CHAPTER ONE
INTRODUCTION AND LITERATURE REVIEW

1.1 Infectious diseases:
Infectious diseases are the diseases that result from the interaction between invading pathogenic organisms and the immune mechanism of a person (Haslett et al., 1999). The strategic details of this interaction are unique not only for each class of organisms but also for individual species within the same class (Fauci et al., 1998). The outcome of this interaction can result in no demonstrable effect, illness or death, and is dependant on the number and virulence of the organism, physiological and anatomical effects that they induce, and the effectiveness of the host’s natural defences (Fauci et al., 1998).

There are many factors that influence the likelihood of infection, such as, geography, environment and behaviour. One such example is *Plasmodium falciparum*, which can be significantly affected by climate, season and time of the day. There are specific host factors that influence the infection with a pathogen, such as, age, immunization history, level of nutrition, pregnancy and emotional state (Fauci et al., 1998).

Although most of the infectious diseases are curable, and despite decades of dramatic progress in their treatment and prevention, they still remain a major cause of death and debilitation and are responsible for worsening the living conditions of millions of people around the world (Fauci et al., 1998).

Infectious diseases can be caused by bacterial infections, parasitic infections, viral infections, and fungal infections.

1.1.1 Bacterial infection:
Bacterial infections are the result of the invasion of a host system by bacteria. Bacterial infections are divided into pyogenic (pus-forming) bacterial infections (e.g. *Streptococcus pneumoniae*) and non-pyogenic bacterial infections (e.g. *Mycobacterium tuberculosis*).
1.1.1.1 Tuberculosis:

The term tuberculosis is generally restricted to disease caused by *M. tuberculosis* and *M. bovis*. *Mycobacterium tuberculosis* is usually acquired by inhalation of droplet nuclei (Bass *et al.*, 1990). The organism may survive for weeks in dried secretions in the environment. Much less commonly, drinking cows’ milk, which is infected with *M. bovis*, can cause tuberculosis. Both species of mycobacteria are equally pathogenic.

In 1993, the World Health Organization (WHO) declared tuberculosis as a global emergency since it is the leading cause of death globally associated with infectious diseases (Suffys *et al.*, 1997). The disease is now included with the major tropical diseases affecting a large group of the world population especially in co-infection with the human immunodeficiency virus (HIV).

Tuberculosis is now estimated to kill around 3 million people a year (WHO, 1998a). One-third of the world population is estimated to be infected with *M. tuberculosis* (WHO, 1998b). This infection results in 8-10 million new cases of active TB (Chin *et al.*, 2000) and about 2 million of them are in sub-Saharan Africa (WHO, 1998a). One-third of the increase in incidence during the last 5 years can be attributed to HIV infection, which weakens the immune system of the human body and makes a person infected with the tubercle bacillus 30 times more likely to become ill with tuberculosis (WHO, 1998a). A figure of 82860 cases has been recorded during the period from 1993-the second quarter of 1998 by the Sudan National Tuberculosis Programme Surveillance (Elsony *et al.*, 2000).

In the hosts’ body, mycobacteria spread by direct extension through the lymphatic channels and blood stream and via the bronchi and gastrointestinal tract. Once established in the tissues, the mycobacteria reside intracellularly in monocytes, reticuloendothelial cells and giant cells. The localization of these bacteria is the feature that makes chemotherapy difficult.

Concerning the susceptibility to infection, the likelihood of developing tuberculosis following exposure depends upon a number of factors: race (North American Indians,
black Africans, and Asians are more susceptible), age (young children and old people are more susceptible than young adults), previous exposure to mycobacteria, and immunological and other host defence factors (Sutherland, 1976).

1.1.1.1 Immune response against M. tuberculosis:
Protection against mycobacterial infection is crucially dependant on intact macrophage and T-cell function. On entry into the body, mycobacteria are taken up by mononuclear phagocytes and processed prior to presentation to T cells. Several pieces of evidence point to the important role played by cluster dominant 4 (CD4) and CD8 T cells and TH1 cytokines in controlling mycobacterial infection (Chapel et al., 1999). Presentation of mycobacterial antigens to T cells at the site of infection triggers clonal expansion and cytokine release. The pattern of cytokine release is an important determinant in controlling infection. A predominant TH1 cytokine profile characterized by interferon-γ (IFN-γ), tumor necrosis factor (TNF), and interleukin-2 (IL-2) leads to macrophage activation and granuloma formation, which enables immunocompetent individuals to develop the disease (Chapel et al., 1999).

1.1.2 Parasitic diseases:
Parasitic diseases result from infection by protozoa, helminths, and some arthropods. There are hundreds of organisms identified as parasites; nearly every phylum of animals and plants has its own parasites. Although parasitic diseases are more common in warm climates, they are not synonymous with tropical diseases.

According to the WHO estimations, of more than 50 million deaths worldwide in 1997, one-third were due to infectious diseases, such as tuberculosis, diarrhea and malaria (WHO, 1998a)

1.1.2.1 Schistosomiasis:
Schistosomiasis is acquired through water-contact. It is an important parasitic disease, second only to malaria in the public health impact of vector-borne tropical diseases (Bergquist, 2001). Schistosomiasis is the result of an invasion of the human body by the members of the trematodes of the genus Schistosoma. There are three major types
of *Schistosoma* spp.; *S. haematobium*, *S. mansoni* and *S. japonicum*; the first two species are endemic in Sudan (Chitsulo *et al.*, 2000).

Recent environmental changes that are related to water resources development, and increases in population densities have led to the spread of schistosomiasis to previously low-endemic and non-endemic areas (WHO, 1998a).

Schistosomiasis is endemic in 74 countries most of them are in Africa (WHO, 2002). According to the WHO estimations, 200 million people, worldwide, are infected with schistosomiasis, of whom 120 millions are symptomatic; 20 million have severe disease. More than 600 million people are at risk of infection. 85% of the estimated numbers of infected people are in Africa (WHO, 2002). Map 1.1 shows the global distribution of *S. mansoni* schistosomiasis.

On one hand, *S. mansoni* is found in 54 countries including Sudan, Egypt, Libya, sub-Saharan Africa, Brazil, and Venezuela. On the other hand, *S. haematobium* is endemic in 53 countries in the Middle East and most of the African continent (Chitsulo *et al.*, 2000).

1.1.2.1.1 Intestinal schistosomiasis:

*Schistosoma mansoni* species distribution is associated with irrigation schemes such as the Gezira Scheme (Malone *et al.*, 2001), and were also found along the shores of Lake Victoria in Tanzania as well as in other parts of Africa and Sudan.

The parasites' life cycle: the lateral spine ovum is passed in the faeces of infected individuals and gain access to the fresh water where the ciliated miracidium inside it is liberated. The miracidium then enters its intermediate host, a pulmonate freshwater snail that belongs to the genus *Biomphalaria*, in which it multiplies producing large number of cercaria, which then liberates into the water.
Map 1.1 The global distribution of *S. mansoni* schistosomiasis

(From: Cambridge University schistosomiasis research group website: http://www.path.cam.ac.uk/~schisto/Background/Distribution.html).
Cercariae are elongated, actively motile larvae with a forked tail and head glands that secrete cytolytic substances, they are the infectious stage. They penetrate human epidermis transforming into tailless-cercariae and are then termed schistosomules. After few days in the dermis, schistosomules enter a venule and are arrested in the lungs, where they grow. Schistosomules then migrate via the systemic circulation to the liver and enter portal veins where final maturation takes place. Thereafter, the adult *S. mansoni* migrates by the bloodstream to the mesenteric veins.

Attached to the endothelium of the veins by their suckers, male and female worms pair up. They feed on red blood cells and excrete a dark haemozoin pigment. A month after skin penetration, they start laying eggs, about 300 eggs/day (Haslett et al., 1999). Figure 1.1 shows life cycle.

1.1.2.1.1 Immune response against *S. mansoni*:

The immunological characteristics of *S. mansoni* infection are increased IgE production, eosinophilia, IL-4, and IL-5 production. This response is regulated by the TH2 subset of CD4+ T lymphocytes. Parasite specific IgE antibodies play an important role in protection against *S. mansoni*. IgE antibodies react with helminth antigens and lead to the release of pharmacologically active mediators from mast cells, eosinophils, and basophils to which the IgE is bound. These mediators cause local accumulation of leukocytes and augment their ability to damage the helminth. They induce local inflammation and act on smooth muscle to aid in expulsion of parasites (Chapel et al., 1999).

1.1.2.2 Leishmaniasis:

Leishmaniasis is a group of diseases that are caused by protozoa of the genus *Leishmania*. These diseases are transmitted by the female of the genus *Phlebotomus* in the Old World and via the sandflies female of the genus *Lutzomyia* in the New World.

In the human body, the leishmaniae are found in cells of the reticulo-endothelial system as oval forms known as amastigotes. *Leishmania spp.* may cause generalized visceral
Schistosomiasis

The infected individual

Adult worms develop in host veins

4-12 weeks

Sexual replication

Up to 48 hours

Cercariae released

Asexual replication

4-8 weeks

Miracidium enters snail

Eggs → water miracidium hatches

3-5 years

Figure 1.1 Life cycle of *Schistosoma mansoni* (from: Bell, D. R. (1999). Lecture notes on tropical medicine. 4th edition. Blackwell Science, pp 223)
infection, Kala-azar, or purely cutaneous infection known as oriental sore in the Old World. In South America cutaneous leishmaniasis may remain confined to the skin or metastasis to the nose and mouth (Haslett et al., 1999). Map 1.2 shows the visceral and cutaneous leishmaniasis distribution in Sudan.

1.1.2.2.1 Visceral leishmaniasis:
Visceral leishmaniasis is caused by *Leishmania donovani* complex, which belongs to the family Trypanosomatidae. In the wooded areas of the central savannah belt of Sudan, the visceral leishmaniasis dominant vector is the female of the species *Phlebotomus orientalis* (Elnaiem et al., 1998).

This disease is prevalent in the Mediterranean and Red Sea littorals, Sudan, parts of East Africa, Asia Minor, mountainous regions of southern Arabia, eastern parts of India, China, and South America (Haslett et al., 1999).

Visceral leishmaniasis spreads over a wide belt from the Atbara river in the north-east along the Sudanese-Ethiopian border to South of the Sobat river and Nassir and Malakal and extending west across the White Nile. Other foci are the Kapoeta area, the Nuba Mountains and scattered areas in the Darfour region (Osman et al., 2000). Until recently, at least 1000 cases of visceral leishmaniasis occurred each year in Gedaref State, eastern Sudan (Osman et al., 1998).

Concerning *Leishmania* spp. life cycle, the female sandfly becomes infected by taking up the amastigotes with its blood meal, the amastigotes being in the blood or skin of the host. The amastigotes are liberated from the cells in which they are usually found, in the stomach of the sandfly, and begin to multiply by simple fission. The amastigotes become elongated and fusiform, and a flagellum appears. This organism is the promastigote and is the stage of the parasite that develops when amastigotes from infected animals or patients are grown in culture. The motile promastigotes migrate from the gut to the mouthparts of the sandfly, and injected into a new host during the fly feeding. The promastigotes attach themselves to the surface of macrophages, where phagocytosis takes place.
Map 1.2 Visceral and cutaneous leishmaniasis distribution in Sudan (Osman et al., 2000).
The promastigotes then transform into flagellated amastigotes, which multiply asexually in resting macrophages. Rupture of parasitized cells eventually results in the dissemination of the amastigotes to the cells of the reticuloendothelial system (Haslett et al., 1999).

1.1.2.1.1 Immune response against L. donovani:
After introduction into the human body, the promastigotes use their major surface molecules, the lipophosphoglycan (LPG) and gp63, to attach to the receptors expressed on the surface of macrophages, such as the complement receptor 3 (CR-3), CR-1, manose-fucose receptor, and fibronectin receptor. This ligand-receptor interaction is followed by phagocytosis of the parasite and formation of a parasitophorous vacuole that fuses with lysosomes. Within the phagolysosome, the promastigotes are transformed into flagellated amastigotes, which multiply asexually in resting macrophages. However, amastigotes can be killed if macrophages are activated by interferon gamma (IFN-γ). Leishmania parasites can rapidly change their surface coat to elude the immune response in a process known as antigenic modulation. Within minutes of exposure to antibodies, leishmania parasites can remove “cap off” their surface antigens, so becoming refractory to the effects of antibodies and complement (Chapel et al., 1999).

1.1.2.3 African trypanosomiasis:
Trypanosomiasis is caused by the extracellular protozoan parasite Trypanosoma. The tse-tse flies of the genus Glossina are the intermediate hosts. There are two species responsible for the human African trypanosomiasis: Trypanosoma brucei rhodesiense and T. b. gambiense, both of them belong to the family: Trypanosomatidae. Trypanosomiasis in Sudan has been predominantly of Gambian type (Hutchinson, 1975).

African trypanosomiasis has re-emerged as a major health threat to rural Africans, with an epidemic extending from the southern part of Sudan through Uganda and Congo to Angola resulting in more than 10000 new infections per year (Welburn et al., 2001) (Map 1.3).
According to the WHO estimations, 55 million people are at risk of sleeping sickness in Africa (WHO, 1998a). In Sudan a recent study, carried out in southern Sudan at Bahr El Jebel, villages around Torit, and El Rajaf area had showed the prevalence rates of: 20.2%, 15.7%, 43.9% respectively. The same study was performed in northern Sudan at displaced peoples camps in Khartoum, and prisoners from Khartoum prisons, who originated from endemic areas, showed the prevalence rates of: 28.4%, and 26.4% respectively (Abd El Gadir, 2000).

Concerning the parasites life cycle (Figure 1.2), trypanosomes are transmitted from host to host by a bloodsucking arthropod vector (i.e. Glossina sp.). The trypanosomes are taken up by the vector with a blood meal, and usually undergo one or more cycles of development and multiplication in the alimentary tract of the tse-tse flies, before infective forms are transmitted to a new host via saliva.

When the parasites encounter a host, they spread to the lymph nodes, blood stream and in terminal stages to the central nervous system where they produce the typical sleeping sickness syndrome: lassitude, inability to eat, tissue wasting, unconsciousness and death.

1.1.2.3.1 Immune response against Trypanosoma spp.: Trypanosoma mechanisms of survival involve three ways: antigenic variation, resistance to macrophage killing, and resistance to complement-mediated lysis (Chapel et al., 1999).

Parasite induced TNF-α initiates inflammatory processes, and IFN-γ provokes immunosuppressant (MacLean et al., 1999), but both also regulate growth of the parasite itself. Cytokines might play an important role during trypanosome infections (Lejon et al., 1999). Interleukin-1 (IL-1) production might cause fever and influences sleep control, IL-4 might play a role in B-cell proliferation together with IL-6, which also is involved in T-lymphocyte activation, growth and differentiation.
Tsetsefly Stages

1. Tsetse fly takes a blood meal (injects metacyclic trypanosomatids).
2. Procyclic trypanosomatids leave the midgut and transform into epimastigotes.
3. Epimastigotes multiply in a salivary gland. They transform into metacyclic trypanosomatids.

Human Stages

4. Tsetse fly takes a blood meal (bloodstream trypanosomatids are ingested).
5. Bloodstream trypanosomatids transform into procyclic trypanosomatids in tsetse fly's midgut. Procyclic trypanosomatids multiply by binary fission.
6. Injected metacyclic trypanosomatids transform into bloodstream trypanosomatids, which are carried to other sites.
7. Trypanosomatids multiply by binary fission in various body fluids, e.g., blood, lymph, and spinal fluid.

Figure 1.2 Trypanosoma spp. life cycle (From: www.dpd.cdc.gov/dpdx/HTML, 2002)
Interleukin-8 was also found in the serum of early stage patients and in the cerebrospinal fluids of late stage patients (Lejon et al., 1999). Interleukin-8 major bioactivities are chemotaxis of neutrophils and T cells, and induction of adhesion molecule expression on the cell surface of the latter cells. Interleukin-10 has been shown to be increased in serum of *T. b. gambiense* patients and might also contribute to immunosuppression (Lejon et al., 1999).

Human serum contains trypanolytic factors for *T. b. brucei* (the cattle pathogen) (Rickman & Robson, 1970). The factors include: trypanosomes lytic factor (TLF-1 and TLF-2), and human haptoglobin-related protein (Hpr). Haptoglobin-related protein (Hpr) appears to be involved in the free radical-mediated killing of trypanosomes. Endocytosis of the Hpr-Hb complex by the trypanosome causes iron toxicity through the formation of reactive free radicals. Lipid peroxidation disrupts the lysosomal membranes, and the trypanosome is autodigested.

### 1.1.2.4 Onchocerciasis:

Onchocerciasis is a major cause of blindness in the tropics. In humans this is caused by filarial nematodes of the species *Onchocerca volvulus*. It is estimated that over 20 million people are infected (Haslett et al., 1999). In parts of West and Central Africa, onchocerciasis affects the whole adult population and blindness rates of 10% are common, reaching 35% in some parts of Ghana. Due to onchocerciasis, huge tracts of fertile land lie virtually untilled, and individuals and communities are impoverished (Haslett et al., 1999).

The epidemiological patterns of onchocerciasis vary considerably between geographical zones, probably because of the existence of *O. volvulus* strains of different pathogenicity (Southgate, 1987), and/or the entomological resources (*Simulium sp.*) (Vargas & Diaz- Najera, 1980).

In Sudan, human onchocerciasis is known to occur in three main areas, known as the southern, northern, and eastern foci (Mukhtar et al., 1998). Map 1.4 shows onchocerciasis foci in Sudan. In 1996, rapid epidemiological assessment (REA) survey
Map 1.4 Onchocerciasis foci in Sudan (Sudan Medical Journal, Vol. 21, supplement, 1985, pp.102).
revealed that 22% of the people in the Bahar El Arab area (in the south-western part of Sudan) had palpable nodules, 28% had onchocercal skin lesions or itching; and blindness was seen in 10% - 12% of infected subjects (Mukhtar et al., 1998).

Northern Sudan focus is situated between the fourth and fifth Nile cataracts, in Abu Hamad area of the Nubian Desert. In this focus the transmission is seasonal (between November and January). About 33.6% of local inhabitants are skin-snip positive. REA survey revealed that 16% of local inhabitants had palpable nodules, 28% had onchocercal skin lesions or itching, and no patient had onchocercal eye disease (Mukhtar et al., 1998).

The eastern focus is situated close to the Upper Atbarra River. In this area, the skin-snip positivity may reach 50% in villages within the focus but nodule rates and microfilarial loads in the skin are low. Most infected subjects present with sowda (local name for the severe localized pruritus confined to one limb).

The Onchocerca sp. microfilariae are picked up from the skin of the human host by blood-feeding Simulium damnosum flies. The flies of the family: Simuliidae breeds in rapidly flowing, well-aerated water, the Simulium larvae being attached to submerged vegetation or rocks (Haslett et al., 1999). The Adult flies bite during daytime both inside and outside houses. Simulium sp. bites the human skin allowing microfilariae to migrate into the small pool of blood before it is ingested. Once in the midgut of the fly, the microfilariae larval first stage (L1) rapidly migrate into the haemocoel and into the cells of the thoracic flight muscles where they develop and moult twice to give the second (L2) and third (L3) larval stages. The third stage larvae (L3) then migrate to the mouthparts, where the final infective form awaits another blood meal to infect another human being (Figure 1.3).

Most of the severe damage that leads to blindness is due to the migration of microfilariae of O. volvulus to the eye.
Figure 1.3 *Onchocerca volvulus* life cycle (Sudan medical journal, Vol. 21, supplement, 1985, pp.2).
1.1.2.4.1 Immune response against *O. volvulus*:

The immunoglobulins, IgE and IgG were reported to play an important role in immunity against filariasis (Hussain & Ottesen, 1986).

The detection of IgE in the circulation is difficult because this immunoglobulin usually binds to the surfaces of basophils and mast cells where it acts as receptor for specific antigens. IgE coated mast cells interact with specific antigens and release potent mediators that have a chemotactic effect on eosinophils; this increases the vascular permeability, which may lead to the accumulation of IgG, macrophages, lymphocytes, and plasma cells to attack the parasite (David, 1982).

The killing of the microfilariae was reported to be by eosinophils through antibodies mediation, which were specified as IgG type (Ghalib *et al.*, 1985).

1.2 The inflammatory response:

The inflammatory response is represented by a wide range of physiological changes that are initiated after an infection. These physiological changes are: fever, acute-phase response serum proteins and leukocytes proliferation (Fauci *et al.*, 1998). The response of the immune system against a pathogen leads to an inflammatory response.

Inflammation is caused by the accumulation of fluids and cells after injury or infection. The innate (natural) immune response of macrophages includes the release of inflammatory mediators such as cytokines [interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α)], and prostaglandins. These factors elicit changes in local blood vessels by dilation of local arterioles and capillaries, plasma escapes from them, edema fluid accumulates in the area and fibrin forms a network to prevent pathogens spread.

Some of the plasma proteins play a role in acute inflammation and they are called acute phase reactant proteins. The acute phase reactant proteins (APRPs) are the cause of a
wide range of physiological changes, which are initiated after the encounterment of a non-tolerated agent by the immune system. The response of the immune system is called the acute phase response (Baumann & Gauldie, 1994).

The aim of the acute phase response is to destroy the pathogen, prevent tissue damage and activate the repairing processes, which are vital for the organism to return to its normal function. One of the most important aspects of the acute phase response is the radically altered biosynthetic profile of the hepatocytes. The liver normally produces a characteristic range of plasma proteins at steady concentrations. Many of these plasma proteins have important roles in the acute phase response, which follows inflammation. The majority of the APRPs are synthesized by hepatocytes. The rest are synthesized by other types of cells such as monocytes, endothelial cells, fibroblasts and adipocytes (Baumann & Schendel, 1991).

1.3 Haptoglobin (Hp):
Haptoglobin is a positive acute-phase reactant protein and is characterized by a molecular heterogeneity with three major phenotypes (Bowman, 1993).

The term haptoglobin is composed of two parts: hapto+globin, it means to attach+globin, which describes the protein–protein interaction between haemoglobin and this plasma protein (Bowman & Kurosky, 1982).

Haptoglobin is an α₂-sialoglycoprotein (Bowman, 1993). As a glycoprotein, Hp contains N-linked oligosaccharides attached to the β-chains. These carbohydrate side chains are characterized by terminal α₂-6-linked sialic acid residues.

Most of the attention has been paid to determine haptoglobin phenotypes as genetic fingerprints used in forensic medicine for paternity testing and individualization (Langlois & Delanghe, 1996). The theoretical exclusion rate of the Hp system is ~0.184 while the blood group systems (ABO, MNSs, Rh... etc) exclusion rate is rather lower than this; therefore, the Hp system supplements the blood groups and human leukocyte
antigens (HLA) used in cases of disputed paternity. Further, the Hp system enhances the safety of diagnosis of zygosity in twin studies (Galatius-Jensen, 1960).

Plasma Hp physiological half-life is 4-5 days (Moretti et al., 1963). Haptoglobin concentration in normal serum varies within the range of 30–190 mg/100ml Hb (Nyman, 1959).

Haptoglobin level does vary with age. Galatius-Jensen (1958) reported that no Hp was detectable in the sera of 90% of newborns. The binding of haemoglobin by haptoglobin is characterized by very high affinity (> 10^{10} \text{ mol}^{-1}) and stability (McCormick & Atassi, 1990); haptoglobin binds haemoglobin in equimolar ratio with avidity, which is irreversible under physiological conditions. Hp-Hb complexes express peroxidase activity at low hydrogen iron concentration (Kawamura et al., 1972).

1.3.1 Regulation of haptoglobin synthesis:

Synthesis of Hp is considerably lower in fetal than in adult liver, the result of a difference in transcriptional rate (Bowman, 1993). A hepatic soluble fetal nucleoprotein of 53kDa is suggested to be involved in the transcriptional regulation of the haptoglobin gene of prenatal hepatic development (Bogojevic et al., 2002). This nucleoprotein was identified by Western Blotting analysis as a protein within the same molecular mass and epitopes as transcription factor p53 (Bogojevic et al., 2002).

The hepatic synthesis of Hp is induced by cytokines, such as interleukin-6 (IL-6), interleukin-1 (IL-1), and tumor necrosis factor (TNF) (Bowman, 1993). Three IL-6 responsive regulatory regions were identified on the human Hp gene promoter: A, B, and C (Bowman, 1993).

During the acute-phase reaction, a nuclear transcription factor is induced by IL-6. This nuclear transcription factor replaces proteins bound to regions A and C in the non-induced state (Bowman, 1993). Region B binds several nuclear proteins, all different from the nuclear transcription factor, and forms complexes that are identical in induced and non-induced cells (Bowman, 1993).
The term anhaptoglobinemia means that the expression of the Hp gene is absent (Hp0-0 phenotype). A condition present in ~1 in 1000 Caucasians (Schultze & Heremans, 1966). In blacks, especially of West African origin (Nigeria, Cameroon), anhaptoglobinemia is more frequent (> 30%).

Hypohaptoglobinemia has been reported in a few non-black families carrying a "silent gene" with no gene product, Hp0 (Giblet, 1968).

1.3.2 Haptoglobin polymorphism and genetics:
Haptoglobin gene is located on chromosome 16q22 (Schultze & Heremans, 1966; Bowman, 1993).

Haptoglobin consists of two polypeptide chains: α-chain and β-chain (Bowman, 1993). There are three major phenotypes of haptoglobin: 1-1, 2-1 and 2-2. The β-chain (245 amino acids) is invariant in all the phenotypes. The variation in α-chain produces the different haptoglobin phenotypes. The α-chains are divided into α1 (83 amino acids) and α2 (142 amino acids). The α1 is further subdivided into two types: α1S (S = slower) and α1F (F = Faster). The fast and slow forms of α1 (so-called from their electrophoretic mobility) differ in the amino acid at position 54, lysine in (F), glutamic acid in (S) as stated by Black and Dixon in 1968. This replacement is a result of a point mutation in the original Hp allele (Smithies et al., 1962). Hpα2 allele originates from a fusion of an Hpα1F allele and Hpα1S allele (Smithies et al., 1962). The α2-chain is a slow-migrating chain. Chains full description is in table 1.1.
Table 1.1: Amino acid composition of human Hpα and β-chains (From: Kurosky *et al.*, 1980).

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1.3.3 Haptoglobin phenotypes:

1.3.3.1 Haptoglobin phenotype 1-1:

It is a small protein (86 kDa). Its formula is:

\[(\alpha^1\beta)_2\] (Lange, 1992).

Structure:

\[H\beta = \bullet\]

\[\text{Figure 1.4 } H_{1-1} \text{ structure (Langlois & Delanghe, 1996).}\]

Both \(\alpha\)-chains belong to \(\alpha^1\) variety; it could be \(\alpha^{1S}\) or \(\alpha^{1F}\).

It has been shown that phenotype 1-1 is associated with:

- Allergic dermatitis (Lange, 1992).
- Breast and cervix carcinoma (Bartel et al., 1985).
- Acute myeloid leukemia (Mitchell et al., 1988).
- Sodium sensitivity in cardiovascular disorders (Kojima et al., 1994).
- Essential hypertension (John et al., 1985).
- Association with the falciparum malaria infection in the Sudan (Elagib et al., 1998).

1.3.3.2 Haptoglobin phenotype 2-1:

\(H_{2-1}\) is a heterozygote; molecular mass ranges between 86-300 kDa. Its formula is:

\[(\alpha^1\beta)_2+(\alpha^2\beta)_n\]

\(n = 0, 1, 2, 3, \ldots, \text{etc. (Lange, 1992).}\)
This phenotype is characterized by polymerization (Lange, 1992). The $Hp\alpha^2$ gene exists only in humans. This phenotype is associated with ovarian carcinoma in patients with family history of this tumor (Frohlander & Stendahl, 1988).

1.3.3.3 Haptoglobin phenotype 2-2:

The molecular mass of Hp2-2 ranges between 170-900 kDa. Its formula is:

$$(\alpha^2\beta)_n$$

$n = 3, 4, 5, \ldots$, etc. (Lange, 1992).
This phenotype is associated with:

- Developing refractory hypertension (Delanghe et al., 1995).
- Psychiatric disorders (Lange, 1992; Maes et al., 1994) and high susceptibility to schizophrenia (Maes et al., 2001).
- In 2002, Delanghe and Langlois found in healthy males that the haptoglobin phenotype (2-2) is associated with higher serum iron, higher transferrin saturation, and higher ferritin than Hp1-1 and Hp2-1. Higher serum ferritin correlates with monocytes L-ferritin content, which is also highest in Hp2-2 subjects due to endocytosis of multimeric Hp-Hb2-2 complexes by the recently identified Hb scavenger CD163 in macrophages. This iron delocalization pathway, occurring selectively in Hp2-2 subjects, has important biological and clinical consequences.
As a disease marker, haptoglobin levels increase during inflammation or tissue breakdown. Haptoglobin level in blood has been reported to be elevated in patients with infectious diseases and cancer (Bowman & Kurosky, 1982).

1.3.4 Haptoglobin functional properties:

Human haptoglobin has served as an unexpected source of information relating the mechanisms involved in genetic rearrangements to the linear sequence of amino acids within a protein (Bowman & Kurosky, 1982).

Haptoglobin functions are:

1.3.4.1 Binding free haemoglobin:

Free haemoglobin (Hb) in blood is toxic to the kidney (Giblett, 1968). The haptoglobin β-chain (Hpβ) binds free haemoglobin (Bowman & Kurosky, 1982) forming a soluble, stable Hp-Hb complex (McCormick & Atassi, 1990). Haemoglobin is an oxygen-binding tetrameric (α2β2) protein containing a protoporphyrin ring complexed with Fe^(3+)(McCormick & Atassi, 1990). The β globin chain of human Hb contains two specific binding sites for haptoglobin, at amino acid residues β11-25 and β131-146, whereas the α globin chain has one Hp-binding region, comprising residues α121-127 (McCormick & Atassi, 1990). Haemoglobin αβ dimers bind stoichiometrically to Hpαβ subunits (Bowman & Kurosky, 1982). And so the Hp-Hb complex enhances the peroxidase activity of Hb.

After destruction of erythrocytes, free Hb in the circulation passes through the glomerular filter, and renal damage may occur. Hp reduces the loss of Hb and iron, because the Hp-Hb complex is not filtered through the glomeruli but is transported to the liver (Giblett, 1968). The complex is destroyed in the parenchymal cells of the liver.

In physiological conditions, serum Hp is saturated when ~500-1500 mg/L free Hb is present (Thomas, 1992). Haemoglobin binding depends not only on serum Hp concentration but also on Hp type (Javid, 1965). The “clearance” of Hb, released into
the circulation after intravascular hemolysis, is less effective in Hp2-2 individuals (Javid, 1965). Existing literature about the phenotype dependency of Hp–Hb binding is often confusing, if not conflicting (Langlois & Delanghe, 1996). Because the $\alpha^1$-chains of Hp are smaller than the $\alpha^2$-chains, 1g of Hp1-1 contains more $\alpha\beta$ subunits than 1g of Hp2-1 or Hp2-2; therefore, 1g of Hp1-1 can bind more Hb than 1g of one of the other phenotypes (Langlois & Delanghe, 1996).

1.3.4.2 Protection against free radicals:

Free radicals, such as superoxide ($O_2^-$) and hydroxyl ($OH^-$) can damage cells by peroxidation of membrane lipids (Gutteridge, 1995). Free Hb promotes the accumulation of hydroxyl radicals, because iron can generate OH by means of the Fenton reaction: $H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + OH$ (Sadrzadeh et al., 1984). Hence binding of free Hb by Hp reduces the damage.

Haptoglobin is the major haemoglobin binding protein and is associated with haemoglobin catabolism. This protein is produced mainly in the liver but is also expressed in alveolar macrophages and eosinophils in diseased lungs of human beings (Yang et al., 2000). Oxidants generated by activated macrophages are involved in respiratory distress syndrome, acute tubular necrosis, and atherosclerosis (Moison et al., 1993). These dangers are reduced by the Hb-binding capacity of Hp.

Another protection event is associated with numerous types of lung injuries, such as tuberculosis, traumatic injury, cystic fibrosis, and other lung disorders, during which occurs the extravasations of erythrocytes into the lower respiratory tract. After hemolysis, the catalytically active iron present in haemoglobin can induce the formation of reactive oxygen species and lead to oxidative damage in lung tissues (Ghio et al., 2000). Heme iron catalyzes the oxidation of low-density lipoproteins, which can damage vascular endothelial cells (Gutteridge, 1995). Both free haemoglobin and erythrocytes have been shown to induce lung injury in experimental animal models (Ghio et al., 2000).
The expression of the Hp gene in the lung increases several folds upon exposure to inflammatory stimuli, which suggests that Hp may play a protective role(s) in the lung but this is not yet known (Yang et al., 2001). However, Hp–Hb binding is phenotype-dependent (Javid, 1965).

Breakdown of erythrocytes in the interstitial (e.g., intracerebral) fluid results in Hb-mediated hydroxyl (OH⁻) formation. Consequently, the antioxidative capacity of body fluids is less efficient in Hp2-2 individuals (Lange, 1992).

1.3.4.3 Inhibition of nitric oxide (NO):
Nitric oxide is a highly reactive substance. It is produced by several types of human cells, such as activated macrophages (Green, 1995). While large amounts of (NO) are cytotoxic and are associated with nonspecific defence against microorganisms (Green, 1995), low amounts have a relaxing effect on the blood vessels and thus it is identified as the endothelial-derived relaxing factor (EDRF). Purified Hp has no inhibition effect on (EDRF) but the Hp-Hb complex has.

1.3.4.4 Inhibition of prostaglandin synthesis:
Prostaglandins are fatty acids derivatives. They are found in greatest concentrations in the seminal fluid of the human body and in minute amounts in other tissues. Almost all prostaglandins are synthesized at their sites of action by prostaglandin synthetase. Prostaglandins are inflammation mediators. Hp is a prostaglandin synthesis inhibitor. Hp2-1 and Hp2-2 have less inhibitory effect on prostaglandin synthesis than Hp 1-1 (Lange, 1992).

In preterm infants with patent ductus arteriosus, use of prostaglandin synthetase inhibitors, such as indomethacin, has been proposed in an attempt to achieve medical ligation of the ductus (Lucas et al., 1980). Hp is a potent prostaglandin synthetase inhibitor but is absent from neonatal blood. Accordingly the use of Hp for the treatment of patent ductus arteriosus has been suggested (Lucas et al., 1980).
1.3.4.5 Bacteriostatic effect:
Heme iron is vital for bacterial growth. Hp establishes an iron-restrictive environment, as a part of non-specific defence against bacterial invasion. Rats inoculated intraperitoneally with pathogenic *Escherichia coli* plus Hb were fully protected against lethality by simultaneous administration of Hp (Eaton *et al.*, 1982). Some bacteria e.g., *Neisseria meningitides, Campylobacter jejuni, Bacteroides fragilis,* and *Vibrio vulnificans* possess specialized iron-acquisition systems for survival in the host. These microorganisms are capable of heme uptake from either Hb or the Hp-Hb complex.

1.3.4.6 Angiogenesis:
Angiogenesis play an important role in a variety of physiological and biological conditions, such as tumor growth, wound healing and chronic inflammatory diseases. Hp is one of the serum angiogenic factors, which is required for endothelial cells proliferation and differentiation in the formation of new blood vessels. Hp2-2 is more angiogenic than the other phenotypes (Cid *et al.*, 1993).

Increased serum Hp concentrations in chronic inflammatory and/or ischemic conditions are important for tissue repair and promoting the growth of collateral vessels (Cid *et al.*, 1993). Furthermore, the neovascular growth-stimulating properties of Hp play a role in the development of maculopathy (Kliffen *et al.*, 1995).

1.3.4.7 Antibody-like properties:
While Hp2-1 and Hp2-2 behave like antibodies, because of their agglutination ability for *Streptococcus pyogenes* group A, carrying the T4 antigen, Hp1-1 has no agglutination effect and can be used as agglutination inhibitor. Despite all this Hp is not a true antibody because it has not got the highly variable antigen-binding sites of an antibody and does not activate complement (Prokop & Köhler, 1979).

1.3.4.8 Interaction with leukocytes:
In human blood plasma, many glycoproteins with α-2-6-linked sialic acids are present, but only IgM and Hp can selectively bind CD22 (Hanasaki *et al.*, 1995).
1.3.4.9 Arterial restructuring:
Collagen turnover and cell migration are fundamental aspects of arterial restructuring. de Kleijn et al., (2002), found that there is an expression of haptoglobin mRNA in adventitial fibroblasts of rabbit arteries. They studied haptoglobin’s *in vitro* role. Their study revealed that the stimulation of haptoglobin expression by lipopolysaccharides in mice fibroblasts stimulates the migration of wild-type fibroblasts but had no effect on the migration of haptoglobin knockout fibroplasts.

*In vivo* studies showed that flow-induced arterial restructuring was delayed in haptoglobin knockout mice. Since cell culture showed that haptoglobin is involved in the breakdown of gelatin, haptoglobin-restructuring function might be explained by facilitating cell migration through accumulation of temporary gelatin matrix. This function may also apply to other functional and pathological restructuring processes such as angiogenesis, tissue repair, and tumor cell invasion (de Kleijn et al., 2002).

1.3.5 Geographical distribution:
The geographical distribution of the three phenotypes is under strong genetic pressure (Schultze & Heremans, 1966). The gene frequencies of Hp show marked geographical differences, with the lowest Hp^1_a allele frequency in Southeast Asia and the greatest frequency in Africa and South America (Schultze & Heremans, 1966). Appendix I, table (2) shows haptoglobin phenotypes geographical distribution.
1.4 Objectives of study:

1. To determine the relationship between haptoglobin phenotypes and susceptibility to some major infectious diseases in the Sudanese population.

2. To determine the differences between Hp phenotypes (if present) among patients with a mycobacterial infection (tuberculosis), with parasitic infections [helminths infections (schistosomiasis and onchocerciasis) and protozoal infections (leishmaniasis and trypanosomiasis)].