father

To my Friends

Acknowledgement

All my praise and thanks to Allah who help me and give me confidence to complete this study .

With my great respect I want to thanks my supervisor ***Dr. Noon Babiker*** for her guidance , helpful ,suggestion ,solving problem and her precious advice as well as continuous assistance through the whole process of the research .

Thanks are also to all staff of clinical chemistry department for their effort .

I am very great full thanks to laboratory staff Saba medical lab for their great support to collect sample for this study .

Finally thanks for my friends for their support and device.

Abstract

Background

CRP was the first acute phase protein to be described and is sensitive systemic marker of inflammation and tissue damage especially in liver disease . hepatitis B viruses Is a common health problem throughout the world and one of the major risk factors for liver disease.

General objective:

To assess High Sensitive C-reactive protien of Hepatitis B in sudanese Patients In Khartoum State.

Specific Objective:

- To assess CRP levels an study Group
- To correlate between CRP level and duration of disease (acute and chronic Group)

Material and Methods

This is case control study conducted in Saba clinic in Khartoum state during march to June 2015 . Sixty hepatitis B positive patients as test group with fifty healthy subject s control group were enrolled in this study .Plasma CRP level was estimated using Nycocard CRP Single test .Data analyzed using SPSS computer program.

Result

The result of showed the mean plasma level of CRP was significantly increased in case group (p.value 0.001) compare with control group The mean plasma level of CRP was significantly increased in hepatitis B virus case group) classified based on duration of disease (p.value 0.001). Pearson correlation showed , there was

weak correlation between CRP level and duration of Hb Ag virus disease, (r 0.554 , p.value: 0.113),and negative correlation between CRP level and acute duration of Hb virus disease, (r : -0.015, p.value:0.937)

Conclusion

In conclusion CRP is useful for of hepatitis B patients, since they tend to have higher CRP level compared with control group. The Study conducted there is a significant increase in mean of CRP level in chronic hepatitis B patients compared with acute.

مستخلص الدراسة

الخلفية

بروتين سي التفاعلي هو أول بروتين للمرحلة الحادة ويمكن وصفه وهو العلامة النظامية الحساسة لإلتهاب وتلف الأنسجة وخاصة في أمراض الكبد ، والتهاب الكبد الفيروسي ب وهي مشكلة صحية شائعة في جميع أنحاء العالم، وأحد عوامل الخطر الرئيسية للإصابة بأمراض الكبد.

الهدف العام:

لدراسة بروتين سي التفاعلي كمؤشر في المرضى الذين يعانون التهاب الكبد من النوع (ب) في ولاية الخرطوم.

الهدف المحدد:

- دراسة تقدير مستويات بروتين سي التفاعلي للمجموعة.
- لمقارنة الحالات بين مستوى بروتين سي التفاعلي (الحادة والمزمنة للمجموعة)
- لربط بين مستوى بروتين سي التفاعلي لتطور المرض في التهاب الكبد المزمن ب

المتطلبات العملية وطرق البحث

هي دراسة الحالة والتي أجريت في عيادة سابا في ولاية الخرطوم خلال (مارس- يونيو 2015)
وعددها ستون حالة إيجابية لإلتهاب الكبد (ب) مجموعة الاختبار كما أن هنالك خمسون حالة وضعت كمجموعة ضابطة لمجموعة الاختبار.

ومستوى البلازما بروتين سي التفاعلي باستخدام جهاز Nycocard لإختبار وقياس بروتين سي التفاعلي.
تم تحليل البيانات باستخدام برنامج الحزم الإحصائية المجتمعية بواسطة الحاسوب.

النتائج:

وأظهرت نتيجة التكرارات زيادة متوسط مستوى البلازما من بروتين سي التفاعلي بشكل ملحوظ في مجموعة الاختبار (القيمه الاحصائيه الدلاليه 0.001). وقد ارتفع متوسط مستوى البلازما وأن بروتين سي التفاعلي في زيادة بشكل ملحوظ في التهاب الكبد الفيروسي ب في مجموعة الاختبار وتصنف على أساس مدة المرض p القيمه الاحصائيه الدلاليه 0.001).

أظهر ارتباط بيرسون، أن هنالك ارتباط سلبي قوي بين مستوى CRP ومدة المرض في فيروس الكبد الوبائي في مجموعة الاختبار في المصابين بهذا المرض بصورة حادة بـ(0.937 -:r،0.015. القيمه الاحصائيه الدلاليه).

وكذلك أظهر ارتباط بيرسون، أن هنالك ارتباط ايجابي متوسط بين مستوى CRP ومدة المرض في فيروس الكبد الوبائي في مجموعة الاختبار في المصابين بهذا المرض بصورة مزمنة بـ
(0.554 -:r،0.113 القيمه الاحصائيه الدلاليه).
وعليه أوصت دراسة على أن الآلية الكامنة وراء ارتباط بين بروتين سي التفاعلي وفترة مدة التهاب الكبد ب.

الخاتمة

يعتبر بروتين سي التفاعلي مؤشر مفيد لمرضى التهاب الكبد ب، لارتفاعها عند المرضى بالمقارنة مع المجموعة الضابطة.

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List Abbreviations

HAV	Hepatitis A virus
HBV	Hepatitis B virus
HCC	Hepato Cellular Carcinoma
HCV	Hepatitis C virus
HBcAg	Hepatitis B core antigen
HBIG	Hepatitis B Immune Globulin
HBsAg	Hepatitis B surface antigen
HIV	Human immune deficiency
hs-CRP	High sensitive C-reactive protein
IFN	Inter Firon
IL	Inter Leiuken
ROS	Reactive oxygen species
PRR	Pattern recognition receptor
TH	T helper
TNF	Tissue Necrosis factor

1. Introduction

1.1Introduction:

Hepatitis is an infection of the live by hepatitis virus and it is a systemic disease primarily infecting the liver. most of acute viral hepatitis in children and adults are caused by one of the following agents: Hepatitis A virus (HAV) the cationic agent of viral hepatitis type A infectious hepatitis C virus (HCV) : the agent of hepatitis type A (infectious hepatitis) Hepatitis B virus (HBV) : which is associated with viral hepatitis B (serum hepatitis) Hepatitis C virus (HCV) :the agent of hepatitis C (common cause of post transfusion hepatitis) hepatitis D Virus (HDV): etiologic agent of delta hepatitis ; cases infection only in presence of HBV, hepatitis E virus (HEV): the agent of enteric ally transmitted hepatitis (Jawetz , et al 2007).

Other viruses are associated with hepatitis cannot be ascribed to known agents, and the associated disease is designated non-A-E hepatitis.

Additional well – characterized viruses that can cause sporadic hepatitis , such as yellow fever virus , cytomegalovirus, Hepatitis viruses produce acute inflammation of the liver, resulting in a clinical illness characterized by fever, gastrointestinal symptoms such as nausea and vomiting , and jaundice.

Regardless of the virus type, identical histopathology lesions are observed in the liver during acute disease (Jawetz., et al 2007).

Because few drugs are useful against viral infections, prevention of infection by the use of vaccines is very important. Prevention of viral diseases can be achieved by the use of vaccines that induce active immunity or by the administration of preformed antibody that provides passive immunity (Okamoto et al ., 1990).

Hepatitis B immunoglobulin (HBIG) is used in the prevention of hepatitis B in people who may have been exposed to the virus either by needle- stick or as a neonate born of a mother who is a carrier of HBV. The preparation contains a high titer of antibody to hepatitis B virus and is obtained from human to avoid hypersensitivity reactions; HBIG is often used in conjunction with hepatitis B vaccine, an example of passive – active immunization (Okamoto et al ., 1990).

C-reative protein (CRP) is a protein found in the blood, the levels of which rise in response to inflammation , it is an acute phase protein . its physiological role is to bind to phosphocholine expressed on the surface of dead or dying cells and some type of bacteria, in order to activate the complement system the CIQ complex (Thompson et al ., 1999) CRP is synthesized by the liver in response to factors released by fat cells (adipocyte). It is a member of the pentraxins family of proteins. It is not related to C- peptide or protein C.C- reactive protein was the first pattern recognition recptor (PRR) to be identified (Mantovani et al ., 2008).

1.2 Rationale

Although enormous strides have been made in recent in the prevention and control of hepatitis , it still remain a serous global problem due to its high morbidity and mortality world wide . approximately 2 billion people world wide have been infected with hepatitis B virus (HBV)and about 350 million live with chronic HBV infection which an estimated 6000.000 people die each year these chronically infected individual are at greater risk of developing progressive hepatic fibrosis , cirrhosis and subsequent complications including hepatocellular carcinoma.

Recently studies hypothesis CRP another important mediator of the host immune defense which is produced predominantly by liver in inflammatory condition , accordingly present research conducted to study CRP as predictor marker for prognosis of hepatitis B patients.

1.3 Objectives

1.3.1 General objective:

To Assess of High sensitive C-reactive protein in hepatitis B in Sudanese patients.

1.3.2 Specific Objective:

- To assess CRP levels in study Group of hepititis B .
- To correlate between CRP level and duration of disease (acute and chronic and Group).

2. Literature Review

2.1 Hepatitis B virus :

HBV is a member of the hepadna virus damily . it is a 42 nm developed vision , with an icosahedra nucleocapsid core containing a partially double-stranded circular DNA genome ; the envelope contains a protein called the surface antigen (HBsAg), which is important for laboratory diagnosis and immunization , within the core is a DNA –dependent DNA polymerase . the genome contains four genes (four open reading frames) that encode five proteins . namely , the S gene encodes the surface antigen , the C gene encodes the core antigen and the E antigen , the P gene encodes the polymerase and the X gene encodes the X protein. The X protein is an activator of viral RNA transcription . the DNA polymerase has both RNA- dependent (reverse trascriptase) and DNA –dependent activity (Levinson : 2008).

Electron microscopy of a patient's serum reveals three different types of particles ; a few 42-nm virons and many 22-nm spheres and long filaments 22 nm wide , which are composed of surface antigen . HBV is the only human virus that produces these spheres and filaments in such large number in the patient's blood . the ratio of filaments and small spheres to virions is 1000:1, in addition to HBsAg, there are two other important antigens: the core antigen (HBcAg) and the e antigen (HBcAg). The core antigen , as the name implies , forms the nucleocapsid core of the viron whereas the e antigen is secreted from infected cells into the blood . the e antigen is an important indicator of transmissibility , for vaccine purposes, HBV has one serotype based on HBsAg . However . for epiemiologic purposes , there are four serologic subtypes of HBsAg based on a group – specific

antigen , “a” and two sets of mutually exclusive epitopes , d or y and w or r. this leads to four serotypes – adw, adw, ayw, and ayr- which are useful in epidemiologic studies because they are communicated in certain geographic areas, the specificity of HBV for liver cells is based on two properties: virus-specific receptors located on the hepatocyte cell membrane (facilitate entry) and transcription factors found only in the hepatocyte that enhance viral mRNA synthesis (act postentry). Humans are the only natural hosts of HBV; there is no animal reservoir (Jawetz et al 3007)

2.1.1 Transmission:

The three main modes of transmission are reported via blood , during sexual intercourse, and prenatally from mother to newborn . the observation that needle – stick injuries can transmit the virus indicates that only very small amounts of blood are necessary . HBV infection is especially prevalent in addicts who use intravenous drugs . screening of blood for the presence of HB.sAg has greatly decreased the number of transfusion-associated cases of hepatitis B . However; because blood transfusion is a modern procedure, there must be another natural route of transmission . it is likely that sexual transmission and transmission from mother to child during birth or breast feeding are the natural routes. Note that enveloped viruses, such as HBV, are more sensitive to the environment that nonenveloped viruses and hence are more efficiently transmitted by intimate contact , e.g., sexual contact.

Nonenveloped viruses, such as HAV, are quite stable and are transmitted well via the environment, e.g., fecal – oral transmission (Aach et al ., 1991).

2.1.2 Epidemiology :

Hepatitis B is worldwide but particularly prevalent in Asia Globally, more than 300 million people are chronically infected with HBV and about 75% of them are Asians . there is a high incidence of hepatocellular carcinoma (Hepatoma) in many Asian countries; which that indicates that HBV may be a human tumor viron. Immunization against HBV in Taiwan has significantly reduced the incidence of hepatoma in children ; it appears that the HBV vaccine is the first vaccine to prevent a human cancer (Okamoto et al ., 1990)

2.1.3 Pathogenesis and immunity:

After entering the blood, the virus infects hepatocytes , and viral antigens are displayed on the surface of the cells. Cytotoxic T cells mediate an immune attack against the viral antigens , and inflammation and necrosis occur. Immune attack against viral antigens on infected hepatocytes is mediated by cytotoxic T cells. The pathogenesis of hepatitis B is probably the result of this cell – mediated immune injury , because HBV itself does not cause a cytopathic effect, antigen – antibody complexes cause some of the early symptoms, e.g., arthralgias , arthritis, and urticaria and some of the complications in chronic hepatitis , e.g., immune-complex glomerulonephritis, cryoglobulinemia , and vasculitis. About 5% of patients with HBV infection become chronic carriers; in contrast , there is no prolonged, carrier state in patients with HAV infection. A chronic carrier is someone who has HBsAg persisting in their blood for at least 6 months . the chronic carrier state is attributed to a persistent infection of the hepatocytes, which results in the prolonged presence of HBV and HBsAg in the blood, the main determinant of whether a person clears the infection or becomes a chronic carriers is the adequacy of the cytotoxic T-cell response. HBV exists primarilty as an

episome in the cytoplasm of persistently infected cells; a small number of copies of HBV DNA are integrated into cell DNA. A high rate of hepatocellular carcinoma occurs in chronic carriers. The HBV genome has no oncogene and hepatocellular carcinoma appears to be the result of persistent cellular regeneration that attempts to replace the dead hepatocytes . Alternatively. Malignant transformation could be the result of insertional into the hepatocyte DNA. Integration of the HBV DNA could activate a cellular oncogene, leading to a loss of growth control . Chronic carriage is more likely to occur when infection occurs in a newborn than in an adult , probably because a newborn's immune system is less competent than an adult's. Approximately 90% of infected neonates become chronic carriers. Chronic carriage resulting from neonatal infection is associated with a high risk of hepatocellular carcinoma. Lifelong immunity occurs after the natural infection and is mediated by humeral antibody against HBs.Ag. Antibody against HBsAg (HBsAb) is protective because it binds to surface antigen on the virion and prevents it from interacting with receptors on the hepatocyte . (HBsAb is said to neutralize the infectivity of HBV) Note that antibody against the core antigen (HBcAb) is not protective because the core antigen is inside the virion and the antibody cannot interact with it (Murphy et al)

2.1.4 Clinical Findings:

Many HBV infections are asymptomatic and are detected only by the presence of antibody to HBsAg. The mean incubation period for hepatitis B is 10-12 weeks , which is much longer than that of hepatitis A(3-4 weeks) .

The clinical appearance of acute hepatitis B is similar to that of hepatitis However , with hepatits B, symptoms tend to be more severe and life threatening hepatitis can occur. Most carriers are asymptomatic , but some have chronic native hepatitis ,

which can lead to cirrhosis and death, patents co infected with both HBV and HIV may have increased hepatic damage if human immunodeficiency virus (HIV) is treated prior to treating HBV . this occurs because the “immune reconstitution ” that results when HIV is treated successfully leads to increased damage to the hepatocytes by the restored , competent cytotoxic T cells. For this reason , it is suggested that HBV be treated prior to trading HIV (Garson et al ., 1990).

2.1.5 Laboratory Diagnosis :

The most important laboratory test for the detection of early HBV infection is the immunoassay for HBsAg . HBsAg appears during the incubation period and is detectable in most patients during the prodrome and acute disease . it falls to undetectable levels during convalescence in most cases; its prolonged presence (at last 6 months) indicates the carrier state and the risk of chronic hepatitis and hepatic carcinoma . HBsAb is not detectable in the chronic carrier state . Note that HBsAb is , in fact, being made but is not detectable in the laboratory tests because it is bound to the large amount of HBsAg present in the blood. HBsAb is also being made during the acute disease but is similarly undetectable because it is bound in immune complexes (Choo et al ., 1994).

Note : people immunized with HBV vaccine have HBsAb but not HBcAb because the immunogen in the vaccine is purified HBsAg.

Note: that there is a period of several weeks when HBsAg has disappeared but HBsAb is not yet detectable . this is the window phase . At this time, the HBcAb is always positive and can be used to make the diagnosis . HBsAb is present in those with acute infection and chronic infection , as well as in those who recovered from acute infection . the IgM From of HcAg is present in acute infection and disappears approximately 6 month after infection . the test for HBcAg not readily

available ; HBcAg the incubation period and is present during prodome and early cute disease and in certain chronic carriers . its presence indicate a high likelihood of transmissibility and conversely, the finding of HBcAb indicates a lower likelihood , but transmission can still occur . DNA polymerase activity is detectable during the incubation period and early in the disease , but the assay is not available in most clinical laboratories . the detection of viral DNA in serum is strong evidence that infectious virions are present (levinson :2008) .

2.1.6 Treatments

Alpha interferon (intron-A)is clinically useful for the treatment of chronic hepatitis B infections . Some nucleoside analogues, such as lamivudine (Epivir – HBV) , that inhibit the reverse transcriptase of HIV also are effective against the DNA polymerase of HBV . Adefovir (Hepsera) is a nucleotide analogue of adenosine monophosphate that also inhibit the DNA polymerase of HBV. These drugs reduce hepatic inflammation & lower the level of HBV in patients with chronic active hepatitis . Neither interferon nor the nucleoside analogues cure the HBV infection. In most patient when the drug is stopped,HBV replication resumes(Levinson;2008).

2.1.7 Prevention

Prevention involves the use of either the vaccine or hyperimmune globulin or both. the vaccine .e.g.Recombivax.contains HBsAg produced in yeasts by recombinant DNA techniques. The vaccine is highly effective in preventing hepatitis B & has few side effects . It is indicated for people who are frequently exposed to blood or blood products. Such as certain health care personnel (e.g medical student , surgeons & dentists).patients receiving multiple transfusions or dialysis,patients with frequent sexually transmitted disease & abusers of illicit intravenous

drugs.Travelers who plan a long stay in areas of endemic infection. Such as many countries in Asia& Africa , should receive the vaccine.

The U.S.Public Health services recommends that all newborns &adolescents receive the vaccine. At present , booster doses after the initial three –dose regimen are not recommended. Widespread immunization with the HBV vaccine in Taiwan has significantly reduced the incidence of hepatocellular carcinoma in children. A vaccine called Twinrix that contains both HBsAg& inactivated HAV provides production against both hepatitis B & hepatitis A. Hepatitis B immune globulin (HBIG) contains a high titer of HBsAb because it is prepared from sera of patients who have recovered from hepatitis B . It is prepared from sera of patients who have recovered from hepatitis B . It is used to provide immediate, passive protection to individuals known to be exposed to HBsAg-possitive blood , eg, after an accidental needle stick . However; the recommendation regarding one common concern of medical students, the needle-stick injury from a patient with HBsAg-positive blood ,is that both the vaccine & HBIG be given ((at separate site). This is true even if the patient's blood is HBeAb positive.

Both the vaccine & HBIG should also be given to a newborn whose mother is HBsAg-positive. These are good examples of passive-active.

Immunization , in which both immediate and long – term protections are provided (Choo et al ., 1994).

All blood for transfusion should be screened for HBsAg . No one with a history of hepatitis (of any tyoe) should donate blood, because non-A, non-B viruses may be present(Choo et al ., 1994)

2.2 C-reactive protein

2.2.1 Definition :

C-reative protein (CRP) is a protein found in the blood, the levels of which rise in response to inflammation , it is an acute phase protein . its physiological role is to bind to phosphocholine expressed on the surface of dead or dying cells and some type of bacteria, in order to activate the complement system the CIQ complex (Thompson et al ., 1999) CRP is synthesized by the liver in response to factors released by fat cells (adipocyte). It is a member of the pentraxins family of proteins. It is not related to C- peptide or protein C.C- reactive protein was the first pattern recognition recptor (PRR) to be identified (Mantovani et al ., 2008).

2.2.2 History and nomenclature of CRP:

CRP was named because it was first discovered as a substance in the serum of patient with acute inflammation that reacted with the C- (capsular) polysaccharide of pneumococcal.

Discovered by tiller and Francis in 1930, it was initially thought that CRP might be a totemic secretion as it was elevated in people with a variety of illness including cancer;

However discovery of hepatic synthesis demonstrated that it is a native protein (Faraj and Salem , 2012).

2.2.3 Genetic and Biochemistry of CRP:

The CRP is located on the first chromosome (Iq21-q23), CRP is a224-resdue protein with a monomer molar mass of 2516 Da . The is an an annular pentameric disc in shape and a member of the small pentraxins family (faraj and salem ,2012).

2.2.4 Function of CRP

The acute phase response develops in a wide range of acute and chronic inflammatory conditions like bacterial , viral , or fungal infections; rheumatoid and other inflammatory disease ; malignancy; and tissue injures and necrosis . these conditions cause release of interlukin-6 and other cytokines that trigger the synthesis of CRP and fibrinogen by the liver.

During the acute phase response levels of CRP rapidly increase within two hours of acute insult reaching a peak at 48 hours. With resolution of the acute phase response. CRP declines with a relatively short half – life of 18 hours. Measuring CRP level is screen for infectious and inflammatory diseases Rapid and marked increases in CRP occur in inflammation. Infection, trauma, tissue necrosis, malignances and auto immune disorders because there are a large number of disparate conditions that can increase CRP production, and an elevated CRP production, an elevated CRP level dose not diagnose specific disease. An elevated CRP level can provide support for the presence of an inflammatory disease, such as rheumatoid arthritis, polymalgia rheumatic or giant cell arthritis (Pepys and Hirschfield, 2003).

CRP is a member of the class of acute – phase reactants, as its levels rise dramatically during inflammatory of IL-6, which is produce predominantly by macrophages as well as adiposity CRP assist complement binding to foreign and damaged cells and enhances phagocytosis by macrophages (opsonin mediated phagocytosis), which express a receptor for CRP . It is also believed to play another important role in innate immunity , as an curly defense system.

Against infections CRP rises up to 50.000 fold acute inflammation , such as infection vise above normal limit with in hours and peak at 48 hours.

Its half life is constant , and therefore level is mainly determinated by the rate of production and hence severity of precipitating cause – serum amy laid A. is a related acute phase marker that responds rapidly in similar circumstances (perpys and Hirsch fild, 2003)

The physiological role of CRP is to bind to phosphocholine expressed on the surface of dead or dying cells, in order to activate the complement system . CRP binds to phosphocholine on microbes and damaged cells and enhances phagocytosis by macrophages. Thus , CRP participates in the clearance of necrotic and apoptotic cells (Pepys and Hirschfield, 2003).

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2.2.5 Genetic and biochemistry of CRP :

The CRP gene is located on the first chromosome (1q21-q23). CRP is a 224 – residue protein with a monomer molar mass of 25106 Da . the protein is an annular pentameric disc in shape and a member of the small pentraxins family (Faraj and Salem , 2012).

2.2.6 Clinical significance of CRP :

sclerodermas polymyositrs , and dermatomyositis often elicit little or response. CRP level also tend not be devoted in SLE unless sororities or synvitis is present.

Elevation of CRP in the absence of clinically significant inflammation can occur renal failure . CRP level is an independent risk factor for atherosclerotic disease patients with high CRP concentrations are more likely to develop stoke , myocardial infraction and sever peripheral vascular disease (Danesh et al , 2004).

2.2.7 Diagnostic use of CRP:

there are two different tests first CRP the stranded test measures a much wider range of CRP levels but is less sensitive in the lower ranges the high sensitivity CRP (hs-CRP) test can more accurately detect lower concentration of the protein (it is more sensitive) which makes it more useful than the CRP test in predicting a healthy persons risk for cardiovascular disease (Faraj and Salem , 2012) CRP is used mainly as a marker or inflammation Apart from liver failure ; there are few known factors that inter few with CRP production (Pepys and Hirschfield , 2003). Measuring and charting CRP values can prove useful in determining disease progress or the effectiveness of treatments. Blood usually collected in serum – separating tube, is analyzed in a medical laboratory or at the point of care . Various analytical methods are available for CRP determination, such as ELISA, immunoturbidimetry, rapid immunodiffusion, and visual agglutination. A high sensitive CRP (hs- CRP) test measures low levels of CRP using laser nephlometry, the test gives results in 25 minutes with sensitivity down to 0,04 mg/L , immunoturbidimetry (Immunoturbidimetric Method) this reagent is intended for the in vitro quantitative determination of CRP concentration in serum or plasma on automated clinical chemistry analyzers . Normal concentration in healthy human serum is usually lower than 10mg/L , slightly increasing with aging . Higher levels are found in last pregnant women , mild inflammation , and viral infections (10-40)mg/L ., active inflammation, bacterial infection (40-200mg/L), sever bacterial infection and accurate reflection of the acute phase response than the ESR. The half – life of CRP is constant . therefore CRP levels are mainly determined by rate of production. In he first 24 hours ESR may be normal and CRP elevated . CRP returns to normal more keikly than ESR in response to therapy (Clyne and Olshaker , 1999).

2.2.8 Immunity of CRP:

The host response to hepatitis involves various component of the immune system , including T-lymphocyte immune- regulator cytokines which have distinct role in the outcome of the disease. While th1 cytokine profile suggests a cell mediated immunity and is assonated with recovery (Hultgren et al ,1998) .Th2 cytokines Response takes place in development of persistent like long infection. Thus ; it can be stated that imbalance of pro inflammatory th1 and anti inflammatory th2 cytokines production may play an important role in the pathogenesis of viral hepatic infections.

Moreover , chronic inflammatory response involving inflammatory cytokines has been reported to be associated with liver injury . these cytokines recruit inflammatory cells , promote fibro genesis and further activate oxidative brust (Ramadori et al , 2001).

Although it is well known that reaction oxygen species (Ros) induction lies at the center of com of complex net work of cytokines , been through and oncogenes , this net work has not been thoroughly investigated in HBV related liver disease(Ramadori et al ,2001) death each veat (Shepard et al ,2006) (one of the foremost charachteristic . feature of these viral infections is that majority of the total affected population becomes chronically infected i.e life long infections.

The exact mechanism testing in such a high chronic transformation still to be revealed , however , in ability of host immune system to eliminate the organism is considered as an important the organism is considered as an important cause of such persistent form of infection (Modlin et al 1993)

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Response takes place in development of persistent like long infection . this ; it can be stated that imbalance of pro inflammatory Th2 and anti inflammatory Th2 cytokines production many play an important role in the pathogenesis of viral hepatic infections.

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Moreover , chronic inflammatory response involving inflammatory cytokines has been reported be associated with liver injury. These cytokines recruit inflammatory cells, promote fibro genesis and further activate oxidative burst. (Ramadori et al 2001)

Although it is well known that reactive oxygen species (ROS)induction lies at the center of complex net work of cytokines, growth factors and ox genes , this network has not been thoroughly investigated in HBV related liver disease.

Successful outcome of the disease requires a strong virus. Specific cytotoxic response by removing infected hepatocytes and secreting cytokines that inhibit viral replication. While Th1 cytokines (IL-2, IFN- γ , TNF-α) are required for host antiviral immune response and are related with tissue injury, Th2 cytokines (IL4 –IL6-IL10) on the other hand typically regulate humeral . immune response and their rising levels are after associated with persistent infections. (Hutgren, 1998)

Cytokines released by one type of Th population can down regulate the function of another Th population .

Earlier studies have reported a significant decreased Th1 and increased Th2 cytokines level in patients with chronic HBV (Jirillo et al , 1995) (Song et al , 2003) . additionally, recent studies have indicated the involvement of Th_2 cytokines in attributing towards the risk of developing HCC as serum levels of IL10-IL6 were observed to be frequently elevated in patient with HCC (Song et al , 2003).

Another important mediator of host immune defense is CRP which is produced predominantly by the liver in pro inflammatory conditions (Pepys et al, 2003)

Normal baseline levels of circulating CRP is low , but may increase by 10.000. Fold within hours of inflammation (Sharive et al , 1996) . Although the physiological role of CRP is unclear , it is considered as potential predicative biomarker for several pathological conditions (Frank et al , 2004) (Di Napoli , 2005). Recently studies have attempt patients to correlate the serum levels of CRP as tumor marker in HCC patients (Lin et al, 2000) (Nagaoka et al , 2000).

2.3 Circulating biomarker and their possible role in pathogenesis of chronic hepatitis infections:

- The evaluated the plausible role of circulating biomarkers immune pathogenesis of chronic hepatitis considered apriority in clinical hematology. total viral loud of chronic hepatitis B virus (HBV)patients was quantified and correlation studies were performed with circulating levels of Th1/Th2 cytokines ; creative protein and circulating nucleosomes ; glutathione reducase (GR) and superoxide dismutase-. the study is first among its kind that validates strong positive correlation of viral load with 1L.4 , 1L6 , GR in HBV however , role of these biomarkers with potential diagnostic or prognostic significance might be helpful in clinical assessment of high risk individuals , thereby , designing interventral strategies , towards development of personalized Medicare . the result of study also offer valuable insights of immune signaling mediators engaged in development of hepatocelluter carcinoma (S.Khan et al ,2011).
- one of the foremost characteristic feature of these viral in feetion is that majority of the total affected population becomes chronically indected i.e life loing infecrions. The exact mechanism tesulting in such a high chronic transformation still to be revealed , however , inability of host immune system to eliminate true organism is considered as an important cause of such persistent form of infection (Modlin et al , 1993).

3-Material and Methods

3.1 Study Design

This is Case control study

3.2 Study area and study period

This study was carried out in Saba clinic in Khartoum state conducted during the period of march to June 2015.

3.3 Study population

sixty hepatitis B patients as test group with fifty healthy subjects as control group were enrolled in this study.

3.4 Inclusion Criteria

All the patients with hepatitis B positive.

3.5 Exclusion criteria

Patients with inflammatory disease like rheumatoid arthritis (RA) osteoarthritis (OA) , hypertensive , systemic lupus erythromtosus (SLE) , autoimmune disease , tuberculosis , diabetes ,stroke , renal disease were excluded from this study .

3.6 Collection of sample

After informed consent and use local antiseptic for skin (70%) ethanol .Sample were collected by using dry , disposable plastic syringe , tourniquet was used to make the vein more prominent , blood sample (3 ml) was collected in heparin

containers from each volunteer under septic condition. All blood samples were centrifuged at 4000 rpm to obtain plasma samples and analysis.

3.7 Ethical considerations

Study was approved from ethical Committee of the Sudan University of Science and technology , verbal informed consent was obtained and all patients were informed by aims of the study .

3.8 Quality control

The precision and accuracy of all methods used in this study were checked each time a batch was analyzed by including commercially prepared control sera.

3.9 Statistical analysis

The data was recorded and analyzing using statistical package for social science (SPSS-version 17) on programmed computer.

The mean and standard deviation of variables were calculated test group and control group and P.value for comparison was obtained $\leq$ 0.05 was considered significant.

3.10 Biochemical Measurement

1. Intended use

Nyco Card CRP single test is in vitro test for the repaid determination of CRP (C-reactive protein) in human serum , plasma and blood .

2. Test principle

NycoCard CRP single test is a solid phase , sandwich-format , immunometric assay . in the test well of the device there is a membrane coat with immobilized CRP-specific monoclonal antibodies .

A diluted sample was applied to the test device. When the sample flows through the membrane , the C-reactive protein are captured by the antibodies .CRP trapped on the membrane will then bind the gold –antibodies conjugate added , in a sandwich –type reaction .unbound conjugate is removed by the washing solution .A paper layer underneath the membrane absorbed excess liquid . In the presence of a pathological level of CRP in the sample , the membrane appears red-brown with color intensity proportional to the CRP concentration of the sample . the color intensity is measured quantitatively with the NycoCard READER 11 . (see appendix)

3. Materials required

(Not supplied with the kit)

-Pipette (50 ML) and pipette tips

-Capillary tube holder

-NycoCard READER11

-The C+/Control positive is produced from collected blood collected from voluntary blood donors to Scandinavian blood banks . each units is separately controlled and found negative for hepatitis B antigen , hepatitis C , antibodies and HIV 1&11 antibodies . However ,universal precautions (treating all human source material as if they were potentially infectious) should be exercised .

3.11 Test Characteristics

1. Analytical specificity

Monoclonal antibodies specific to human CRP are used in the test . No other human blood component are found to cross react with CRP in the NycoCard CRP single test system .

2. Precision

In professional use, a coefficient of variation (CV) of` less than 7% IS usually obtained.

3.12 Internal quality control

The C+/control positive should be used to confirm the efficacy of the reagent and correct performance of the test .test this control according to the same procedure as for a patient sample .

Note ; Use the CRP Serum/plasma menu for reading the result with NycoCard READER11. The measured value should be within the acceptance limits stated on the vial labeled.

3.13Test procedure

1-Dilute sample

Fill a 5 ML with patient sample op C+/control positive to TD/test device , And drop the capillary into the tube with R1/Dilution liquid .Close the tube and mix well for 10second

2-Apply sample

Apply 50MLdiluted sample or diluted C+/control positive to TD/test device .Allow the sample to soak into the membrane (approx .30second).

Note : Avoid air bubbles on the membrane .Do not touch the membrane with the pipette tip .

3-Apply R2/Conjugate

Apply one drop R2/Conjugate to the TD/Test Device .Allow the reagent to soak into the membrane (approx 30 seconds) .

Note : The droplet bottle should be held vertically , about 1cm above the membrane

4-Apply R3/Washing solution

Apply one drop R3/washing solution to the TD/test device. Allow the reagent to soak into the membrane (approx 20 second).

Note : The droplet bottle should be held vertically , about 1cm above the membrane .

5-Read the result

Read the result within 5 minutes using the NycoCard READER11 .follow the READER11 user instruction manual .

Note :Use the CRP menu for reading whole blood samples , and the CRP serum /plasma menu for reading serum or plasma and the c+/control positive.

3.14 Interpretation of Results

Interpret the NycoCard CRP test results with careful consideration to the patient's medical history, clinical examinations and other laboratory results. If the test result is questionable or if clinical signs and symptoms appear inconsistent with the test result , re-test the sample or confirm the result using another method .Analyze control materials frequently to verify the performance of the N ycoCard *READER II test system.

4. Result

This study was carried out in clinic (Saba clinic) in Khartoum state conducted during the period of March to June 2015.

Table (1) Showed the mean plasma level of CRP was significantly increased in case group with (p.value 0.001).

Table (2)Showed The mean plasma level of CRP was significantly increased in hepatitis B virus (case group) classified based on duration of disease (p.value 0.001).

Figure (1) Showed mean of hs CRP mg/L level in study group (test group and control group) .

Figure : (2) Showed mean of hs CRP mg/L level in study group a classified based on duration of disease (acute 30% – chronic 30% with p.value)

Figure (3) Showed negative correlation between CRP level and acute duration of HbsAg virus disease, (r : -0.937 , p.value: 0.015)

Figure (4) :Showed significant moderate correlation between CRP level and chronic duration of Hbs Ag virus disease, (r : 0.554 , p.value: 0.113).

Table(1) Showed comparison between mean of hs CRP mg/L level in study group .

Variable	Test group	Control group	P.value
CRP mg/L (M±SD)	29.4±26.0	6.9±1.5	0.001

P-value ≤ 0.05 is considered significant

Table (2) Showed comparison between mean of hs CRP mg/L level in study group a classified based on duration of disease (acute – chronic with p.value)

Variable	Chronic	Acute	P.value
CRP mg/L (M±SD)	49.6 ± 22.9	9.17 ± 2.99	0.001

P-value ≤ 0.05 is considered significant

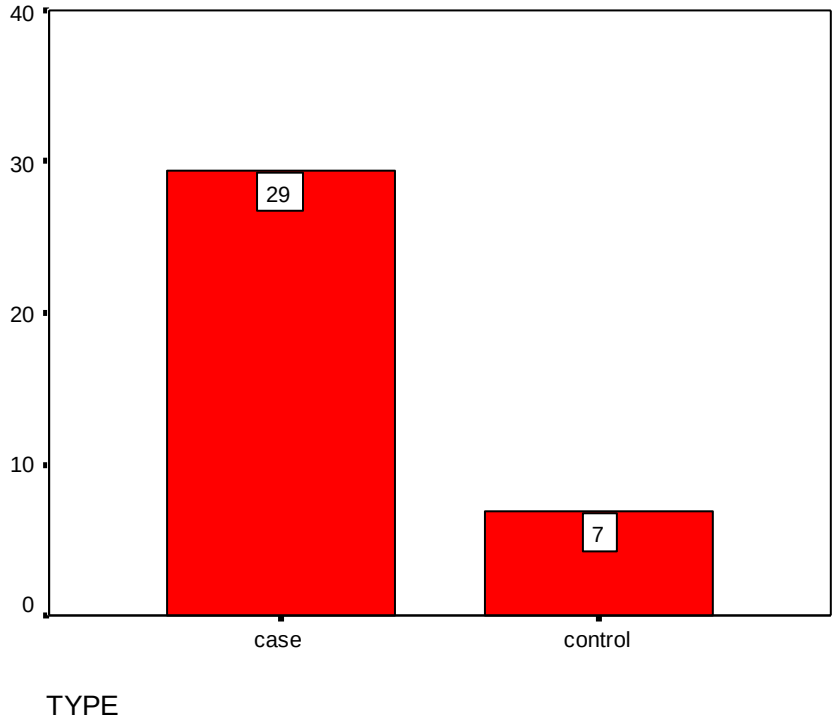

Figure (1) Showed mean of hs CRP mg/L level in study group (test group and control group)

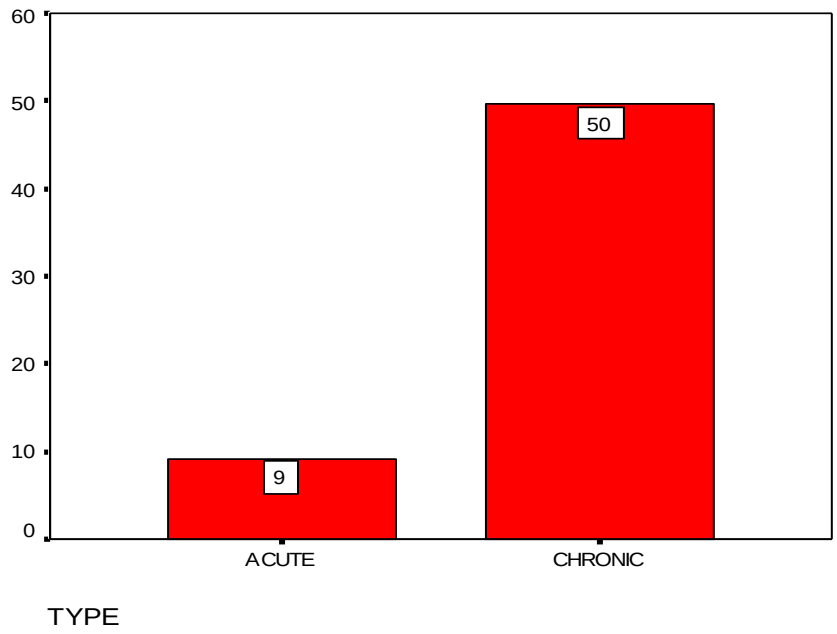

Figure : (2) Showed mean of hs CRP mg/L level in study group a classified based on duration of disease .

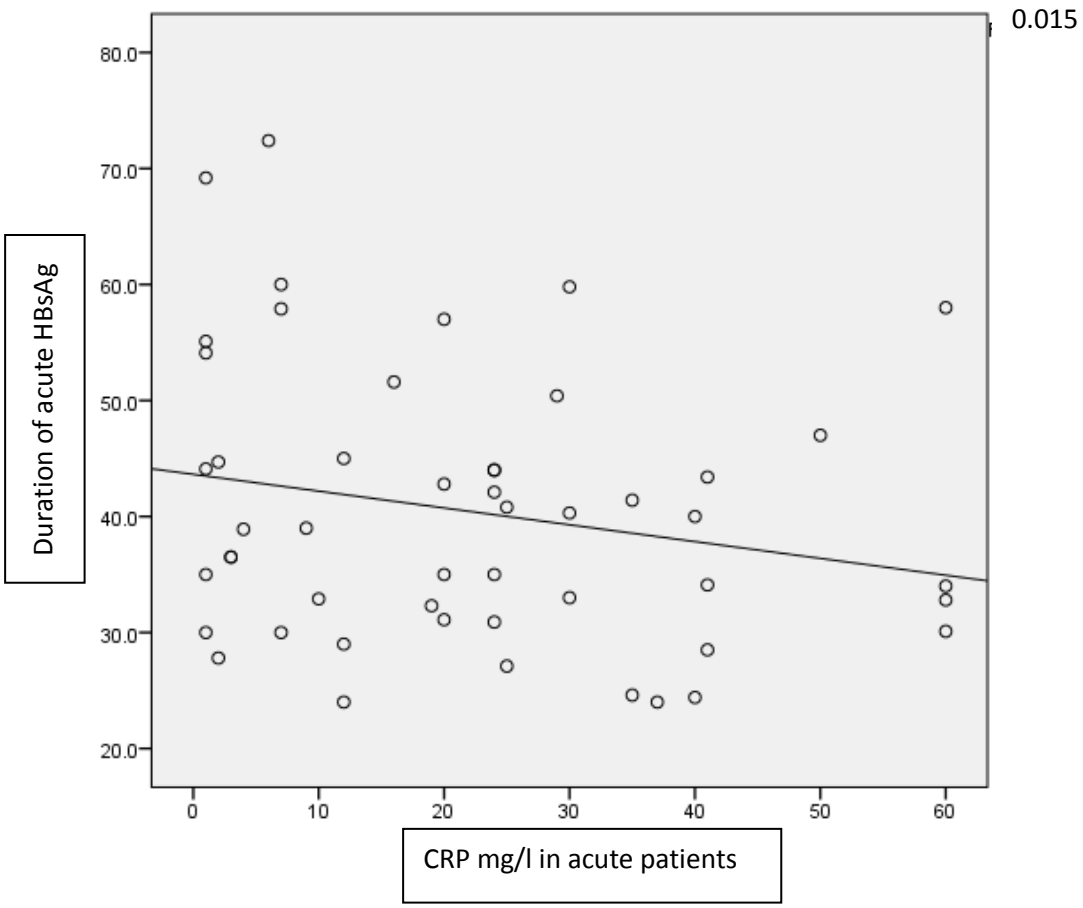

Figure (3) Scatter plot show strong negative correlation study between CRP mg/l level in acuteHbs Ag patients (r :- 0.937 , p.value: 0.015).

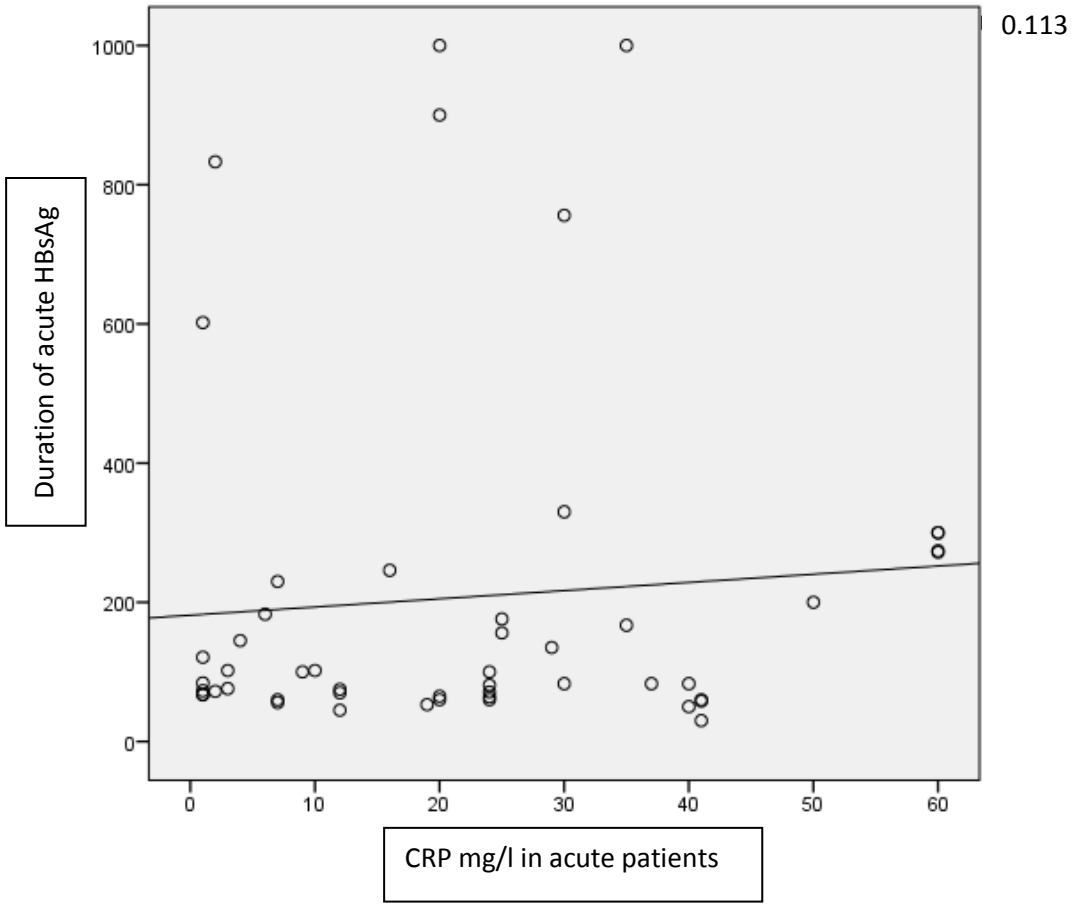

Figure (4) : scatter plot show moderate positive correlation study between CRP mg/l level in chronic HbsAg patients (r: 0.554 , p.value: 0.113)

5. Discussion, Conclusion and Recommendation

5.1 Discussion

Nowadays hepatitis B virus (ABV) is one of the important cause of death all over the world specially in Asia and Africa , because of its infects hepatocytes and viral antigens are displayed on the surface of the cells and CRP is one the important mediator of host immune defense which produced predominantly by the liver in inflammatory conditions. Many studies have shown the elevated level of CRP in hepatitis B patients (Sharive et al , 1996). there fore this descriptive cross sectional

study was carried out to study hs CRP levels of hepatitis B in sudanese patients in Khartoum state during the period of April to July 2015 , in addition to compare between CRP level cases (Acute and chronic).

Analysis frequency revealed that hs CRP elevated more common in case (45.5%) and the result showed significant increase in mean concentration of hs CRP in case compared to control with (P.value 0.001) , the possible justification for our result is the case susceptible to Hepatitis B virus and control.

Our finding was agreed with previous study performed in Korea , that significant elevated or increasing mean CRP case (66.3%) than control (79%) .Also agreed with study performed in Ruche that significantly increased in the hepatitis B virus patients group compared to control group (p.value 0.001) (Kostic et al , 2008).

The present study showed significant moderate positive correlation between CRP level and duration of disease (chronic HbAg), (p. value : 0.554 , r: 0.113) , and showed

strong negative correlation between CRP level and acute HbAg (p. value :- 0.937, r: 0.015). a similar observation was made by previous study as mentioned that elevated level of CRP in chronic patients showed significant association between duration of HB virus disease and CRP level (Shrive et al, 1996). Any patient have high CRP with bad prognosis and prolonged duration of disease with high CRP indicate bad prognosis of hepatitis. The study notes all the patients with short duration of disease low CRP that means CRP affect by duration.

Finally, the present study showed significant increase in mean CRP level in chronic hepatitis B patients compared to acute hepatitis B virus (p.value 0.00) .

This finding confirm association hs CRP level with age of infection (duration period). The production of CRP one of host immune produced predominantly by the liver regulated by cytokines principally interleukin (IL_6 - IL_{10} - IL_4 - IL_1 TNF α) although several alteration in cytokines have been found in patients with chronic hepatitis.

Our result were confirmed by previous study in Japan found that hs CRP level is higher in chronic hepatitis B than acute hepatitis B patients, thus plasma CRP concentration significantly increase in mean of chronic compared with acute.

5.2 Conclusion

In conclusion CRP as useful for hepatitis B patients, since they tend to have higher CRP level compared with control group. The Study conducted there is a significant increase in mean of CRP level in chronic hepatitis B patients compared with acute. There was weak positive correlation between CRP level and chronic Hb Ag virus disease, and negative correlation between CRP level and acute Hb Ag virus disease

5.3 Recommendation

1- routine test of CRP in patients with Hbs virus highly recommended.
2- Vaccination against Hb virus usually needed to reduce the incidence of infection.
3- Further studies are neededto underlying the mechanism of association between CRP and duration period of hepatitis B virus e.x IL6 -IL10 – IL4 – TNF-α – SOD (superoxide dismutase) .
4- Research study design should be performed cohort study to associate between CRP level and progression of disease in chronic hepatitis B patients.

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