

Introduction and Literature Review

1.1 Introduction

Healthcare personnel (HCP) are defined as persons (employees, students, contractors, attending clinicians, public-safety workers, or volunteers) whose activities involve contact with patients or with blood or other body fluids from patients in a health-care, laboratory, or public-safety setting. The potential exists for blood and body fluid exposure to other workers, and the same principles of exposure management could be applied to other settings. An exposure that might place HCP at risk for HBV, HCV, or HIV infection is defined as a percutaneous injury (a needles tick or cut with a sharp object) or contact of mucous membrane or no intact skin (exposed skin that is chapped, abraded, or afflicted with dermatitis) with blood, tissue, or other body fluids that are potentially infectious. In addition to blood and body fluids containing visible blood, semen and vaginal secretions also are considered potentially infectious. Although semen and vaginal secretions have been implicated in the sexual transmission of HBV, HCV, and HIV, they have not been implicated in occupational transmission from patients to HCP. The following fluids also are considered potentially infectious: cerebrospinal fluid, synovial fluid, pleural fluid, peritoneal fluid, pericardial fluid, and amniotic fluid. The risk for transmission of HBV, HCV, and HIV infection from these fluids is unknown; the potential risk to HCP from occupational exposures has not been assessed by epidemiologic studies in health-care settings (Miller et al , 2001).

A blood-borne disease is one that can be spread through contamination by blood and other body fluid. Since it is difficult to determine what pathogens any given blood contains (Jason , 2014).

The pathogens of primary concern are the human immunodeficiency virus (HIV) and hepatitis B virus (HBV). Workers and employers are urged to take advantage of available work practices to prevent exposure to blood and other body fluids. (Miller, et al, 2001)

AIDS or acquired immune deficiency syndrome, is caused by a virus called the human immunodeficiency virus, or HIV. Once a person has been infected with HIV, it may be many years before AIDS actually develops. HIV attacks the body's immune system, weakening it so that it cannot fight other deadly diseases. AIDS is a fatal disease, and while treatment for it is improving, there is no known cure. The HIV virus is very fragile and will not survive very long outside of the human body. It is primarily of concern to custodians, healthcare professionals, first aid responders, and researchers working with human blood or other potentially infectious materials (OPIM). HIV is a devastating disease; therefore, all precautions must be taken to avoid exposure (Miller et al, 2001). HBV infection is a well recognized occupational risk for HCP. The risk of HBV infection is primarily related to the degree of contact with blood in the work place. "Hepatitis" means "inflammation of the liver," and, as its name implies, Hepatitis B is a virus that infects the liver. While there are several different types of Hepatitis, Hepatitis B is transmitted primarily through blood contact. HBV initially causes inflammation of the liver, but it can lead to more serious conditions such as cirrhosis and liver cancer. The Hepatitis B virus is very durable, and it can survive in dried blood for over seven days. For this reason, HBV is a major concern for employees such as custodians, laundry personnel and other employees who may come in contact with blood or potentially infectious (Miller et al, 2001)

1.2 Literature Review:

1.2.1 Hematology:

Hematology is the branch of medicine concerning the study of blood, the blood-forming organs, and blood diseases. Hematology tests include laboratory assessments of blood formation and blood disorders.

Hematology is practised by specialists in the field who deal with the diagnosis, treatment and overall management of people with blood disorders ranging from anemia to blood cancer.

1.2.1.1 Some examples of hamatology tests :

- CBC : complete blood count - A count of the total number of red blood cells(RBC), white blood cells (WBC) and platelets present in blood.
- Blood film - Blood is smeared over a glass slide that is stained with specific dyes and viewed under a microscope. The number, shape and size of blood cells and the presence of any abnormal cells or immature cells are noted. Staining may flag up abnormally shaped red blood cells such as sickle cells or spherocytes.
- Blood indices : MCV , MCH , RDW ,MPV and PDW .
- Staining may also detect blood parasites such as malaria, toxoplasmosis, and microfiliariasis.
- Assessment and staining of immature platelets or megakaryocytes may also be performed.
- Assessment of granulocytosis.
- The erythrocyte sedimentation rate (ESR) may be tested.
- The bone marrow may be examined.

- Iron status and anemias are assessed using tests such as serum ferritin, vitamin B12 and folate levels.
- The Coombs' test or antiglobulin test may be used for blood typing and blood matching prior to blood transfusion, for example.
- Platelet function in bleeding and coagulation may be checked using a test called prothrombin time.
- D-dimer assessment may be performed to check for thrombotic disorders.
- Electrophoresis may be used to examine proteins in the blood such as hemoglobin and to check for hemoglobinopathies such as thalassemia or sickle cell anemia.
- The enzyme G6PD may be assessed in sickle cell disease.
- Fine-needle aspiration (FNA) of lymph nodes and tumors.
- Examination of spleen biopsy.
- Immunocytochemical techniques for detecting antigens both inside and on the surface of cells.
- Assessment of chronic myeloproliferative disorders using bone marrow and blood examination.
- Karyotyping to look for chromosomal disorders and abnormalities, (Ananya ,2013).

1.2.2 Laboratory worker safety :

Persons working in clinical diagnostic laboratories are exposed to many risks. Whether laboratorians work in haematology lab or elsewhere in the laboratory, the human diagnostic laboratory is a challenging environment. The more that laboratorians become aware of and adhere to recommended, science-based safety precautions, the lower the risk.

The goal of a safety is to lower the risk to as close as possible to zero, although zero risk is as yet unattainable as long as patient specimens and live organisms are manipulated.

Protection of laboratorians, coworkers, patients, families, and the environment is the greatest safety concern.

In the hematology laboratory, the major causes of injuries are likely to be exposure to blood and body fluids; needle sticks, aerosols from centrifuge or removal of tube stoppers, tube breakage; or contaminated gloves.

The primary mistake may be assuming that a given specimen contains no infectious agents and then working with little attention to risk for infection. (Miller *et al*,2012).

This scenario can be particularly problematic in laboratories developing new technologies, such as molecular and biochemical technologies, and in point-of-care diagnostics performed by staff unaccustomed to testing that requires biosafety considerations and use of barrier techniques such as personal protective equipment. (Miller *et al*,2012).

no national surveillance system is in place to which medical laboratory exposures and subsequent work-related infections are reported. Increased attention has been focused on laboratory biosafety but has been largely limited to precautions required for agents of bioterrorism. (Miller *et al*, 2012).

1.2.3 A Culture of Safety:

The concept of a "culture of safety, "as describe in these research, encourages all diagnostic laboratories to promote an organizational culture of systematic assessment of all work processes and procedures to identify associated risks and implement plans to mitigate those risks.

In addition to the often unknown biohazard risk associated with handling diagnostic specimens, each section of the diagnostic laboratory has procedures and processes for handling known infectious agents that convey excessive risk for exposure and possible infection and/or occupational injury. These risks typically are associated with design flaws or lack of or inadequacy of safety procedures and training. (Miller *et al*,2012).

In addition, the day-to-day operations of diagnostic laboratory differ markedly from those of an academic or research laboratory and require different biosafety guidelines; these differences prompted the focus of this research on medical laboratory communities, their occupational risks, potential for exposure, and opportunities to mitigate those risks. (Miller *et al*,2012).

Successful establishment of a culture of safety requires that laboratory safety become an integral and apparent priority to the organization, embraced first and foremost by top management and with the concomitant infrastructure support required to foster safe behaviors among its employees. As required by the Clinical Laboratory Improvement Amendments, a laboratory director needs to assume the responsibility for

- establishing and enforcing a policy for a culture of safety within the laboratory;
- identifying as many hazards as possible and specifying practices and procedures that will minimize or eliminate those hazards;
- ensuring that all personnel are instructed in and engaged in performing risk assessments and demonstrating that they can identify laboratory hazards in their individual work environments;

- ensuring that all personnel are trained and competent in the standard practices and techniques that minimize identified workplace hazards.

providing an avenue for personnel to identify hazards and to present risk-mitigation strategies to management; and educating clinicians and nurses about safe specimen procurement and transport to ensure their safety and that of the laboratory personnel who receive the clinical samples. (Miller *et al*,2012).

1.2.4 Routes of Laboratory Infection:

The four most predominant routes are

- parenteral inoculations with syringe needles or other contaminated sharps.
 - spills and splashes onto skin and mucous membranes.
 - ingestion or exposure through mouth pipetting or touching mouth or eyes with fingers or contaminated objects.
 - animal bites and scratches inhalation of infectious aerosols.
- (Miller *et al*,2012).

1.2.5 Blood borne pathogens (BBPs)

BBPS are microorganisms such as viruses or bacteria that are carried in human blood and can cause disease in people. There are many different blood borne pathogens including malaria, syphilis, and brucellosis, but Hepatitis B (HBV), Hepatitis C (HCV), and the Human Immunodeficiency Virus (HIV) are the three diseases specifically addressed by the blood borne pathogens standard. People suffering from these infections may have the agent of disease present In their blood. In some cases the organisms persist in the blood for long periods and in sufficient numbers to represent a high risk of transmission. If others are exposed to their blood - or other bodily fluids - the infectious

agent may be transferred into their bodies and infect them. The main risk of occupationally acquired blood borne infection relates to viruses that persist in the blood . In these cases, the infectious agent is usually a blood-borne virus (BBV). The individual infected with the virus may not show symptoms or even be aware that they are carrying it.

Body fluids that may contain BBVs

- Blood
- Cerebrospinal fluid
- Pleural fluid
- Breast milk
- Amniotic fluid
- Vaginal secretions blood
- Peritoneal fluid
- Pericardial fluid
- Synovial fluid
- Semen
- Other bodily fluids containing

Urine, faeces, saliva, sputum, tears, sweat and vomit, present a minimal risk of blood-borne virus infection unless they are contaminated with blood. However, they may be hazardous for other reasons.

BBVs of major concern are the human immunodeficiency virus (HIV) which causes Acquired Immune Deficiency Syndrome or AIDS), and Hepatitis B and C, which may result in chronic infection. These viruses represent a significant risk of blood-borne transmission.

Viruses of major concern are the human immunodeficiency virus (HIV, which causes Acquired Immune Deficiency Syndrome or AIDS .There are other causes of viral hepatitis. Hepatitis A and E are mainly spread by the faecal-oral route, do not result in chronic infection and hence do not present a significant risk of blood-borne infection. The Hepatitis D virus is a defective virus, which can only infect and replicate in the presence of Hepatitis B virus. I will therefore concentrate only on HIV, hepatitis B viruses (Aberg , 2009)

1.2.6 Universal Precautions:

Universal Precautions is Occupational Safety and Health Administration OSHA's required method of control to protect employees from exposure to all human blood. The term, "Universal Precautions," refers to a concept of blood borne disease control which requires that all human blood and certain human body fluids be treated as if known to be infectious for HIV, HBV or other blood borne pathogens. (Aberg , 2009)

The Occupational Safety and Health Administration requires a written Exposure Control Plan. The purpose of this plan is to insure that all medical personnel are provided with a safe workplace and are made aware of potential workplace hazards resulting from exposure to blood and other potentially infectious materials. (Aberg , 2009)

1.2.7 Engineering controls:

Refers to controls (sharps disposal containers, self-sheathing needles, safer medical devices, such as sharps with engineered sharps injury protections and needleless systems) that isolate or remove the blood borne pathogens hazard from the work. (Aberg , 2009)

1.2.8 Transfusion-Transmitted Diseases:

A Transfusion transmitted infection (TTI) is a virus, parasite, or other potential pathogen that can be transmitted in donated blood through a transfusion to a recipient. Many infectious agents are transmitted through transfusion of infected blood; these include hepatitis B virus, hepatitis C virus, human immunodeficiency viruses 1 and 2, human T-cell lymphotropic viruses (HTLV-I and II), cytomegalovirus, parvovirus B19, West Nile virus , dengue virus, trypanosomiasis, malaria .these also can be infectious for laboratory worker (Nordgrist , 2014).

1.2.9 AIDS (Acquired immune deficiency syndrome or acquired immunodeficiency syndrome):

Is a disease caused by a virus called HIV (Human Immunodeficiency Virus). The illness alters the immune system, making people much more vulnerable to infections and diseases. This susceptibility worsens as the disease progresses. HIV is found in the body fluids of an infected person (semen and vaginal fluids, blood and breast milk). The virus is passed from one person to another through blood-to-blood and sexual contact. In addition, infected pregnant women can pass HIV to their babies during pregnancy, delivering the baby during childbirth, and through breast feeding.

HIV can be transmitted in many ways, such as vaginal, oral sex, anal sex, blood transfusion, and contaminated hypodermic needles.

Both the virus and the disease are often referred to together as HIV/AIDS. People with HIV have what is called HIV infection. As a result, some will then develop AIDS. The development of numerous opportunistic infections in an AIDS patient can ultimately lead to death.

According to research, the origins of HIV date back to the late nineteenth or early twentieth century in west-central Africa. AIDS and its cause were first identified and recognized in the early 1980s.

There is currently no cure for HIV/AIDS. Treatments can slow the course of the disease - some infected people can live a long and relatively healthy life (Nordgrist , 2014).

1.2.9.1 Human Immunodeficiency Viruses (HIV-1 and HIV-2):

There are two types of human immunodeficiency virus, HIV-1 and HIV-2. HIV- 1 is responsible for the large majority of global HIV

infections and cases of AIDS, whilst the relatively less common HIV-2 is mainly restricted to West Africa.

HIV-1 and HIV-2 are very similar in almost every respect, although Accumulating evidence indicates that progression of disease is slower in HIV-2 infection. Unless specifically highlighted, the properties of these viruses are presented under the generic term 'HIV'. (Nordgrist , 2014).

1.2.9.2 HIV can be transmitted through:

- Sexual transmission. It can happen when there is contact with infected sexual secretions (rectal, genital or oral mucous membranes). This can happen while having unprotected sex, including vaginal, oral and anal sex or sharing sex toys with someone infected with HIV.
- Prenatal transmission. The mother can pass the infection on to her child during childbirth, pregnancy, and also through breastfeeding.
- Blood transmission. The risk of transmitting HIV through blood transfusion is nowadays extremely low in developed countries, thanks to meticulous screening and precautions. Among drug users, sharing and reusing syringes contaminated with HIV-infected blood is extremely hazardous.

Individuals who give and receive tattoos and piercings are also at risk and should be very careful. (Nordgrist , 2014).

1.2.9.3 The virus cannot be transmitted from:

Shaking hands, hugging, casual kissing, sneezing, touching unbroken skin, using the same toilet, sharing towels, sharing cutlery, mouth-to-

mouth resuscitation or other forms of "casual contact" (Nordgrist , 2014).

1.2.9.4 HIV blood test:

Diagnosis is made through a blood test that screens specifically for the virus. If the HIV virus has been found, the test result is "positive". The blood is re-tested several times before a positive result is given to the patient. For those whose tests came back positive, they will be asked to undergo some other tests to see how the infection has progressed, and also to decide when to start treatment. If a person has been exposed to the virus, it is crucial that they get tested as soon as possible. The earlier HIV is detected, the more likely the treatment will be successful. Also, precautions can be taken to prevent the virus from spreading to other people. After infection with HIV, it can take up from three weeks to three months for the virus to show up in testing. Re-testing may be necessary.

If a patient's most at risk moment of becoming HIV infected was within the last three months, he/she can have the test immediately. However, a good doctor will urge (Nordgrist , 2014).

1.2.9.4.1 Antibody tests-window period:

From 6 weeks after exposure (i.e. rapid, Tri-Dot and Centaur tests). The most common test for HIV is an antibody test. These tests for a person's immune response to having come into contact with HIV. An antibody is a protein produced by your body when it recognizes an infection. The main antibody test is called ELISA (Enzyme-Linked Immunosorbent Assay). If blood tested is from a finger prick, then 'rapid' tests can give the results in 10-30 minutes. If the blood is taken with a syringe, it is usually then sent to a lab, and results can take

anything from a few hours to a few weeks, depending on the urgency of the sample, and procedures of that clinic. (collins , 2011).

If this result is negative or non-reactive, then you are HIV-negative.

If the result is positive though, this does not mean that you are HIV-positive. A small percentage of people can test positive with ELISA who are not HIV-positive (called a ‘false-positive’ result).

All positive results need to be confirmed by a second, more sensitive antibody test called Western Blot. The Western Blot test takes longer (usually a week or so) and is the most accurate at identifying genuine positive results. The Western Blot test used to confirm a positive result is 100% accurate. (collins , 2011).

1.2.9.4.2 p24 antigen tests and combined antibody/antigen tests window period:

3–4 weeks after exposure .Some HIV tests combine an antibody (Ab) test with an antigen (Ag) test.An antigen is a name for genetic material caused by a virus or other infection. In these tests the antigen being tested is p24 (protein 24), a major protein that is part of HIV.p24 antigen tests can be used 3–4 weeks after exposure – so can give an earlier result than an antibody test. There are combined with antigen tests because the window period that p24 is detected is very short. (collins , 2011).

1.2.9.4.3 Viral load tests (PCR)–window period:

1–4 weeks after exposure Viral load tests are also called PCR (Polymerase Chain Reaction) tests. They either test for HIV RNA or HIV DNA. Rather than look for an immune response, they look directly for HIV, usually in a blood sample. In a new infection, viral load can reach very high levels within a few weeks of infection. More rarely this can be within if few days.(collins , 2011).

1.2.9.4.4 HIV Screening Test after Diagnosis:

While being treated for HIV, your doctor will perform several tests to monitor your health, determine when you need to start treatment, and check how well treatment is working, the main test is :

1.2.9.4.4.1 CD4 count:

CD4 is a protein that lives on the surface of infection-fighting white blood cells called T-helper cells. HIV targets these immune cells. To monitor the health of immune system, doctor will check CD4 count - the number of CD4 cells in a sample of blood. CD4 count should be tested every three to six months during treatment. (Johnson , 2013)

1.2.10 Hepatitis B:

Hepatitis B is a viral infection that attacks the liver and can cause both acute and chronic disease. The virus is transmitted through contact with the blood or other body fluids of an infected person. More than 780 000 people die every year due to the consequences of hepatitis B. Hepatitis B is an important occupational hazard for health workers. Hepatitis B is preventable with the currently available safe and effective vaccine. Hepatitis B is a potentially life-threatening liver infection caused by the hepatitis B virus. It is a major global health problem. It can cause chronic liver disease and chronic infection and puts people at high risk of death from cirrhosis of the liver and liver cancer. A vaccine against hepatitis B has been available since 1982. Hepatitis B vaccine is 95% effective in preventing infection and its chronic consequences, and was the first vaccine against a major human cancer (WHO , 2014)

1.2.10.1 Transmission:

In highly endemic areas, HBV is most commonly spread from mother to child at birth, or from person to person in early childhood. Perinatal or early childhood transmission may also account for more than one third of chronic infections in areas of low endemicity, although in those settings, sexual transmission and the use of contaminated needles, especially among injecting drug users, are the major routes of infection. The hepatitis B virus can survive outside the body for at least 7 days. During this time, the virus can still cause infection if it enters the body of a person who is not protected by the vaccine. The hepatitis B virus is not spread by contaminated food or water, and cannot be spread casually in the workplace. The incubation period of the hepatitis B virus is 75 days on average, but can vary from 30 to 180 days. The virus may be detected 30 to 60 days after infection and persists for variable periods of time (WHO , 2014).

1.2.10.2 Diagnosis:

It is not possible, on clinical grounds, to differentiate hepatitis B from hepatitis caused by other viral agents and, hence, laboratory confirmation of the diagnosis is essential. A number of blood tests are available to diagnose and monitor people with hepatitis B. They can be used to distinguish acute and chronic infections. Laboratory diagnosis of hepatitis B infection focuses on the detection of the hepatitis B surface antigen HBsAg by the same technique which mentioned above in the diagnosis of HIV. Acute HBV infection is characterized by the presence of HBsAg and immunoglobulin M (IgM) antibody to the core antigen, HBcAg. During the initial phase of infection, patients are also seropositive for HBeAg. Chronic infection is characterized by the persistence (>6 months) of HBsAg (with or without concurrent

HBsAg). Persistence of HBsAg is the principal marker of risk for developing chronic liver disease and hepatocellular carcinoma (HCC) later in life.

The presence of HBeAg indicates that the blood and body fluids of the infected individual are highly contagious (WHO , 2014).

1.3 Rational

- Clinical laboratory personnel, like all healthcare workers, have a high risk of occupational exposure to blood-borne infections from clinical specimens, more so in developing countries, where unsafe practices are common and the dealing with the sample as if it is safe and the careless of using simple safety procedures and personal protective equipments without taking an infectious probability of the sample . The most common and important ones are HIV ,HBV infections.
- Since the beginning of the epidemic studies , almost 78 million people over the world have been infected with the HIV virus and about 39 million people have died of HIV. In sudan 2014 , the Number of people living with HIV 53,000 . Adults aged 15 to 49 prevalence rate 0.2%, Adults aged 15 and up living with HIV, 49,000 , Women aged 15 and up living with HIV 23,000, Children aged 0 to 14 living with HIV4,300 . Deaths due to AIDS 2,900 .
- Hepatitis B is a serious and common infectious disease of the liver, affecting millions of people throughout the world. More than 2, 000 million people alive today have been infected with HBV at some time in their lives . Sudan is classified among the countries with high hepatitis B virus seroprevalence . Exposure to the virus varied from 47%–78%, with a hepatitis B surface antigen prevalence ranging from 6.8% in central Sudan to 26% in southern Sudan. Hepatitis B virus was the commonest cause of chronic liver disease and hepatocellular carcinoma and was the second commonest cause of acute liver failure in the Sudan.

1.4 Objectives:

1.4.1 General objective:

- To evaluate the frequency of HIV and HBV among patients referred to routine Hematology Laboratory in al Khartoum Teaching Hospital .

1.4.2 Specific objectives

- To estimate the frequency of HIV and HBV.
- TO associate between HIV and HBV and history of blood transfusion.
- To associate between HBV with history of vaccination against HBV.
- TO determine the frequency of HIV and HBV according to gender.

2. Materials and Method

2.1 Study design:

This is across sectional study conducted in Khartoum State in patients attending Khartoum Teaching Hospital during (June 2014).

2.2 Study population:

Patients referred routine Haematology laboratory . Sample size is ninety (n=90) blood specimens, thirty seven male and fifty three female .

2.3 Inclusion criteria :

Samples requested for complete blood count (CBC) test was included within the study .

2.4 Exclusion criteria :

Sample requested for HIV and HBV test , and patients who complain of liver disease were exeluted from the study.

2.5 Data collection :

Data were collected using questionnaire which was specifically designed to obtain information including age, sex , gender, medical history ,history of blood transfusion , HBV vaccination .

2.6 Sample collection:

Three ml of venous blood specimens were collected in EDTA tube container. The samples were transported to Haematology Laboratory within 10-15 minutes of collection for CBC test and then separated plasma using centerfuge in plain container (1500 round per minutes) for 5 minutes and the plasma cooled at refregator 1-2 days before tested for HIV and HBV.

2.7 Data analysis:

Data were analyzed by using the SPSS computer programme version 11.5 with confidence level 95% and significant Level < 0.05.

2.8 Ethical considerations:

Ethical approval of Khartoum Teaching Hospital was taken . all obtained information was kept as highly confidential data and specimens results were not permitted . the consent of study subjects was taken after being informed with all detailed objectives of the study and its health benefit future.

2.9 Methods:

2.9.1 ELISA:

This high sensitivity kit is an enzyme –linked immunosorbent assay (ELISA) for qualitative detection of antigens to HBs and HIV and antibodies to HIV type 1 and 2 in human serum or plasma .The kits is intended for screening of blood donors and for diagnosis of patient related to infection with HIV-1,HIV-2 and Hepatitis B virus .

2.9.2 HIV test:

2.9.2.1 Principle:

HIV (1+2)Ag /Ab ELISA kits was a two step incubation, (sand wish) enzyme immuno assay ,which uses polystyrene micro well strips precoated with recombinant HIV antigens (recombinant HIV-1 and recombinant HIV-2)And anti-HIV antibodies. As a first step ,biotinylated anti-HIV antibodies together with the patient plasma sample was added into wells. During incubation ,the specific HIV1/2 antibodies if present in sample well be captured inside wells . Stimultaneoulsy, if HIV Ag is present in sample, it will also be capture

as a double antibody “sandwich” complex comprising of the coated antibodies biotinylated Abs .

The microwells was then washed to remove unbound serum protein .the detection of the captured HIV Ag- biotinylated Abs complex or HIV 1 /2 Abs is achieved during the second incubation step by adding of the enzyme Horseradish peroxidase (HRP)which has been conjugated to second HIV 1+2 recombinant Ags and to avidin.

2.9.2.2 Component:

- 96 Microwell plate.
- Negative control (protein buffer tested non reactive for HIV1/2)
- Antibody positive control -1 (HIV 1)
- Antibody positive control -2(HIV 2)
- Antigen positive control
- HRP-Conjugate reagent (Horseradish peroxidase conjugated HIV 1/2 Ags)
- Horseradish peroxidase conjugated avidin)
- Biotin –conjugate reagent (anti HIV Abs)
- Stock wash buffer
- Chromogen solution A and B
- Stop solution (diluted sulfuric acid solution)
- Cardboard plate cover

2.9.2.3 Additional materials and instruments:

- Fresh distilled water
- Gloves and timer
- Single pipette
- Tips
- Dry incubator

- Micro plate reader wavelength 450 nm

2.9.2.4 Reagent preparation:

the reagent and sample was allowed to reach the room temperature (18-30C).

The wash buffer was diluted 1 to 20 with distilled water .

2.9.2.5 Assay procedure:

Biotin –conjugate reagent was added	20 micro l
Sample was added	100microl
Incubation was	60 minutes at 37 c
Hrp-conjugate was added	50 micro l
Incubation was	30 minutes
Washing was	5 times
Coloring was	50 micro l a +50 micro l b for minutes
Incubation was done for	30 minutes
Stopping reaction was	50 micro l stop solution
The absorbance was read by	450nm or 450/630nm

The absorbance was read with 15 minutes after stopping the reaction

2.9.2.6 Interpretation of result:

The result was calculated by relating each sample's optical density (OD) value to the cut-off value (C.O.) of the plate.

Calculation of the C.O. value = $\bar{N}_c + 0.12$

* \bar{N}_c = the mean absorbance value for three negative controls.

Negative result (sample OD/C.O. < 1) sample giving absorbance less than the cut –off value was negative for this assay.

Positive result (sample OD/C.O. > 1) sample giving an absorbance equal to or greater than the C.O. value was considered initially reactive.

2.9.3 Hepatitis B:

2.9.3.1 Test principle:

This kits uses antibody ‘sandwich’ ELISA method in which, polystyrene microwell strips was pre coated with monoclonal antibodies specific to HBsAg .patient’s plasma sample is added to the microwells . During incubation, the specific immunocomplex formed in case of presence of HbsAg in the sample, was captured in solid phase .after washing to remove plasma protein, second antibody conjugated the enzyme horseradish peroxidase (the HRP-conjugate) and directed against to different epitope of HBsAg IS added into the wells.

During the second incubation step ,these HRP-conjugated antibodies will be bound to any anti-HBs-HBsAg complex previously formed during the first incubation ,and the unbound HRP-conjugate is then removed by washing .Chromogen solutions containing tetramethylebenzidine (TMB) and urea peroxidase was added to the wells. In the presence of the antibody-antigen-antibody(HRP)’sandwich’ immunocomplex,the colorless chromogens was hydrolyzed by the bound HRP-conjugate to a blue-colored product . The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of color intensity can be measured and it is proportional to the amount of Ag captured in the wells, and its amount in sample respectively. Wells containing samples negative for HBsAg remain colorless.

2.9.3.2 Component:

- 96 micro well plate
- Negative control (protein buffer tested non reactive for HBsAg)
- Positive control (HBs diluted in protein –stabilized buffer)
- HRP-conjugate reagent (horseradish peroxidase –conjugate anti-HBs antibodies)
- Sample diluent
- Wash buffer
- chromogen solution A and B
- Stop solution (sulfuric acid)
- Cardboard plate cover

2.9.3.3 Additional materials required:

- Fresh distilled water
- Gloves and timer
- Single pipette
- Tips
- Dry incubator
- Micro plate reader wavelength 450 nm

2.9.3.4 Reagent preparation:

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Sample diluents was added	20 micro l
Sample was added	100 micro l
Incubation was	60 minutes
Hrp-conjugate was added	50 micro l

Incubation was for 30 minutes
Washing was 5 times
Coloring was added 50 micro l A + 50 micro l B
Incubation was for 30 minutes
stopping reaction was added 50 micro l stop solution
the absorbance was read by 450nm or 450/630 nm

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Negative result (sample OD/C.O. < 1) sample giving absorbance less than the cut-off value was negative for this assay.

Positive result (sample OD/C.O. > 1) sample giving an absorbance equal to or greater than the C.O. value was considered initially reactive.

3. Results

Figure (3.1) shows general information about patients ,thirty seven were males and fifty three females.

Figure (3.2) shows the distribution of the study population according to history of vaccination against viral disease (HBV) , three were vaccinated and eighty-seven were not .

Figure (3.3) distribution of study group according to possible HBV infection , positive was one (1.1%) and negative was eighty nine (98.9%).

the HIV positive result distribution among the study population was zero (0%).

The distribution of HBV in study population according to gender affect , positive result one (zero male and one female) and negative eighty nine (thirty seven male and fifty two female) , this difference was insignificant (p value 0.4), as in table (3.1)

The HBV distribution among study population in affect of blood or blood transfusion history , positive was one (1.1%) with no history of blood transfusion while negative were fifteen (16.7%) with blood history and seventy four (82.2%) with no history ,this difference was insignificant (p value 0.6) , as in table (3.2) .

The distribution of HBV among the studying group according to vaccination against HBV , positive (one) not vaccinated and negative (eighty nine) three vaccinated and eighty sex were not , this difference was insignificant (p value 0.8) ,as in table (3.3) .

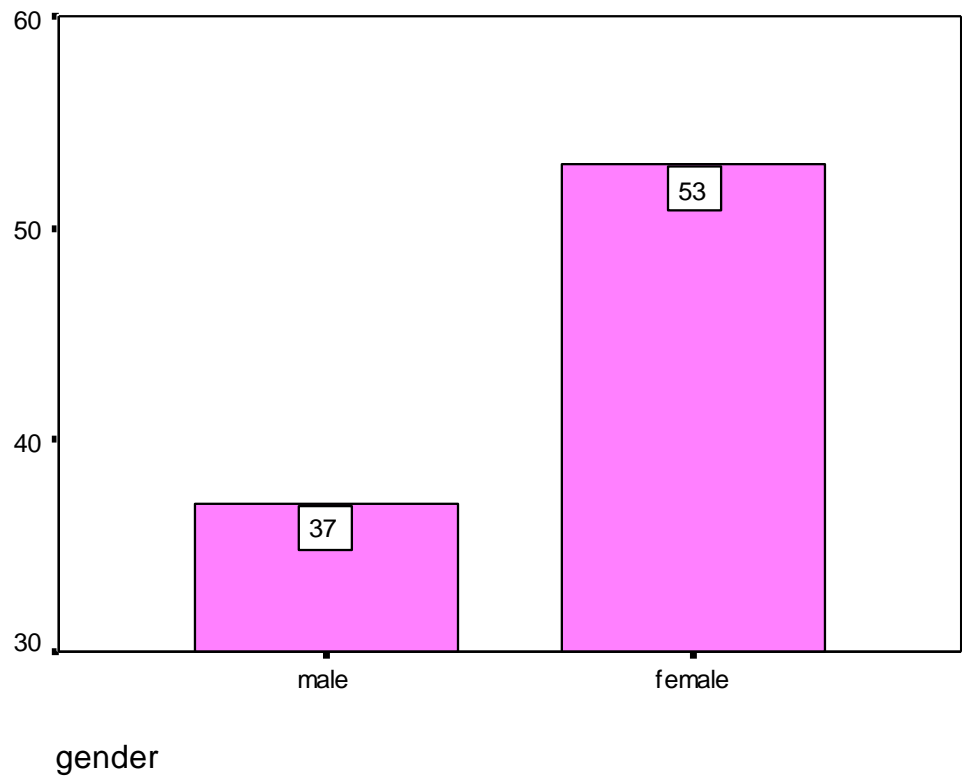


Figure (3.1) Distribution of study population according to gender

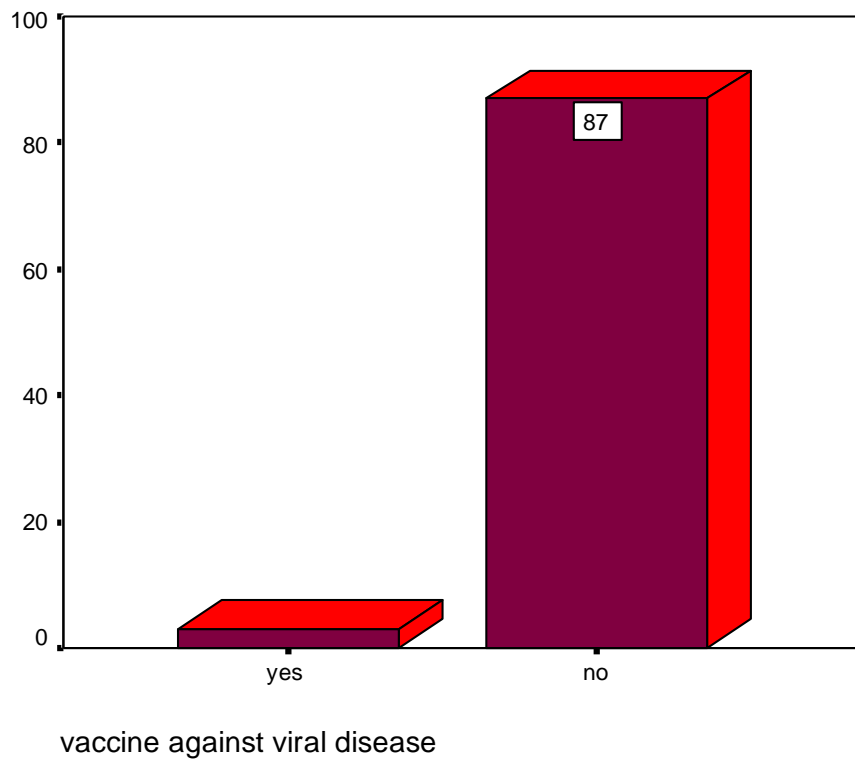


Figure (3.2) Distribution of study population according to vaccination against viral disease (HBV)

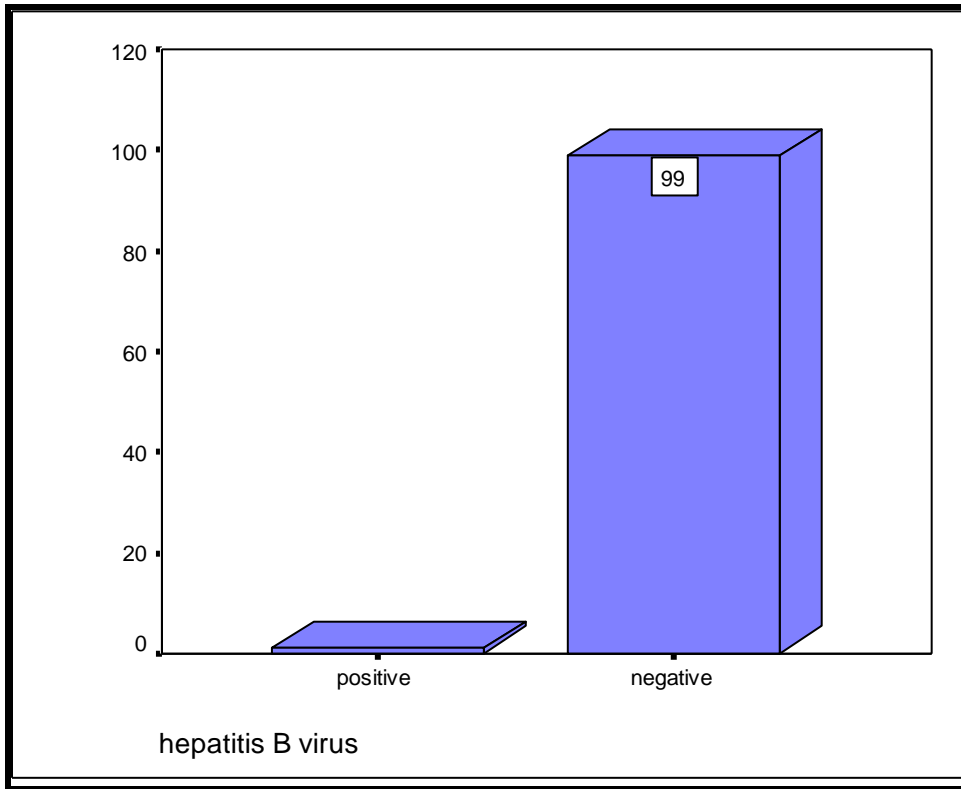


Figure (3.3) Hepatitis B virus distribution among study population

Table (3.1) Frequency of Hepatitis B virus in study population according to gender

	Gender			
Result	Male	Female	Total	P. value
Positive	0 (0 %)	1 (1.1%)	1 (1.1 %)	0.4
Negative	37 (41.1%)	52(57.8%)	89 (98.9 %)	
Total	37 (41.1%)	53 (58.9%)	90 (100%)	

Table (3.2) Hepatitis B virus frequency among the study population according to blood or blood product transfusion history

	HBV			
Blood transfusion History	Positive	Negative	Total	p.value
Yes	0 (0.0%)	15 (16.7%)	15 (16.7%)	0.6
NO	1 (1.1%)	74 (82.2%)	75 (83.3%)	
Total	1 (1.1%)	89 (98.9%)	90 (100%)	

Table (3.3) Frequency of Hepatitis B virus distribution among the study population according to vaccination against HBV

	Vaccination			
HBV	Yes	No	Total	P.value
Positive	0	1 (1.1%)	1 (1.1%)	0.8
Negative	3 (3.3%)	86 (95.6%)	89 (98.9%)	
Total	3 (3.3%)	87(96.7)	90 (100%)	

Chapter Four

Discussion, Conclusion and Recommendation

4.1 Discussion:

Clinical laboratory workers are in direct contact with blood specimens on daily basis. The risk of getting infected by a serious viral disease is underestimated by many of them, especially when there is lack of information regarding the prevalence of blood borne pathogens in the local community they serve. The risk is aggravated by the low awareness about the universal safety precautions and absence of post exposure prophylaxis strategies.

The study revealed the following : the Prevalence of HBV in this study was (1.1%) , This is lower than (7.8%) that reported by Elmadhoun *et al*(2012) , who concluded that the risk of blood borne viral disease in blood specimens is remarkable even in non-suspected specimens and protective measures against occupational exposure must be taken in order to prevent infection among laboratory workers . this result lower than that reported by Abou *et al* (2009) , who concluded that the seroprevalence of HBV (6.25%) was an intermediate and low rates among blood donors in Nyala .and also lower than that reported by McCarthy *et al* (1994) south sudan (26%) , Elfaki *et al* (2008) West Sudan (10%) , Nagi *et al* (2007) Shendi North sudan (5.1%) , Mudawi *et al* (2007) and Gezira Central Sudan (5.6%) .The finding agrees with study of Adekanle *et al* (2015), who concluded that The hospital workers of Nigerian Tertiary Hospital have low perceived risk of HBV infection . Also agree with the study of Nkrumah B (2011). This study showed that The distribution of HBV according to

gender affect was insignificant (p value 0.4) . These finding disagree with study of Hadler *et al* (1985) , in which there study demonstrated that the HBV prevalence infection (14%) in hospital workers. Prevalence of infection was strongly related to race (Asian greater than Black greater than White), sex (male greater than female) and increasing age . strongly correlated with frequency of contact with blood during work, Workers having frequent blood contact had the highest estimated infection rate , also correlated closely with degree of blood-needle contact .the finding are also disagreed with the study of El Beltagy *et al* (2008) who conclude that The prevalence of HBV infection among male Saudi blood donors in the Northwest region of Saudi Arabia is higher compared to that reported from the Central Capital area (Riyadh).The present study revealed that an insignificant difference in HBV result according to blood or blood transfusion affect (p value 0.6). Blood or blood product did not affect the prevalence of HBV result. this result is contra electing this finding of Nanteza *et al* (2008) , who demonstrated that The prevalence of HBV infection was 12.9% and Blood transfusion is a factor associated with HBV infection among children admitted in Mulago Hospital. Also Ali *et al* (2009) , who reported that there is moderate to high prevalence of hepatitis B and hepatitis C in different areas of Pakistan and the modes of transmission implicate contaminated needle use in medical care and drug abuse and unsafe blood and blood product transfusion as the major causal factors .this contra acting the finding of this study . This study showed that an insignificant difference in HBV result according to vaccination against HBV affect (p value 0.8) , this finding disagree with the result reported Luo *et al* (2012), who concluded that Hepatitis B vaccination has effectively reduced the infection and chronicity rates of HBV and related complications.

The present study revealed that the Prevalence of HIV in this study was (0.0%) . This was lower than that reported by Wadie (2012) ,who demonstrated that HIV seroprevalance was 0.5% in blood specimens received at a Clinical Laboratory in Atbara, Sudan. and lower than Elfaki *et al* (2008), who demonstrated that The screening result for antibodies against HIV was (0.8%) among blood donors at ElObeid Teaching Hospital, West Sudan .and the finding is lower than that reported from Motayo *et al*(2015) ,who concluded that the Prevalence rates to HIV is (6.2%) in first time blood donors in Abeokuta, Nigeria and the finding is lower than that reported from Tajeldin *et al* (2012) , who also demonstrate that the seroprevalence of HIV was 3% among blood donors in Kassala. This study showed that gender not affect the HIV result .this finding is disagree with that result reported from Fernando *et al* (1994) who conclude that the seroprevalence of HIV among clinical laboratory specimens was almost eight times higher in men (3.9%) than in women (0.5%).

The low HBV and HIV positive result may be due to the small sample size which did not reflect the accurate frequency risk . On the other hand the patients in Khartoum Teaching Hospital were from different region .

4.2 Conclusion:

The study concluded that:

- The prevalence of Hepatitis B Virus and Human Immune Deficiency Virus positive result was low.
- There were no significant changes in HBV result related to gender , blood or blood product transfusion and vaccination history .
- There were no changes in HIV result related to gender , blood or blood product transfusion history .

4.3 Recommendations:

- There is a need for risk management model system which focus on risk assessment, monitoring and evaluation to enable it to become more responsive to increasing demands for safety and accountability imposed on our current health care systems. The risk management focus must become more strategic, systems based and individual provider based in order to provide its greatest value to the organization employers and the patients those organization serve.
- Medical worker must have an overall strategic safety programs including effective safety training programs which help to establish a safety culture in which employees themselves help in promoting safety procedures while on the job .they should be properly trained for importance of work place safety.
- Further studing with large sample size in the different Country States Hospital should be done to measure the risk of HIV, HBV and other viruses, and share all occupational employer in surveys studies for the blood born disease.
- Although the prevalence of HBV was found to be low in the current study. There is a need to educate the community and health worker about immunization .

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