DISEASE PREVALENCE AND CLINICAL PATHOGENESIS OF ARTHROPOD-BORNE INFECTIONS AND OTHER CLINICALLY SIMILAR MICROBIAL INFECTIONS IN THE ARABIAN PENINSULA

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DECLARATION

I hereby declare that this thesis is my original work and it has been written by me in its entirety. I have duly acknowledged all the sources of information, which have been used in the thesis.

This thesis has also not been submitted for any degree in any university previously.

Mohamed Yousif

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SUMMARY (329 words)

Arthropod-borne diseases can result in numerous outbreaks causing high number of morbidity and mortality worldwide. The clinical similarities between some of the diseases studied (malaria, DENF, CHIKF, brucellosis, GAS, and leptospirosis) makes it difficult, if not impossible, to make a definitive diagnosis based on clinical and/or laboratory investigations only. There is no routine laboratory biomarker which can predict a specific diagnosis for these types of infections or monitor the effectiveness of standard management.

Febrile patients were clinically assessed. Skin rash, joints pain, and chills were reported mainly in virally infected patients. Headache, back pain and joints pain were reported mainly in bacterially infected cases. Nausea, headache, and back pain were reported predominantly in malaria positive cases.

Viral infections showed thrombocytopenia ≤ 100 µL and significate elevation in liver enzymes reported in DENV+CHIKV co-infection. In bacterial infections, GAS and meningiococcal meningitis patients showed CRP levels ≥ 100 mg/dl, brucellosis cases reported ESR levels around 60 mm/hr, low Hb and PLT count were reported in malaria infection.

Furthermore, 42 different biomarkers were studied from 100 DENV samples, 42 malaria samples, 22 meningeococcal meningitis samples and from 32 GAS samples. Skin rash, joints pain, elevated liver enzymes, increased MIP-1α and MCP-1 were distinctive features of DENV infection with significant association between DENV NS1 and DENV specific IgM, for bacterial infections older age, fever and low levels of Eotaxin, GRO-α, PlGF-1, RANTES, and SDF-1α were the distinctive parameters. In malaria, thrombocytopenia, elevated liver enzymes and increased IFN-γ were distinctive features in non-sickle cell disease patients. IL-17A was significantly correlated with parasitemia level during acute malaria infection. More than 80% of recruited patients with acute febrile illnesses were classified as febrile unknown cases. Distinctive clinical features, laboratory parameters, and unique immune-profiles were idemtified for DENV, GAS and malaria for better diagnosis and management. Advanced moleculer investigations as well as implementation of surrvailance programms and door-to-door campaings of public education should be introduced in the kingdom to enhance rapid and accurate diagnosis of diseases studied.

LIST OF ABBREVIATIONS

AB	Antibody
Ae.	*Aedes*
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
A*STAR	Agency for Science, Technology and Research Singapore
A*STAR BSF	A*STAR Biopolis Shared Facilities
BBB	Blood Brain Barrier
BDNF	Brain-derived neurotrophic factor
bNGF	Beta nerve growth factor
BPNF	Proprioceptive Neuromuscular Facilitation
BRU	Brucellosis
°C	Degree Celsisus
CDC	Centre of Disease Control and Prevention
CHIKF, CHIKV	Chikungunya fever, Chikungunya virus
CNS	Central Nervous System
CSF	Cerebro-Spinal Fluid
DC	Dendritic Cells
DENF, DENV	Dengue fever, Dengue virus
DHF	Dengue Hemorrhagic Fever
CRP	C Reactive Protein
DSS	Dengue Shock Syndrome
EDTA	Ethylenediaminetetraacetic acid
EGF	Human epidermal growth factor
ELISA	Enzyme Linked Immunosorbent Assay

Eotaxin	Eosinophil chemotactic protein
ESR	Erythrocyte Sedimentation Rate
FGF-2	Fibroblast growth factor-2
GAS	Group A Streptococcus
GBS	Guillian Barré Syndrome
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GRO-α	Growth factor oncogene-alpha
Hb	Haemoglobin
HCV	Hepatitis C Virus
HGF	Hepatocyte growth factor
HIV	Human Immunodefiency Virus
Hpi	Hours post-infection
IDC	Infectious Diseases Clinic
IFN,IFN-α, IFNβ, IFN-γ	Interferon, IFN-alpha, -beta, -gamma
Ig	Immunoglobulin
IL	Interleukin
IL-1RA	Interleukin-1 receptor antagonist
IP-10	Interferon gamma induced protein-10
ISGs	Interferon stimulated genes
LEP	Leptospirosis
LIF	Leukocyte inhibitory factor
LP	Lumber Puncture
MAL	Malaria
MCP-1	Monocyte chemoattractant protein-1
MENA	Middle East and North Africa

MERS	Middle East Respiratory Virus
MIP-1α	Macrophage inflammatory protein -1 alpha
MLVA	Multi-Locus Variable Number Tandem Repeat Analysis
MOH	Ministry Of Health
NEA	National Environmental Agency
N. meningitides	*Neisseria meningitides*
NTD	Neglected Tropical Diseases
PCR, qRT-PCR	Polymerase Chain Reaction, quantitative Reverse Transcription Polymerase Chain Reaction
PDGF	Platelet derived growth factor
Pf, Pv	*P. falciparum*, *Plasmodium vivax*
PIGF	Placental growth factor
PLT	Platelets
PMNs	Polymorphonuclear leukocytes
PUO	Pyrexia of Unknown Origin
RANTES	Regulated on Activation, Normal T cell Expressed and Secreted
RCF	Relative Centrifugal Force
RDT	Rapid Diagnostic Test
RNA (RI-RNA)	Replicative Intermediate Ribonucleic Acid
RPM	Revolutions Per Minute
SARS	Severe Acute Respiratory Syndrome
SCD	Sickle Cell Disease
SCF	Stem Cell Factor
SDF-1α	Stromal cell- derived factor -1 alpha
SLE	Systemic Lupus Erythematosus

SMA	Severe Malarial Anemia
STSS	Streptococcal Toxic Shock Syndrome
Th1, Th2	T helper 1 , T helper 2
TMB	Tetramethylebenzidine
TNF-α	Tumer necrosis factor – alpha
TPO	Thrombopoietin
RBCs	Red Blood Cells
RT-PCR	Real-Time Polymerase Chain Reaction
VEGF	Vascular endothelial growth factor
WBCs	White Blood Cells
WNV	West Nile Virus
ZIKV	Zika Virus

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their corresponded *P* values during the acute phase of malaria infection

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Chapter 1. General introduction

1.0. Disease epidemiology in Southern Saudi Arabia

Arthropod-borne infections have been regarded as a problem in tropical countries, but they have re-emerged and expanded to new parts of the world over the last two decades (WHO, 2014). According to WHO, arthropod-borne infections pose a threat to the global public health concerning the number of people affected with relation to geographic spread. The constant changing dynamics of arthropod-borne infections in the world demand an active approach towards getting up to date information about their circulation, prevalence, pathogenesis, and clinical manifestations.

Arboviruses are transmitted by arthropod vectors and maintained in nature in complex cycles. Infected arthropod vectors transmit pathogens by feeding on animal`s blood (Wernery and Kaaden, 2002). The re-emergence of arbovirus diseases such as Chikungunya virus (CHIKV) and Dengue virus (DENV) in South East Asia, Brazil, the Arabian Peninsula and others, indicates that better methods of investigations and control stratigies are needed to reduce the risk of transmission by *Aedes albopictus (Ae. albopictus) and Aedes aegypti (Ae. Aegypti)* (Halstead SB, 2015) (Figure 1.0.1). Other arboviruses such as Sindbis virus, Rift Valley fever, have been reported in Saudi Arabia on several occasions (Alzahrani et al., 2010).

CHIKV, DENV, and West Nile Virus (WNV) make up a part of a vast number of neglected human pathogenic arboviruses whereby their combined figures for mortality and morbidity exceed those of Ebola, Middle East Respiratory Syndrome virus (MERS-CoV), and Severe Acute Respiratory Syndrome (SARS) (Liang et al., 2015). Infectious diseases common in Saudi Arabia include malaria, Dengue fever, tuberculosis, human immunodeficiency virus (HIV), Hepatitis C Virus (HCV) and emerging Alkhurma hemorrhagic fever. Alkhurma hemorrhagic fever has been recently described as an emerging vector-borne disease where the tick is the main vector (Shibl et al., 2012).

The emergence and re-emergence of these diseases such as arboviruses, malaria and others are natural phenomena related to species evolution and adaptation. Various ecological changes that have been produced by man tend to increase vector prevalence, creating newer strains that adapt new cycles (Ernest et al., 2017).

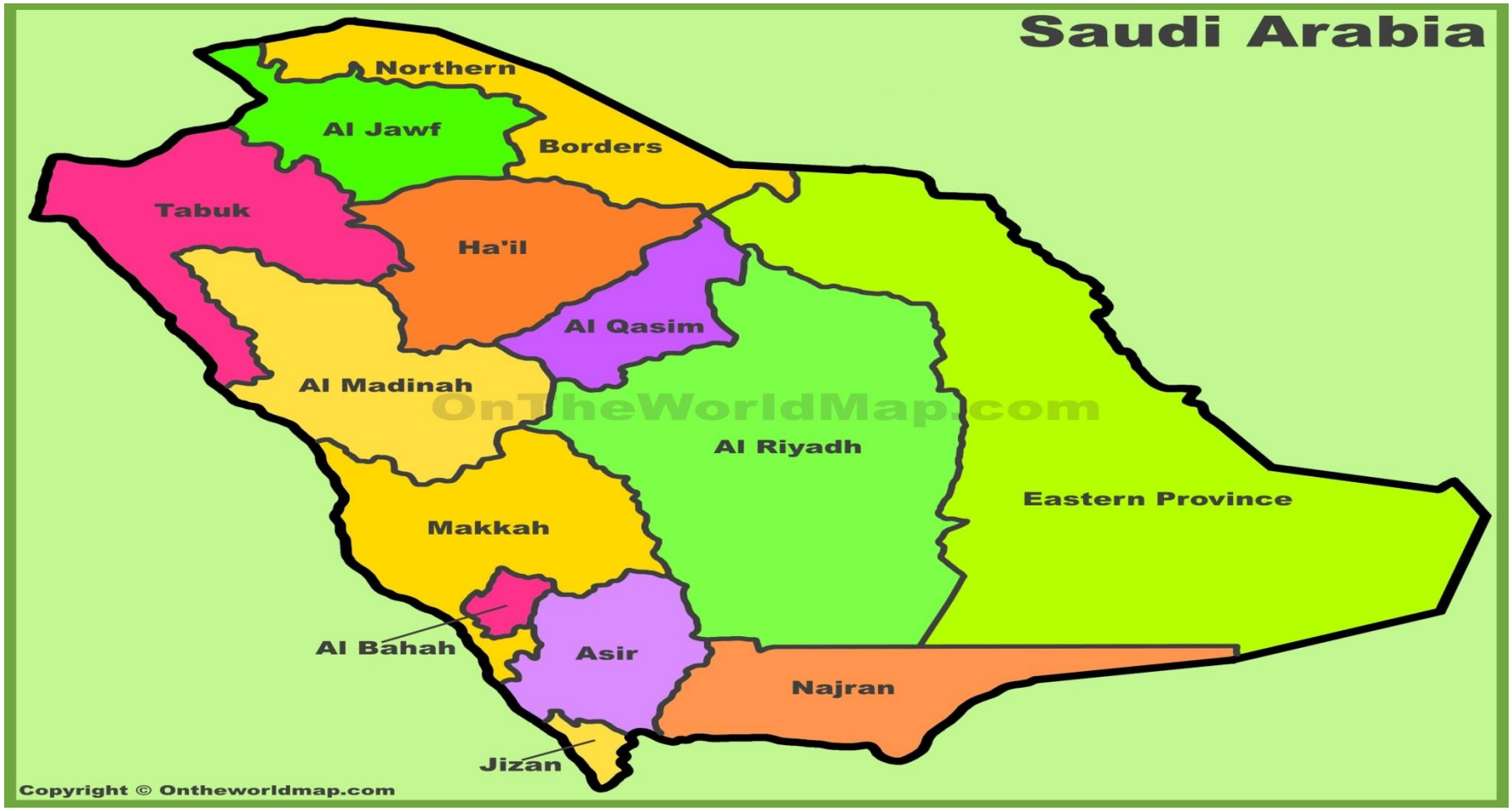

Figure 1.0.1. The map of the Kingdom of Saudi Arabia (KSA), showing the kingdom in geographical regions and location of Jizan province (Yellow).

Emerging infectious diseases in Saudi Arabia result from contributory factors such as tourism and the Hajj pilgrimage as well as the Ummrah that sends an influx of people who come from different regions and continents to the holy place in Makkah (Shibl et al., 2012). Recently, the sudden movement of refugees from Yemen to the Kingdom of Saudi Arabia has increased. In the meantime, the observed number of people visiting the IDC were also rising. This added more burden on the Ministry of Health (MOH) in Saudi Arabia (Ahmed, 2015). The adaptive nature of most of the infectious diseases creates emerging public health challenges that were not present in South Arabia. A cross-sectional study was carried out in Jizan region, Kingdom of Saudi Arabia from April 2010 to March 2011, 553 hospitalized DENF suspected cases, out of which 264 cases (47.7%) were confirmed to have DENF by NS1 DENV antigen detection with ELISA kit, it was also noticed that the highest reported number of cases were in April. The presence of foreign workers from different counteries like Indonesia, Pakistan, Yemen, India and other tourists coming from Africa to Saudi Arabia plays a major role in the distribution of DENV infection in the area, beside the use of water containers in which the transmitting mosqiutes breed. The rainy and humid climate of Jizan ameliorate vector reproduction and expansion (Magda et al., 2014).

Arboviruses, for examples, have the potential to travel over vast distances and move into new countries, meaning that the potential for pandemic spreading is very likely similar to the spread of Zika virus (ZIKV), lately (Figure 1.0.2).

Epidemiological evaluation of arthropod-borne diseases is currently based on pathogen exposure sites identification using county spatial unit, the study discussed the need for advanced methods like postal code or sensus tract scales that enable access to the communities (Eisen and Eisen, 2007). Other methods currently being used are mapping of breeding sites and using of mosquito-capture strategies (Wahid et al., 2017). These methods have proved quite important in the control of transmission risk, but they have various limitations. These methods are not effective in large-scale areas with dense populations.

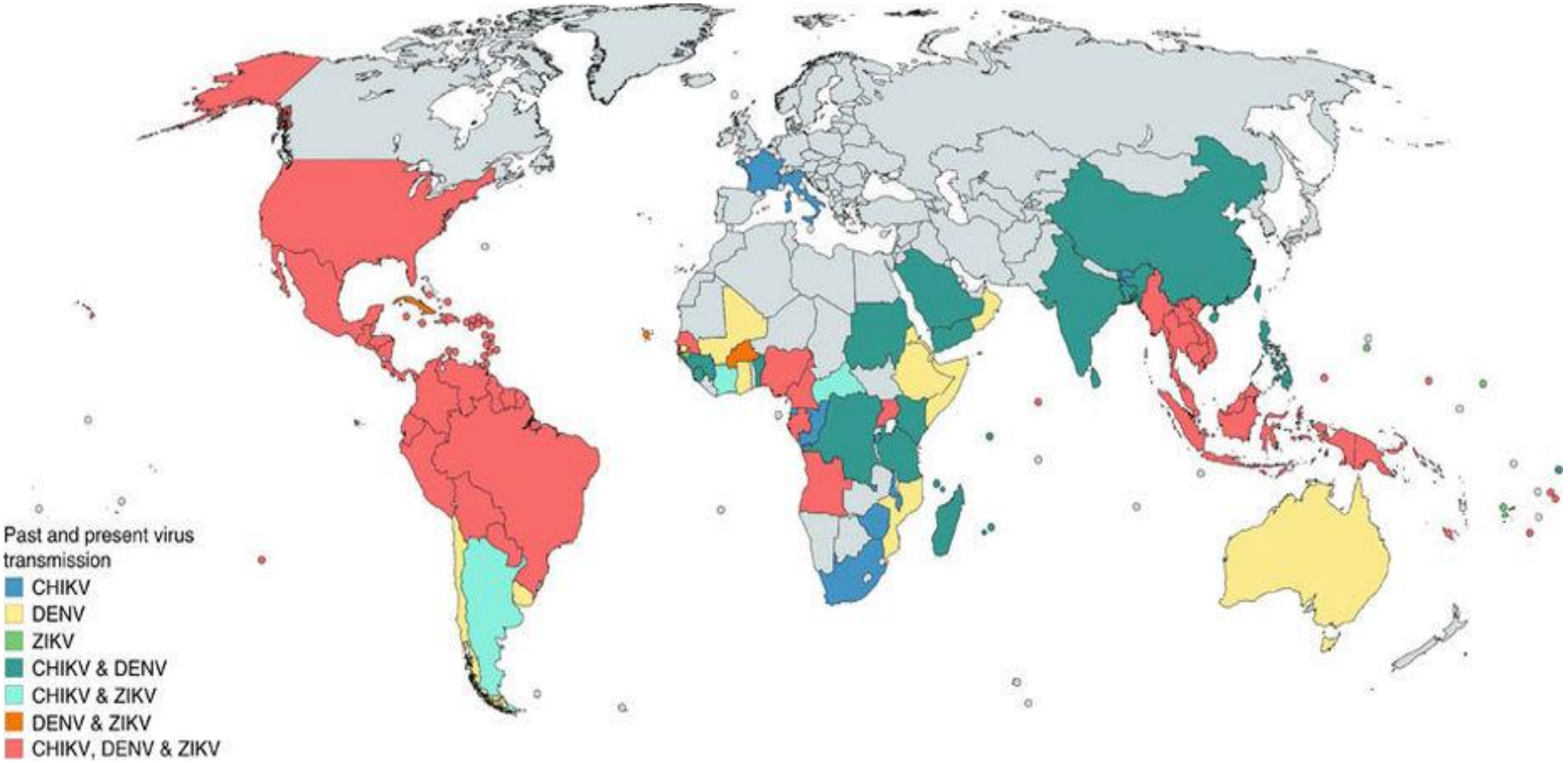

Figure 1.0.2. The world map shows countries in past and present with viral transmission between (1979-2014) in which CHIKV (blue), DENV(yellow), ZIKV (light green), CHIKV & DENV (deep green), CHIKV & ZIKV (blue), DENV & ZIKV (orange), CHIKV, DENV & ZIKV (pink). Both Americas and South East Asia are severely affected by the spread of CHIKV, DENV & ZIKV. Africa shows a mixture of all disease. India and China both shows a widespread of CHIKV & DENV only. Australia shows a predominant DENV spread. Europe shows spread of CHIKV in some countires such as France and Italy. Russia shows no reports of these disease pathogens. It was generated using the free online tool https://mapchart.net/detworld.html and is based on data provided by the CDC, PAHO, WHO, the National Institute for Communicable Disease (NICD-NHLS).

Figure 1.0.3 The world map shows the prevelance of Rheumatic Heart Disease (RHD) caused by GAS per countires, J R Carapetis, A C Steer, E K Mulholland & M Weber,2005. This figure explains the complications due to GAS infection (Post Streptococcal infection).

1.1. Clinical manifestations and other parameters of arboviruses

Most arboviruses have proven very difficult to diagnose based on clinical grounds only. This is because clinical manifestations show substantial overlap. Various studies have shown that the most common clinical presentations of CHIKV, ZIKV, DENV, include fever, asthenia, arthralgia, anorexia, nausea, myalgia, headache, bilateral conjunctivitis, reduced PLT count, and reduced leukocytes (Schaller et.al. 2016; Cao-Lormeau, 2016; Beltrán-Silva et al., 2016).

1.1.1. Dengue virus (DENV)

DENV is the most common arthropod-borne virus that belongs to the Flaviviridae family of genus *Flavivirus*. There are four genetically and antigenically distinct serotypes named DENV-1, DENV-2, DENV-3, and DENV-4. DENV is a single strand of RNA, 11 kb RNA genome with positive polarity. The viral genome encodes for ten protiens and it consist of three structural protiens (C, prM, and E), and seven non-structural protiens; NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 (Guzman et al., 2010).

DENV transmitted by a bite of infective female mosquitoes that belongs to *Ae. Aegypti* species, sometimes *Ae. albopictus* (Gubler, 1998). *Ae. albopictus* is also a vector of the disease but transmits DENV to a lesser extent (Lambrechts et al. 2010). Dengue Haemorrhagic Fever (DHF), which is commonly related to secondary DENV infection with a serotype different than the primary causative one, it was mainly noted in children below 15 years old present in endemic areas where recurrent infections act as a major risk factor for complicated DENV infection (Ole et al.,2007; Muhammad, 2014).

DENV infection is endemic in tropical and subtropical countries with more than one serotype can be detected in positive cases. The common detected serotype was serotype 2 (40.6%) by phylogenetic analysis (Shubham et al., 2018). Presenting symptoms in acute DENV infection include rash, aching muscles, weakness, vomiting, prostration, and fever (WHO, 2009; Afreen et al., 2014). Some symptoms of acute arthritis are more expressed in CHIKV infection than DENV infection (Laoprasopwattana et al., 2012; Kularatne et al., 2009), whereas in DENV infection the fever progress to shock and bleeding due to plasma leakage (WHO, 2009).

The DENV four serotypes (DENV-1, DENV-2, DENV-3 and DENV-4) do not confer immune protection to one another while it provide long-term serotype specific protection (Heinz and Stiasny, 2012; Gublar, 2002). The DENV serotypes can be differentiated into specific genotypes according to their genomic sequences, genotypes found to be responsible of the virus virulence characters, as found to be associated with Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS), phylogenetic studies were needed to find out new emergent genotypes and it is epidemiological impact (Rico-Hesse, 2003). In Saudi Arabia, the first reported case of DENF was in Jeddah in 1994, the most circulating DENV serotypes at that time were DENV-1 and DENV-2 (Zaki et al., 2008). The first outbreak of DENF was in Mekkah in 2004, where DENV-2 and DENV-3 were the most circulating serotypes (Khan et al., 2004). DENV is endemic mostly in the western parts of Saudi Arabia (Jeddah, Makkah and Al-Madinah) acoording to the Saudi MOH, supported by 4312 laboratory-confirmed cases of DENV infection in 2015, more than 73% of cases were reported in Jeddah (Ministery of Health, Department of Statistics, 2015). Recently, the four serotypes of DENV reported circulating in Jeddah and Makkah (Al-Saeed et al., 2017; AshShi, 2017). Pilgrims are the main cause of DENV-1 and DENV-2 emergence in Jeddah from endemic countries (EL-Kafrawey et al, 2015, Al-Saeed et al., 2017). Phylogenetic analysis of DENV-3 sequences showed multiple introduction of DENV-3 in Jeddah since 1997 to 2014. Pilgrims from Pakistan, India, and Singapore plays a major role in the introduction of DENV-3 in Saudi Arabia. Epidimiological surveillance programmes in Saudi Arabia are required to detect imported DENV strains from endemic countries during Hajj and Ummranh for better control strategies and vaccines development (Hashem et al., 2018).

DENV epidemiology in the Middle East and North Africa (MENA) region is not well described due to many reasons; poor clinical reporting, unavailability of DENV confirmatory laboratory tests, limited human and vector surveillances, and serological cross reactivity with other flaviviruses (Elduma and Osman, 2014; Aghaie et al., 2014; Ben Hassine et al., 2014). In Pakistan, DENV serotype 1-4 were reported, beside the highest number of prevelance studies and DENV confirmed cases among MENA countries (Koo et al., 2013; Khan et al., 2013; WHO, 2013). Seroprevelance studies with IgG measures of more than 20% reported in the Red Sea region which include Djibouti, Saudi Arabia, Somalia, Sudan, and Yemen. There is a wide distribution of DENV in this area since 1983 in Yemen and 1969 in Eygpt

but outbreaks and prevelance studies were available (Bhatt et al., 2013; Jimenez-Lucho et al., 1984; WHO, 2015).

Transmission of DENV to humans happens through a bite of an infected female mosquito. If an infected mosquito happens to incubate the virus for 4-10 days, the mosquito will transmit the virus for life (CDC, 2014; WHO, 2016). Infected human becomes the main carrier and multiplier of the virus whereby, they serve as a source of infection to other uninfected mosquitoes. (CDC, 2014) argued that patients who are infected with the virus can transmit the infection for a maximum of 12 days via the female *Aedes* mosquitoes from the first time their symptoms appear. Human movement across countries and continents plays a significant role in the spread of DENV infection (Semenza et al., 2014; Nakamura et al., 2012). Today, the DENV is a leading cause of hospitalization among Asian and Latin American population and is a leading cause of death among children (WHO, 2017). The presence of infection in travellers was very common, represent the second common cause of acute febrile illness after Malaria (WHO, 2016).

1.1.1.1. Clinical features

The common manifested clinical symptoms of DENV are flu-like illness among patients of all age groups. Other clinical features include high grade fever and at least a combination of one of the followings, severe headache, joint pain, rash, gum bleeding, petechiae, severe eye pain, and muscle pain (Faiz et al., 2014). Dengue fever (DENF) has been shown to have three clinical manifestation forms as argued by (Al-Garadi, 2015). The incubation period of the disease varies from few days to two weeks (Figure 1.1.2.3.1), the mild form of the disease presents as an acute febrile illness characterized by body aches, retro-orbital pain, arthralgia, and headache in more than 90% of cases. These are sometimes accompanied by vomiting or nausea, remitting fever, pain in the muscles, and maculopapular rash (Figure 1.1.1.1.1). DSS and DHF forms are the most severe, but they are less common than the mild form. The two conditions are characterized by thrombocytopenia, fever, increased vascular permeability and haemorrhagic manifestations (Al-Garadi, 2015).

Children and people experiencing DENV infection for the first time often manifest with milder illness than those who have been exposed to the disease

before. Severe DENV symptoms include severe abdominal pain and persistent vomiting, bleeding from the nose, drowsiness, difficulty in breathing, pale clammy skin, and black tarry stools. DENV complications include excessively permeable small blood vessels, which allow leakage of the fluid components through the blood capillaries causing ascites and pleural effusions (Sam et al., 2013). This persistent excessive permeability can lead to failure of the circulatory system leading to shock and can cause death without immediate appropriate treatment.

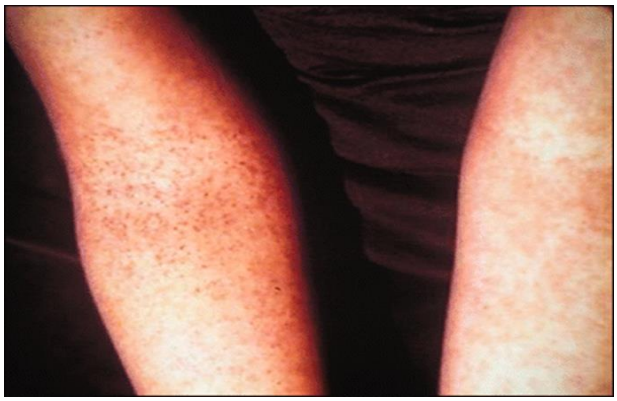

Figure 1.1.1.1.1. DENF skin rash. Generalized morbiliform, erythematous rash with spared areas, involve mainly the limbs. It was proceded by flushing symptoms and erythema in face, neck and chest (Huang et al., 2016). Figure is adopted from (Ashlesha et al, 2010).

1.1.1.2. Seasonality

It was published on a longitudinal study of 10 years from the 1997-2006, the seasonality trend of DENF and DHF. Their results indicated that seasonality of these diseases varies according to regions. For example, Southeast Asia experienced DENF in June and September. The study revealed that seasonality pattern during outbreaks years was different from what observed in other years. For example, DENF outbreak years recorded high detection rates of infection from April to August and low case detection in January. In general, during high DENV activity, the major peaks start at April until August (Eli et al, 2008).

1.1.2. Chikungunya virus (CHIKV)

The CHIKV is an RNA single-stranded alphavirus (Togaviridae family), that was firstly discovered in Tanzania and northern Mozambique (Robinson, 1955; Ross, 1956) in an epidemic described clinically by rapid onset fever, joints pain, muscle pain, headache, eye pain, and skin rash (Rohatgi et al., 2014). The outbreak

occurred in two areas, the river valley and Makonde Plateau, the area was resident by around 150.000 people distributed in small villages, 115 cases admitted to hospitals and others observed in the villege, the water supply was poor and resulted in large numbers of *Ae. aegypti* found around resident houses that promote transmission of the disease. The mortality rate reached 47-50% at that time (Lumsden, 1955).

In 2013, the disease was globally known, entered the western parts of the world where it was distributed in 45 countries, the first CHIKV infected case was reported in Saint Martin by the French National Referrence Center for Arboviruses (Halstead SB. 2015; Pan American Health Organization 2016). In 2015, WHO has stated that in the new world, within the first year of introduction of CHIKV, the virus caused more than one million of cases that is accompanied by sequelae, which includes persistent arthralgia, chronic pain, and rheumatoid arthritis. The rapid circulation of CHIKV has now threatened Europe and parts of Asia through people who are returning from already infected areas (Liang et al., 2015). The disease activity was different from region to another, in Africa it was linked to enzootic transmission cycle with small number of infected cases, in Asia there is large periodic outbreaks with no known enzootic cycle identified (Jupp and McIntosh 1988). Thailand experienced the first urban outbreak in early 1962, where 70.000 outpatient child were affected by CHIKV infection with an estimated outbreak rate around 31% (Halstead et al., 1969). There are poor data about the epidimiolgical characters of CHIKV in the MENA region which involve Djibouti, Egypt, Iraq, Iran, Kuwait, Pakistan, Saudi Arabia, Somalia, Sudan, and Yemen (Kuno, 2015). The first confirmed CHIKV outbreak was in Yemen in 2011 with more than 15.000 suspected cases (Malik et al., 2014). In Saudi Arabia, the first reported case of CHIKV infection was in Jeddah, confirmed by qRT-PCR (Hussain et al., 2013). In Sudan, anti-CHIKV antibodies were detected among Sudanese populations since 1970s, could be due to geographical location of sudan near to an endemic regions like East Africa (Markoff, 2013; Farnon et al., 2010).

There is large range of *Ae. spp* in Africa that transmit CHIKV beside *Ae. aegypti* and *Ae. Albopictus,* this includes *Mansoniauniformis*, *Culexannulirostris*, and *Anopheles* species (Jupp and McIntosh, 1990). The virus transmitted from human to human by bites of infected female mosquitoes. The mosquitoes can be found biting

out on people throughout the day with peaks in the morning and late afternoon. The onset of illness occurs within 4 to 8 days after the bite of an infected mosquitoe.

The pathogenesis determinants of CHIKV are the non-structural proteins nsP2 and nsP3, as they potentiate antiviral suppression response (Fros et al., 2010; Akhrymuk, 2012).

CHIKV associated with syndromes presented with exanthema and arthritis (Navarrete-Espinosa et al., 2016). On the other hand, CHIKV is classified into three genotypes namely West African, East/Central/South African (ECSA) and Asian (Powers et al., 2000). The virus genomic sequence in each genotype is different from other by up to 15% (Sharp et al., 2014). CHIKV pose a global risk with Europe, Africa, and Southeast Asia being endemic areas (Eo et al., 2016).

1.1.2.1. Clinical features

CHIKV infection is an asymptomatic in 15% of the population, others present with some clinical symptoms include high-grade fever and joints pain. Secondary symptoms include muscle pain, skin rash, joints swelling, and headache (Halstead, 2015). The disease is not fatal. However, sever manifestations may occur in the presence of co-morbidities (Pialoux et al., 2007). The incubation period of this virus is 1-12 days and the mean duration is three days (Burt et al., 2012), symptoms begin to appear after this period. The disease is usually symptomatic in about 75 - 96% (Appassakij et al., 2013). It has been determined in susceptible populations that the infection rate can go high to reach 40 - 85%. The viral load reaches > 10^9 viral genome /mL and remains until the appearance of IgG antibodies after 7 days from the infection, which allows better transmission of the virus to mosquitoes and back to humans again (Kam et al., 2012; Marcus et al., 2008).

Usually, prodromal symptoms are rare. At the acute stage, the onset of illness start abruptly with sudden presence of high fever, myalgias, severe arthralgias, and skin rash (Mohan et al., 2010). In the Makonde language *Chikungunya* means "what bends you" which reflect the severity of joint pain. Other symptoms like abdominal pain, constipation, and sore throat may be present.

At some point, mucocutaneous manifestations like scaling, intertrigo, morbilliform, xerosis, and urticaria have been described in patients with CHIKF and

usually present in association with the fever attack (Figure 1.1.2.1.1). High grade fever develops and takes place for a short period, it usually resolves in 3 - 4 days. In some patients with CHIKF, a biphasic pattern of fever have been described. The acute febrile episode followed by a recurrence of fever lasts for a few days (Mohan et al., 2010). The fever accompanied by polyarticular, bilateral and symmetrical arthritis, large joints beside wrists, phalanges, and ankles are commonly involved, periarticular swelling and sever pain are noticed in the affected joints, persistant arthralgia noticed in 63.6% of adults (Borgherini et al., 2008). Joints clinical features may precede the fever strike by few hours and may persist for three days to three weeks (Thiberville et al., 2013). Chondritis was also noticed, the external ears are commonly involved which are suggestive of Chikungunya fever (CHIKF), Tenosynovitis of the wrist tendons, fingures and lower legs have been observed. Regression of fever is associated with development asthenia and anorexia (Javelle et al., 2014). Even though, death caused by CHIKV complications is rare. Most patients feels better within a week of infection, but joints pain can persist for several months.

CHIKV complications occurs among new-borns, specially during the last trimester of pregnancy, adults above 65 years, chronic alcohol abusers, and people with a compromised immune system or chronic diseases are also at risk of developing complications from CHIKV (Economopoulou et al., 2009). Several studies reported neurological complications from CHIKV like encephalopathy, polyneuropathy, and Guillian-Barre Syndrome (GBS). Other symptoms including maculopapular rash, bilateral lymphedema, pruritus, facial erythema, aphthous-like ulcers, and haemorrhage also have been reported (Sharda and Meena, 2007). In neonatal CHIKV infection, the neonate is at high risk of getting the disease from an acutely infected mother in 1 day before the delivery and up to 5 days of life, it was reported in about 50% of cases (Ramful et al., 2007). The neonates either gets the infection by vertical transmission (congenital) or through arboviruses, mostly present with fever, poor breast feeding, rash, and pain. Sever clinical manifestations were observed during perinatal period. Neonatal CHIKV infection present with neurological complications in form of encephalopathy, which manifest as global developmental delay with permenant disabilities present in around 50% of cases (Gérardin et al., 2014).

In laboratory findings; lymphopenia, thrombocytopenia, increase level of the alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were noticed. The C-reactive protein was mildly increase and reaches 50-60 mg/L, high level of creatine phosphokinase was also reported. In some cases, X-ray imaging and MRI of the involved joints displayed the presence of bony erosions, joint effusions, synovial thickening, and tenosynovitis (Manimunda et al., 2010).

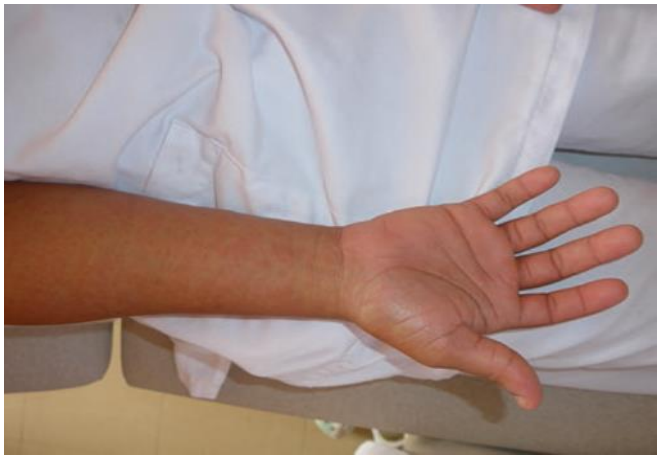

Figure 1.1.2.1.1. CHIKF skin rash. Macular or maculopapular petechial rash, sometimes associated with itching develop acutely within 2-3 days from the onset of fever, distributed mainly in the face, trunk and exterimities. Other cutaneous presentations like vesicles, blisters, exfoliative dermatitis, hyperpigmentation, purpuric lesion, mucosal involvement, and photosensitivity were noticed (Rivaldo and Karen, 2017; Ramesh et al., 2017). The figure is obtained from (Chioma, 2016).

1.1.2.2. Seasonality

CHIKV displays cyclical, secular, and seasonal trends. The outbreaks are rare as compared to those of DENF. In endemic areas, the infection can last several years to decades before a second outbreak occurs (Simon et al., 2008). In endemic areas, the CHIKV infection occurs throughout the year (Mohan et al., 2010; Chevillon et al., 2008). However, cases that have been published showed that the symptoms of virus infection manifested among travellers returning from endemic areas. For example, (Charrel et al., 2008) reported an outbreak in Italy in July 2007. The outbreak was the first in history and occurred among travellers who returned from endemic areas in India. Charrel and his colleagues study showed that CHIKF is more expected from July towards October when travellers are returning from endemic areas during summer holiday visits.

1.1.2.3. DENV and CHIKV mode of transmission to humans

Transmission of DENV and CHIKV occurs via mosquitoes bite (Figure 1.1.2.3.1 and 1.1.2.3.2) in which the virus is injected into the bloodstream and incubated for approximately 4 - 10 days. Humans are the definitive host of DENV, and the source of the virus transmission to uninfected vectors. Once a new DENV infection is stablished, another transmission can occur to a new human in approximately 4 - 5 days of the incubation period (CDC, 2014). *Ae. aegypti* feeds all daylong, with a maximum feeding time in the early morning. The vector habits in urban areas and breeds in human-made containers, which explain the spread of the infection among large population (Van den Hurk et al., 2012; De Castro Medeiros et al., 2011). On the other hand, the secondary vector for DENV and CHIKV is *Ae. Albopictus* (Heilman et al., 2014). This vector is native to Asia, but it has spread to the rest of the world through international trade. *Ae. albopictus* is highly adaptive to new environments, this explains the spread and establishment of the mosquito to several non-native countries.

Unlike malaria parasite's life circle, in CHIKV infection infected mosquitoes harbour the virus for the rest of their life considering that the main vector, *Ae.aegypti* bites people during the day within a short period of time when feeding (Heilman et al., 2014). Urban dwelling vector suggests that CHIKV demonstrates high transmission rate due to availability of population concentration. Apart from transmission through vectors, CHIKV can be transmitted from mother to child during birth time, but rare cases of this transmission have been reported. The target cells for CHIKV infection are the connective tissue fibroblasts, osteoblasts, muscle cells and macrophages. CHIKV musculoskeletal associated diseases are due to direct replication of the virus in the affected tissues. CHIKV antigen RNA can be detected in the joints, muscles, and skin within weeks of symptoms (Ozden et al., 2007; Hoarau et al., 2010).

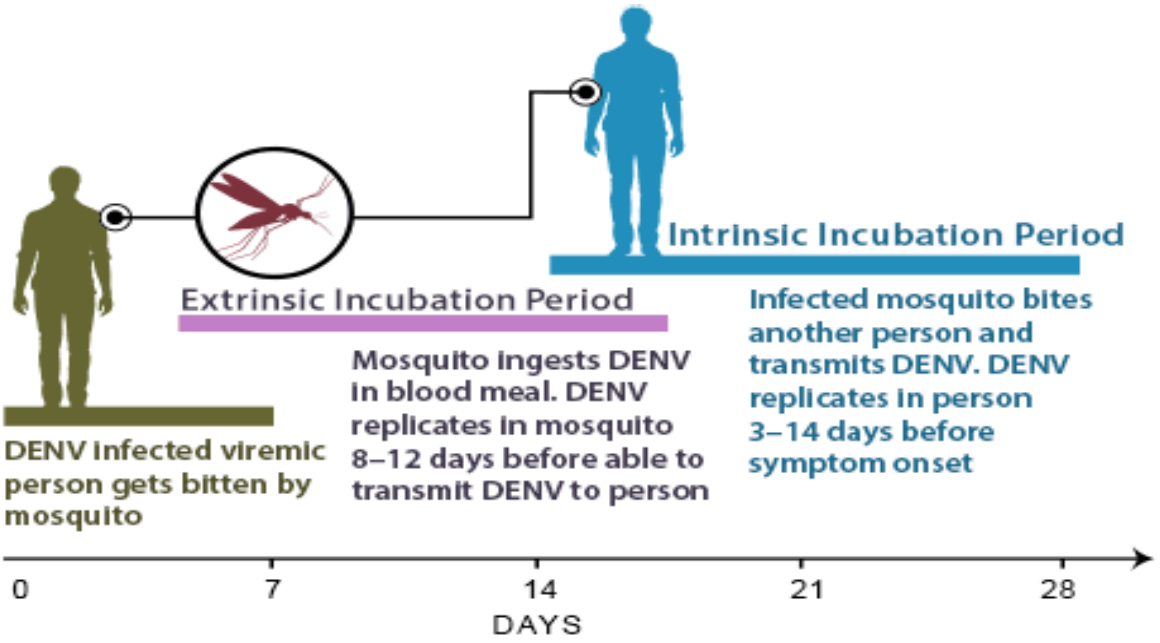

Figure 1.1.2.3.1. Schematic diagram illustrating the transmission cycle of DENV and disease stages. First week, development of viremia; next two weeks, replications in mosquito and transfer DENV to person(s); incubation period in infected individuals (3-14 days before the start of symptoms (WHO, Dengue Clinical Case Management E-learning).

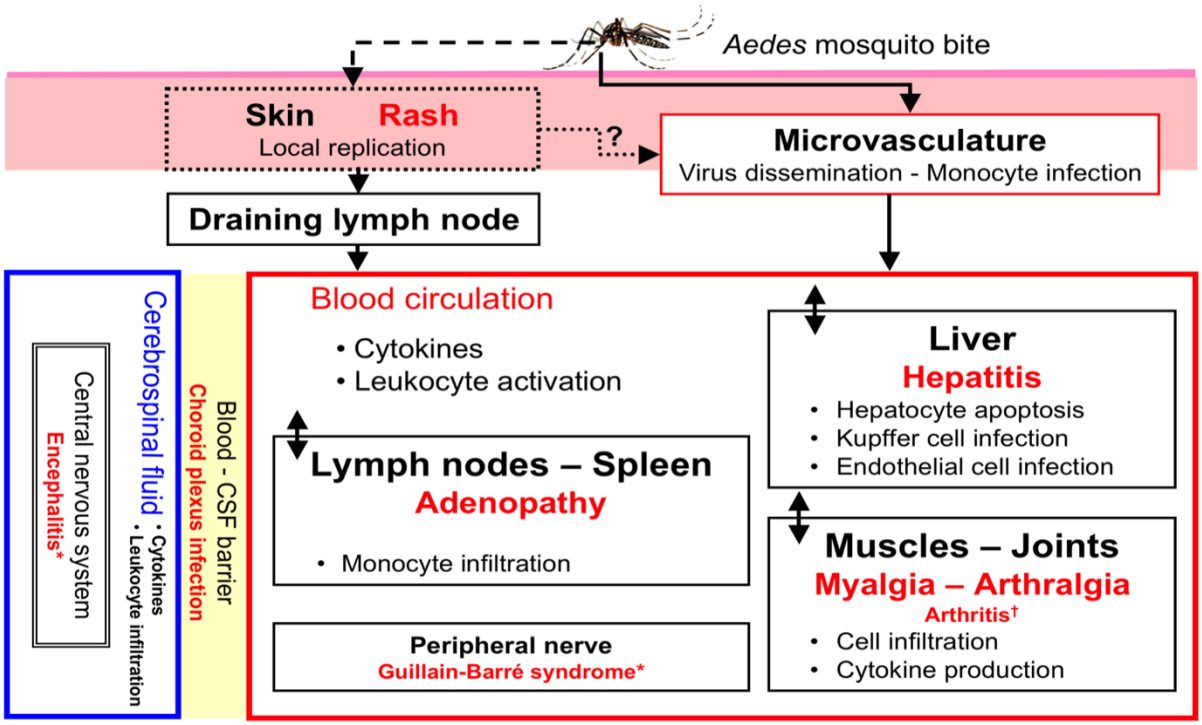

Figure 1.1.2.3.2. Schematic diagram illustrating the transmission cycle of CHIKV infection. Following mosquito bite, the virus enters the subcutaneous capillaries and infect skin macrophages or fibroblasts and endothelial cells. The virus is then transported to secondary lymphoid organs and other organs (liver, muscle, joints, and remote lymphoid organs), followed by high infiltration of mononuclear cells, including macrophages. This will lead to hepatocyte apoptosis and lymphadenopathy. Viral replication in other sites such as muscles and joints is associated with very strong pain, which may lead to arthritis. Guillain-Barré syndrome (GBS) and encephalitis are very rare events. Image was obtained from an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited (Dupuis-Maguiraga L, Noret M, Brun S, Le Grand R, Gras G, Roques P. (2012).

1.1.3. Group A Streptococcus (GAS)

GAS refers to a Gram's positive bacterium arranging in chains found in skin and throat (Ralph and Carapetis, 2013). People who are infected with this bacterium in either skin or throat do not carry symptoms of illnesses because most infections are mild (Piver, 2015). GAS causes infections with clinical signs and symptoms resembles those seen in DENV and CHIKV infections.

According to the Centre for Disease Control and Prevention (CDC, 2016), GAS is a bacterium capable of causing different infections that range from minor illnesses to serious, fatal diseases. They commonly cause bacterial pharyngitis, scarlet fever, and impetigo (Henningham et al., 2012). In the recent past, GAS is responsible for the development of Streptococcal Toxic Shock Syndrome (STSS). M protein and T typing have been used to characterize and identify the bacterium (Henningham et al., 2012; Johnson et al., 2006). In about less than 1% of all cases of bacterial meningitis, GAS can cause meningitis if it gains access to the meninges, following otitis media or sinusitis, through rapid multiplication in the CSF, where poisonous extracts released and leads to swelling and inflammation. The resultant effect leads to increase pressure on the brain that results in meningitis-like symptoms such as a headache, photophobia, focal neurological deficits, and neck stiffness (Diederik et al., 2002).

In Saudi Arabia the case fatility rate due to invasive GAS infections range from 16% to 21% (Al Majid et al., 2008). In a retrospective study conducted in King Fahad Medical City (KFMC), Riyadh, Saudi Arabia from 2007 to 2015, 33 patients with documented bacteraemia due to GAS were recruited, the reported overall mortality rate is 12.1% (Fawzyh et al., 2017). In South Africa, the incidence rate of invasive and non-invasive GAS infections in Eastern Cape province decreased from 2003 to 2015, specific increase in GAS infections incidence rate with increase in age as patients older than 65 years old reported 14.06 per 10^5 person-years. Factors like insuffient documentation and underdetectable GAS patients due to improper diagnosis and antibiotics overuse without microbiological culture were reported (Dylan et al., 2018), while in Kenya the incidence rate of invasive GAS for children < 5 years was 35 per 10^5 person-years (Seale et al., 2016). A molecular epidemiological study on GAS in Tunisia provide that biotype 3, T3/13/B3264 and

emm118 were the most frequent types. *emm1* and *emm18* types were associated with invasive GAS infections (Hraoui et al., 2011).

1.1.3.1. Clinical features

The GAS clinical symptoms vary with a range of symptoms, infected persons may show mild illnesses (Piver, 2015). The common mild illnesses are streptococcal throat or impetigo skin infection (Figure 1.1.3.1.1). When severe illness reported, it occurs in form of necrotizing fasciitis or STSS. Even though, rare complications with severe GAS infection can be life threatening, severe GAS diseases will occur, if a GAS bacterium enters the blood stream, lungs, or muscles. The bacteria are usually not found in these organs, but when GAS enters there, the clinical term used for the situation is "invasive GAS disease". Necrotizing fasciitis and STSS are the most severe GAS infections (Machado, 2011). Necrotizing fasciitis can destroy the patient's fat, muscles, and skin tissues. On the other hand, "invasive GAS disease" results in STSS is responsible of severe drop in blood pressure which found to be related to *M/emm* types GAS virulence factors (Tyrrell et al*.,* 2010). Studies showed that more than 20% of patients suffering from necrotizing fasciitis die and it was found that STSS is fatal in approximately 63% of patients (Machado, 2011). These statistics shows that GAS that causes "invasive GAS disease" is lethal to infected patients. However, the "invasive GAS disease" remains a rare condition.

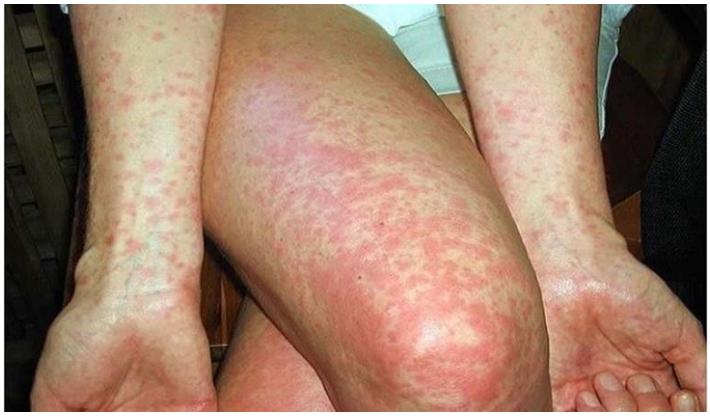

Figure 1.1.3.1.1. Scarlet fever skin rash. Bright red erythematous patches, papular itchy lesions, blanchable on pressure gives characteristic "sandpaper" feel in the skin associated with flushed cheeks start in the trunk, face, and neck then spread to invove the limbs, more prominent in flexural areas. Macules with generalized erythema were noticed. The skin lesions develop within 1-4 days from the onset of illness, desquamation of palms and soles develop in several days after fading of the skin rash in 6-9 days (Michael et al., 2016). The picture is adopted from (Amy Willis, https://metro.co.uk/2016).

1.1.3.2. Mode of transmission to humans

The mode of transmission of GAS is through mucus contact. The sites infected are usually nose or throat, although there are some occasions where direct contact with GAS infected wounds is infectious to healthy individuals (Walker et al., 2014).

GAS colonization was detected in about 12% of 152 indiviuals in contact with patients diagnosed with invasive GAS infection with a significant risk of secondary infection (Shimizu and Tokuda, 2010). The risk factors for sever GAS infections, namely, necrotizing fascities are diabetes, peripheral vascular disease, alcohol intake, smoking, obesity, chronic liver disease, trauma, and immune compromised patients (Roje et al., 2011).

1.1.3.3. Seasonality

The streptococcal throat season is very common in late winter and early spring. The strep throat usually expected to occur approximately seven times a year for the most frequent reported cases per paediatrician, suggesting that it is not uncommon to find strep throat occurring several times a year. Elsewhere, (Gurol et al., 2017) conducted a study of GAS and influenza virus co-infection and determined that the peak for co-infection is between January and April.

1.1.4. Brucella (BRU)

Brucellosis is a known zoonotic disease that caused by a Gram-negative bacteria, *Brucella spp* can infect both animals and humans. Transmission to humans is by direct or indirect contact with animals or infected animal's products (Alsoghair, 2017). It affects both males and females across all age groups. Human brucellosis remains one of the most common zoonotic diseases globaly accounting for approximately half a million cases annually (Wernery, 2014). In the Arabian Peninsula, brucellosis infection reported in small ruminants, humans, ruminating animals and dromedaries (Wernery and Kaaden, 2002). In Upper Egypt, the seoprevelance of brucellosis from 2005-2008 in sheep 1.16 (95% CI 1·05–1·27) , 0.44 (95% CI 0·34–0·54) in goats, 0.79 (95% CI 0·71–0·87) in cows and 0.13 (95% CI 0·08–0·18) in buffaloes (Hegazy et al., 2011). The seroprevelance of brucellosis in Awassi sheep in the northern part of Jordan was 2.2% (95% confidence interval (CI) 0·5–3·5), while in individual animal and flock levels were 45% (95% CI 32–58) (AL-Talafhah et al., 2003). There was serological evidence of brucellosis in humans in Lebanon, Palestine and Bahrain,

instead there was no evidence of seropositivity in ruminants, due to small populations of ruminants in these countries compared to other countries of the Middle East (Musallam et al., 2016). The estimated seroprevelance of brucellosis in Jordan reported one in five cattle herds and one in three small ruminant flocks were infected, the percentage of vaccinated small ruminants flocks in 2013 were only 1.5% (Musallam et al., 2015). In a cross-sectional study conducted in the West of Iran from 2014 to 2015 to estimate the seroprevelance of brucellosis among high risk individuals like butchers, slaughterhouse workers, and veterinarians reported 13.3% were sero-positive with the highest rate noticed among butchers followed by veterinarians, and slaughterhouse workers (Mojgan et al., 2018).

Most cases of brucellosis in the Arabian Peninsula arise due to the consumption of unpasteurized milk, some cases identified in gatherings such as Hajj pilgrims due to consumption of camel milk (Gautret et al., 2013). People who have frequent contact with animals such as employees working with animals, veterinarians, and butchers are at high risk of brucellosis.

Saudi Arabia has been reported to be one of the most endemic countries with brucellosis, the incidence rate was 12.5 per 100.000 of population in 2012, with the highest incidence witnessed in the Al-Qassim region (Aloufi et al., 2016). The peak incidence of brucellosis infection occurs when animals are producing their young ones. Reported incidence rate in Al-Qassim region between 2010 and 2014 was 66.1 per 100.000 population (Alsoghair, 2017). Outbreaks had been reported in areas where families consume infected products from the same animal.

According to (Atluri et al., 2011), the virulence of the infecting strain depends on the ability of the organism to survive within the body macrophages. According to virulence, the Brucella strains can be classified into *B. melitensis, B. abortus,* and *B. suis*. This is in the order of most virulent to least virulent strain. Infection with *Brucella spp* results in increased number of macrophages within the body system in the early stages, which declines eventually. The infection spreads to all organs of the body through bacteraemic spread mechanism with the liver, spleen and lymph glands being the significantly affected organs. Multi-Locus Variable-number tandem repeat Analysis (MLVA) genotyping assay have been widely used by scientists to study and identify the brucella strains of both humans and animals (Ferreira et al., 2012).

1.1.4.1. Clinical features

Brucellosis is a systemic infection that presents in many forms, vary from mild to severe acute infections (Corbel, 2006). These symptoms appear early during the infection period. The initial clinical symptoms are a combination of fatigue, fever, back pain, lack of appetite, abdominal pain, hepatosplenomegaly, increased sweating, muscle and joints pain, testicular pain, skin rash, and hot flushes (Galinska and Zagórski, 2013). Patients may show recurrent night sweats, joints pain and swelling, and organ swelling which results in impaired function. Organs that are commonly affected include testicles, spleen, liver, and heart. The disease effect on the organs considered as complications of brucellosis infection (Al Dahouk et al., 2013). Persistent complications results in chronic fatigue, neurological symptoms, and depression.

Laboratory diagnostic methods for brucellosis infection are variable, time consuming, and sometimes of low specificity. It needs clinical correlation for early start of management (Al Dahouk et al., 2013).

1.1.4.2. Mode of transmission to humans

When domestic farm animals such as goats, sheep, cows, and camels are infected with Brucella, the milk they produce will, therefore, be contaminated with the bacteria (Galinska and Zagórski, 2013). Humans can also acquire the infection if they inhale the bacteria. The risk of inhalation is associated with people who are working within laboratories that study or deal with Brucella organisms. People who are working in meat processing plants are expose to Brucella bacteria droplets. Another rare mode of transmission is infection through skin breaks or mucus membranes (Galinska and Zagórski, 2013; Avila-Calderón et al., 2013).

Infection can also come from wild animals that are used as food through hunting. Human-human brucellosis transmission is rare but can occur during unprotected sexual intercourse (Meltzer et al., 2010). Transmission can also occur through transplacental transfer and during breast feeding. Transmission from human to human with blood transfusion and bone marrow transplantation were also reported (Felipe et al., 2017). Possible complications from brucellosis include heart's inner lining infection. Untreated brucellosis endocarditis can lead to death due to heart valves destruction.

1.1.4.3. Seasonality

Unlike vector-borne viruses such as DENV and CHIKV, brucellosis present through out the year. In fact, infected individuals harbour the bacteria for months and even years (Islam et al., 2013). Many studies also reported increase incidence of brucellosis infection with breeding seasons of animals due to increase human exposure to it is suspected contaminated secretions with peak incidence in summer and autumn (Pengwei et al., 2016; Maryam and Saeid, 2017). Due to the difficulty in diagnosing brucellosis acutely during infection time, it is therefore difficult to estimate the seasonality of high infections with patients who may show signs and symptoms several months after the infection (Maria and Javier, 2009).

The test of choice to diagnose chronic and relapsed brucellosis is the use of anti-Brucella antibodies, this is because clinical symptoms are highly non-specific (Šiširak et al., 2010).

1.1.5. Leptospirosis (LEP)

Leptospirosis is a common zoonotic disease caused by spirochetes bacteria of genus Leptospira, it is often infectious. Leptospira is a naturally occuring microorganism that is well maintained within wild and domestic animals. Human infection happens when one is bitten by an infected animal or by direct contact with urine of an infected animal (Beaute et al., 2013). Indirect exposure to Leptospira can happens through coming into contact with contaminated dump water or damp soil.

Leptospirosis represents one of the most important causes of febrile illnesses, whom management and diagnosis in environment with low resources remains a significant challenge (Conroy et al., 2014). Leptospirosis and DENV infection remains to be dangerous diseases, and plays an important cause of morbidity and mortality worldwide. Leptospirosis remains an important differential diagnosis in clinical practice among patients suffering from acute febrile illness especially when in the context of decreased malaria transmission in many counteries of the globe (Conroy et al., 2014; Murray et al., 2012). Leptospirosis accounts for a significant percentage of febrile illnesses in hospitalized patients (Rafiza et al., 2013).

The disease causes a wide range of symptoms among humans, the majority of the infected persons always remain asymptomatic or develop a mild, spontaneously recovered illnes (Devars et al., 2013; Adler and de la Peña, 2010; Evangelista and Coburn, 2010).

Leptospirosis has been reported to be endemic throughout the world except for the Polar Regions. Self-limited systemic failure in the early stages of leptospirosis infection cannot be distinguished from influenza, DENV, malaria, and CHIKV infections (Devars et al., 2013).

Genetic characterization and identification of the *Leptospira* isolates can be done through sequencing and Multi-Locus Variable Number Tandem Repeat Analysis (MLVA) (Bourhy et al., 2012). The MLVA has generated four classes of *Leptospira* human pathogens: *L. kirschneri, L. interrogans, L. borgpetersenii*, and *L. borgpetersenii* group B (newly described) (Bourhy et al., 2012), *Leptospira mayottensis* a phylogroup was recently added (Bourhy et al., 2014). Further serological typing of the isolates indicates that the species are further subgrouped into four serogroups. The mini serogroup represents the major human pathogenic *Leptospira* (Bourhy et al., 2010).

Leptospiral pathogenesis mechanisms are poorly known, although significant development was achieved in understanding the bacteria virulence factors such as molecular profiling of the pathogenic species (Ciamak, 2018). The *Leptospira* genome is composed of 3.9 - 4.6 Mbp which varies across different species. The genome contains two circular chromosomes with a third replicon found in *L. biflexa* but not in pathogenic species (Evangelista and Coburn, 2010). More researches are undergoing to understand the pathogenic pathways and the virulence factors of these pathogens (Adler, 2014).

1.1.5.1. Clinical features

The clinical symptoms of leptospirosis range in severity from mild illness to multi system syndrome. This infection is characterized by fever, headache, and photophobia. Skin manifestations include erythematous skin rash with petechial eruption mainly in the palate and retro-orbital pain (Figure 1.1.5.1.1). Other symptoms include conjunctival suffusion, nausea, dry cough, and muscle pain in the lumbar areas (Peter, 2011; Hartskeerl et al., 2011).

Complications of leptospirosis include kidney failure, inflammation of the membranes that surrounds the spinal cord and the brain leading to meningitis (Daherede et al., 2010). Leptospirosis complications may cause respiratory distress, liver failure accompanied by hemorrhagic diathesis and even death in rare cases (Hartskeerl et al., 2011; Beaute et al., 2013). Other complications include icteric

leptospirosis that causes Wiel's disease which includes icterus phase causing jaundice, renal failure, haemorrhagic fever features, and systemic inflammatory syndrome or shock.

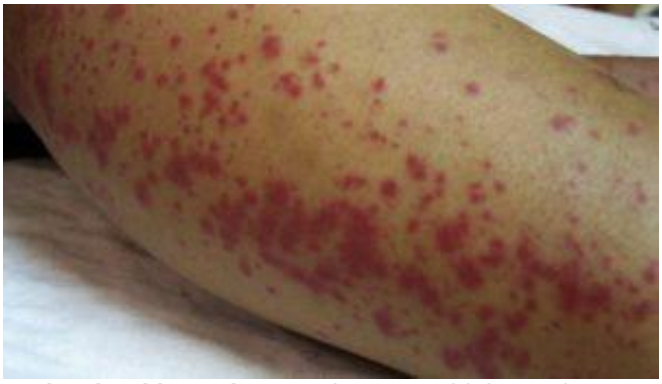

Figure 1.1.5.1.1. Leptospirosis skin rash. Transient petechial eruptions, red-brown small papules-macules can involve the palate, associated with urticarial and conjunctival suffusion. Raised, tender erythematous plaques 1-5 cm in diameter are characteristic of *L. interrogans*, appears in the chins fade after 4-7 days, termed pretibial or Fort Bragg fever (Justin et al., 2015). The Figure is adopted from (Francisco, 2016).

1.1.5.2. Mode of transmission to humans

Leptospirosis caused by bacteria that spread through the urine of infected animals, hence classified as a zoonotic disease. The bacteria transmitted to humans through contact with infected animal urine or contaminated water, both domestic and wild rodents can transmit leptospirosis (Forbes et al., 2012). Leptospira bacteria enters the infected animal's bloodstream through mucous membranes (Costa et al., 2015; Lau et al., 2010).

The Leptospira bacteria that causes Weil's disease transmitted by rodents. Rats are the primary carriers of this disease. Exposed drinking water, poor sanitation, poverty, flooding, and overcrowding among urban slum populations result in recurrent leptospirosis infection (Felzemburgh et al., 2014). Serovar Canicola is a leptospirosis disease transmitted from dogs to humans that affects the kidneys.

1.1.5.3. Seasonality

A retrospective study conducted to describe the seasonality and patterns of human leptospirosis between 1998 and 2008. Factors included in the study were temperature, rainfall, and global solar radiation (Desvars et al., 2011).

According to their results, the authors concluded that leptospirosis has a seasonal distribution in Europe. This seasonality allows health care providers to

develop preventional programs and alert the public about the presence of Leptospira organisms. In Europe, Leptospira infection occurs before and during the rainy seasons, when there is a likelihood of contacting infected water.

1.1.6. Meningiococcal meningitis (MEN)

Meningitis is an inflammation of the membranes surrounding the brain and the spinal cord (meninges) which can be caused by many pathogens such as bacterial, viral, fungal, and parasitic. However, head injuries, cancer and certian drugs may result in meningitis (CDC, 2018). Severe bacterial meningitis affects the central nerves system and can lead to permanent disability or death. The mortality rate of bacterial meningitis due to *Streptococcus pneumonia* in adults range between 37% and 51% according to countries availability of optimal medical services. Several types of bacteria cause this disease (Brouwer et al., 2010). The most common causes include *Streptococcus pneumonia*, *Haemophilus influezae,* and *Neisseria meningitidis,* the causative agent of MEN is *Neisseria meningitidis* (Edmond et al., 2010). Viruses, parasites, and fungi can cause their respective meningitis, but bacterial meningitis is the most severe one. Bacterial meningitis and septicemia in children and young adult are mostly caused by *Neisseria meningitides*. Approximately 500.000 reported cases and 50.000 deaths were reported every year worldwide (McGill et al., 2016; WHO, 2002). MEN mainly caused by 6 serogroups; A, B, C, W, X, and Y (Broker et al., 2012). In European countries the incidence of MEN decreased from 1999 to 2006 by one half, after the introduction of serogroup C conjugate vaccines. The common *N. meningitidis* serogroup reported in these countries were B strains, 0.2-14 cases per 100.000 populations was the reported incidence rate (Jafri et al., 2013).

In 2016, the WHO reported 26,029 cases of meningitis in the African meningitis belt of sub-Saharan Africa which extend from Senegal in the west to Ethiopia in the east and involve 26 countries, estimated number of deaths were 2080 with 8.0% case fatality ratio (WHO, 2016). The primary cause of bacterial meningitis in Middle East and North Africa is *N. meningitidis* (Ceyhan, 2012). In Tunisia, the case fatality ratios of MEN were around 18% with 85% of cases were reported in children below 5 years of age, the common detected serogroup was MenB. Pilgrims and children with high risk medical conditions in Tunisia receive polysaccharide vaccine (ACWY) (Borrow et al., 2017). The incidence rate of MEN in Morocco was 2-3.6 case per 100,

while the case fatality ratios were 7-13%. Morocco is MEN endemic area with sporadic outbreaks, MenB was the commonly detected MEN serogroup which identified in 95% of cases in a study conducted from 2011 to 2015 (Borrow et al., 2017). The African meningitis belt is characterized by high incidence rate of MEN during the first 6 months of the year due to associated dryness and high temperatures. In this regions the incidence rate can reach 1000 cases per 100.000 populations (Harrison et al., 2009). The most common serogroups of *N. meningitidis* reported in the African meningitis belt were serogroup A followed by W135 with serogroup X noticed in recent outbreaks (Chang et al., 2012; CDC, 2016). In 2015, Niger experienced one of the largest MEN outbreaks in the African meningitis belt caused by serogroup C (WHO, 2015). In Burkina Faso, Chad, Niger, and Nigeria the prominent *N. meningitidis* serogroup before the appearance of MenAfriVac vaccination in 2009 was MenA (Lingani et al., 2015). In South Africa the incidence rate of MEN was 0.44 per 100,000 in 2013 and 0.36 per 100,000 in 2014. MenW was the main identified serogroup followed by MenB (Meiring et al., 2015). In 1988, a serogroup A MEN epidemic reported in Chad and Sudan, 7500 and 18.000 cases were identified respectively (Salih et al., 1990). MenW serogroup present in Saudi Arabia after the MEN outbreak in Hajj in 2000 (Taha et al., 2002). MenW can affect all age groups and it was considered as a global medical emergency. Quadrivalent ACWY conjugate vaccine was introduced in 2015 and showed direct protection (Ladhani et al., 2016).

In Saudi Arabia, the presence of large number of muslims durig Hajj and Ummrah in Mekkah and Medina under certain conditions such as, high temperatures, physical prostration during these religious rites, crowded hostels were the major risk factors for MEN dissemination (Memish, 2010; Ahmed et al., 2006). International outbreaks caused by pilgrim carriers returning back from Hajj to their countries represent a global threat and have a major impact in the epidimiology of the disease (Saber et al, 2016). In 1987, the first reported international MEN outbreak in Hajj caused by serogroup A, South Asian pilgrims were believed to be the source of the infection as they represent 10% of the pilgirim at that time and reported the highest attack rate during the epidemic (Moore et al., 1989; Novelli et al., 1987). 1841 laboratory confirmed MEN cases reported mainly in Mecca, Medina, and Jeddah soon after the Hajj many cases reported in nearby Gulf countries, Europe, and North America among pilgirims returned from Saudi Arabia and their

family contacts (Al-Gahtani et al., 1995; Jones et al., 1990). In 1992, 102 MEN serogroup A confirmed cases were identified, 59% were religious visitors in Mekkah. The overall case fatality rate was 14.7%, among pilgrims from Pakistan the percentage reached 26.7% (Bushra et al., 1995). Saudi Arabia experienced another two outbreaks in 2000 and 2001, in which the reported annual incidence was 1.42 and 1.32 cases per 100.000 populations, respectively (Memish et al., 2013). W135 was the most involved serogroup reported in more than one-third of cases, while serogroup A reported in less than a quarter (Lingappa et al., 2003). In 2000, more than 400 cases reported during Hajj and in around 16 counteries (UK, Belgium, US, France, Morocco, Kuwait, Saudi Arabia, Oman, Indonesia, Singapore, Denmark, Finland, Sweden, Norway, Germany, and the Netherlands) (Mayer et al., 2002). More than 102 casess were reported in 2001 with 33 deaths, more than 50% were caused also by *N. meningitidis* serogroup W135 (Shibl et al., 2013; WHO, 2001). In 2010, the annual incidence decreased to 0.01 cases per 100.000 populations (Memish et al., 2013). *N. meningitides* serogroup A and W135 were the commonly reported in Saudi Arabia, MenB and MenC were also detected (Ceyhan et al., 2012; Jafri et al., 2013).

Quadrivalent meningococcal vaccine (ACYW153) introduced in Saudi Arabia by 2001, it was a mandatory requirement for all local and international pilgrims, pilgrims from outside Saudi Arabia would not receive an entery visa without vaccination (Ceyhan et al., 2012). MEN antibiotic prophylaxis given at ports for pilgirim from the African meningitis belt (Alqarni et al., 2015). These precautions decreased the incidence of MEN disease significantly during the period from 2002 to 2011 where only 184 laboratory confirmed reported cases in 2011 of which 9% were noticed among Hajj or Umrah pilgrims (Memish et al., 2013). Only 14 cases of MEN were reported in 2012 to 2015 all of which were outside Makkah and the Hajj season (Saber et al., 2016).

1.1.6.1. Clinical features

The clinical signs and symptoms of MEN can appear immediately or can last a few days before showing, the usual incubation period range between 3 - 7 days. The initial symptoms include a mixture of high-grade fever, headache, confusion, photophobia, petechial skin rash, heomorrhagic eruptions, nausea, vomiting, siezures, and signs of meningeal irritation (Figure 1.1.6.1.1) (Stephens et al., 2007;

Kim, 2010). Among children, this infection presents with signs of distress, excessive crying, irritability, refusal of feeding, jerky movements, and stiffness or floppy posture. Complications among children involve increase intracranial pressure. Bacterial meningitis caused by *Streptococcus pneumonia* specificly associated with neurological complications, the mortality rate was approximately 100% in untreated patients (Kim, 2010).

Approximately 68% of the infected cases die from complications. Those who survive experience permanent disabilities such as hearing loss, epilepsy like conditions, problems with concentration and memory, learning difficulties, and of loss of vision (Van de Beek et al, 2012). The complications can also lead to loss of limbs resulting from necessary amputations. Other people may develop arthritis-like symptoms and kidney problems.

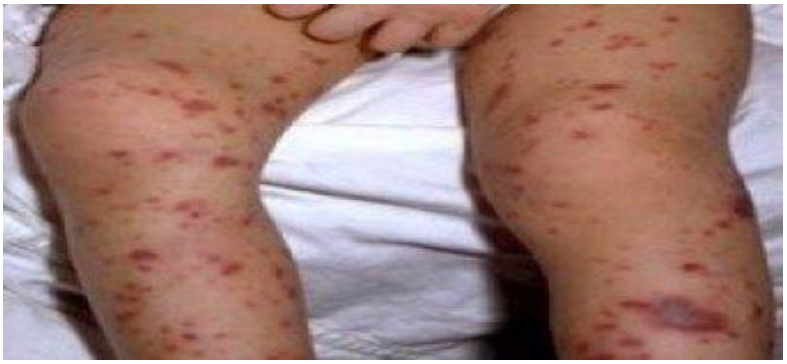

Figure 1.1.6.1.1. Meningococcoal meningitis skin rash. Dissaminated macular, maculopapular erythematous rash, purpuric, petechial, large hemorrhagic areas can progress to gangrenous, necrotic lesions in untreated patients. Start in the peripheries then spread to involve all the body, the face usually spared. It is non-blanchable skin rash by the glass test (Jean et al., 2013). The figure is obtained from (Sarah, 2017).

1.1.6.2. Mode of transmission to humans

MEN is an infectious disease that occur when the bacteria spread from one person to another, the spread is mainly through sneezing and coughing. Mothers who harbour the disease may pass the infectious agent to their newborn babies during birth (CDC, 2017).

The spread of meningitis depends on the bacterial agents involved. For example, meningitis caused by *Listeria monocytogenes* spread through food in which carriers are the source of the infection (Carpentier and Cerf, 2011).

1.1.6.3. Seasonality

Seasonality in meningitis belt is highly specific. Trends of bacterial meningitis seasonality in the African meningitis belt were studied and reported that, this disease occurs in patterns throughout the year (Traore et al., 2009). The peak season for MEN is during December to April (Paireau et al., 2016). However, pneumococcal meningitis can appear to have a higher frequency as compared to MEN. Different regions in the African meningitis belt might have differences in seasonalities. However, quantifications of all regions was not done (Traore et al., 2009). Seasonality changes through years were different, as some years do not have the same seasonal trends. These are the factors need to be considered when designing prevention and control strategies.

1.1.7. Malaria (MAL)

Malaria is a vector-borne disease caused by a protozoan parasite *(Plasmodium spp*.), which remains to be a significant disease that affects more than 100 tropical countries (Figure 1.1.7.1). Malaria is a major problem that affects country's economy due to the massive fatalities, treatment expenses, lost days of labor, and the subsequent adverse effect on development. In 2013, WHO reported malaria disease burden of about 207 million cases and an estimated 627,000 deaths in 2012. The CDC statistics indicated that within 2016, approximately 216 million cases of Malaria occurred and about 445,000 people died. However, the disease burden is reducing drastically in many places due to the control and preventive measures that have been put in place. For example since 2000, malaria mortality reduced by 48% globally and 29% in sub-Saharan Africa (WHO, 2013; CDC, 2018).

Malaria transmission occurs in 97 countries globally with 80 endemic countries in 2010, which puts almost 3.4 billion people at risk with heavy concentration noted in Sub-Saharan Africa (Griffin et al., 2016). Human malaria is known to be caused by mainly five species of the Plasmodium parasite, namely, *P. falciparum, P. ovale, P. vivax, P. knowlesi,* and *P. malariae*. Identification and differentiation of the parasite from one another was difficult due to the genotypic and phenotypic plasticity that affects control and surveillance strategies. The Polymerase Chain Reaction (PCR) allows researchers to apply genetic markers to differentiate sibling species from one another (McNamara et al., 2006).

Unlike Jizan region, aridity and hot climate in some parts of the Arabian Peninsula reduces the malaria risk dramaticly (Goldewijik et al., 2010). Yemen represents more than 80% of malaria positive cases (Snow et al., 2013). The peninsula shown to have a different malaria epidemiology with an important historical contribution that helps in understanding the failures and successes of malaria elimination. The Anopheles female mosquito is responsible for the distribution and circulation of these protozoan parasites that cause human malaria (Beck-Johnson et al., 2013; Paaijmans et al., 2010).

There are almost 400 species of the Anopheles mosquitoes known to exist, but only 30 to 40 species are responsible for the distribution of the causative agents of human malaria. *Anopheles arabiensis* is the most dangerous vector that transmits the most dangerous malaria protozoan parasite, *P. falciparum* (Yaw et al., 2012). However, in the Arabian Peninsula, *Anopheles sergentii* has established its presence (Robert et al., 2014). Malaria transmission occured through the bites of infected female Anopheles mosquitoes that are active during dusk and dawn. Transmission of malaria is more intense in areas where the mosquitoes species known to have a long lifespan and a regular habit of biting humans than areas where it is lifespan is short. *Anopheles gambiae* and *Anopheles funestus* are other examples, they are responsible for transmitting malaria in Africa (WHO, 2013).

Malaria requires mandatory treatment when signs and symptoms appear. If left untreated, patients may develop complications that would lead to death within a short time. Malaria is severe among children due to thier under-developed immune system.

1.1.7.1. Clinical features

Malaria, like other diseases discussed here, shows a variety of signs and symptoms. The initial symptoms include interchanging extreme fever, chills, and sweating. These symptoms may appear within 7 to 30 days after a mosquito bite depending on the malaria parasite species. Other clinical intestinal symptoms are diarrhoea, vomiting, and nausea. Associative symptoms include migraine headache, pallor, increased heart rate, and mental confusion. Malaria is grouped into two categories or phases, first phase is described as uncomplicated malaria, where treatment occurs through outpatient care facilities. The second phase is the complicated one.

Complicated malaria often shows signs of organ dysfunction in which patients adopt prone position, impaired consciousness, abnormal bleeding, multiple convulsions, and clinical jaundice, which is an evidence of vital organs dysfunction (Alessandro and Lorenzo, 2012; CDC, 2015). Among the five *Plasmodium spp* that cause malaria, *P. falciparum* cause the most severe malaria complications associated with organs dysfunction and death.

1.1.7.2. Mode of transmission to humans

Mosquito of the Genus *Anopheles*, transmit five different species of Plasmodium parasite that cause malaria in human. The link between the human and the mosquito completes the parasite's life cycle. Uninfected mosquitoes get infected through consuming infected blood meals from humans. Uninfected humans acquired the infection through mosquitoes bites (CDC, 2015). Malaria parasite can infects the host and the vector equally. Malaria life cycle passes through different stages: the sporozoites (which is injected by the female anopheles mosuitoes when feeding from humans), merozoites (stage affecting RBC's and causing their burst and appearance of symptoms), trophozoites (the multiplying form in erythrocytes), and gametocytes (the sexual stages). Gametocytes are then taken by the female *Anopheles* from infected individuals during blood meal and form another life cycle (Figure 1.1.7.2.1) (Brian et al., 2008; Laurence et al., 2002). The sporogonic growth of Plasmodium parasite within mosquitoes depends on several factors including temperature and humidity. Temperature accelerates the parasite growth within the mosquito (Paul and Edwin, 2010). Anopheles female mosquito is highly susceptible to Plasmodium parasite. The mosquito considered as having an innate susceptibility to Plasmodium parasite (Mike et al., 2004).

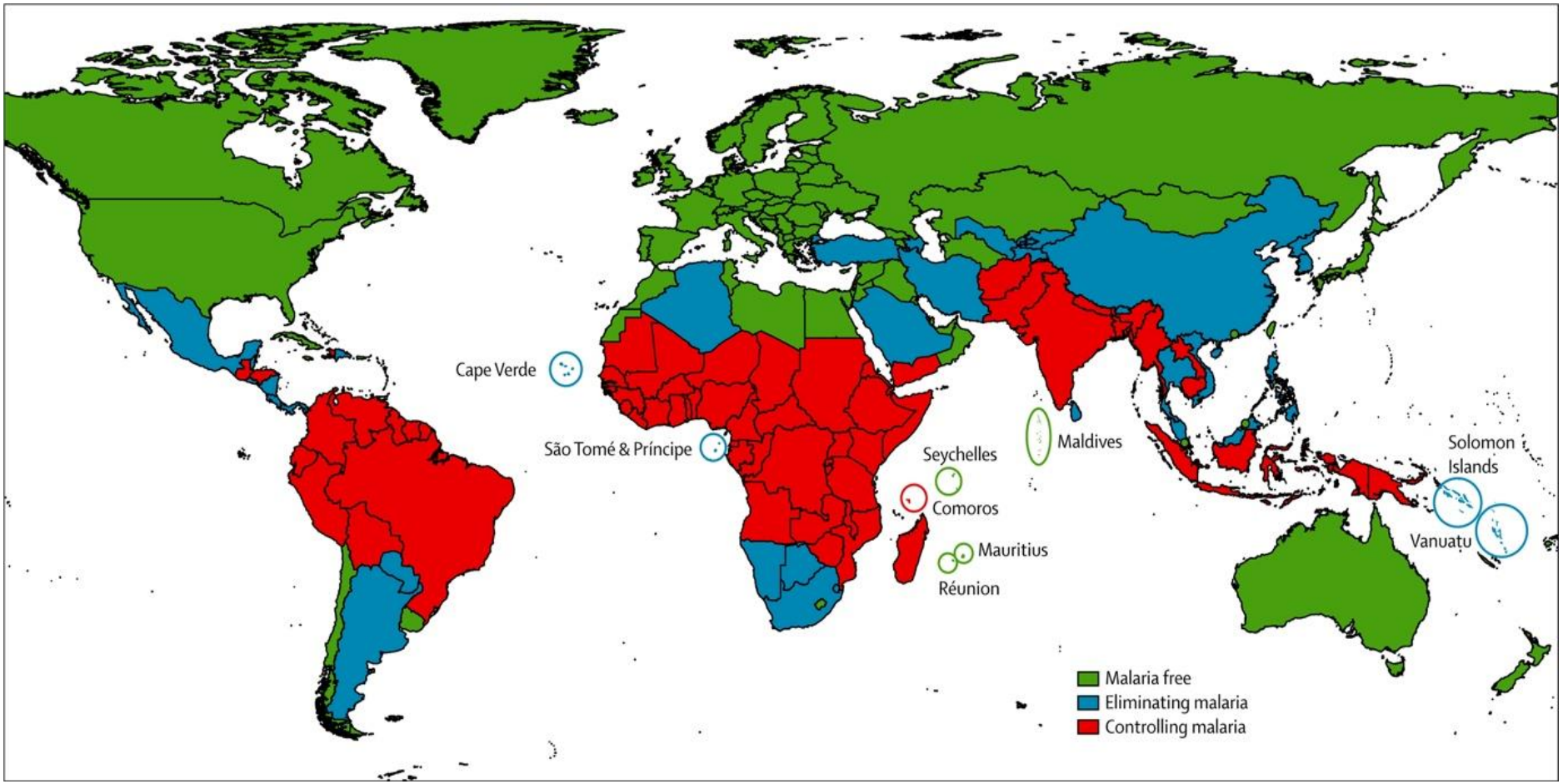

Figure 1.1.7.1 The world map shows prevalence of malaria in different countries; areas where malaria shows very high prevalence (Red), area where malaria shows less prevalence (blue), and countries of no records of reported cases (green) (Chris Cotter et al., 2013).

1.1.7.3. Seasonality

As compared to DENF, CHIKF, leptospirosis, brucellosis, and meningococcal meningitis, malaria shows high and specific seasonality. Malaria shows seasonality in most of the endemic areas (Roca-Feltrer et al., 2009). The program of choice for preventing and controlling malaria depends on seasonality. High peaks of malaria infection coincides with the presence of the transmitting mosquitoes (Charrel et al., 2007).

Mosquitoes breed during rainy seasons and it is present in high numbers towards the end and after the rainy season in endemic areas (Lindblade et al., 2013). Unfortunately, malaria-endemic areas are characterized by high rainfall. Malaria transmission may occur all year-round (Eckhoff, 2011). Malaria transmission can be grouped into marked and unmarked seasonality. Unmarked seasonality occurs in non-endemic areas. This seasonality also described for infected patients traveling from high endemic areas.

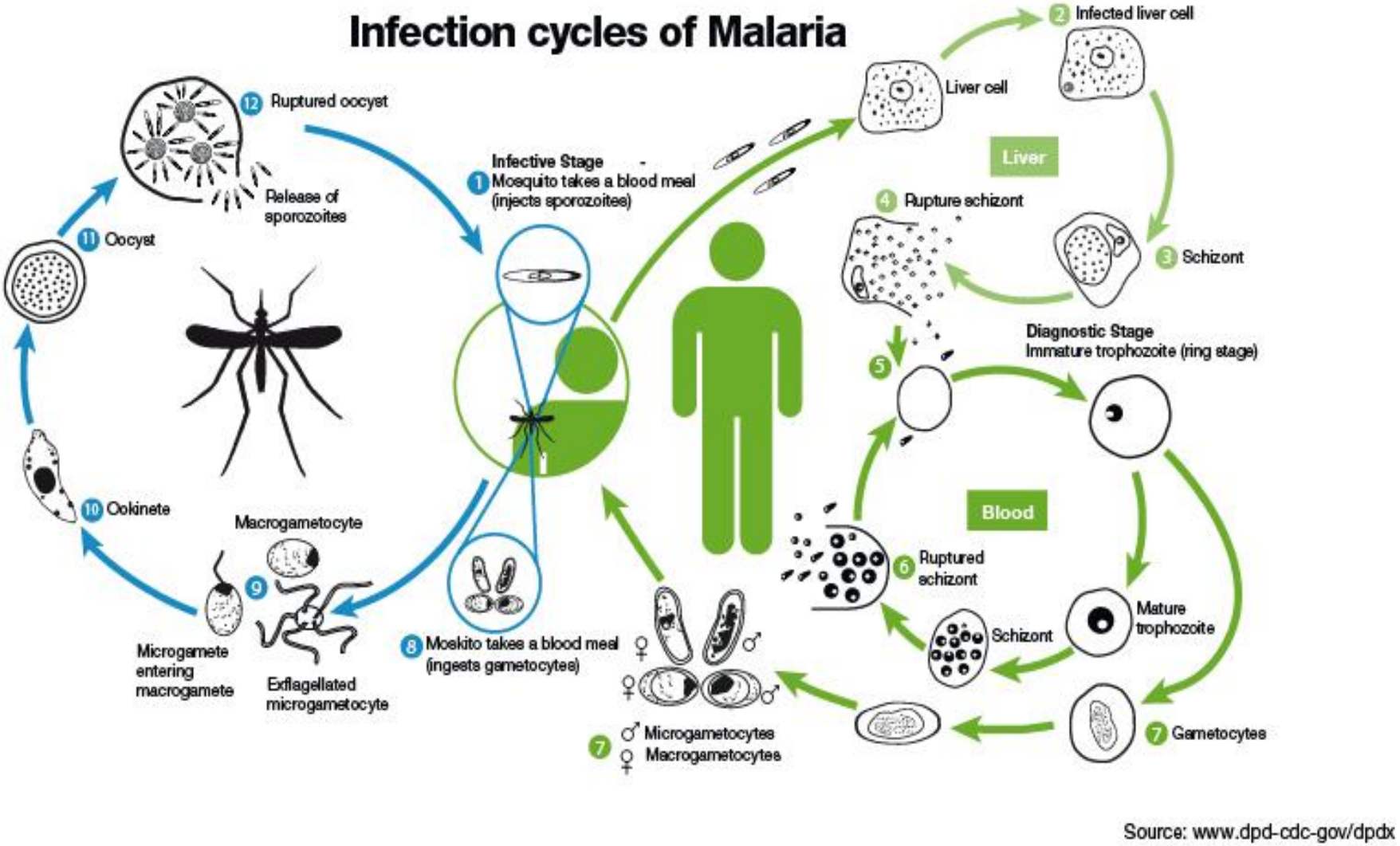

Figure 1.1.7.2.1. Schematic diagram illustrating the transmission cycle of malaria and disease stages (www.dpd-cdc-gov/dpdx).

1.2. The impact of Interferon stimulated genes (ISGs) in viral biology

Interferon stimulated genes (ISGs) are induced by viral antigens through specific receptors, hunderads of ISGs are produced which have a significant antiviral effects. ISGs are characterized by it is viral inhibitory activity, it has the ability to arreset the virus at any step of it is life cycle (John and Charles, 2011). *Myxovirus resistance (Mx)* genes are virus entery inhibitors. The human cells express Mx1 and Mx2 protiens which inhibit any virus component from cell entery before genome replication. Mx1 mutations results in the loss of human antiviral activity against LaCrosse and influenza A viruses, the involved mechanism is not clear (Gao et al., 2010). USP18 mediate long-term desensitization to IFNs, which predict poor treatment response of hepatitis C virus (HCV) infection to IFN-α therapy (Sarasin-Filipowicz et al., 2008). Mx2 inhibits HIV-1 and HIV-2 replication by preventing the virus from nuclear entery and chromosomal integration, this inhibitory function can be lost with mutations in the virus capsid protein (Kane et al., 2013; Goujon et al., 2013).

Interferons were classified into type I (IFNα/β), type III (IL28A, IL28B, IL29) IFNs, and type II IFN (IFNγ) (Borden et al., 2007). Each interferone can induce specific ISGs set through the JAK-STAT pathway, or it can be stimulated directly in response to viral infections (Sen and Peters, 2007). About 25 years ago, the first ISGs were discovered (Larner et al., 1984). The microarray study adentified 50-1000 ISGs including 200-500 genes of many cell types (de Veer et al., 2001). The antiviral effects of ISGs were not clealy identified untill now. According to several discovary-based screens, antiviral response against DENV mediated by Viperin, ISG20, IFITM2 and IFITM3 ISGs (Jiang et al., 2010). The inhibitory effect of ISGs specific group against certain virus may overlap with other viruses profiles, some ISGs showed activiation of virus replication in a virus-specific manner (John and Charles, 2011). Detailed identification and characterization of ISGs antiviral mechanisms will influence the evolution of new antiviral therapy.

1.3. Current problems and unmet needs

Emerging vectors-borne infectious diseases in Saudi Arabia prevalence was influenced by the global influx of people and vector adaptation to new regions with

new species evolution had the major impact (Chapter 1.0). Moreover, these diseases were neglected in Kingdom of Saudi Arabia over the last decade due to limited diagnostic tools and specialized clinics; the diagnostic tools are only available in the large central hospitals laboratories. Poor Ministry of Health (MOH) data availability regarding the magnitude of these epidemics and lack of geographical locations of the endemic areas are also reasons why this research has been carried out. Furthermore, the deficiency in knowledge and orientation of the health care providers in terms of clinical manifestations and the necessary laboratory investigations parameters of these diseases had played a role in diseases spread.

1.4. Project aims and approaches

The overlapping of clinical presentations and laboratory findings in many diseases limits the ability to narrow down the clinical differential diagnosis in certain diseases. Therefore, this research project aim to profile and characterize febrile diseases based on their immune mediators, along with the classical clinical and laboratory diagnosis in order to study the unique and common immune mediators of each particular disease.

Chapter 3.1. This chapter will focus on:

I. The routine laboratory tests conducted in all acute febrile patients recruited in the research project.

Chapter 3.2. This chapter will focus on:

I. DENV, CHIKV, and DENV+CHIKV co-infection confitmatory tests results.

Chapter 3.3. This chapter will focus on:

I. Traditional culture methods for identification of GAS, Leptospira spp, Brucella spp, and *N. meningitides*.
II. All mentioned pathogenic organisms culture and biochemical confirmatory tests results.

Chapter 3.4. The chapter will focus on:

I. Malaria identified species, parasitemia level, and ICT test results.

Chapter 4.1. This chapter will focus on:

I. Studying the longitudinal characterization of each disease group (viral, bacterial, and parasitic) to understand the incidence, prevalence, and epidemiological factors impacting disease causation for each disease group eg. factors impacting disease distribution for DENV, CHIKV and malaria.
II. Studying each disease group in accordance to gender and to different age groups. The age groups are classified in to five age groups (0-1, 1-4, 5-14, 15-44, and 45 and above).

Chapter 4.2. This chapter will focus on:

I. Understanding the similarities and differences in laboratory parameters such as neutrophils percentages, lymphocytes percentages, C Reactive Protein (CRP) levels, haemoglobin (Hb) levels, platelets count (PLT), liver enzymes, and Erythrocytes Sedimentation Rate (ESR), as well as the similarities and differences in patient's clinical presentations such as fever, rash, headache, joints pain, nausea, and vomiting for each disease group.
II. Comparing the differences between viral, bacterial, and malarial infections based on clinical signs, symptoms, and the relevant diagnostic tests.
III. Raising the public awareness with regards to neglected diseases in the kingdom and to bring up proper treatment suggestions and patients managements protocols for quicker diagnosis of each disease group to reduce the number of morbidity and mortality.

Chapter 4.3. This chapter will focus on:

I. Identifying the profiles of immune-mediators in DENV, GAS, Meningiococcal meningitis, and MAL patients through the use of multiplex microbead-based immunoassays.
II. Identifying the common immune mediators found in each disease group (bacterial, viral and malaria)
III. Identifying the unique immune mediators for each disease.

Chapter 4.4. This chapter will focus on:

I. Meta-analysis of results to identify correlations between DENV parameters (NS1, IgM, IgG, and viral load) and differential immune mediators with the corresponded *P* values.
II. Identifying correlations between *N. meningitidis* serotypes (W135, A, B, X) and differential immune mediators with the corresponded *P* values.
III. Identifying associations between Plasmodium species, parasitemia level and positive malaria thick blood film with differential immune mediators and the corresponded *P* values.

Chapter 2. Materials and methods

2.1. Materials

2.1.1. Generals reagents

The materials that were mostly used were either purchased or supplied exclusively from different enterprises like Bio-Rad Laboratories Hercules, California, United States, bioactiva diagnostic GmbH-Bad Homburg vor der Höhe Deutschland. A*STAR Biopolis Shared Facilities (BSF), Diagnostic Automation Inc, Research Support Centre, Singapore. Cepheid, Jeddah, Kingdom of Saudi Arabia and Oxoid Ltd, England WA142DT. EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany. Luminex Corporation, Biotechnology Company, Austin, Texas, United States. Roche Diagnostics GmbH., Germany. Qiagen, Netherlands. Murex Diagnostics Ltd, USA.

2.1.2. Patients enrollment

serum samples between Jan 2014 to Jan 2018 were collected from febrile patients attending the infectious diseases clinic (IDC) at King Fahad Central Hospital, Jizan, southwest of the Kingdom of Saudi Arabia, control samples were collected from healthy individuals and tested negative for diseases. The collection of all samples considered ethically approved by the Ethical Committee Panel with IRB number 17/EA/002/1436H in King Fahad Hospital (Saudi Arabia), by obtaining consent from the patients before collection of the samples. The study subject both the experimental and control participants to the same diet and conditions to allow for consistency of the findings and avoid the possibility of bias.

2.1.3. Serum samples

The samples were randomly selected to ensure that the results that obtained were not biased and the infectious diseases suspected samples collected after consent as mentioned above, which took place from January 2014 to Jan 2018 in which 2048 serum samples obtained along with 100 plasma samples used as healthy controls. The serum specimens were kept in freezers at - 80^{o}c until they were required. The samples were stored in different physical locations to reduce the chances of loss caused by electric breakdown, which would alter the temperatures.

2.2. Methods

2.2.1. Clinical assessments

The diseases that were analysed in the study includes DENF, CHIKF, GAS, meningiococcal meningitis, brucellosis, leptospirosis, and malaria. The research was conducted in the IDC, all of the patients presented to the clinic with acute (within one week) or subacute (within 4 weeks) febrile illness were included in the study and approached as suspected cases of one of the above mentioned infectious diseases. Personal data includes; patient full name, age, occupation, home address, nationality, recent travel, and history of exposure to mosquitoes. The main complaint for all patients was fever, which was differentiated in accordance to the duration, severity, pattern, associations, and relieving factors. Malaria, DENF, CHIKF, GAS, meningiococcal meningitis and leptospirosis infections associated with acute fever. Malaria have characteristic tertian or quatrain pattern associated with chills and sweating. DENF characterized by very high-grade fever, which may reach 41ºC, associated with aching pain in the back muscles and extremities (break-bone fever). The fever reported by brucellosis suspected patients usually sub-acute, chronic or intermittent and associated with drench sweating mostly at night, other complains like headache, nausea shared by all these infectious diseases with variation in presentation.

Backache was the main complain of chronic brucellosis patients, malaria and DENF. Patients suffering from CHIKF presented with polyarticular arthralgia with > 10 joints involved, the pain was noticed during mornings with restriction of movement, DENV supposed patients presented with generlized muscle pain. Patients may present with erythematous maculopapular rash in meningiococcal meningitis, DENF and CHIK. Patients with leptospirosis manifested with petechial eruptions in the soft palate.

Vomiting was reported by malaria and sever GAS infection. Some patients presented with retro-orbital pain, as it is characteristic of DENF. On clinical examination, high temperature was recorded in many patients, signs of anaemia were noticed with few cases reported with jaundice and conjunctival suffusion detected in leptospirosis cases. The level of consciousness was assessed and signs of meningeal irritation were checked for identification of meningitis suspected cases.

On examination, many skin manifestations were observed such as erythematous maculopapular rash, petechial and pupuric rashes. The purpuric rash was assessed using the tourniquet test, in which the blood pressure cuff inflated in the upper arm and fixed in between the systolic and diastolic blood pressure for five minutes, if > 10 petechiae are noticed the test is considered positive, which makes DENF and CHIKF highly suspicious. Abdominal examination revealed hepato-splenomegaly in brucellosis suspected patients, while moderate-massive splenomegaly was found in malaria suspected cases and tender hepatomegaly in doubtful sever malaria or leptospirosis sufferers.

Joints were examined for signs of arthropathy or septic arthritis, which indicate viral haemorrhagic fevers or brucellosis. Central nervous system examinations and cardiovascular examinations were carried-out only in suspected cases of brucellosis complications. Chest was examined in suspected GAS infection, along with lymph nodes examination and pharyngeal assessment, scarlitiniform skin rash and presence of any pusy pustules or features of cellulitis.

A list of differential diagnosis for any patient presented to the clinic with acute febrile illness was completed following history taking and clinical examination. The list includes the seven infectious diseases of the study beside other suspected conditions like; Typhoid fever, acute viral upper respiratory tract infections, urinary tract infections, acute gastroenteritis, and connective tissue diseases (acute systemic lupus erythromatosis, rheumatoid arthritis, vasculitis).

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2.2.2. Lumber puncture (LP) procedure

LP was performed by the following technique, the patient positioned in lateral decubitus or on sitting position, under aseptic condition L3 - L4 inter-space was located by finding the right and left posterior superior iliac crests, then the interspaces were assessed above and below L3 - L4 interspace to find the widest one followed by marking the correct space for needle entry. A spinal tray was prepared near the patient, which contains sterile gloves, skin swabs, sterile fenestrated drapes plastic tubes with an adhesive tape manometer and stopcock. A 10 mL of local anaesthesia, lidocaine was in-filtered in the skin. After anaesthetising the whole area, a 20 - 22 gauge needle was placed with the thumb guidance to pass through the skin in the marked space, the bevel was in a direction parallel to the longitudinal dural fibers. The needle inserted was pointed towards the umbilicus

smoothly, after 4 - 5 cm, the stylet was removed and observed for fluid release, sometime a specific "pop" was felt with needle entry through the dura. If no fluid is returned, the stylet was replaced and the needle either advanced or pulled-back slowly with observation of fluid release. CSF opening pressure was measured while the patient legs straightened in lateral recumbent position. When the fluid appears out of the needle, the manometer connected by stopcock and the height of the fluid identified.

The determination for the presence of bacterial, viral, fungal, parasitic or tuberculous cause was done based on cytological evaluation of the CSF and bacterial identification in appropriate culture media.

2.2.2.1. Processing of CSF specimens

8 µL of CSF sample was used, centrifugation was done for 3 minutes at 275 × g (6-place rotor for 1,500 min-1). The supernatant was drawn off by sterile pasteur pipette. Subsequently, the sediments were mixed vigorously, once mixed 1 - 2 drops of sediments were used for Gram staining, another one drop used for streaking in primary culture media. Cytological examination was done before centrifugation and heating of the CSF by wet preparation under light microscopy.

2.2.2.2. Interpretation of CSF analysis results, biochemical and cytological

Collected CSF samples from suspected patients were processed as discussed above. Opening pressure was determined, cellular and biochemical tests were performed. The results were interpreted as shown in (Table 2.2.2.2.1). Confirmatory microscopic investigations were done for identification of the causative organism.

Table 2.2.2.2.1. CSF macroscopic and microscopic results interpretation.

	Appearance	Opening pressure mm h_2O	WBC count Cells/µl	Glucose mg/dl	Protein mg/dl
Normal CSF	Clear	80 - 180	0-5 lymph	50-75	15-40
Bacterial meningitis	Cloudy	> 250	100-5000 > 80% PMNs	< 40	> 100
Viral Meningitis	Clear	90 - 200	10-300 Lymphocytes	Normal	normal
Tuberculous meningitis	Clear/opaque	180 -300	100-500 lymphocytes	Reduced < 40	>100

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2.2.3. Serum immune mediators quantifications

A 45 immune mediators were detected and measured from positive serum samples for DENV, GAS, MEN, and MAL, by using human cytokine 45-plex immunoassay kits (Procarta) and utilizing Luminex FlexMap 3D® instrument for data extraction. The procedure was performed according to the manufacture instructions. Cytokines levels measured were 20 pro-inflammatory cytokines, which include GRO-α, IFN-γ, IL-7, IL-8, IL-18, IL-1α, IL-1β, TNF-α, GM-CSF, IL-2, IL-15, IL-17A, IL-21, IL-22, IL-23, IL-27, IL-31, TNF-β, IL-6, and IL-20p70. The anti-inflammatory cytokines analysed were, IL-10, IL-4, IL-13, and IL-1RA. The chemokines included IP-10, MIP-1α, MIP-1β, RANTES, MCP-1, Eotaxin, and SDF-1α. Growth factors measured were PDGF-BB, PIGF-1, EGF, VEGF-A, VEGF-D, bNGF, SCF, BDNF, FGF-2, HGF, and LIF. The analysis was done by Bio-plex Manager™ 6.0 software (Bio-Rad), based on standard curves plotted through a five-parameter logistic curve setting. Signal of IFN-α, IL-5, and IL-9 were found to be below detection limit and hence excluded from subsequent analysis.

2.2.4. Statistical analysis

The analysis was performed by using GraphPad Prism 6 (GraphPad Software Inc), were tables and graphs generated. Mann-Whitney 2-tails analysis and Kruskal-Wallis with Dunn's comparison were used for comparisons between groups.

Chapter 3: Results

3.1. Routine laboratory investigations

Laboratory testing was carried out for all febrile patients samples including healthy control group. The tests include:

- Complete blood count; haemoglobin level, lymphocytes percentages, Neutrophiles percentages and PLT count
- C Reactive Protein level (CRP) level
- Estimated Sedimentation Rate (ESR)
- Liver enzyme, ALT
- Liver enzyme, AST

Confirmatory tests for suspected viral, bacterial or malaria infections were performed.

3.2. Confirmatory investigations

3.2.1. DENV

3.2.1.1. DENV NS1 antigen detection

DENV NS1 was detected by one-step sandwich-format micro-plate enzyme immunoassay for qualitative and semi-quantitative detection of DENV NS1 antigen in human serum or plasma (Bio-Rad Laboratories Hercules). NS1 Antigen captured by the murine monoclonal antibodies (MAb). The test was performed from serum samples of patients and healthy control individuals in accordance to the manufacture instructions. The samples were incubated directly and simultaneously with conjugate inside micro-plate wells sensitised with anti-NS1 MAb at 37ºC for 90 minutes. Immune-complex Mab-NS1/peroxidise was formed in NS1 antigen positive samples. The plate was then washed, followed by identification of the immune-complexes by colour development reaction through distribution in a chromogenic solution found in each well. Later, the micro-plate was incubated at room temperature for 30 minutes; acidic solution was added to stop the enzymatic reaction. A spectrometer set at 450/620 nm was used for reading of the optic density which was commensurate to the current level of NS1 antigen in the samples. A sample is considered positive or negative for NS1 antigen by comparing the optical density reading of the sample with the optical density of the calibrator.

3.2.1.2. DENV antibodies quantifications

DENV IgM, IgG

In this study DENV IgM detection was tested by ELISA, Enzyme Immunoassay for qualitative findings of IgM class antibodies against DENV in human serum (bioactiva diagnostic GmbH-Bad Homburg vor der Höhe). It is an Enzyme Linked Immunosorbent Assay technique, which uses 12 break apart 8-well snap-off strips coated with DENV antigen type 2. The test was performed for samples and control using the serum according to the manufacture instructions. The human serum samples were firstly diluted to 1/100 dilution with IgM sample diluent by adding 10µL of the sample to 1 ml of IgM sample diluent in a tube and thoroughly mix with a vortex. The control and diluted samples allocated to their respective wells, A1 well left blank for substrate, then the wells were covered by foil and incubated for 1 hour at 37ºC. Later, wells contents were aspirated and each well was carefully washed 3 times with 300 µL washing solution, the remaining was then removed by tapping strips in tissue paper. A 100 µL of DENV anti-IgM was conjugate then added, except for A1 well. The plate was then covered with foil and incubated at room temperature for 30 minutes. The washing step was repeated in the same manner as before. A 100 µL of Tetramethylebenzidine (TMB) substrate solution was finally added into each well and incubated at room temperature in the dark for 15 minutes ensued by dispense of 100 µL stop solution in all wells. The absorbance level of each specimen was measured by spectrometer set at 450/620 nm within 30 minutes.

Specific IgG immunoglobulin class against DENV was detected using ELISA (bioactiva diagnostic GmbH-Bad Homburg vor der Höhe). Enzyme Immunoassay for qualitative determination of IgG class antibodies against DENV in human serum or plasma, applied for samples and control using serum according to the manufacture instructions. Micro-titer strip wells pre-coated with DENV antigen type 2 was used, the test conducted on the same manner as described above for detection of DENV IgM. DENV antibodies expressed as antibody titer that defined as the lowest dilution required for detectable signal above the control.

3.2.1.3. DENV PCR

DENV RNA Replicative Intermediate RNA (RI-RNA) for all DENV serotypes was detected via qualitative Real-Time PCR (RT-PCR) by usage of the light/smart cycler system. It is an integrated DNA/RNA amplification and detection system carried in 20

µL serum or plasma according to the manufacture instructions (Cepheid), it contains 16 independently programmable I-core modules, every module performs four-colour real-time fluorometric detection. Serum samples were gathered from the suspected DENV patients in the acute stage of infection.

Table 3.2.1.1. ELISA results for detection of NS1, IgM, IgG antibodies and RT-PCR for DENV RNA in serum of patients with acute DENV infection.

Sample No.	NS1	IgM	IgG	Viral load (PFU/mL)	Sample No.	NS1	IgM	IgG	Viral load (PFU/mL)
1	1	1	1	231	45	0	0	1	10.67
2	0	0	1	9	46	0	0	1	5.87
3	1	0	1	8	47	1	1	0	902.65
4	0	0	1	5.86	48	1	0	1	74
5	0	1	0	6	49	0	0	1	3.089
6	0	1	0	12	50	1	0	1	6.953
7	0	1	0	1.3	51	1	0	1	8
8	1	1	1	37.31	52	1	1	0	706.48
9	1	1	1	15	53	0	0	1	12
10	0	0	1	ND	54	0	0	1	6.43
11	0	1	1	5.43	55	1	1	0	8.976
12	1	1	1	311.62	56	1	1	0	5.183
13	0	0	1	6.53	57	1	1	0	3.9
14	0	0	1	8	58	1	1	0	10.64
15	0	1	1	2.458	59	1	1	0	123
16	0	1	1	18.13	60	1	1	0	473.431
17	0	1	1	724	61	1	0	0	25
18	0	1	1	6.92	62	1	1	0	840
19	1	1	0	44.19	63	1	1	0	8.643
20	1	1	0	841.4	64	1	1	0	4. 979
21	1	0	0	48	65	1	1	0	124.73
22	1	0	0	53	66	1	0	1	467.92
23	1	1	0	17	67	1	1	0	806.33
24	1	1	0	6.72	68	1	1	0	56.23
25	1	0	1	18	69	1	1	0	33. 458
26	0	0	1	ND	70	1	0	1	947.54
27	1	1	0	22	71	1	1	0	861
28	0	1	0	7.09	72	1	1	0	36.568
29	1	1	0	168.36	73	1	1	0	573.99
30	1	1	0	400	74	1	0	0	3. 071
31	1	1	0	915	75	1	1	0	66
32	1	0	0	46.159	76	1	1	0	7. 389
33	0	0	1	0.652	77	1	0	0	11. 890
34	0	0	1	23	78	1	1	0	45
35	1	1	0	1.409.2	79	1	1	0	6.35
36	1	0	0	442	80	1	1	0	87.218
37	0	0	1	38	81	1	1	0	74.38
38	1	0	0	51.37	82	1	1	0	186.7
39	1	0	0	25	83	1	1	0	93.654
40	1	0	0	62.632	84	1	1	0	3.79
41	1	0	1	78.6	85	1	1	0	543
42	0	0	1	47	86	1	1	0	871.633
43	0	1	0	13	87	1	1	0	985
44	0	0	1	8.7	88	1	1	0	47.551

Sample No.	NS1	IgM	IgG	Viral load (PFU/mL)	Sample No.	NS1	IgM	IgG	Viral load (PFU/mL)
89	1	1	0	18.67	132	1	0	0	2.620.1
90	1	1	0	976	133	1	1	0	850.62
91	1	1	0	65	134	1	0	0	18
92	1	1	0	614.877	135	1	1	0	152.76
93	1	1	0	26. 443	136	1	1	0	690.34
94	1	1	0	38	137	1	1	0	9.65
95	1	1	0	2.976	138	1	1	0	13
96	1	1	0	17	139	1	1	0	832
97	1	1	0	3.06	140	1	1	0	812.39
98	1	1	0	381.62	141	1	1	0	799.46
99	1	1	0	235.71	142	1	1	0	870.65
100	1	1	0	2.27	143	1	1	0	34.87
101	1	1	0	126.88	144	1	0	1	679.82
102	1	1	0	798.22	145	1	1	0	595.61
103	1	0	0	15	146	1	1	0	2.72
104	1	1	0	956.24	147	1	1	0	884.53
105	1	0	0	76.44	148	1	1	0	72
106	1	1	0	280.64	149	1	1	0	673.49
107	1	1	0	67.82	150	1	1	0	875.32
108	1	0	1	74.31	151	1	1	0	720.48
109	1	1	0	3.67	152	1	1	0	12.64
110	1	1	0	114.95	153	1	1	0	148.91
111	1	1	0	361.55	154	1	1	0	462.75
112	1	1	0	521.82	155	1	1	0	28.63
113	1	0	0	971.73	156	1	1	1	396.86
114	1	1	0	45.61	157	1	1	0	10
115	1	1	1	759.49	158	1	0	1	480.97
116	1	1	0	39	159	1	1	0	3.05
117	0	1	1	ND	160	1	1	0	804.6
118	1	1	0	796.83	161	1	1	0	13
119	1	1	0	8.31	162	1	1	0	142.65
120	1	0	0	618.36	163	1	1	0	98.36
121	1	1	0	471.93	164	1	1	0	5.84
122	1	1	0	1.634.2	165	1	1	0	160.73
123	1	0	0	651.13	166	1	1	0	206.98
124	1	1	0	34.88	167	1	1	0	4.31
125	1	1	0	369.52	168	1	0	1	936.62
126	1	1	1	620.21	169	1	1	0	94.61
127	1	1	0	385	170	1	1	0	21
128	1	0	0	1.642.2	171	1	1	0	4.08
129	1	1	0	95.46	172	1	0	0	1.368.8
130	1	1	0	761.53	173	1	1	0	169.02
131	1	0	0	984.71	174	1	1	0	2.04

Abbreviations: ELIZA, Enzyme Linked Immunosorbent Assay; DENV, Dengue virus; IgM, Immunoglobulin M; IgG, Immunoglobulin G; RT-PCR, Real Time PCR; ND, not determined.

There were 174 confirmed DENV infected patients, 172 out of 174 patients which represent (98.8%) were on acute DENV infection based of DENV RNA identification by RT-PCR (Table 3.2.1.1). The viral load was not determined in two infected cases, one was positive for IgG and negative for both NS1 and IgM which may indicate late

acute phase specimen and in the absence of convalescence phase specimen defined as laboratory-indeterminate case (CDC, 2017), the other one was positive for both IgM and IgG and negative for NS1which may indicate recent probable DENV infection as the IgM may remain in serum up to 3 months after the infection or it was secondary DENV infection. 41 out of 174 (23.5%) patients were IgG positive which indicate early or late secondary DENV infection. Convalsoscence phase specimens were not taken, all the specimens were collected only during acute illness.

3.2.2. CHIKV

3.2.2.1. CHIKV immunogloulins quantifications

CHIKV specific immunoglobulin class IgM in the serum was detected by semi quantitative ELISA test according to the manufacture instructions (EUROIMMUN Medizinische Labordiagnostika AG). The principle of the test based on ELISA technique, it contains micro-titer strips coated with recombinant CHIKV antigens. Plasma samples were diluted with green coloured sample buffer to 1:101 and incubated for 10 minutes at room temperature, then transferred to individual microplate wells according to pipetting protocols. The test plate was coverd by foil and incubated for 60 minutes at 37° C ± 1° C, followed by three times washing by 300 µL wash buffer, the wash buffer was left for 30 to 60 minutes per washing cycle which was subsequently totally removed and dried by absorbent paper. Later, the conjugation was performed by adding peroxidase-labelled anti-human IgM conjugate enzyme in each microplate well and incubated for 30 minutes at room temperature, then washed as above. Lastly, 100 µL of chromogen/substrate solution was pipetted in every microplate well and incubated at room temperature for 15 minutes, followed by 100 µL stop solution, which was added, on the same manner and speed of substrate solution. Subsequently, photometric measurements were made at wavelength of 450 nm within 30 minutes from stop solution addition.

Specific immunoglobulin IgG against CHIKV was detected by ELISA test according to the manufacture instructions (EUROIMMUN Medizinische Labordiagnostika AG). The test principle is the same as for CHIKV IgM detection, except it was only considered positive with 4-fold IgG titer increase in two samples. CHIKV antibodies are expressed as antibody titer, which is defined as the lowest dilution required for detectable signal above the control.

3.2.2.2. CHIKV PCR

CHIKV RNA was detected and quantified via Real-time PCR (RT-PCR) during early viremic period within 7 days from the start of illness, the protocol used was TaqMan® real-time RT-PCR which use the E1 structural gene region targeting primers. The protocol utilized LightCycler® 480 Instrument (Roche Diagnostics GmbH., Germany) according to manufacture instructions. The kit used was QuantiTect Probe RT-PCR (Qiagen, Netherlands). A 10 µL reaction volume was prepared according to the protocol, the concentrations were adjusted and each PCR run involved no-template control (NTC) and a positive control (CHIKV). Then a quantification assay standard curve was generated.

Table 3.2.2.1. ELISA results for detection of CHIKV specific IgM, IgG and RT-PCR resuts for CHIKV RNA detection in serum of patients with acute CHIKV infection.

Sample No.	IgM	IgG	Viral load (PFU/mL)	Sample No.	IgM	IgG	Viral load (PFU/mL)
1	1	0	1000	18	1	1	10
2	1	1	100	19	0	0	100
3	0	0	100	20	0	0	1000
4	1	1	10	21	1	0	100
5	1	1	10	22	1	1	10
6	1	0	100	23	1	0	100
7	1	1	100	24	1	0	100
8	1	1	10	25	0	0	100
9	1	1	100	26	1	1	10
10	1	1	100	27	1	1	10
11	1	0	100	28	1	1	0.1
12	1	1	0.1	29	1	0	100
13	1	1	0.1	30	1	1	10
14	1	0	100	31	1	0	100
15	1	1	100	32	0	0	1000
16	1	1	0.1	33	1	0	1000
17	1	1	10	34	1	1	100

Abbreviations: ELIZA, Enzyme Linked Immunosorbent Assay; CHIKV, Chikungunya virus; IgM, Immunoglobulin M; IgG, Immunoglobulin G; RT-PCR, Real Time PCR.

CHIKV infection was confirmed by RT-PCR in all 34 out of 34 patients (100%), which indicate acute CHIKV infection, results are reflected in (Table 3.2.2.1). Only 5 patient out of 34 infected cases (14.7%) were CHIKV specific IgM negative as the sample will be collected during early infection within less than 1 week before the appearance of IgM. CHIKV specific IgG detected in 19 out of 34 infected cases (55.8%). CHIKV specific IgG detected in 10 to 15 days from the appearance of symptoms.

3.2.3. DENV+CHIKV co-infection

On clinical assessment of 15 patients presented with acute febrile illness, the signs and symptoms of both DENV and CHIKV infection were reported in severe form. The DENV and CHIKV RNA were confirmed and identified by RT-PCR in serum samples (Table 3.2.3.1). The detection of both DENV and CHIKV RNA confirmed the diagnosis of DENV+CHIKV co-infection, it is difficult to predict which infection occurred before the other or both viruses were carried and transmiited by the same vector.

Table 3.2.3.1. DENV+CHIKV co-infection tested by RT-PCR.

Sample No.	DENV VL	CHIKV VL	Sample No.	DENV VL	CHIKV VL	Sample No.	DENV VL	CHIKV VL
1	512.34	100	6	82.21	100	11	792	100
2	680.75	100	7	913.05	10	12	978.13	100
3	754	1000	8	495.77	100	13	603.49	1000
4	875.93	1000	9	743.8	1000	14	1.325.02	1000
5	382.66	100	10	879	100	15	2.858	0.1

Abbreviations: VL, viral load; CHIKV, Chikungunya virus; DENV, Dengue virus; RT-PCR, Real-Time PCR.

3.3. Traditional culture methods

The traditional culture methods for bacterial identifications were used to test for the presence of GAS, Brucella, and leptospirosis organisms in patient's samples depending on the sample type and the collection site.

The specimens were collected and cultured for microbiological culture as shown in the (Table 3.3.1).

Table 3.3.1. Traditional culture media, incubation duration and targeted organisms.

Organism seen in gram film	Standard Media	Incubation		reading	Target Organisms
		Atmos	Time		
GPC ? GAS	Blood agar Chocolate agar Neomycin (+Mtz 5) FAA	CO_2 CO_2 Air AnO_2 AnO_2	24-48 hrs 24-48 hrs 24-48 hrs 24-48 hrs 24-48 hrs	Daily	Any
	3 drops into 5ml sterile water as inoculum for 1st and 2nd line disc sensitivity tests on iso-sens plate. Incubate in air at 37C for 24 hrs				
GNB ? Brucella	BACTEC–Broth Blood agar Chocolate agar	CO_2 CO_2 Air AnO_2	2-14 days 2-14 days 2-14 days 2-14 days	Daily	Any
	3 drops into 5ml sterile water as inoculum from BACTEC Pos bottles into Blood and chocolate plate. Incubate on CO_2 at 37C for 48 hrs				
GNB ? Brucella	semi-solid Korthof`s media	CO_2 CO_2 AnO_2	2-30 days 2-30 days	Daily	Any
GNDC ? *Neisseria*	Blood agar Chocolate agar GC agar	5% CO_2 5% CO_2 5% CO_2	48 hrs 48 hrs 48 hrs	Daily Daily Daily	Any

Abbreviations: GPC; Gram positive cocci, GNB; Gram negative bacilli, GNDC; Gram negative diplococci, Atoms; atmosphere, FAA; fastidious anaerobe agar, MtZ 5; Metronidazole 5 mg.

3.3.1. Identification of GAS

Culture was performed from clinical specimens as well as the control samples in selective media. The used media was Columbia Agar with colistin or nalidixic acid. Samples incubated at 35ºC - 37ºC under anaerobic condition or with presence of 5% CO_2, for optimal condition needed for streptococcal growth (Barbara, 2016). The sample incubated for 24 hrs, then identified by the presence of clear zone of haemolysis of white to grey colour of ≥ 0.5 mm around the colonies. The organism was confirmed as Gram-positive cocci arranged in pairs and chains. Further biochemical tests showed that the colonies were characteristically catalase-negative. Bacitracin sensitivity was performed as a confirmatory test by using a disc contains 0.04 IU of bacitracin placed on the plate and incubated overnight.

Table 3.3.1.1. Biochemical tests for identification of GAS in serum of patients with acute febrile illness.

Sample No.	Colombia agar	Gram stain	Catalase test	Bacitracin disc	Sample No.	Colombia agar	Gram stain	Catalase test	Bacitracin disc
1	BHS	GPC	Neg	S	35	BHS	GPC	Neg	S
2	BHS	GPC	Neg	S	**36**	BHS	GPC	Neg	S
3	BHS	GPC	Neg	S	**37**	BHS	GPC	Neg	S
4	BHS	GPC	Neg	S	**38**	BHS	GPC	Neg	S
5	BHS	GPC	Neg	S	**39**	BHS	GPC	Neg	S
6	BHS	GPC	Neg	S	**40**	BHS	GPC	Neg	S
7	BHS	GPC	Neg	S	**41**	BHS	GPC	Neg	S
8	BHS	GPC	Neg	S	**42**	BHS	GPC	Neg	S
9	BHS	GPC	Neg	S	**43**	BHS	GPC	Neg	S
10	BHS	GPC	Neg	S	**44**	BHS	GPC	Neg	S
11	BHS	GPC	Neg	S	**45**	BHS	GPC	Neg	S
12	BHS	GPC	Neg	S	**46**	BHS	GPC	Neg	S
13	BHS	GPC	Neg	S	**47**	BHS	GPC	Neg	S
14	BHS	GPC	Neg	S	**48**	BHS	GPC	Neg	S
15	BHS	GPC	Neg	S	**49**	BHS	GPC	Neg	S
16	BHS	GPC	Neg	S	**50**	BHS	GPC	Neg	S
17	BHS	GPC	Neg	S	**51**	BHS	GPC	Neg	S
18	BHS	GPC	Neg	S	**52**	BHS	GPC	Neg	S
19	BHS	GPC	Neg	S	**53**	BHS	GPC	Neg	S
20	BHS	GPC	Neg	S	**54**	BHS	GPC	Neg	S
21	BHS	GPC	Neg	S	**55**	BHS	GPC	Neg	S
22	BHS	GPC	Neg	S	**56**	BHS	GPC	Neg	S
23	BHS	GPC	Neg	S	**57**	BHS	GPC	Neg	S
24	BHS	GPC	Neg	S	**58**	BHS	GPC	Neg	S
25	BHS	GPC	Neg	S	**59**	BHS	GPC	Neg	S
26	BHS	GPC	Neg	S	**60**	BHS	GPC	Neg	S
27	BHS	GPC	Neg	S	**61**	BHS	GPC	Neg	S
28	BHS	GPC	Neg	S	**62**	BHS	GPC	Neg	S
29	BHS	GPC	Neg	S	**63**	BHS	GPC	Neg	S
30	BHS	GPC	Neg	S	**64**	BHS	GPC	Neg	S
31	BHS	GPC	Neg	S	**65**	BHS	GPC	Neg	S
32	BHS	GPC	Neg	S	**66**	BHS	GPC	Neg	S
33	BHS	GPC	Neg	S	**67**	BHS	GPC	Neg	S
34	BHS	GPC	Neg	S	**68**	BHS	GPC	Neg	S

Abbreviations: BHS, Beta-haemolytic streptocci; GPS, Gram positive cocci; Neg, Negative; Pos, Positive; S, sensitive.

GAS bacteria was isolated in 68 blood samples of patients with acute febrile illness. GAS infected patients mainly presented clinically with features of upper respiratory tract infection, pneumonia, and cellulitis. The diagnosis was confirmed by bacterial growth on conventional culture and biochemical tests in all samples as reflected in (Table 3.3.1.1).

3.3.2. Identification of pathognomic Leptospira spp

Leptospira is a fastidious aerobic organism that requires enriched media for growth. All samples were cultured in liquid and semi-solid Korthof`s media in which a 2 mL of serum was inoculated, antibiotics (sulfamethoxazole, trimthoprime, amphotericin B and fosfomycin) were then added into the media and incubated at 28ºC - 30º C with pH adjusted between 7 to 8, for 28-30 days (Chakraborty et al., 2011). The organism isolated confirmed to be thin, helical and motile with prominent hooked ends under dark-field microscopy.

Table 3.3.2.1. Culture results in patients with acute leptospirosis.

Sample No.	Culture inKorthof`s media	Gram stain
1	Positive growth	G –ve spirochete
2	Positive growth	G –ve spirochete

Abbreviation: G-ve, Gram negative.

Leptospira was isolated in only two patients with clinical manifestations of suspected acute Leptospirosis after culture in special media (Table 3.3.2.1). Charactersric structural features were identified microscobically.

3.3.3. Identification of Brucella spp

Clinical samples taken are blood and bone marrow aspirates for cultures, isolation was done by culture within the first 2 weeks of symptoms as this is recognised as the gold-standard method of diagnosis. All samples were cultured using BACTEC FX blood culture system and incubated for 4 -12 weeks, aerobically at 37ºC; with 5-7% CO_2 under biosafety level 3 conditions (Scholz et al., 2010; Moshe et al., 2017). The organism was confirmed to be Gram-negative cocco-baccilli, non-motile, non-spore forming. Biochemical tests done; h_2s and hydrogen sulphide production, oxidase test, catalase test and urea hydrolysis were confirmed to be positive.

Table 3.3.3.1. Brucella species isolated and it is biochemical tests results in serum of patients with brucellosis.

Sample No.	*B. spp*	Culture in BACTEC FX	5% CO2 requirment	H2S peoduction	Urease
1	*B. melitensis*	Pos	Neg	Neg	weak
2	*B. melitensis*	Pos	Neg	Neg	weak
3	*B. melitensis*	Pos	Neg	Neg	weak
4	*B. abortus*	Pos	Pos	Pos	weak
5	*B. melitensis*	Pos	Neg	Neg	weak
6	*B. melitensis*	Pos	Neg	Neg	weak
7	*B. melitensis*	Pos	Neg	Neg	weak
8	*B. melitensis*	Pos	Neg	Neg	weak
9	*B. melitensis*	Pos	Neg	Neg	weak

Abbreviations: *B. spp, Brucella species, Pos, Positive, Neg, Negative.*

In 9 patients with suspected clinical manifestations of brucellosis, *B. melitensis* isolated in 8 out of 9 patients and *B. abortus* identified in 1 out of 9 patients, these pathogens were isolated in serum and bone marrow aspirate of patients with febrile illness (Table 3.3.3.1).The brucella species were differentiated by specific biochemical tests. *B. melitensis* was the commonly detected strain.

Primary culture and presumptive identification of meningitis causative pathogens

Meningitis can be due to bacterial, viral, fungal or parasitic organisms or due to certain granulomatous conditions**.**

Diagnosis and identification of meningitis causative organisms in suspected individuals was performed by obtaining a lumbar puncture (LP) for CSF fluid withdrawal for culture and further laboratory investigations.

3.3.4. Identification of *Neisseria meningitidis*

All isolated organisms were confirmed to be Gram-negative diplococci. Cultured in an enriched media, chocolate blood agar with 5% CO_2 at 35ºC - 37ºC, under biosafety level 2 conditions. The colonies appeared were large, colourless and opaque. Kovac`s oxidase test was done for detection of cytochrome oxidase by tetramethyl-p-phenylenediaminedihydrochloride reagent, which turned purple with positive tests. Carbohydrate utilization test (glucose, maltose, sucrose, lactose) were also performed in which acid was produced by oxidation of glucose and maltose only, indicted by phenol red which turned yellow in presence of pH ≤ 6.8 (Lynne,

2010). *Nisseria meningitidis* serogroups A in CSF were identified using Wellcogen™ Bacterial Antigen Kit (Murex Diagnostics Ltd, USA).

Table 3.3.4.1. Eitiological diagnosis of meningitis in serum of patients with clinical features of meningeal infection.

Sample No.	Causative organism	*N. meningitides* serotype	Sample No.	Causative organism	*N. meningitides* serotype
1	*N. meningitidis*	W135	32	*N. meningitides*	W135
2	*N. meningitidis*	W135	33	*N. meningitides*	W135
3	*N. meningitidis*	W135	34	*H. influenza*	-
4	*N. meningitidis*	W135	35	*N. meningitides*	A
5	*N. meningitidis*	X	36	*N. meningitides*	B
6	*N. meningitidis*	W135	37	*N. meningitides*	W135
7	Viral †	-	38	*N. meningitides*	W135
8	*N. meningitidis*	A	39	*N. meningitides*	W135
9	*N. meningitidis*	A	40	*N. meningitides*	A
10	*N. meningitidis*	W135	41	*N. meningitides*	W135
11	*N. meningitidis*	B	42	*N. meningitides*	W135
12	*N. meningitidis*	W135	43	*N. meningitides*	B
13	*N. meningitidis*	A	44	*N. meningitides*	A
14	*N. meningitidis*	A	45	*N. meningitides*	W135
15	*N. meningitidis*	W135	46	*N. meningitides*	W135
16	*N. meningitidis*	W135	47	Viral †	-
17	*N. meningitidis*	W135	48	*N. meningitides*	W135
18	*N. meningitidis*	A	49	*N. meningitides*	A
19	*N. meningitidis*	B	50	*N. meningitides*	W135
20	*N. meningitidis*	W135	51	*N. meningitides*	W135
21	*N. meningitidis*	X	52	*N. meningitides*	W135

22	*N. meningitidis*	A	53	*N. meningitides*	A
23	*N. meningitidis*	W135	54	*N. meningitides*	W135
24	*N. meningitidis*	W135	55	*N. meningitidis*	W135
25	*N. meningitidis*	W135	56	*S. pneumonia*	-
26	*N. meningitidis*	W135	57	*S. pneumonia*	-
27	*N. meningitidis*	A	58	*L. monocytogenes*	-
28	*N. meningitidis*	W135	59	*S. pneumonia*	-
29	*N. meningitidis*	W135	60	*S. pneumonia*	-
30	*N. meningitidis*	A	61	*S. pneumonia*	-
31	*N. meningitidis*	A			

Abbreviations: *N. meningitides, Neisseria meningitides; H. influenza, Haemophilus influenza; S. pneumoniae, Streptococcus pneumonia; L. monocytogenes, Listeria monocytogene;* †, the viral causative pathogen was not identified

In 61 patients with acute febrile illness and clinincal suspected manifestations of meningitis, serum samples were cultured and meningitis causative pathogens were identified microscopically and confirmed by biochemical tests (Table 3.3.4.1). *N. meningitidis* identified in 52 out of 61 patients (85.2%). The commonly reported *N. meningitidis* serotype was MEN W135 identified in 32 out of 52 patients (61.5%) followed by MEN A in 14 out of 52 patients (26.9%), MEN B in 4 out of 52 patients (7.69%), and MEN X in 2 out of 52 patients (3.84%). For other meningitis causative pathogens, *S. pneumoniae* identified in 5 of 61 patients (8.19%), *H. influenza* isolated in one patient out of 61 infected cases (1.63%), and *L. monocytogenes* isolated also in only one patient (1.63%).

3.4. Malaria ICT, thick and thin blood films

Malaria antigens in patients serum were detected by ICT (rapid immune-chromatographic assay), utilizing Malaria (P.f./Vivax) WB Rapid Test. The blood samples were placed on the device; positive results indicated by the presence of double lines opposing negative tests, which showed only one line (control line). Thick and thin blood smears study were used to determine the presence of malaria

parasite species and parasitemia level (Casey and Gerard, 2015). A 10 % Giemsa stain was used, each slide needed approximately 3 ml, fixation by methanol drops using pasteure pipette was applied on thin blood films. The slides were left to dry in a ventilated area. The stain was left for 8 - 10 minutes followed by washing with tap water and left afterwards to dry facing downward or in vertical position over a rack (Basic Malaria Microscopy. Part I. Learner's Guide, WHO. 2010). Microscopic examination was performed on thick blood film for detection and identification of malaria parasites, while species identifications were carried on thin blood film due to better morphological differentiation.

In 55 patients with acute febrile illness, malaria was confirmed by ICT. On malaria species identification, *P. faciparam* identified microscopically in 35 out of 55 patients (63.6%). In 20 infected cases the plasmodium species were not identified. Parasitemia level was assessed in each sample. Parasitemia level 3+ reported in 2 out of 55 patients (3.63%), level 2+ in 13 out of 55 patients (23.6%), level 1+ in 23 out of 55 patients (41.8%), and zero in 17 ou of 55 patients (30.9%) (Table 3.4.1).

Table 3.4.1. Microscopic identified plasmodium species, parasitemia level and ICT results in serum of patients with acute fibrile illness.

Sample No.	Malaria *spp*	Para. level	ICT	Thick BF	Sample No.	Malaria *spp*	Para. Level	ICT	Thick BF
1	*P.faciparam*	2 +	1	1	29	Not known	0	1	0
2	*P. faciparam*	2 +	1	1	30	Not known	2 +	1	1
3	not known	0	1	0	31	*P. faciparam*	1 +	1	1
4	not known	0	1	0	32	*P. faciparam*	2 +	1	1
5	*P. faciparam*	1 +	1	1	33	*P. faciparam*	2 +	1	1
6	*P. faciparam*	1 +	1	1	34	*P. faciparam*	1 +	1	1
7	*P. faciparam*	1 +	1	1	35	*P. faciparam*	2 +	1	1
8	not known	0	1	0	36	*P. faciparam*	3 +	1	1
9	not known	3 +	1	1	37	*P. faciparam*	1 +	1	1
10	not known	0	1	0	38	*P. faciparam*	1 +	1	1
11	not known	0	1	0	39	*P. faciparam*	1 +	1	1
12	not known	0	1	0	40	*P. faciparam*	1 +	1	1
13	not known	0	1	0	41	*P. faciparam*	2 +	1	1
14	not known	0	1	0	42	*P. faciparam*	2 +	1	1
15	not known	0	1	0	43	*P. faciparam*	1+	1	1
16	*P. faciparam*	1 +	1	1	44	*P. faciparam*	1 +	1	1
17	*P. faciparam*	1 +	1	1	45	*P. faciparam*	2+	1	1
18	*P. faciparam*	2 +	1	1	46	*P. faciparam*	1 +	1	1
19	not known	0	1	0	47	*P. faciparam*	1 +	1	1
20	not known	2 +	1	1	48	*P. faciparam*	1 +	1	1
21	not known	0	1	0	49	*P. faciparam*	1 +	1	1
22	not known	0	1	0	50	*P. faciparam*	1 +	1	1
23	not known	0	1	0	51	*P. faciparam*	1 +	1	1
24	not known	0	1	0	52	*P. faciparam*	1 +	1	1
25	*P. faciparam*	2 +	1	1	53	*P. faciparam*	2 +	1	1
26	*P. faciparam*	1 +	1	1	54	*P. faciparam*	1 +	1	1
27	not known	0	1	0	55	*P. faciparam*	1 +	1	1
28	not known	0	1	0					

Abbreviations: *P. faciparam, Plasmodium falciparum*; not known, the plasmodium species was not identified; ICT, rapid immunochromatographic assay.

Chapter 4

4.1. Longitudinal characterization of disease patients

During the study period, which extended from January 2014 to January 2018, all patients presented to the IDC with acute febrile illnesses were clinically assessed. Blood samples were collected from selected patients who are suspected to be infected with one of the eight infectious diseases included in this research project and sent to the laboratory for investigations.

The total number of samples collected were 2048 of which, 418 cases (20.4%) were positively diagnosed for DENV, CHIKV, DENV+CHIKV co-infection, malaria, GAS, brucella, meningococcal meningitis, and leptospirosis. However, 1630 cases (79.6%) were not diagnosed with any of the above eight diseases and labelled as febrile unknown patients also known as "Pyrexia of Unknown Origin" (PUO), 100 healthy control cases were included. Demographic data including the age and gender were reported, clinical history and examination were conducted and routine laboratory tests were performed. The positively diagnosed patients shared many symptoms, as all of these cases present with acute febrile illness, there were distinguishing differences in between. Each case was approached individually and suspected cases for the eight infectious diseases subjected to specific laboratory tests for confirmation.

The total number of positive cases were summarized as follows: total number of DENV positive cases included in the study were 174 (78% of viral infections), 34 CHIKV infection positive cases (15% of viral infections), 15 DENV+CHIKV Co-infected patients (7% of viral infections), and 61 meningococcal meningitis (44% of bacterial infections). Malaria positive patients were 55 cases, 2 reported leptospirosis cases (1% of bacterial infections), 68 GAS positive patients (49% of bacterial infections), and 9 cases of brucellosis (6% of bacterial infections) (Figure 4.1.1).

Some of the eight selected diseases included in the study are arthropod-borne infections, which represent a global health problem with variable geographical distribution, prevalence, and pathogenesis. The study was directed towards the description of these diseases in Kingdom of Saudi Arabia – Jizan province, as this area is endemic with Brucella, malaria, and DENV infections. Beside the increase of prevalence of the vector-borne diseases due to climate change and other factors such as human movements across the borders for Hajj, Ummrah or tourism.

Demographically, all positive cases were categorized according to age into five groups; (0-1), (1-4), (5-14), (15-44), and ≥ 45 years old, this classification was applied to each disease separately. Each positively diagnosed case was further differentiated according to gender into male and female (Table 4.1.1).

Table 4.1.1. Total number of samples per disease, age groups in number and percentages; gender in number and percentages, respectively.

	Total Patients (n)	Age groups / n / (%)			Gender / n / (%)		
Dengue (DENV)	174	0-1 1-4 5-14 15-44 ≥45	0 0 2 15 157	0 0 1.15 8.6 90	M F	95 79	54.6 45.4
Chikungunya (CHIKV)	34	01 1-4 5-14 15-44 ≥45	0 0 0 14 20	0 0 0 41.2 58.8	M F	18 16	52.9 47
Group A Streptococcus (GAS)	68	0-1 1-4 5-14 15-44 ≥45	0 0 0 16 52	0 0 0 23.53 76.47	M F	40 28	58.8 41.2
Malaria (MAL)	55	0-1 1-4 5-14 15-44 ≥45	0 0 0 47 8	0 0 0 85.5 14.5	M F	26 29	47.3 52.73
Meningococcal meningitis (MEN)	61	0-1 1-4 5-14 15-44 ≥45	0 0 0 29 32	0 0 0 47.54 52.5	M F	40 21	65.6 34.43
LEPTOSPIRA (LEP)	2	0-1 1-4 5-14 15-44 ≥45	0 0 0 1 1	0 0 0 50 50	M F	2 0	100 0
BRUCELLA (BRU)	9	0-1 1-4 5-14 15-44 ≥45	0 0 0 3 6	0 0 0 33.33 66.67	M F	8 1	88.9 11.11
Chikungunya+Dengue CHIKV+DENV	15	0-1 1-4 5-14 15-44 ≥45	0 0 0 3 12	0 0 0 20% 80%	M F	10 5	66.67 33.33

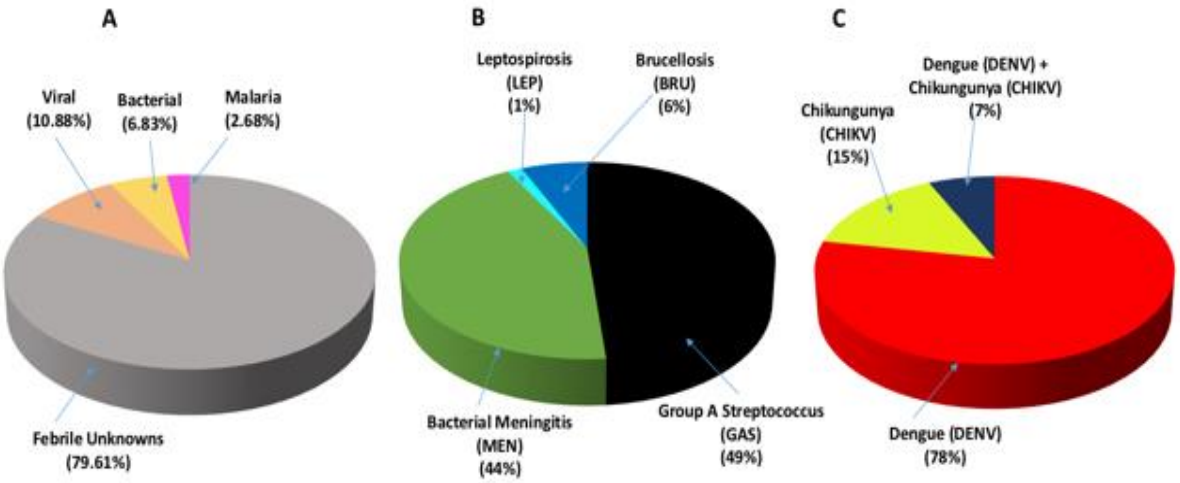

Figure 4.1.1. Diseases percentages per pathogen. A, total cohort group of febrile patients showing 10.88% viral infections, 6.83% bacterial infections, 2.68% malaria (MAL) infections, and 79.61% febrile unknowns; B, percentages of bacterial infections, 1% Leptospirosis (LEP), 6% Brucellosis (BRU), 44% Meningeococcal meningitis (MEN), and 49% Group A Streptococcus (GAS); C, percentages of viral infections, 15% Chikungunya (CHIKV), 7% Co-infection of DENV and CHIKV, and 78% Dengue (DENV) infected patients.

4.1.1. Viral infections diseases distributions and frequencies per year

The total number of DENF cases were 174, which were classified into five subgroups according to age, reflected in (Table 4.1.1). There was no reported cases in the age group (0-1) and (1-4), in the age group (5-4) there were only two cases which constitute (1.15 %) of the total number of DENV positive patients. The largest detected number of positive cases presented was in the age group of ≥ 45 years old which represent (90%), 157 cases followed by the (15-44) age group in which, 15 positive cases were reported (8.6%).

According to gender, males represent (54.6%), while females represent (45.4%) of the total positive cases.

In CHIKV positive cases, 34 positive cases were reported, 14 cases were reported in the (15-44) age group, represent (41.2%), while 20 cases were in the age group of ≥ 45 years, represent (58.8%). According to gender, males represent (52.9%) by 18 case, while females were 16 case in total, represent (47%).

The total number of DENV+CHIKV co-infection positive cases were 15, 12 cases were ≥ 45 years old, represent (80%), while in the (15-44) age group 3 cases were reported, represent (20%). According to gender, males represent (66.67%) with 10 cases, while females represent (33.3%), 5 cases.

The distribution patterns and frequencies of positive viral infections during the study period is shown in (Figure 4.1.1.1). DENV positive cases showed the large number of cases which peaked in August 2014 with 10 positive cases per month, this followed by rapid decrease in number of patients between September 2014 and June 2016.

In CHIKV, the first reported case was in April 2014 with a steady reporting of 4 cases per month between June - December 2014, there was no reported positive case of CHIKV between January 2015 until the end of the study period.

In DENV+CHIKV co-infection, the main disease distribution was reported between March - December 2014 with another single positive reported case in December 2015.

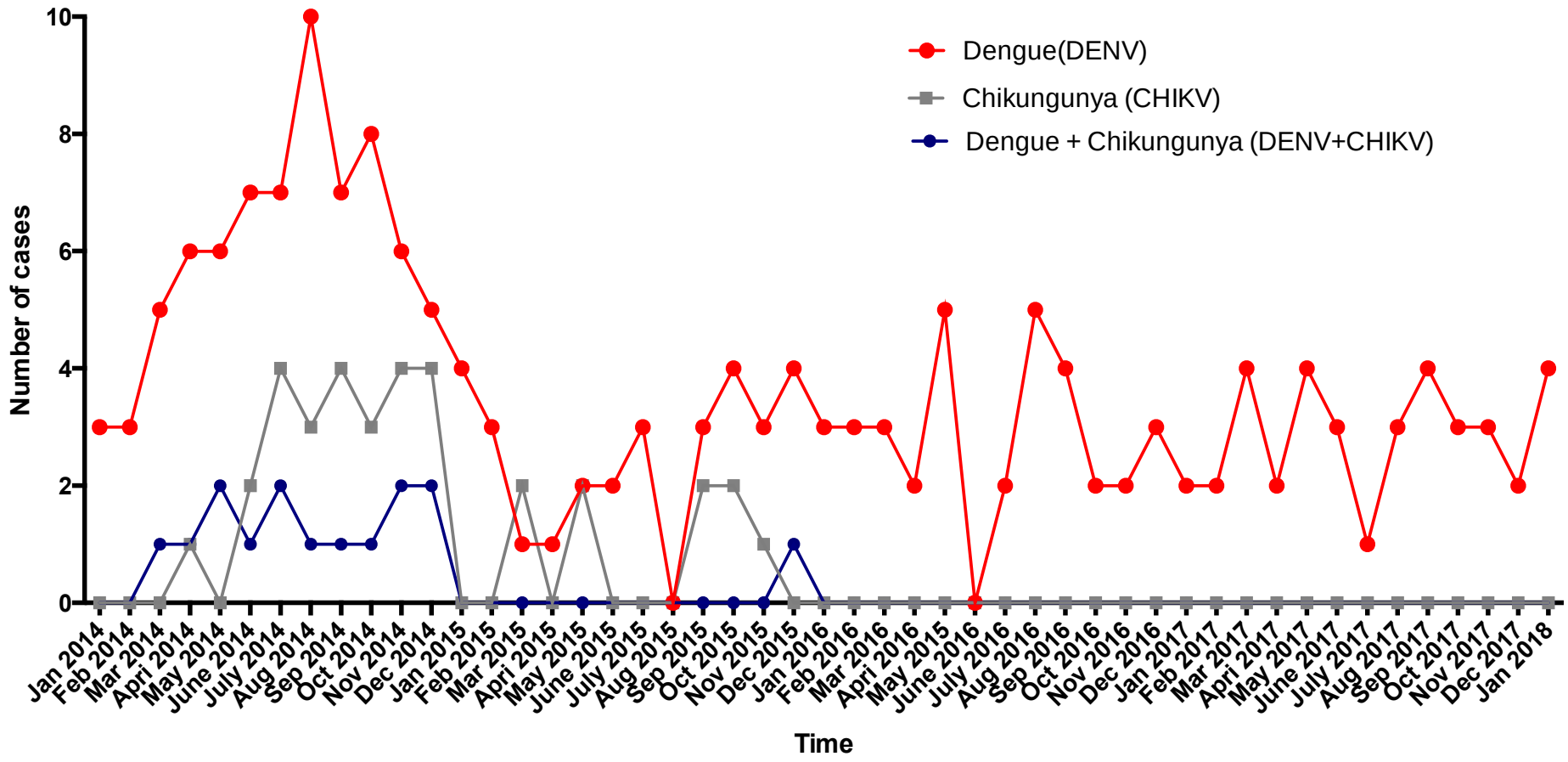

Figure 4.1.1.1. Number of viral positive cases per month and year; DENV (red) showing high peak between January 2014 and decline in November with highest peak in July of the same year with constant decline until the end of the year. In 2015, the highest peak was in October and December.In 2016, the highest number of reported cases were in May and August. In 2017, the highest number of cases were reported in March, May, and September; CHIKV (gray) showing high number of cases reported in July, September, and November. In 2015, highest number of cases reported were in March, May, September, and October. From 2016 until January 2018, no cases were reported; DENV+CHIKV (deep blue) showing highest number of cases reported in May, July, November, and December. From January 2015 until January 2018, there was one case reported in December 2015.

4.1.2. Bacterial infections diseases distributions and frequencies per year

The total number of GAS positive patients was 68. Most of which fall in the age group of ≥ 45 years old with 52 positive cases (76.47%), while in the age group (15-44) 16 positive cases were reported (23.53%). Males were predominant with 40 cases (58.8 %), while females were 28 cases (41.2%). The largest reported number of cases was in GAS noticed in two peaks, the first one between March - June 2014 with the highest peak was in May 2014, followed by sharp decline in July 2014. The second peak was between April 2016 and June 2016 (Figure 4.1.2.1).

The total number of meningococcal meningitis positive cases were 61 of which, 32 cases fall in the age age group of ≥ 45 years old, represent (52.5%), while 29 cases reported in the age group (15-44) years old, represent (47.54%). According to gender, males were 40 cases, represent (65.6%), while females were 21 cases in total, represent (34.4%). Meningococcal meningitis reported cases showed recurrent peaks in May 2014, March 2015, January 2017, and April 2017, respectively with steady reporting of 2 cases per month between October 2015 – July 2016. There was no reported meningeococcal meningitis positive cases from August 2016 untill December 2016 (Figure 4.1.2.1).

In leptospirosis, only two cases were reported during the study period with equal distribution between age groups (15-44) and ≥ 45 years old. Both cases were males and reported in July 2014.

In brucella infection, the total cases reported were 9, most of which fall in the age group of ≥ 45 years old with 6 cases (66.67%), while 3 cases in the age group (15-44) years old (33. 33%). According to gender, males represent the dominant group with 8 cases (88.95%), while there was only one female (11.11%). The number of positive cases peaks in September 2016 with a second peak in November and December 2017 (Figure 4.1.2.1).

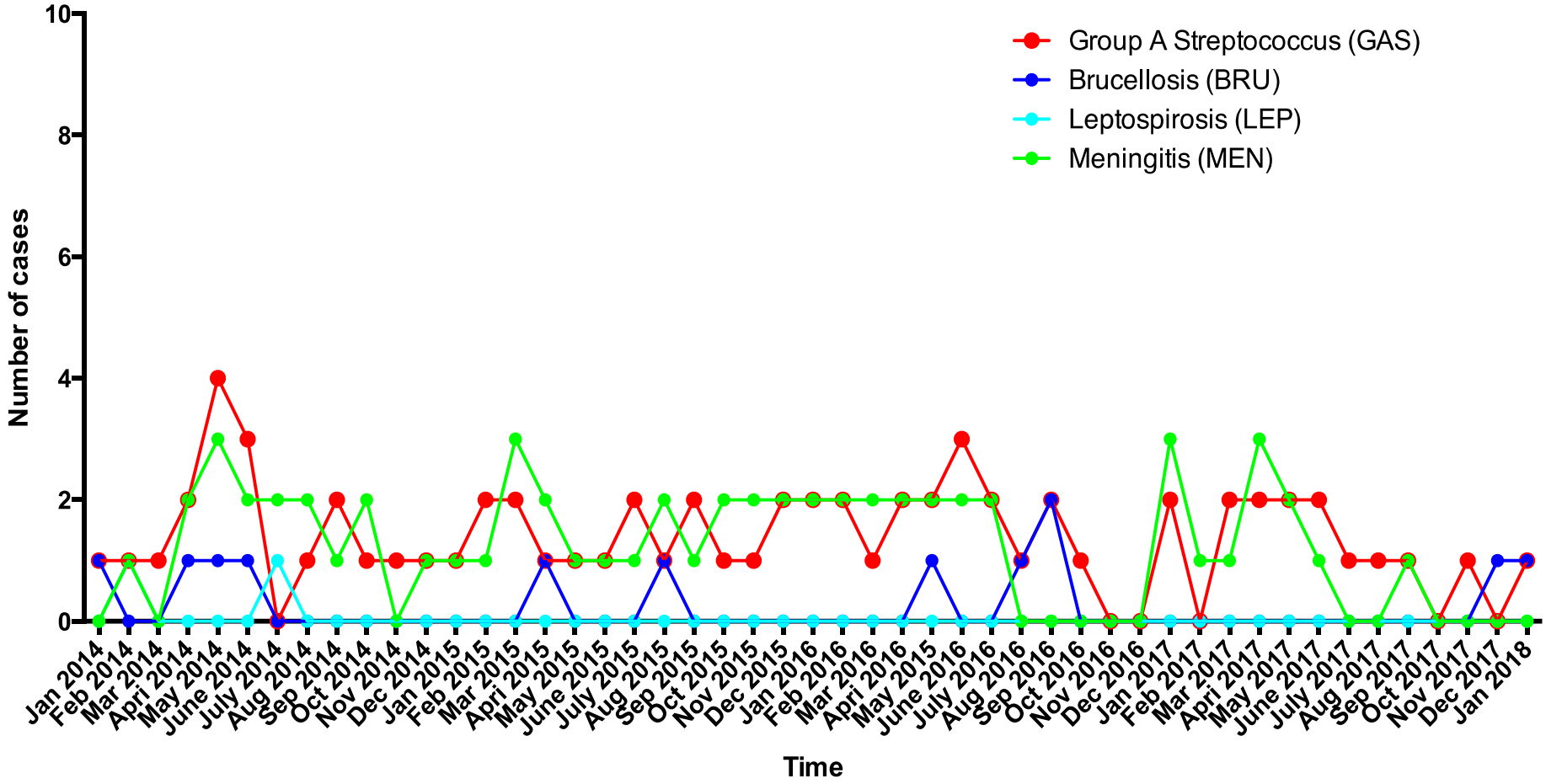

Figure 4.1.2.1. Number of bacterial positive cases per month and year; GAS (red) showing highest number of reported cases in May 2014, In 2015, highest number of cases were reported in February, March, July, September, and December. In 2016, the highest number of cases were recorded in June. In 2017, there were 4 months in which the highest number of cases were recorded, which are January, March, April, May, and June; BRU (Deep blue) between 2014 and 2018, the higher number of reported cases were in September 2016; LEP (light blue), between the 2014 and 2018, total of two cases were reported in February and July in 2014; menineococcal meningitis (green), showing highest number of reported cases in May 2014, March 2015, Jan 2017, and April 2017.

4.1.3. Malaria disease distribution and frequencies per year

The total number of malaria positive cases was 55, most of which fall in the age group (15-44) years old with 47 cases, represent (85.5%), while only 8 cases were reported in the age group of ≥ 45 years old (14.5%). The females represent the dominant group with 29 positive cases (52.73%), while the males were 26 cases, represent (47.3%).

Malaria positive cases distribution during the study period shown in (Figure 4.1.3.1). The main disease distribution was reported in July to September 2014. No reported cases from October 2014 to December 2014. Few malaria cases were reported for the rest of the study period.

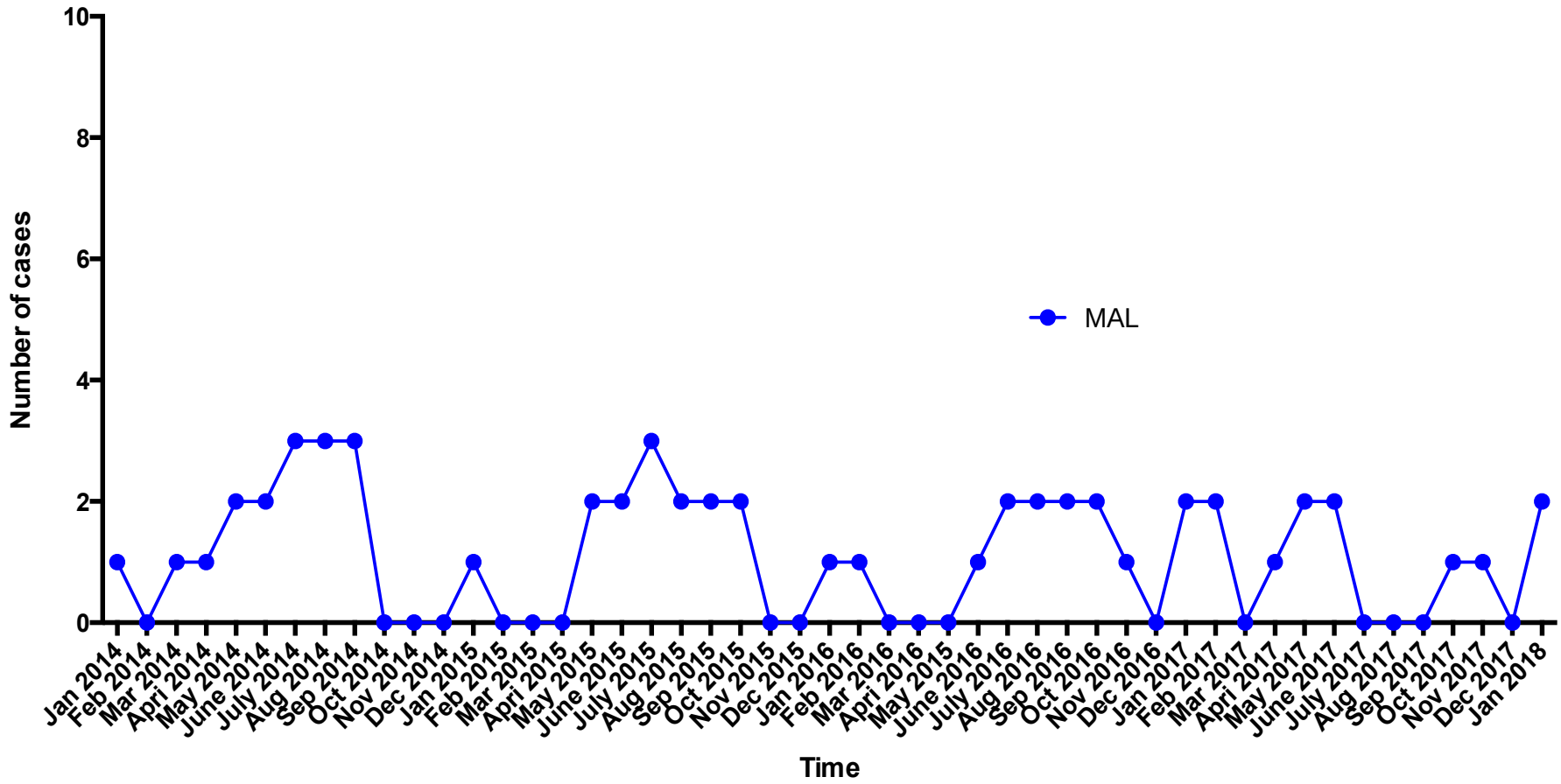

Figure 4.1.3.1. Number of malaria positive cases per month and year. In 2014, the highest numbers of cases reported were in July, Aug, and September. In 2015, another high number of reported cases was also noted in July. In 2016, the highest number of reported cases were noted in July, August, September, and October. In 2017 high number of cases were reported in January, February, March, July, and December.

4.2. Clinical and biochemical indicators of disease patients

The clinical parameters were used involve skin rash, back pain, headache, joints pain, vomiting, nausea, and chills. All patients presented with fever. The laboratory biochemical parameters investigated were neutrophils and lymphocytes percentages, ESR, Hb, ALT, AST, CRP levels, and PLT count.

4.2.1. Clinical and biochemical indicators of viral infections

The percentages of viral infected cases presented with skin rash is displayed in (Figure 4.2.1.1). All cases diagnosed with DENV and CHIKV infections presented with skin rash (98.9%), (100%) respectively with (86.6%) in DENV+CHIKV co infection.

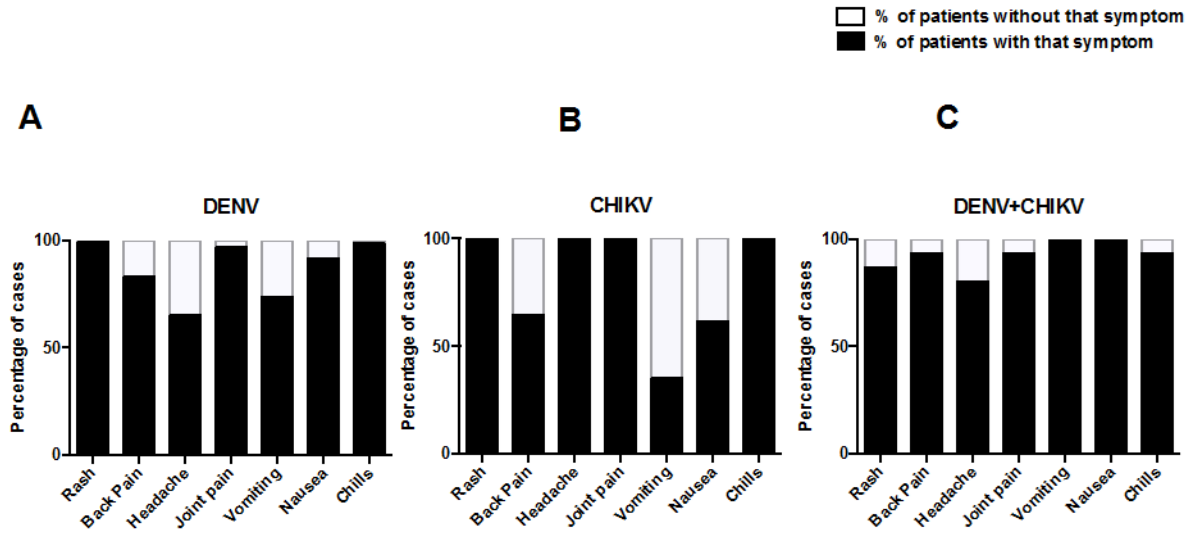

Figure 4.2.1.1. Percentages of clinical parameters presented in viral infections positive cases, who presented with skin rash, back pain, headache, joints pain, vomiting, nausea, and chills respectively.

Back pain was reported in (93%) of DENV+CHIKV co-infection, (82.7%) in DENV and (64%) in CHIKV positive patients, respectively. Headache was reported in all CHIKV patients (100%), (64%) in DENV and (80%) in DENV+CHIKV co-infection, respectively.

Joints pain was reported in all CHIKV and DENV infected cases (100% and 96.5%, respectively) (Figure 4.2.1.1), while it is (93%) in DENV+CHIKV co-infection.

Vomiting was reported all DENV+CHIKV co-infection positive cases (100%), (73.5%) in DENV positive patients and (35.2%) in CHIKV positive patients, respectively.

Nausea was reported in (100%) of DENV+CHIKV co-infected positive patients, (91.3%) in DENV positive cases and (61.7%) in CHIKV infected patients, respectively.

Chills was reported in (93.3%) of DENV+CHIKV co-infection positive cases, (100%) in CHIKV positive patients and (98.2%) in DENV positive patients, respectively.

In the laboratory tests, the CRP level in relation to viral infections is displayed in (Figure 4.2.1.2). The range of the CRP level control cases was < 50 mg/dL. In DENV positive cases, the highest level of CRP reported was around 80 mg/dL, which was observed in one case, while the vast majority of cases resulted in an increased CRP level of around 40 mg/dL, the same results were reported in DENV+CHIKV co infection. In CHIKV positive cases, the highest reported level of CRP was around 40 mg/dL, while in most of the cases the CRP levels were between 20 - 40 mg/dL.

The range of the control PLT counts is between 250,000 - 400,000/µL. In CHIKV positive cases, the PLT count were between 200,000 - 400,000/µL, with lowest count of about 100,000/µL reported in DENV+CHIKV co-infection, where as it was below 200,000/µL in DENV positive cases.

AST level in association with viral infections was shown in (Figure 4.2.1.2). AST level < 50 IU/L was reported in the control cases, increased level of ≥ 100 IU/L was reported in DENV and DENV+CHIKV co-infection positive cases, while in CHIKV positive cases it was < 50 IU/L.

The range of the ALT control level was < 50 IU/L. ALT levels ≥ 100 IU/L were reported in DENV and DENV+CHIKV co-infection positive cases, while it was between 40 - 60 IU/L in CHIKV positive cases, respectively.

The range of the Hb control level was between 12 - 15 g/dL. The lowest Hb levels of ≤ 12 g/dL were reported in DENV+CHIKV co-infection, while it was around 15 g/dL in DENV positive cases with 10 g/dL Hb level reported in other patients. In CHIKV patients, reported Hb levels range between 10 - 14 g/dL.

The range of the ESR control level was around 20 mm/hr (Figure 4.2.1.2), more than 20 mm/hr was only observed in 2 cases. All DENV, CHIKV, and DENV+CHIKV co-infection positive cases reported ESR levels were < 30 mm/hr.

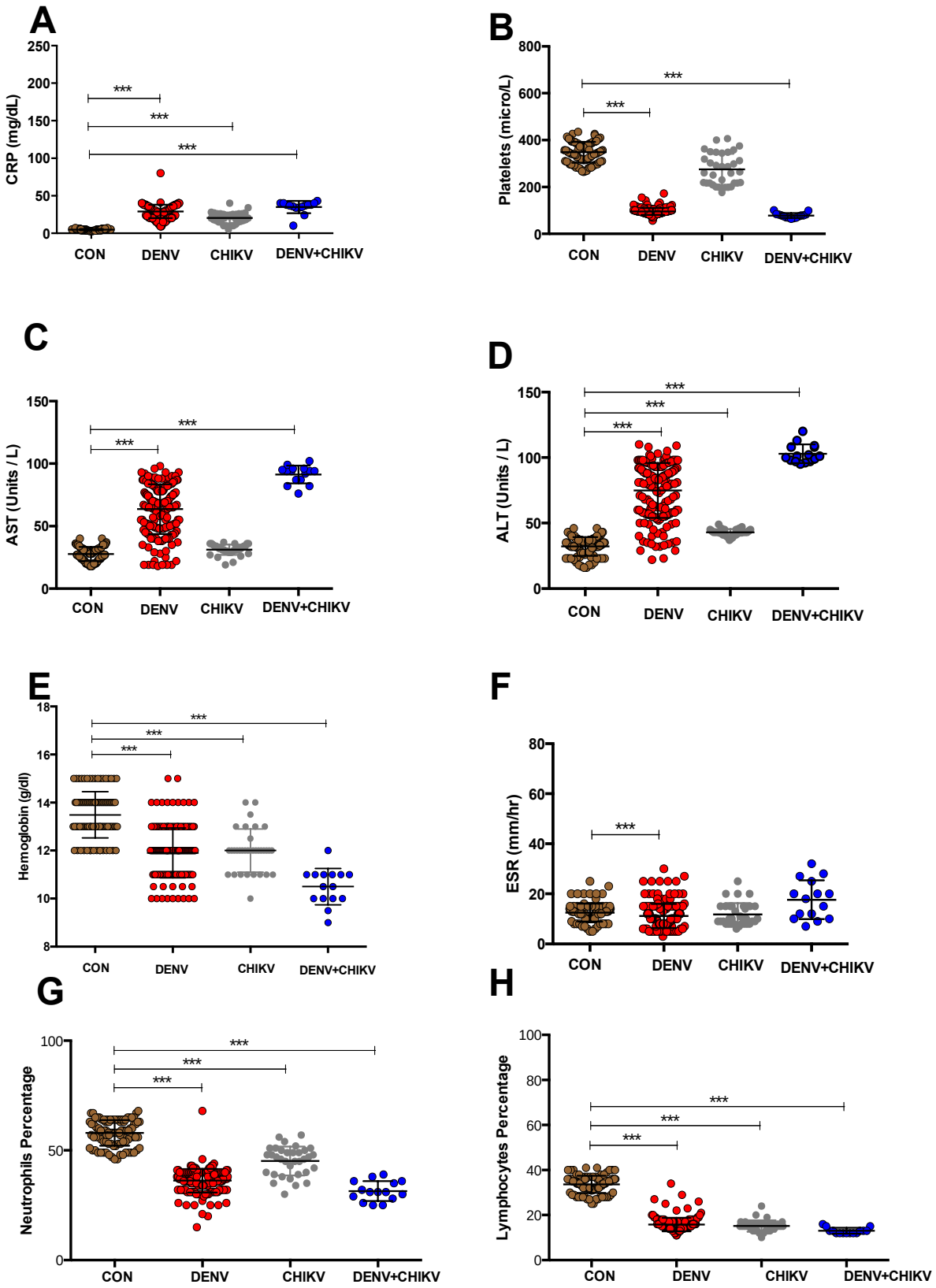

Figure 4.2.1.2. Biochemical tests results of viral infections against control cases levels. (A) CRP detected levels in mg/dL. (B) Platelets count per microliter. (C) AST detected levels in IU/L. (D) ALT detected levels in IU/L. (E) Haemoglobin level in g/dL. (F) ESR levels in mm/hr (G) Neutrophils percentages. (H) Lymphocytes percentages. Kruskal-Wallis tests was used to determine if the measurements of the sample groups differ significantly from each other.

4.2.2. Clinical and biochemical indicators of bacterial infections

The percentages of skin rash presentation in bacterial infections were shown in (Figure 4.2.2.1). All leptospirosis patients presented with skin rash (100%). In meningococcal meningitis the percentage was (32.7%), (7.3%) of GAS patients presented with skin rash, no skin rash was complained by or observed in brucellosis positive cases.

All brucellosis patients presented with back pain (100%). In GAS positive cases only (30.8%) complained of back pain, (39.3%) of meningococcal meningitis positive cases presented with back pain, while no back pain reported in leptospirosis positive cases.

Meningococcal meningitis and leptospirosis patients presented with headache (96.7%) and (100%), respectively. In brucellosis patients headache reported in (88.9%) and in GAS positive cases the percentage was around 51.4%.

Joints pain reported in (88.9%) of brucellosis patients, (32.7%) in meningococcal meningitis positive cases and (16.1%) in GAS positive cases. Joints pain was not complained by leptospirosis positive cases.

All leptospirosis positive cases presented with vomiting (100%). Only (22.2%) of brucellosis patients presented with vomiting, (39.7%) in GAS positive cases and (90.1%) in meningococcal meningitis positive cases, respectively.

Meningococcal meningitis and leptospirosis positive cases presented with nausea, (98.3% and 100%, respectively). In brucellosis positive cases, the proportion of nausea presentation was (33.3%), in GAS diagnosed patients the percentage was (73.5%).

All leptospirosis patients presented with chills (100%), (17.6%) in GAS patients and (13.1%) in meningococcal meningitis positive cases, respectively. Chills was reported in (11.1%) of brucellosis positive cases.

Laboratory tests were as follow, the range of the CRP control levels are shown in (Figure 4.2.2.2). The highest levels of CRP in bacterial infections were reported in GAS patients, CRP levels ≥ 150 mg/dL were observed in 4 cases. Meningococcal meningitis patients represent the second cause of increased CRP levels, which were ≥ 150 mg/dL. CRP level ≥ 60 mg/dL was observed in one leptospirosis case, other reported levels were around 10 mg/dL. In brucellosis patients CRP levels around 50 mg/dL were reported.

Bacterial infections PLT count are displayed in (Figure 4.2.2.2), GAS patients showed the highest PLT count of ≥ 600,000/µL, which was observed in one case, other reported levels range between 180,000 - 600,000/µL. PLT count between 200,000 - 600,000/µL were reported in meningococcal meningitis positive cases, while it were between 150,000 - 300,000/µL in brucellosis patients. In leptospirosis positive cases, PLT count range between 200,000 - 300,000/µL.

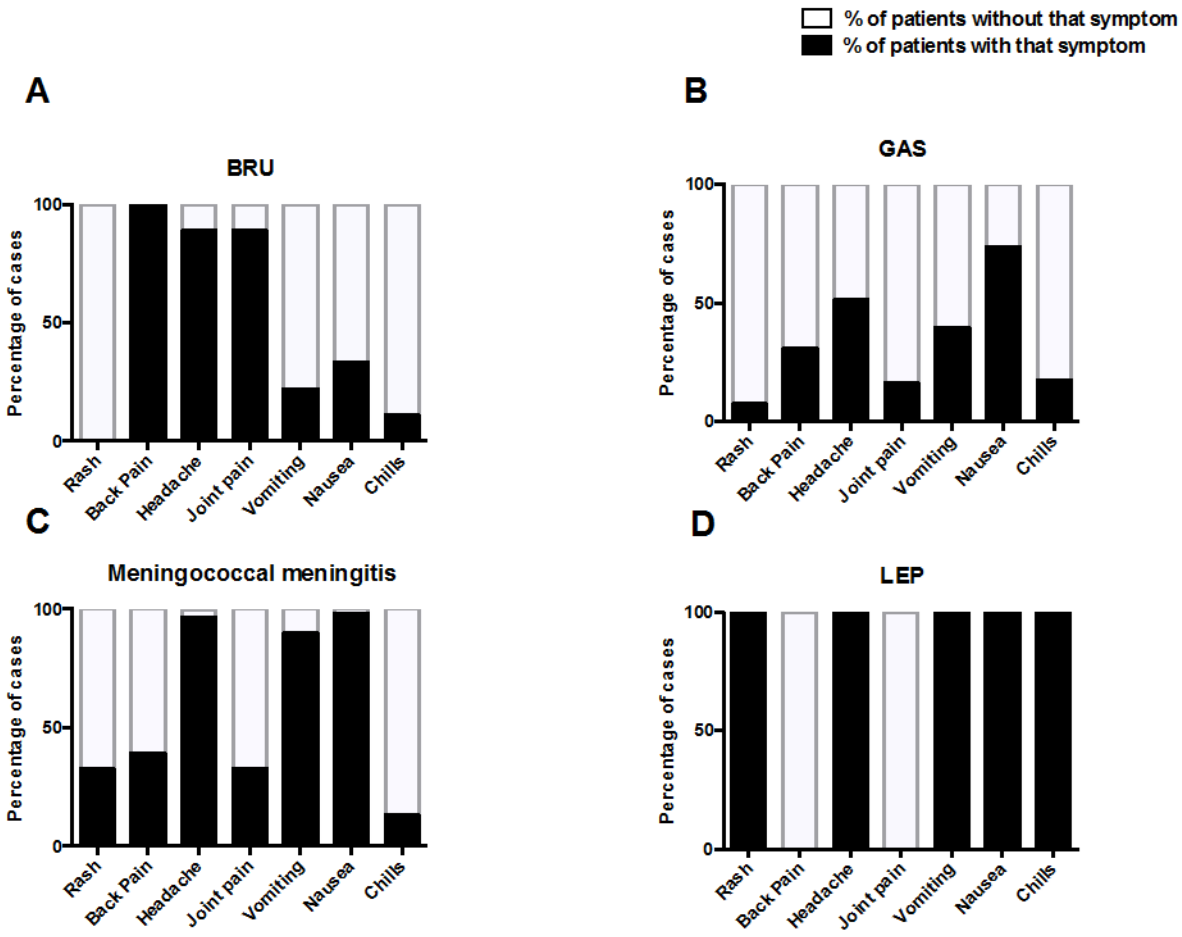

Figure 4.2.2.1. Percentages of clinical parameters presented in bacterial infections positive cases, including; skin rash, back pain, headache, joints pain, vomiting, nausea, and chills in bacterial infected cases. (A) Clinical parameters associated with brucellosis positive cases. (B) clinical parameters presentation in GAS patients. (C) Meningeococcal meningitis positive cases clinical features. (D) Leptospirosis diagnosed cases clinical presentations.

AST levels around 100 IU/L were reported in leptospirosis patients and AST levels > 50 IU/L were reported in brucellosis patients. 100 IU/L level of AST was observed in only one brucellosis patient. Normal levels of AST were reported in GAS patients. In meningococcal meningitis patients, AST levels were between 20 - 50 IU/L were reported.

ALT levels reported in leptospirosis patients were between 90 - 110 IU/L and in brucellosis patients the lowest reported level was 50 IU/L and the highest level was around 110 IU/L. In GAS patients, there were no reported levels > 50 IU/L.

Hb levels between 11 - 15 g/dL were observed in meningococcal meningitis positive cases and 11 - 12 g/dL were reported in leptospirosis patients. In brucellosis patients, reported Hb levels varies between 10 - 13 g/dL, while in GAS positive cases, 10 - 15 g/dL Hb levels were reported.

The ESR levels in brucellosis patients showed the highest reported levels of 60 mm/hr. In GAS patients, ESR levels start from about 5 mm/hr and varies to 50 mm/hr. ESR levels between 1 - 30 mm/hr were reported in leptospirosis patients, while it were between 10 - 30 mm/hr in meningococcal meningitis positive cases, 50 mm/hr level reported in only one meningococcal meningitis patient.

Neutrophils percentages reported in bacterial infections shown in (Figure 4.2.2.2). Meningococcal meningitis and GAS patients showed the highest percentages of neutrophils were around (80 - 100%), while brucellosis positive cases reported percentages < (50%) with one patient showed (60%). In leptospirosis patients (60 - 80%) neutrophils percentages were reported.

Lymphocytes percentages < (40%) reported in brucellosis patients, in meningococcal meningitis and GAS positive cases the reported lymphocytes percentages varies between (30 - 70%), while in leptospirosis positive cases it were around (50%), respectively.

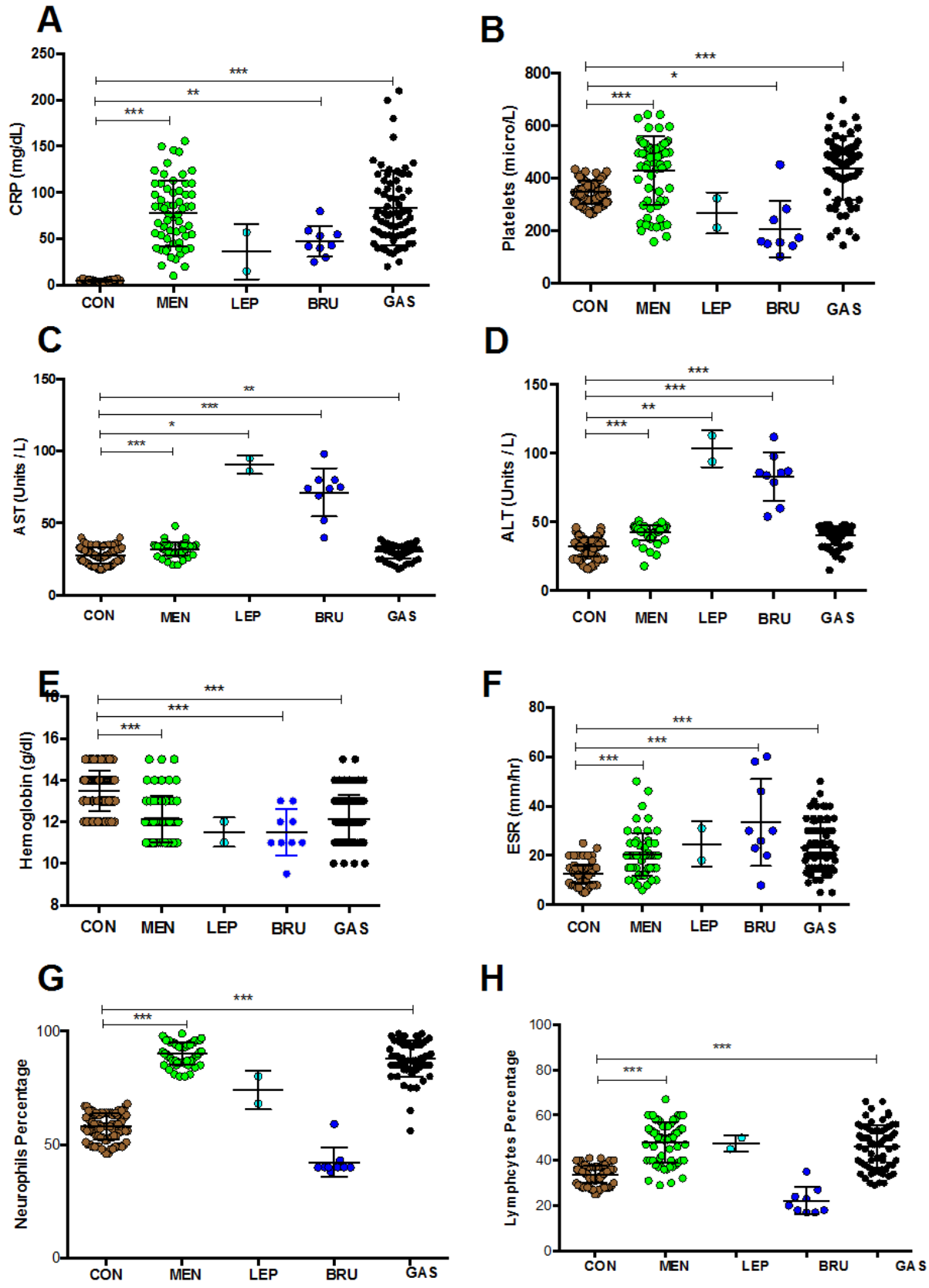

Figure 4.2.2.2. Biochemical tests results of bacterial infections against control cases levels. (A) CRP levels in mg/dL (B) Platelets count in microliter (C) ALT levels in IU/L (D) AST levels in IU/L (E) Haemoglobin levels in g/dL (F) ESR levels in mm/hr (G) Neutrophils percentages (H) lymphocytes percentages. Kruskal-Wallis tests were used to determine if the measurements of the sample groups differ significantly from each other.

4.2.3. Clinical and biochemical indicators of malaria infection

The clinical parameters assessed are displayed in (Figure 4.2.3.1), only (1.8 %) of malaria positive cases presented with skin rash. Back pain was reported in (83.6%) of malaria patients, while headache, chills, and nausea were a complain by malaria positive cases (100%, 98.1%, and 98.1% respectively). Joints pain were reported in (52.7%) of malaria patients, while vomiting was reported in (72.7%).

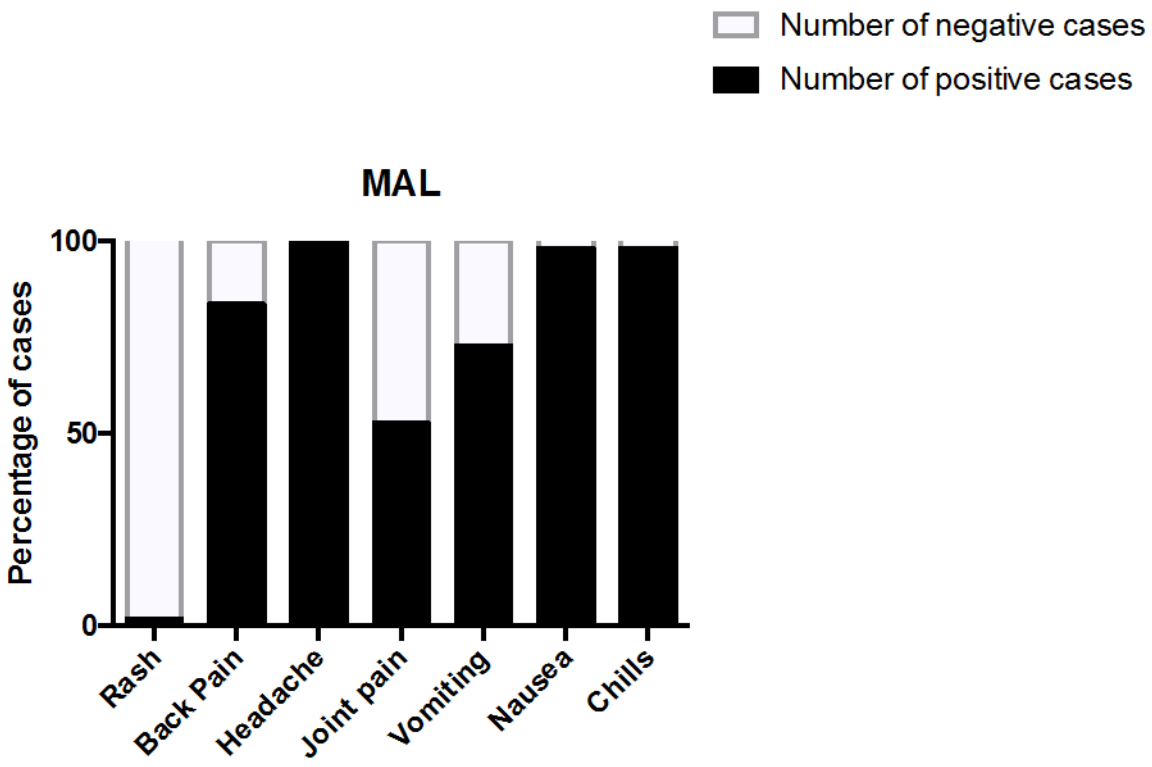

Figure 4.2.3.1. Percentages of clinical parameters presented in malaria positive cases; skin rash, back pain, headache, joints pain, vomiting, nausea, and chills, respectively.

CRP levels reported in malaria patients were shown in (Figure 4.2.3.2). The vast majority of patient's CRP levels were around 20 - 40 mg/dL. Except in 2 patients where CRP levels found to be around 60 mg/dL.

PLT count reported in malaria positive cases range between 100,000 - 250,000/µL, except in few cases were reported PLT count around 500,000/µL.

Normal AST levels reported in malaria patients were shown in (Figure 4.2.3.2). AST levels above 50 IU/L were reported only in few number of malaria patients.

The vast majority of malaria positive cases reported ALT levels < 50 IU/L, 60 - 70 IU/L levels were reported in only few cases.

Hb levels in malaria patients varies between 10 - 15 g/dL, levels < 10 g/dL were reported in few cases. ESR levels < 20 mm/hr were reported in the vast majority of malaria positive cases.

The neutrophils percentages in malaria positive cases showed variations between (50 - 70%) in general, while reported lymphocytes percentages varied between (20 - 40%).

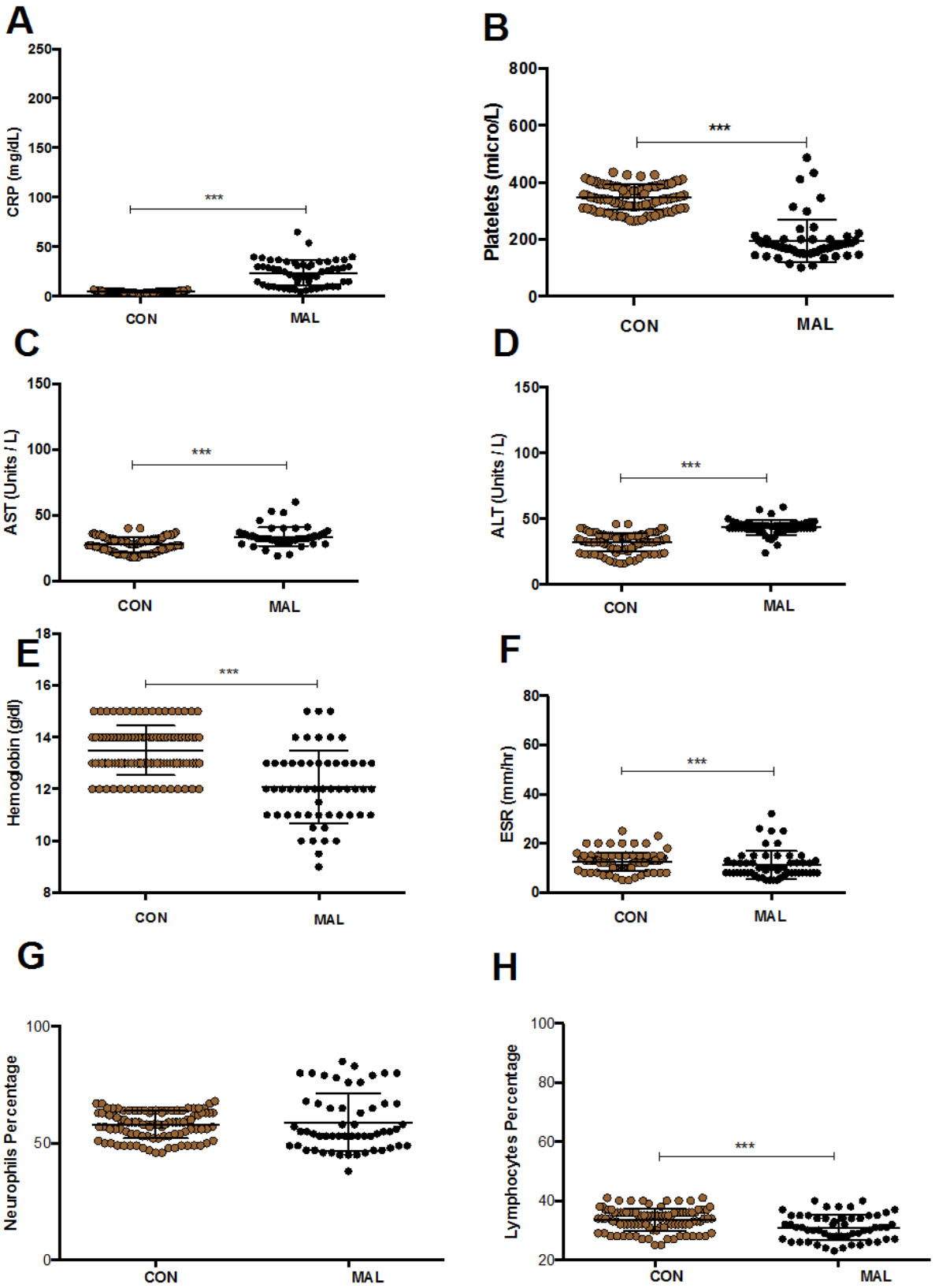

Figure 4.2.3.2. Biochemichal tests results of malaria positive cases against control cases levels. (A) CRP levels in mg/dL of malaria and control (B) Platelets count in microliter of malaria and control (C) AST levels in IU/L of malaria and control (D) Malaria and control ALT levels in IU/L (E) Haemoglobin concentrations in g/dL in malaria and control (F) ESR in mm/hr (G) Neutrophils percentages in malaria and control (H) lymphocytes percentages. Mann-Whitney *U*-tests were used to determine if the measurements of the sample group differ significantly from each other.

4.3. Immune profiles of disease patients

The serum samples of positively diagnosed patients were analysed for quantification of the immune mediators' profiles, the control samples were also included in the analysis for better characteristic clinical observations. The immune mediators selected for the analysis were classified into four groups; Pro-inflammatory cytokines which involve 20 cytokines; GRO-α, IFN-γ, IL-7, IL-8, IL-18, IL-1α, IL-1β, TNF-α, GM-CSF, IL-2, IL-15, IL-17A, IL-21, IL-22, IL-23, IL-27, IL-31, TNF-β, IL-6, and IL-20p70. Anti-inflammatory cytokines which includes 4 cytokines; IL-10, IL-4, IL-13 and IL-1RA. Chemokines which includes 7 cytokines; IP-10, MIP-1α, MIP-1β, RANTES, MCP-1, Eotaxin and SDF-1α, and growth factors, which includes 11 cytokines; PDGF-BB, PIGF-1, EGF, VEGF-A, VEGF-D, bNGF, SCF, BDNF, FGF-2, HGF, and LIF.

4.3.1. Differential immune mediator's profiles in DENV infection

DENV positive patient's immune profiles were tested against healthy controls using serum samples collected during the acute phase of the disease. Profiles showed that DENV infected patients had very high levels of three pro-inflammatory cytokines immune mediators, IFN-γ, IL-6 and IL-18 compared to the healthy control group (Figure 4.3.1.1).

The anti-inflammatory cytokines profile assessment showed that two immune mediators, IL-1RA and IL-10 were highly elevated compared to the healthy control group in DENV infection in opposition to IL-4 and IL-13 (Figure 4.3.1.2).

The chemokines profiling assessment showed that three immune mediators, IP-10, MIP-1α, and MCP-1 were highly elevated in DENV positive samples in opposition to MIP-1β, RANTES, Eotaxin, and SDF-1α (Figure 4.3.1.3).

The assessment profile between DENV infected patients and healthy controls did not detect significant association for growth factors and others cytokines, except for weakly detected low BDNF level (Figure 4.3.1.4).

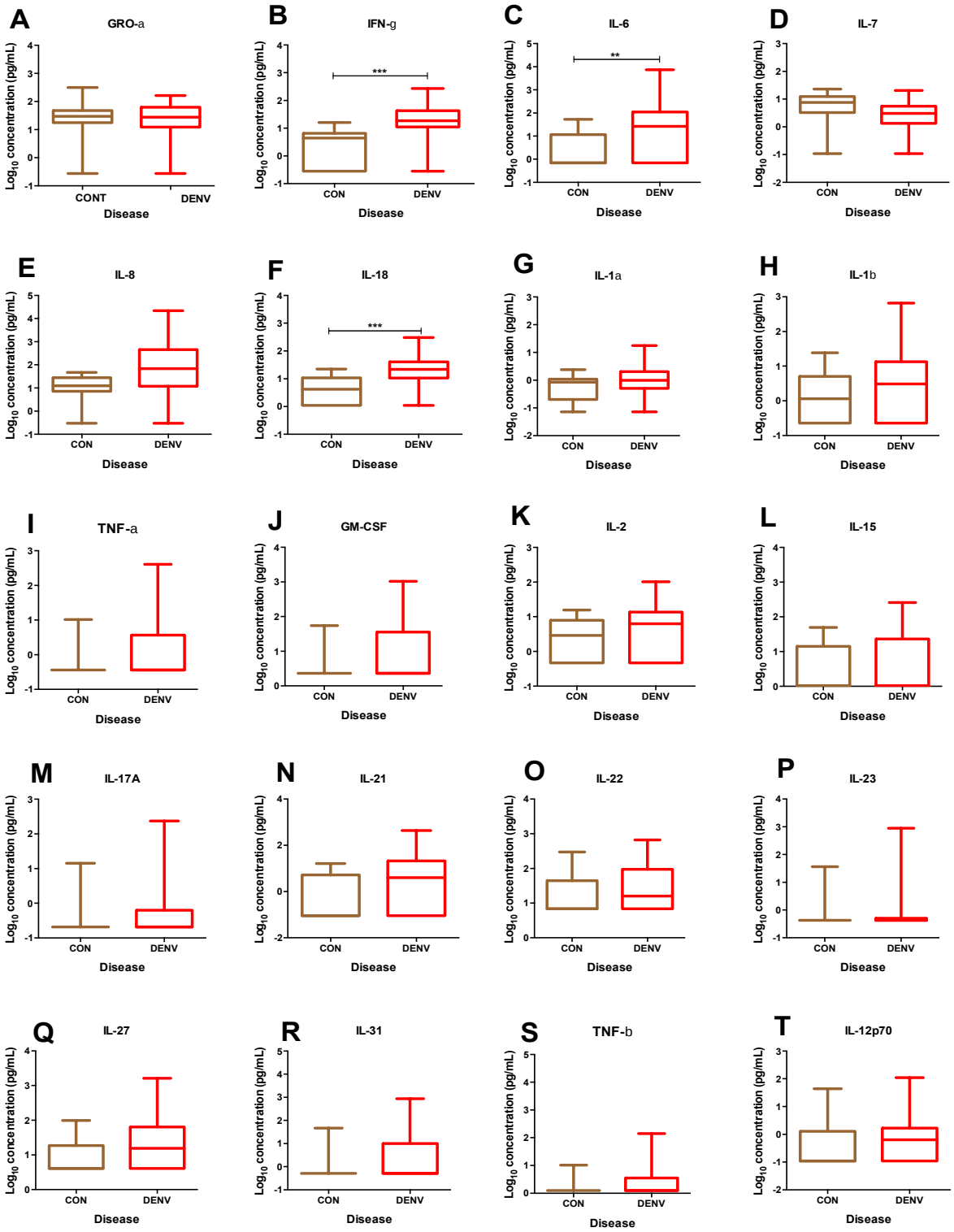

Figure 4.3.1.1. Pro-inflammatory cytokines profile in DENV infection. 20 selected induced cytokines from DENV positive samples compared to healthy control samples are depicted as Tukey box plots. One-way analysis of variance was conducted to logarithmically transformed concentrations with Bonferroni-corrected post hoc t test; results were corrected for multiple testing using Benjamini and Hochberg method. Abbreviations: GRO-α; growth-regulated oncogene alpha, IFN-γ; interferon gamma, IL-1α, TNF-α; tumor necrosis alpha; GM-CSF; granulocyte –macrophage colony – stimulating factor.

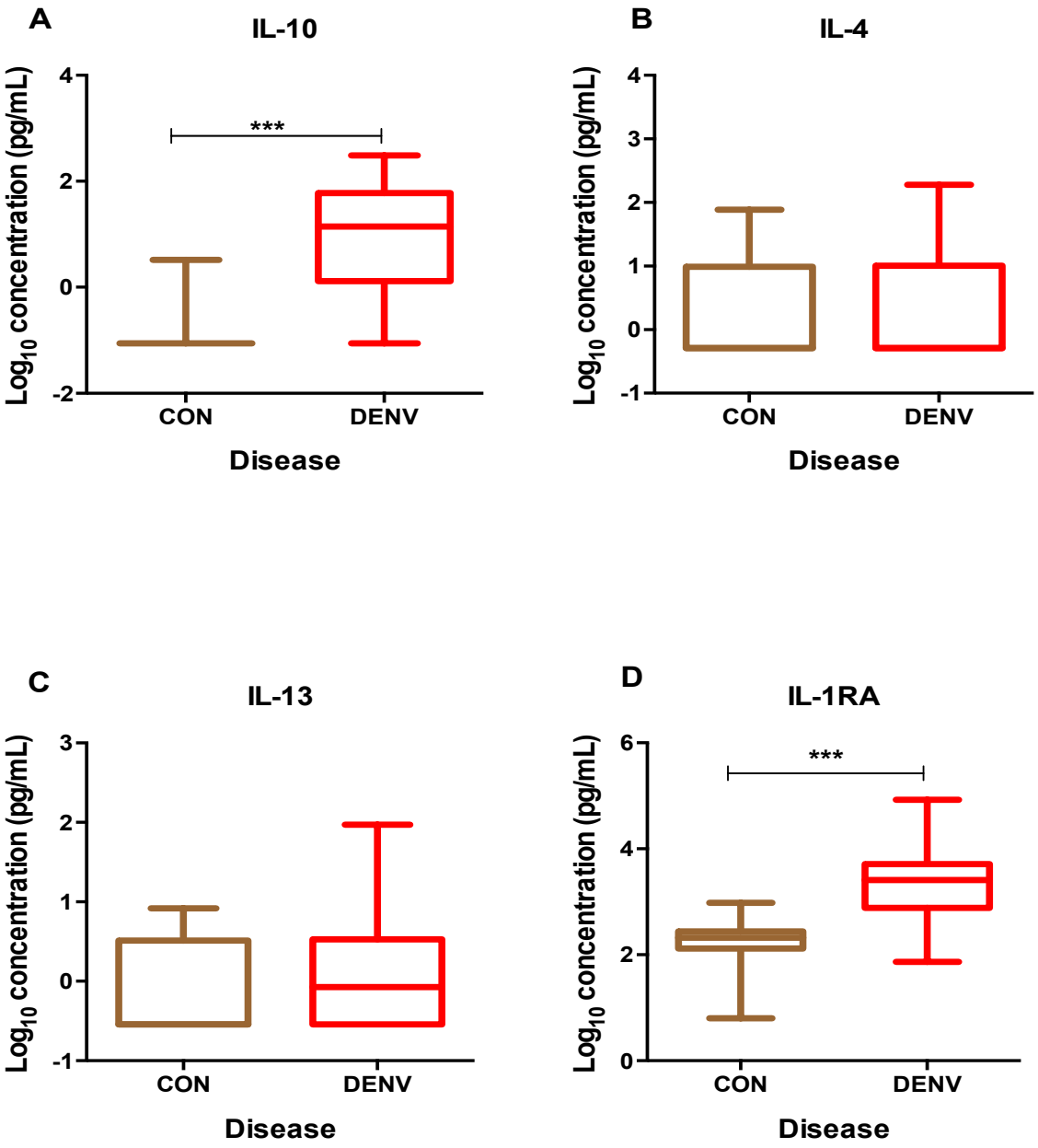

Figure 4.3.1.2. Anti-inflammatory cytokines profile in DENV infection. The level of 4 selected cytokines from DENV positive samples compared to healthy control samples are depicted as Tukey box plots. One way analysis of variance was conducted to logarithmically transformed concentrations with Bonferroni-corrected post hoc *t* test; results were corrected for multiple testing using Benjamini and Hochberg method. Abbreviations: IL-10; interleukin-10, IL-1RA; interleukin-1 receptor antagonist.

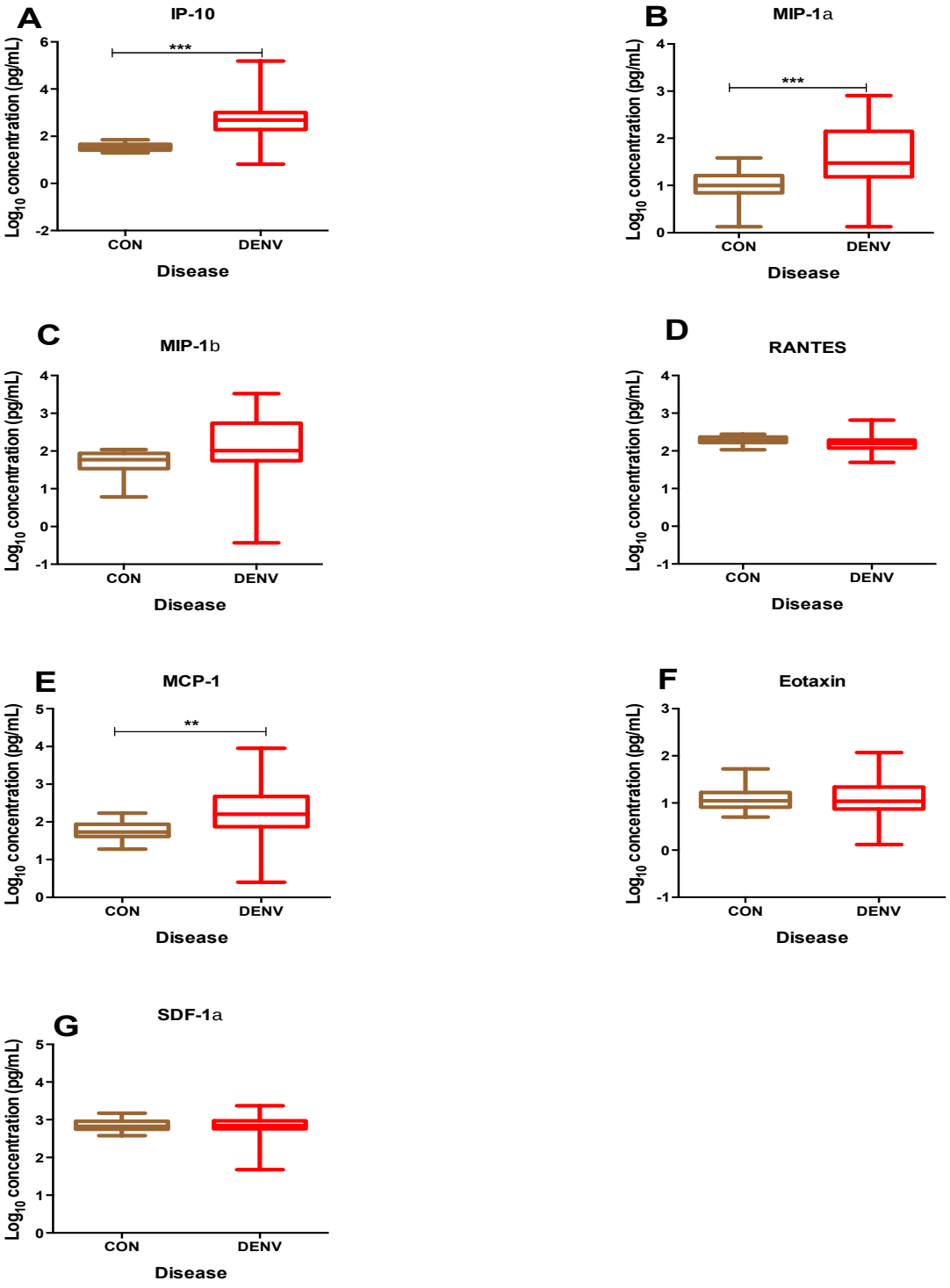

Figure 4.3.1.3. Immune mediators concentrations (Chemokines) in DENV infection. Selected immune mediators compared against control cases were depicted as Tukey box plots. One way analysis of variance was conducted to logarithmically transformed concentrations with Bonferroni-corrected post hoc *t* test; results were corrected for multiple testing using Benjamini and Hochberg method. Abbreviations: IP-10, interferon gamma-induced protein 10; MIP-1α, macrophage inflammatory protein 1α; RANTES, regulated on activation, normal T-expressed and presumably secreted, MCP-1, monocyte chemoattractant protein-1, Eotaxin, eosinophil chemotactic protein**;** SDF-1α**,** stromal cell-derived factor 1α.

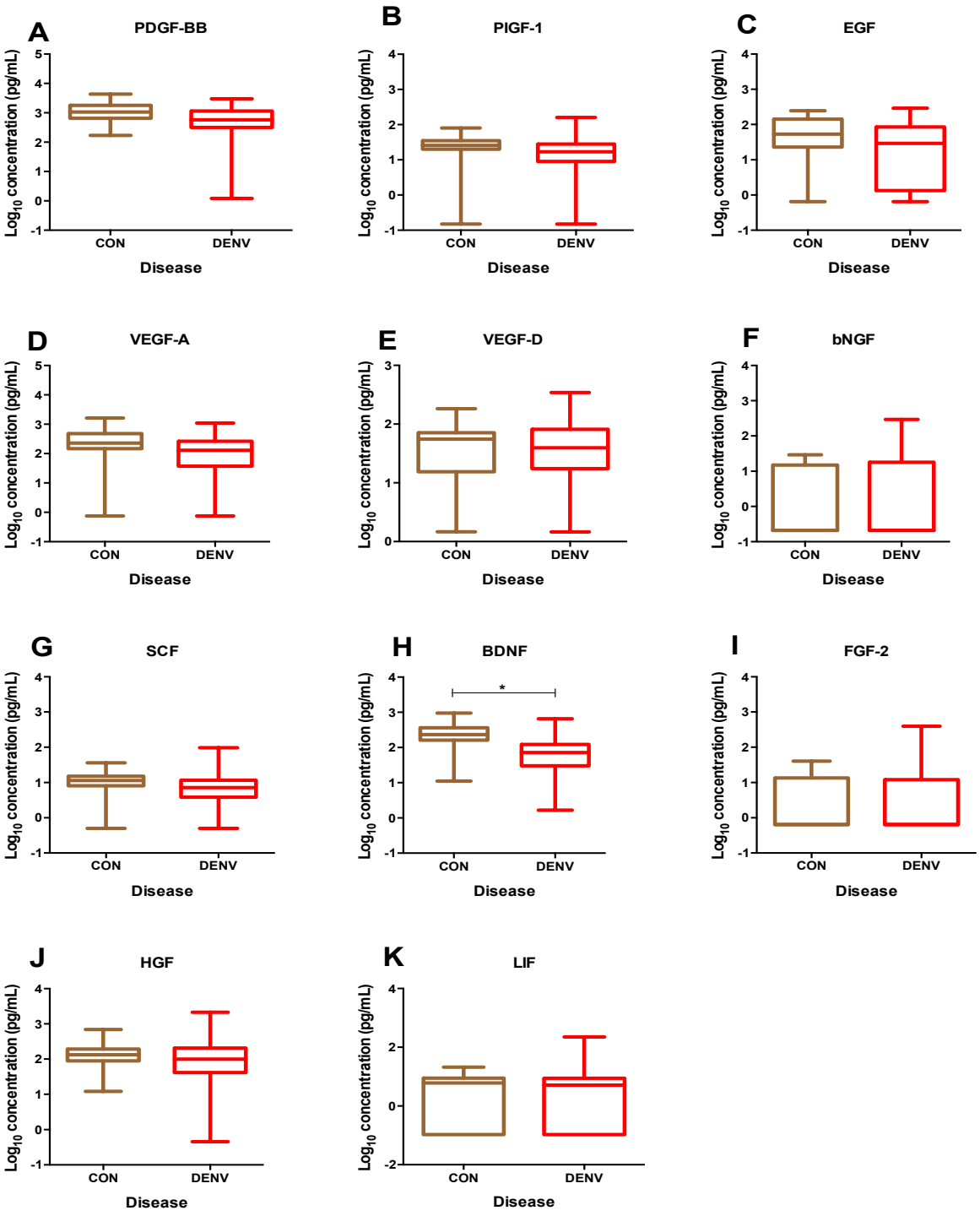

Figure 4.3.1.4. Growth factors and other cytokines profile in DENV infection. Selected immune mediators' concentrations from DENV positive samples were compared against healthy individuals are depicted as Tukey box plots. One way analysis of variance was conducted to logarithmically transformed concentrations with Bonferroni-corrected post hoc *t* test; results were corrected for multiple testing using Benjamini and Hochberg method. Abbreviations: PDGF-BB, platelets derived growth factor; VEGF, vascular endothelial growth factor; PIGF, placental growth factor; EGF, human epidermal growth factor**;** bNGF, beta-nerve growth factor,SCF; stem cell factor**;** BPNF, brain-derived neurotropic factor**;** FGF-2, fibroblast growth factor 2; HGF**,** hepatocyte growth factor; LIF, leukemia inhibitory factor.

4.3.2. Differential immune mediator's profiles in GAS and MEN

Profiles between the GAS and healthy controls immune mediators were assessed using serum collected during the acute phase of the diseases. Profiles showed that individuals infected with GAS had a very high level of 2 pro-inflammatory immune mediators, which are IL-6 and IL-18 compared to the healthy control group, while GRO-α and IL-7 are significantly below the control level. In meningococcal meningitis infection, the profile was assessed against the healthy controls showed that individuals infected with meningococcal meningitis had a very high level of two immune mediators IL-6 and IL-18 (Figure 4.3.2.1).

The anti-inflammatory cytokines profile, showed that only one immune mediator, IL-1RA was expressed in high concentration level in GAS and meningeococcal meningitis infected individuals compared to the healthy controls (Figure 4.3.2.2).

The chemokines group assessed profile for GAS and meningococcal meningitis positive patients showed that three immune mediators; RANTES, Eotaxin, and SDF-1α levels were significantly low in positive cases in comparison to the healthy controls levels (Figure 4.3.2.3).

Growth factors and others cytokines produced in low concentration levels due to both GAS and meningococcal meningitis infections were PDGF-BB, PIGF-1, EGF, and BDNF when compared to the healthy controls (Figure 4.3.2.4).

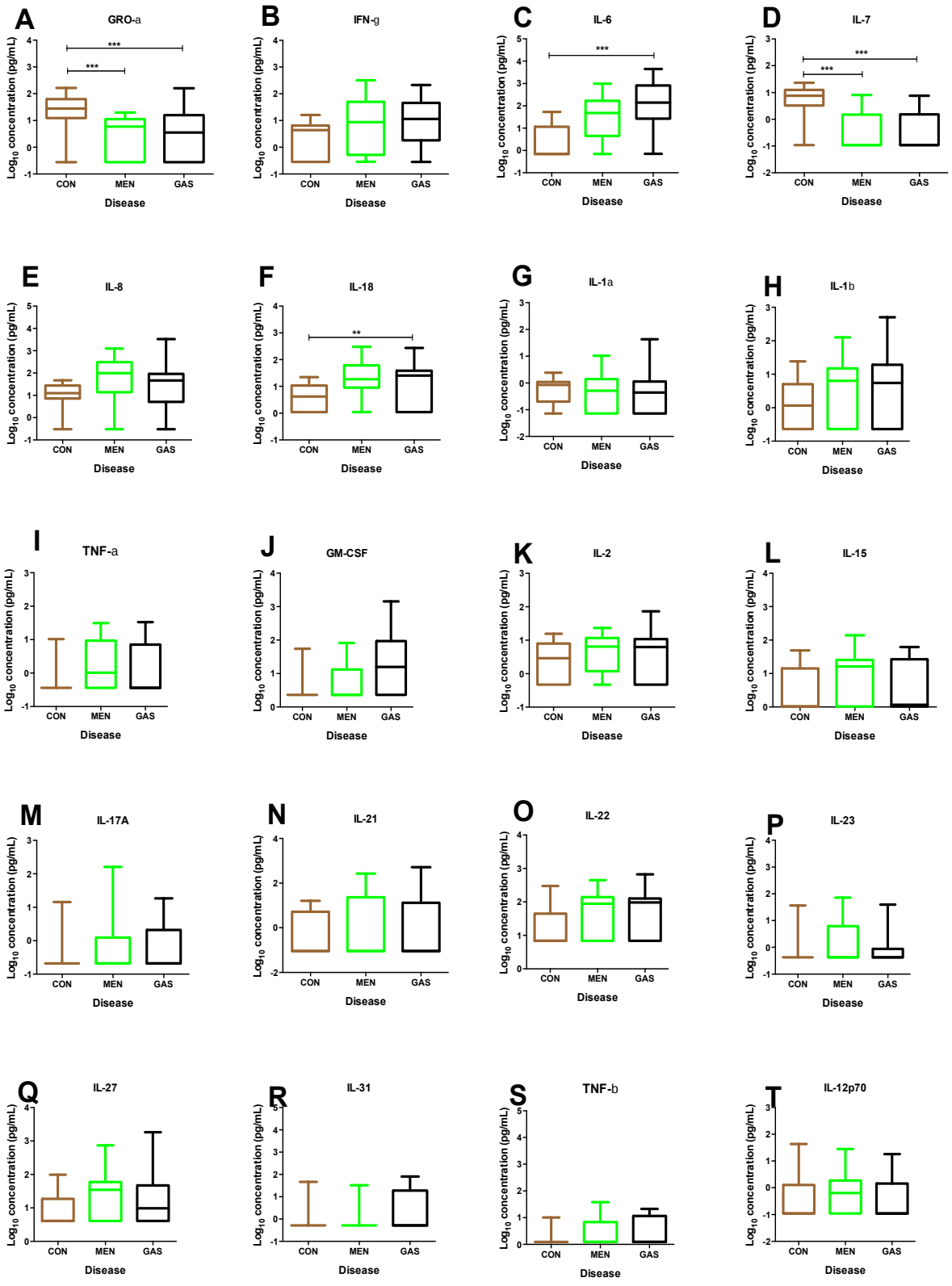

Figure 4.3.2.1. Pro-inflammatory cytokines profile in GAS and MEN infections. Selected induced cytokines from GAS and MEN positive samples compared to healthy control samples are depicted as Tukey box plots. One-way analysis of variance was conducted to logarithmically transformed concentrations with Bonferroni-corrected post hoc t test; results were corrected for multiple testing using Benjamini and Hochberg method. Abbreviations: GRO-α; growth-regulated oncogen alpha, IFN-γ; interferon gamma, IL-1α, TNF-α; tumor necrosis factor alpha; GM-CSF; granulocyte –macrophage colony – stimulating factor.

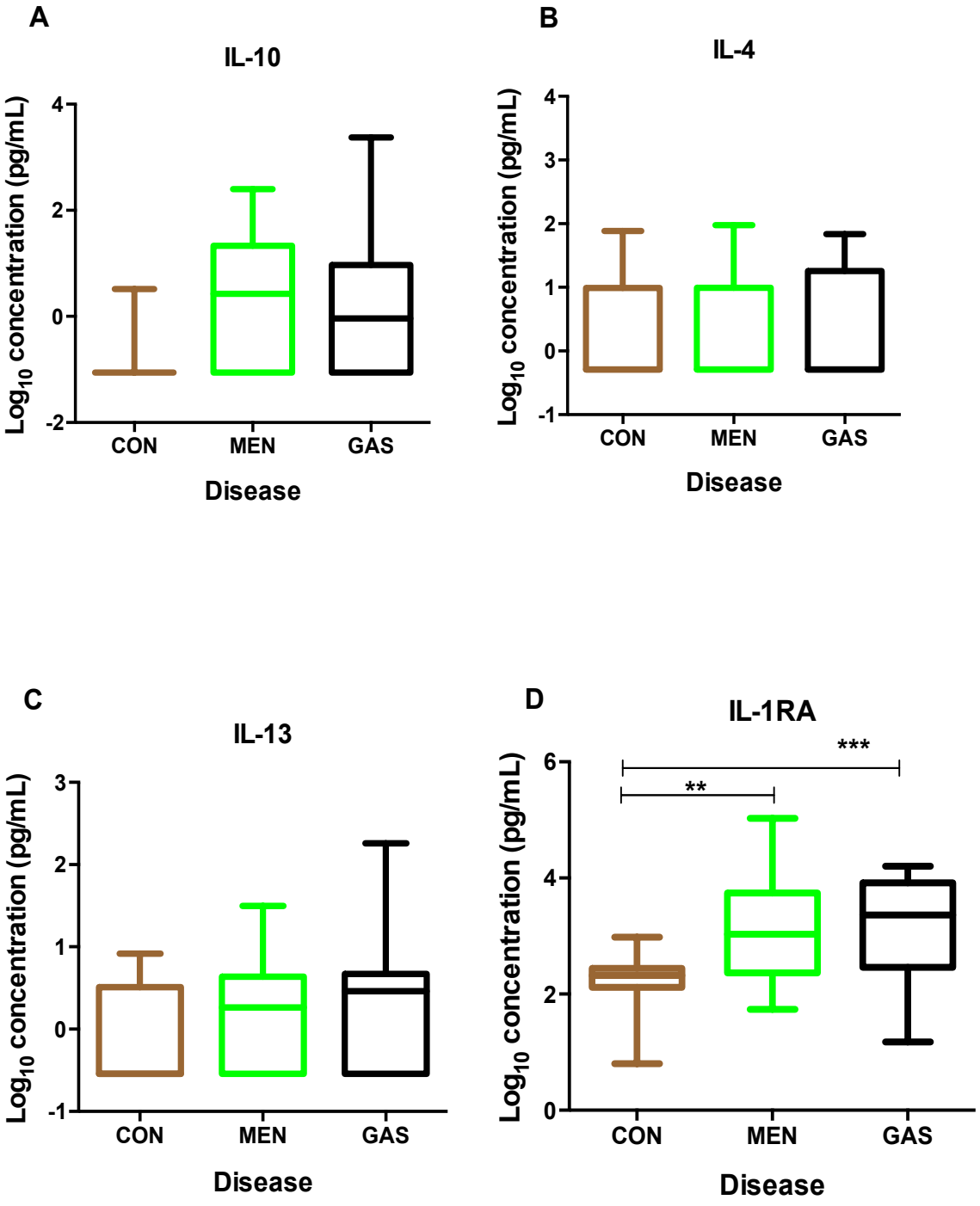

Figure 4.3.2.2. Anti-inflammatory cytokines profile in GAS and MEN infections. The level of 4 selected cytokines from GAS and MEN positive samples compared to healthy control samples are depicted as Tukey box plots. One-way analysis of variance was conducted to logarithmically transformed concentrations with Bonferroni-corrected post hoc *t* test; results were corrected for multiple testing using Benjamini and Hochberg method. Abbreviations: IL-10 interleukin-10, IL-1RA; interleukin-1 receptor antagonist.

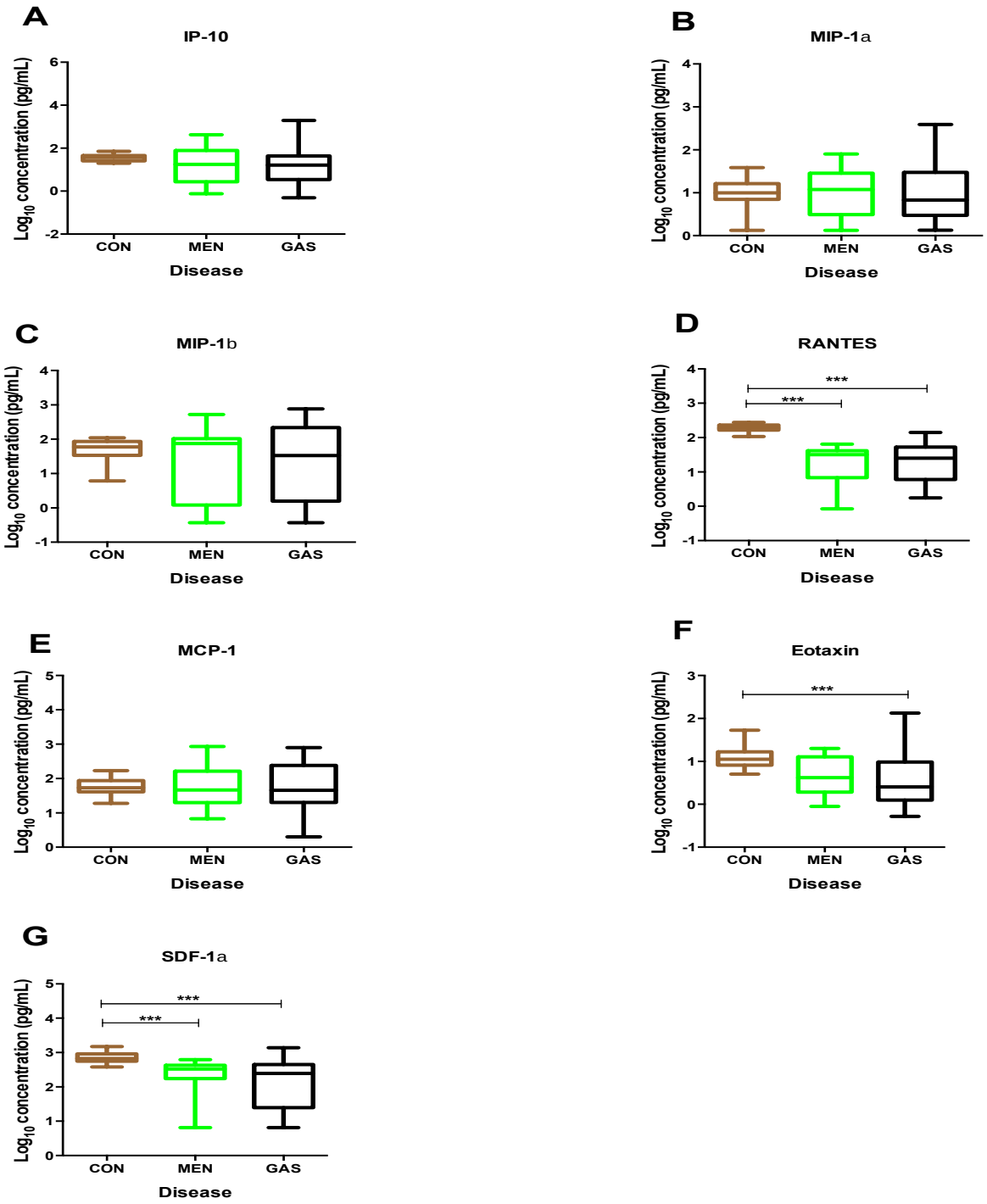

Figure 4.3.2.3. Immune mediators concentrations (Chemokines) in GAS and MEN infections. Selected immune mediators compared against control cases were depicted as Tukey box plots. One-way analysis of variance was conducted to logarithmically transformed concentrations with Bonferroni-corrected post hoc *t* test; results were corrected for multiple testing using Benjamini and Hochberg method. Abbreviations: IP-10, interferon gamma-induced protein 10; MIP-1α, macrophage inflammatory protein 1α; RANTES, regulated on activation, normal T-expressed and presumably secreted, MCP-1, monocyte chemoattractant protein-1, Eotaxin, eosinophil chemotactic protein**;** SDF-1α**,** stromal cell-derived factor 1α.

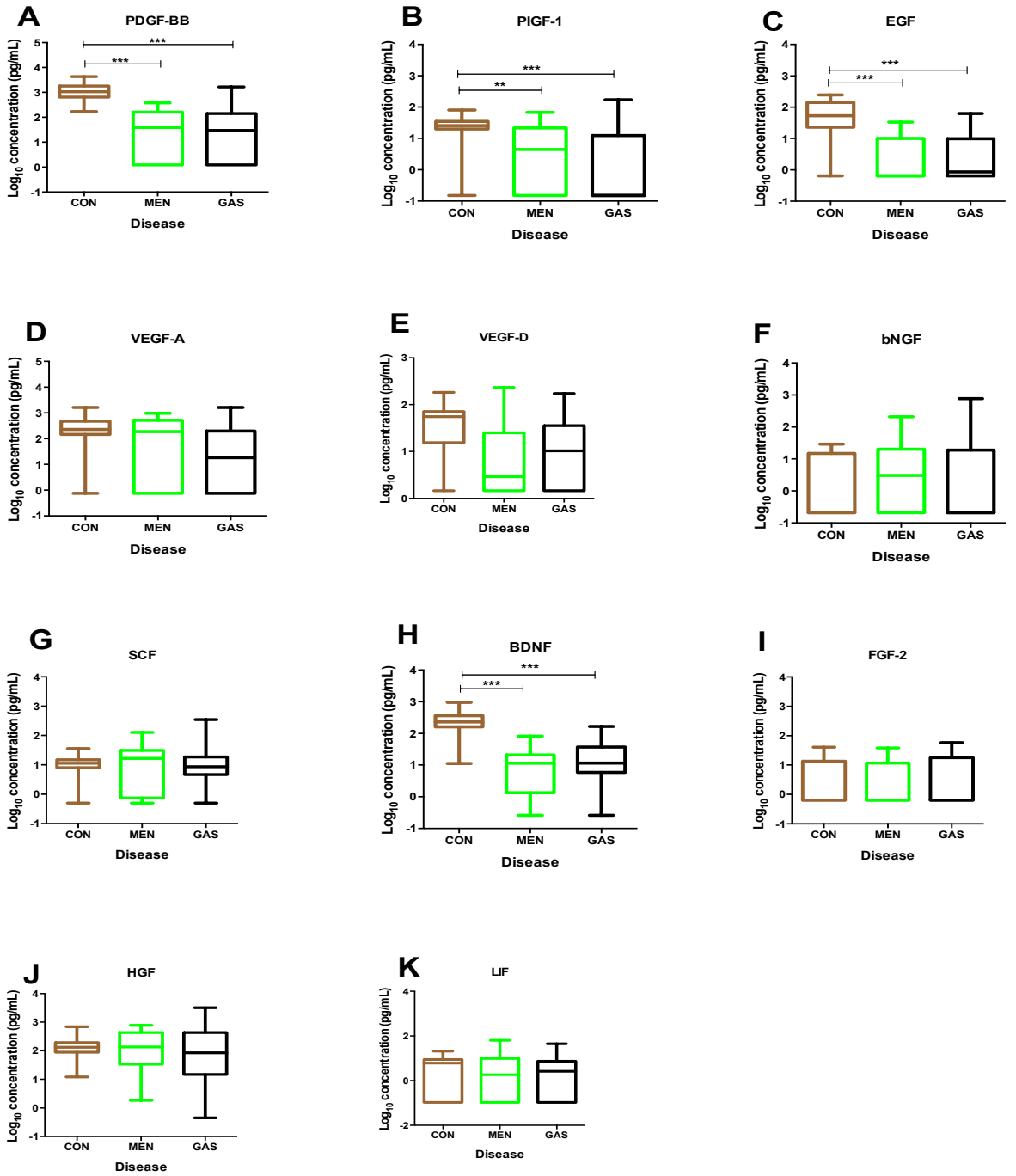

Figure 4.3.2.4. Growth factors and other cytokines profiles in GAS and MEN infections. Selected immune mediator's concentrations from GAS and MEN positive samples were compared against healthy individuals are depicted as Tukey box plots. One-way analysis of variance was conducted to logarithmically transformed concentrations with Bonferroni-corrected post hoc *t* test; results were corrected for multiple testing using Benjamini and Hochberg method. Abbreviations: PDGF-BB, platelet derived growth factor; VEGF, vascular endothelial growth factor; PIGF, placental growth factor; EGF, human epidermal growth factor**;** bNGF, beta-nerve growth factor; SCF; stem cell factor**;** BDNF, brain-derived neurotrohic factor**;** FGF-2, fibroblast growth factor 2; HGF**,** hepatocyte growth factor; LIF, leukemia inhibitory factor.

4.3.3. Differential immune mediator's profiles in malaria

Immune mediator's profile induced by malaria infection were analysed against the healthy controls. The assessment of pro-inflammatory cytokines showed that four immune mediators (IFN-γ, IL-6, IL-18, and TNF-α) present in very high level compared to healthy control group (Figure 4.3.3.1). The anti-inflammatory cytokines study showed a very high level of two immune mediators, IL-10 and IL-1RA compared to the healthy controls (Figure 4.3.3.2). The chemokines inflammatory cytokines analysis showed that only one immune mediator, IP-10 was detected in high level in malaria positive cases compared to the healthy controls (Figure 4.3.3.3). The growth factors and other cytokines profile study demostrated that three immune mediators which are BDNF, PDGF-BB, and EGF, showed significant low levels in malaria positive cases compared to the healthy controls (Figure 4.3.3.4).

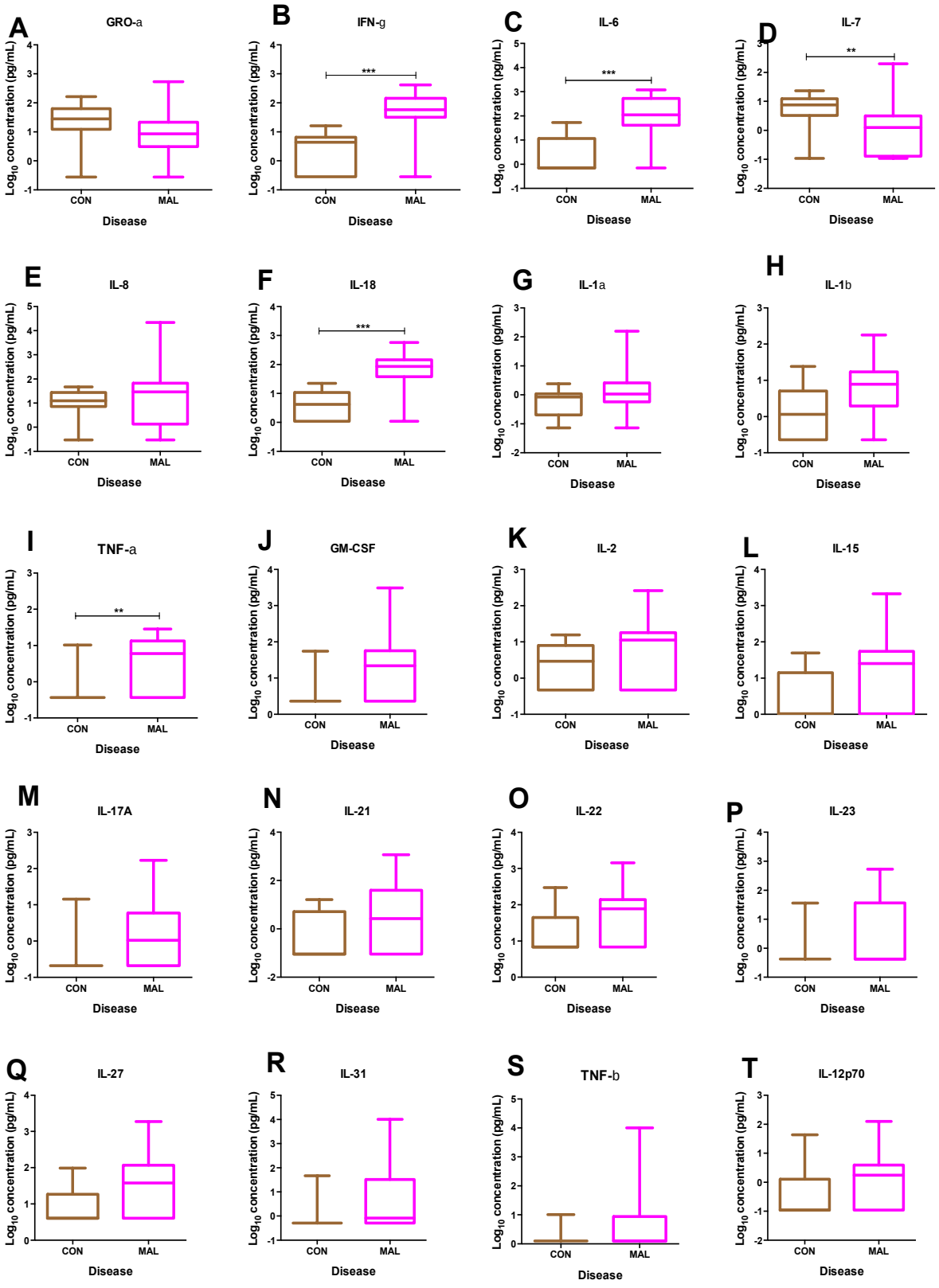

Figure 4.3.3.1. Pro-inflammatory cytokines profile in malaria infection. Selected produced cytokines from malaria positive samples compared to healthy control samples are depicted as Tukey box plots. One way analysis of variance was conducted to logarithmically transformed concentrations with Bonferroni-corrected post hoc t test; results were corrected for multiple testing using Benjamini and Hochberg method. Abbreviations: GRO-α; growth-regulated oncogene alpha, IFN-γ; interferon gamma, IL-1α, TNF-α; tumor necrosis factor; GM-CSF alpha; granulocyte –macrophage colony – stimulating factor.

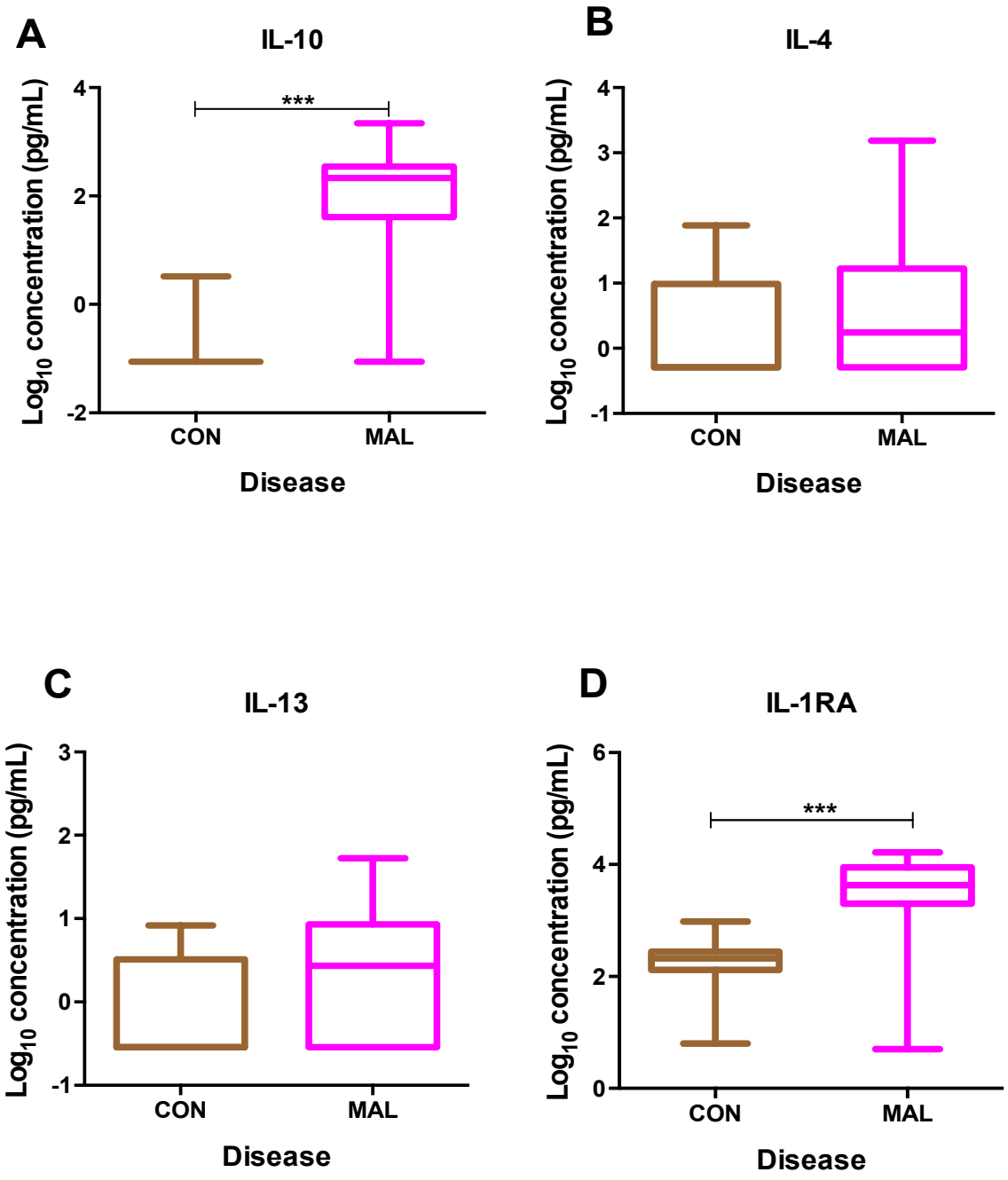

Figure 4.3.3.2. Anti-inflammatory cytokines profile in malaria infection. The level of 4 selected cytokines from malaria positive samples compared to healthy control samples are depicted as Tukey box plots. One way analysis of variance was conducted to logarithmically transformed concentrations with Bonferroni-corrected post hoc *t* test; results were corrected for multiple testing using Benjamini and Hochberg method. Abbreviations: IL-1RA interleukin-1receptor antagonist.

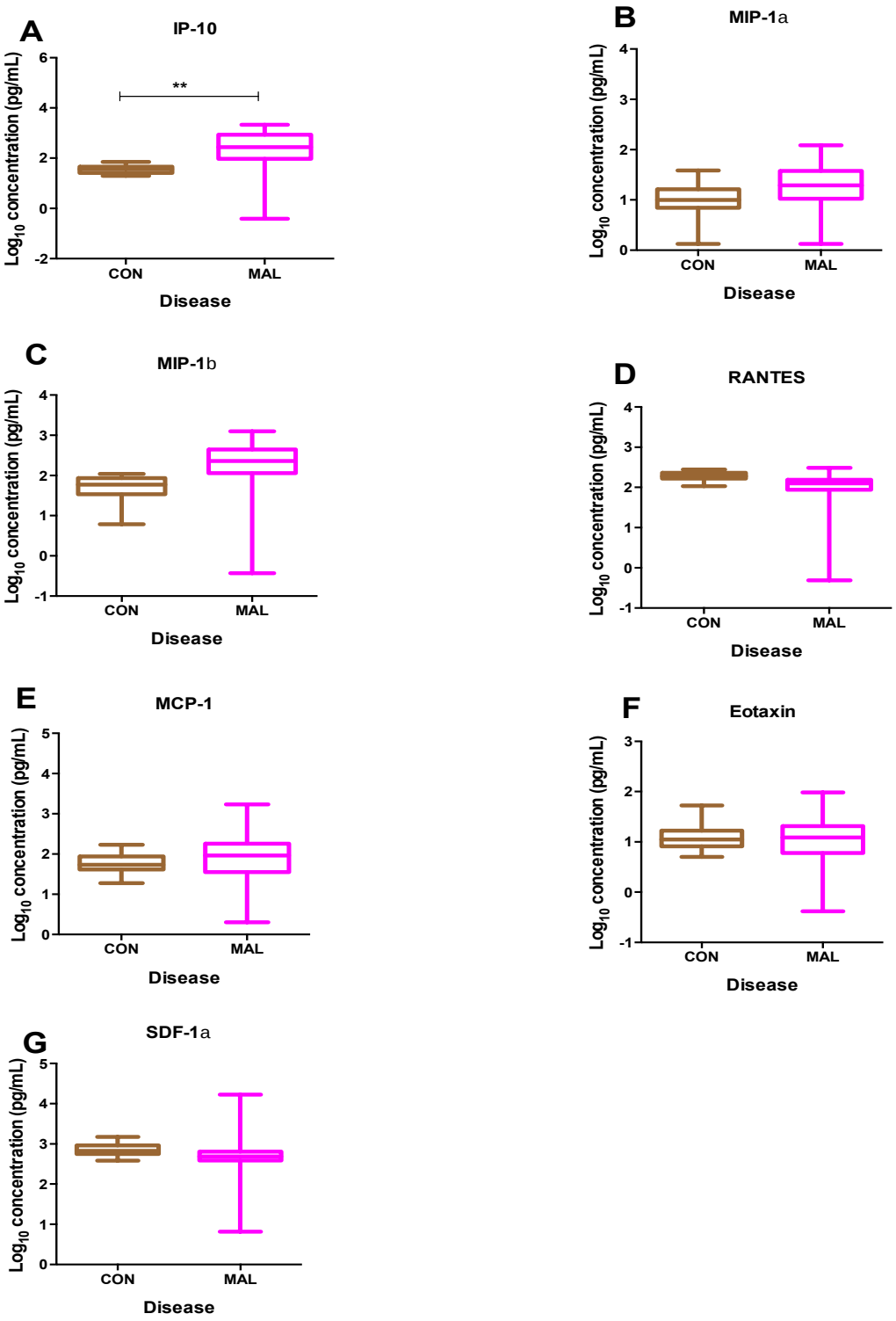

Figure 4.3.3.3. Immune mediators concentrations (Chemokines) in malaria infection. Selected immune mediator's concentrations were compared against control samples depicted as Tukey box plots. One way analysis of variance was conducted to logarithmically transformed concentrations with Bonferroni-corrected post hoc *t* test; results were corrected for multiple testing using Benjamini and Hochberg method. Abbreviations: IP-10, interferon gamma-induced protein 10; MIP-1α, macrophage inflammatory protein 1α; RANTES, regulated on activation, normal T-expressed and presumably secreted; MCP-1, monocyte chemoattractant protein-1; Eotaxin, eosinophil chemotactic protein**;** SDF-1α**,** stromal cell-derived factor 1α.

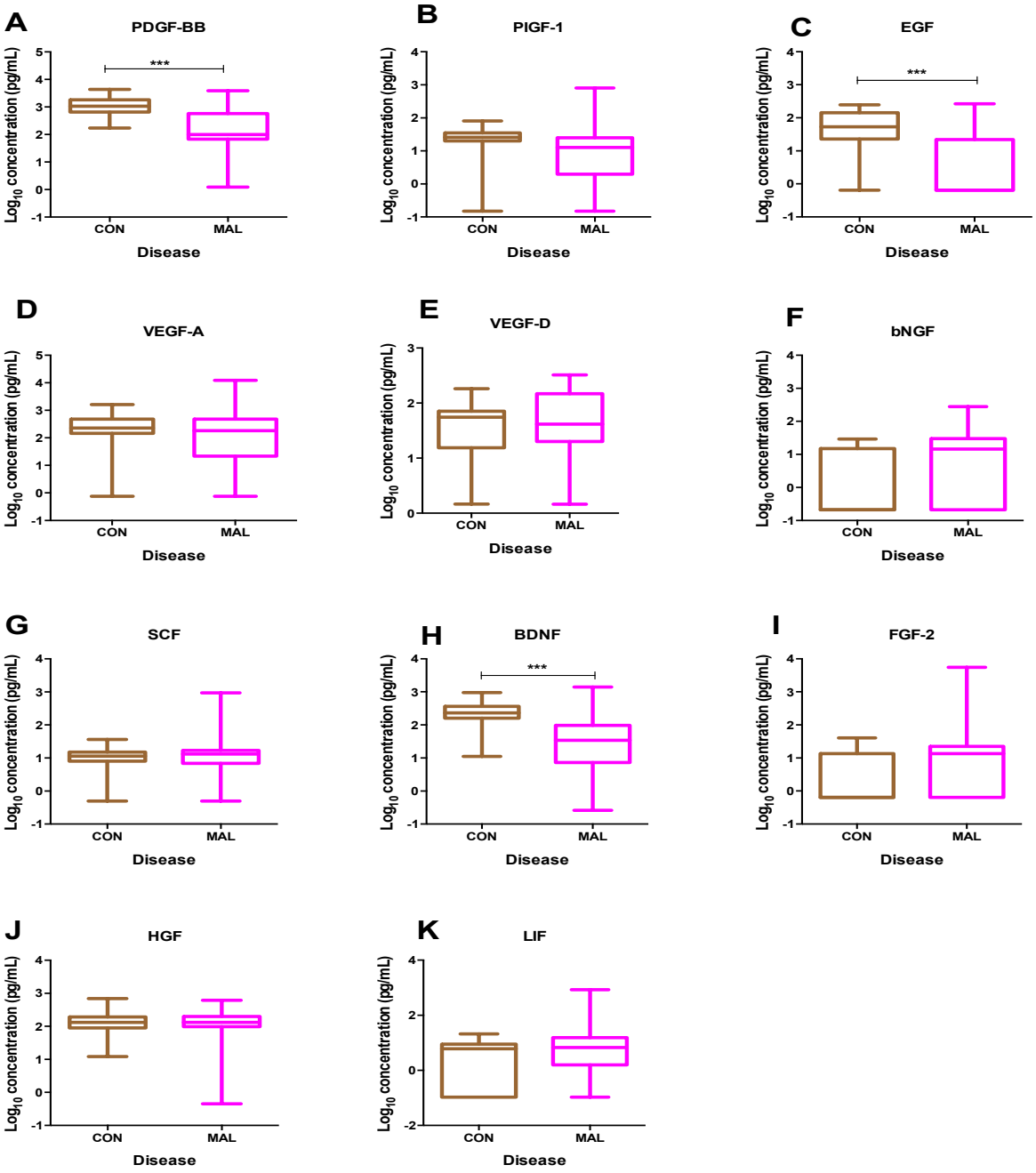

Figure 4.3.3.4. Growth factors and other cytokines profiles in malaria infection. Selected immune mediator's concentrations from malaria positive samples were compared against healthy individuals are depicted as Tukey box plots. One-way analysis of variance was conducted to logarithmically transformed concentrations with Bonferroni-corrected post hoc *t* test; results were corrected for multiple testing using Benjamini and Hochberg method. Abbreviations: PDGF-BB, platelet derived growth factor; VEGF, vascular endothelial growth factor; PIGF, placental growth factor; EGF, human epidermal growth factor**;** bNGF, beta-nerve growth factor; SCF; stem cell factor**;** BDNF, brain-derived neurotropic factor**;** FGF-2, fibroblast growth factor 2; HGF**,** hepatocyte growth factor; LIF, leukemia inhibitory factor.

4.3.4. Common patterns of immune mediators per disease group

All DENV-infected febrile patients had high levels of a set of common immune mediators compared to the healthy controls (Figure 4.3.1.1, 4.3.1.2, 4.3.1.3 and 4.3.1.4). Three pro-inflammatory cytokines (IFN-γ, IL-6, IL-18) were significantly higher in febrile patients than in healthy controls (Figure 4.3.1.1).

This above mentioned phenomenon was also seen in malaria infection. If IFN-γ is excluded, the same similarty can be seen in GAS and meningococcal meningitis infections where both IL-6 and IL-18 significantly elevated (Figure 4.3.2.1 and 4.3.3.1). Thus, those pro-inflammatory cytokines are not very specific in differentiating between the three diseases groups. Among the 20 different pro-inflammatory cytokines, there was no significant observation of a unique cytokine that can diffentiate between the three disease groups, except an elevation of TNF-α which was exclusively repored in malaria.

The anti-inflammatory study showed that IL-10 was only expressed in very high levels in DENV and MAL infections.Among the febrile patients, no bacterial pathogen showed a significant elevation of IL-10. However, IL-1RA was expressed in very high quantities in all viral (DENV), parasitic (MAL) and bacteria (meningeococcal meningitis) pathogens (Figure 4.3.1.2, 4.3.2.2 and 4.3.3.2). There was no observation of a specific anti-inflammatory cytokine that can be up regulated in a particular disease groups.

In the chemokines study, IP-10 was highly elevated in viral (DENV) and parasitic (MAL) infection (Figure 4.3.1.3 and 4.3.3.3). No bacterial infection (GAS and meningococcal meningitis) showed a significant elevation of this particular cytokine (Figure 4.3.2.3). However, MIP-1α and MCP-1 were found to be the only two unique identifiers for viral (DENV) infections as no bacterial or parasitic infections expressed a significant level of those two cytokines. RANTES, Eotaxin, and SDF-1α levels were significantly reduced compared to the control levels in both bacterial infections (GAS and meningococcal meningitis) but not in viral (DENV) or malaria infections (Figure 4.3.1.3, 4.3.2.3, and 4.3.3.3).

BDNF was the only reduced biomarker in DENV. PDGF-BB, BDNF, and EGF were significantly reduced in both bacterial and malaria infections but did not show a significant elevation or reduction in viral infections (DENV). PIGF-1 was significantly reduced in bacterial infections (Figure 4.3.1.4, 4.3.2.4 and 4.3.3.4).

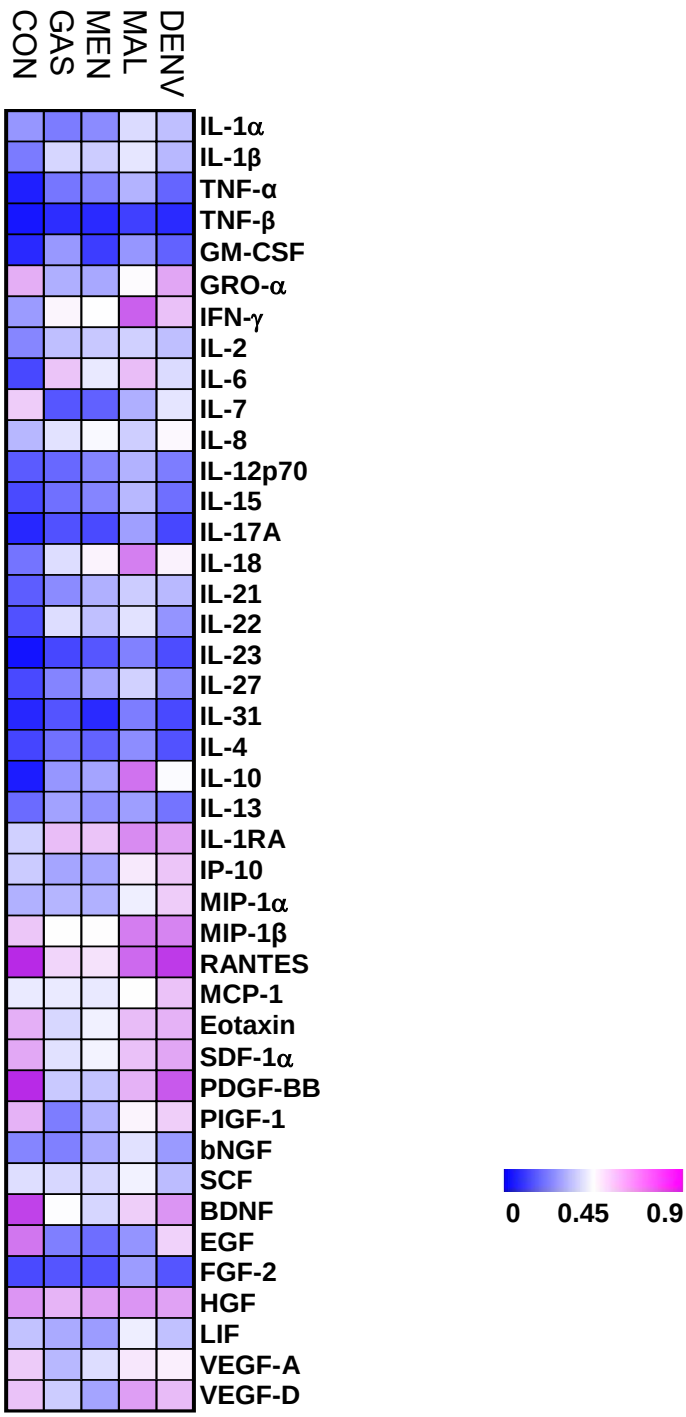

Figure 4.3.4.1. Predictive immune mediators in DENV, malaria, MEN & GAS infections from serum samples collected during acute phase of the disease were analysed and presented in heat map for normalized scores. In the heat-map presentation, the immune mediator concentration was scaled between 0 and 0.9 for each measured immune mediator, and the average scaled value was then computed for each group. Blue color represent the lowest average scaled value; pink color, the highest average scaled value. Abbreviations: BDNF, brain-derived neurotrophic factor; bNGF, beta-nerve growth factor; EGF, epidermal growth factor; FGF-2, basic fibroblast growth factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; GRO-α, growth-regulated oncogene alpha; HGF, hepatocyte growth factor; IFN-γ, interferon gamma; IL-1α, interleukin 1α; IP-10, interferon gamma-induced protein 10; LIF, leukemia inhibitory factor; MCP, monocyte chemoattractant protein; MIP-1α/β, macrophage inflammatory protein-1alpha/beta; IL-1RA, interleukin1 receptor antagonist, PDGF-BB, platelets-derived growth factor; PIGF, placental growth factor; RANTES, regulated on actitation, normal T-expressed, and presumably secreted; SCF, stem cell factor; SDF-1α, stromal cell-derived factor 1α; TNF-α/β, tumor necrosis factor-alpha/beta; VEGF, vascular endothelial growth factor.

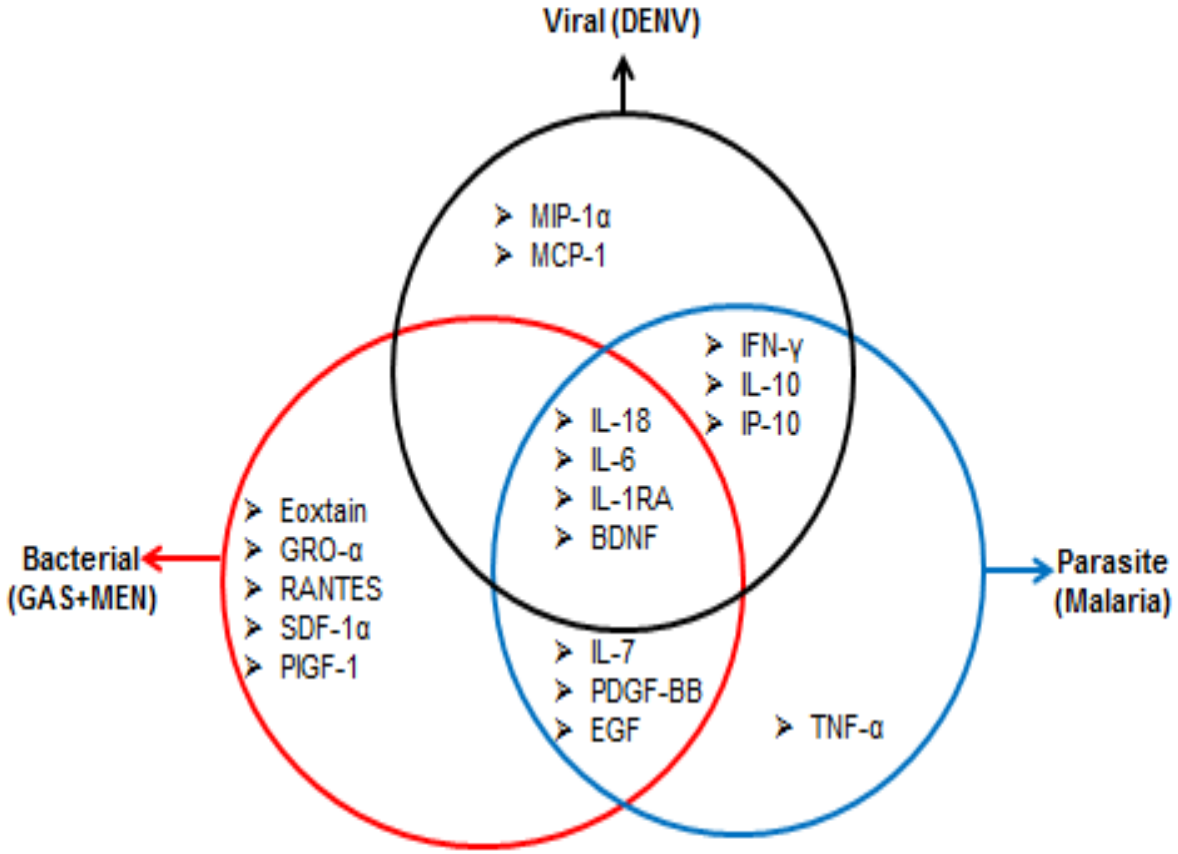

Figure 4.3.4.2. Venn diagram summarizing the statistically significant immune mediators compared to the healthy controls. The black circle, highlight significate cytokines expressed in high amount in viral infections, namely, MIP-1α, MCP-1, IL-18, IL-1RA, BDNF, IL-6, IFN-γ, IL-10, and IP-10; The blue circle, illustrate cytokines that are significantly expressed in high concentrations in Malaria infections, namely, IFN-γ, IL-10, IP-10, TNF-α, IL-18, IL-1RA, BDNF, IL-6, IL-7, PDGF-BB, and EGF; the red circle shows cytokines that can be secreted in high concentrations in GAS and Meningeococcal meningitis, namely, IL-18, IL-1RA, BDNF, IL-6, IL-7, PDGF-BB, EGF, Eotaxin, GRO-α, RANTES, SDF-1α, and PIGF-1. The intercepted circles shows the shared cytokines that are highly expressed in the three types of infections, namely, IL-18, IL-1RA, BDNF, and IL-6. Abbreviations: BDNF, brain-derived neurotrophic factor; bNGF, beta-nerve growth factor; EGF, epidermal growth factor; FGF-2, basic fibroblast growth factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; GRO-α growth-regulated oncogene-alpha; IL-1RA, interleukin1 receptor antagonist; HGF, hepatocyte growth factor; IFN-γ, interferon-gamma; IL-1α, interleukin-1α; IP-10, interferon gamma-induced protein-10; LIF, leukemia inhibitory factor, MCP, monocyte chemoattractant protein; MIP-1α/β, macrophage inflammatory protein-alpha/beta; PDGF-BB, platelets-derived growth factor-BB; PIGF, placental growth factor; RANTES, regulated on activation, normal T-expressed, and presumably secreted; SCF, stem cell factor; SDF-1α, stromal cell-derived factor 1α; TNF, tumor necrosis factor alpha; VEGF, vascular endothelial growth factor.

4.4. Meta-analysis results

4.4.1. DENV NS1, IgM, IgG and viral load association with differential immune mediators

Analysis of correlation between immune-mediators and DENV NS1, DENV IgM, DENV IgG, and DENV viral load (PFU/mL) in serum among patients with acute DENV infection with thier correspondant *P* values are shown in (Table 4.4.1.1). Only IL-7 and IL-21 were significantly correlated with DENV NS1 (*P* value < 0.05). IFN-γ was significantly associated with DENV IgM (*P* value < 0.01). IL-18, IL-21, IL22, and SCF showed positive correlation with DENV IgM (*P* value < 0.05). For analysis of correlation between DENV IgG and immune mediators concentrations, IFN-γ and IL-17A showed strong association with DENV IgG (*P* value < 0.01). GM-CSF, bNGF, and FGF-2 showed significant correlation with DENV IgG (*P* value < 0.05). On analysis of correlation between DENV viral load (PFU/mL) and stimulated cytokines in response to acute DENV infection as shown in (Table 4.4.1.1), no significant association were reported (*P* value > 0.05). The correlation between DENV NS1, DENV IgM, DENV IgG and viral load (PFU/mL) during acute DENV infection were assessed and reflected in (Table 4.4.1.2). There were no significant association between viral load (PFU/mL) and any of DENV NS1, DENV IgM or DENV IgG (*P* value < 0.05). Significant correlation between DENV NS1 and DENV IgM was identified (*P* value < 0.01), while there was neither an association between DENV IgG and DENV NS1 nor with DENV IgG and DENV IgM (*P* value > 0.05) (Table 4.4.1.2).

Table 4.4.1.1. Analysis of immune mediators concentrations among DENF infected patients during the acute phase of the disease with thier corresponded *P* value.

variable	IFN-γ	IL-8	IL-18	IL-10	MIP-1α	BDNF	IP-10	IL-1RA	MCP-1	GRO-α	IL-7	Eotaxin	SDF-1α	PIGF-1	PDGF-BB	RANTES	IL-1α	IL-1β	TNF-α	GM-CSF	IL-2
DENV NS1[a]	0.14	0.72	0.55	0.07	0.69	0.74	0.63	0.28	0.25	0.15	**0.016**	0.34	0.84	0.98	0.95	0.11	0.21	0.20	0.10	0.18	0.14
DENV IgM[a]	**0.001**	0.21	**0.027**	0.69	0.59	0.49	0.59	0.72	0.31	0.48	0.60	0.87	0.17	0.11	0.26	0.18	0.07	0.20	0.21	0.16	0.38
DENV IgG[a]	**0.009**	0.64	0.21	0.30	0.69	0.84	0.97	0,64	0.18	0.21	0.62	0.11	0.33	0.90	0.25	0.66	0.54	0.09	0.15	**0.04**	0.36
Viral load[b] (PFU/mL)	0.70	0.99	0.97	0.99	0.97	0.28	0.99	0.86	0.97	0.36	0.82	0.97	0.17	0.92	0.33	0.97	0.98	0.99	0.74	0.99	0.17

Variable	**bNGF**	**SCF**	**MIP-1β**	**IL-27**	**IL-31**	**TNF-β**	**FGF-2**	**HGF**	**LIF**	**VEGF-D**	**IL-15**	**IL-17A**	**IL-21**	**IL-22**	**IL-23**	**IL-6**	**IL-12p70**	**IL-4**	**IL-13**	**EGF**
DENV NS1[a]	0.10	0.36	0.74	0.93	0.50	0.70	0.37	0.85	0.46	0.43	0.86	0.85	**0.04**	0.76	0.90	0.83	0.58	0.55	0.60	0.21
DENV IgM[a]	0.47	**0.02**	0.65	0.16	0.28	0.86	0.14	0.14	0.18	0.94	0.62	0.10	**0.018**	**0.04**	0.47	0.23	0.61	0.05	0.93	**0.03**
DENV IgG[a]	**0.01**	0.44	0.31	0.11	0.50	*	**0.03**	0.53	0.18	0.56	0.92	**0.006**	0.21	0.86	0.06	0.39	0.20	0.25	0.28	0.60
Viral load[b] (PFU/mL)	0.98	0.82	0.97	0.99	0.98	0.61	0.99	0.74	0.98	0.98	0.98	0.99	0.99	0.87	0.67	0.97	0.99	0.99	0.85	0.61

Immune mediators during the acute phase of DENF and MEN infections were identified and compared to positive NS1, IgM, IgG and viral load in (PFU/mL) in DENV infection. Abbreviations: BDNF, brain-derived neurotrophic factor; bNGF, beta-nerve growth factor; EGF, epidermal growth factor; FGF-2, basic fibroblast growth factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; GRO-α, growth-regulated oncogene alpha; HGF, hepatocyte growth factor; IFN-γ, interferon gamma; IL-1α, interleukin 1 alpha; IP-10, interferon gamma-induced protein 10; LIF, leukemia inhibitory factor; MCP, monocyte chemoattractant protein; MIP-1α/β, macrophage inflammatory protein-1alpha/beta; IL-1RA, interleukin1 receptor antagonist, PDGF-BB, platelets-derived growth factor; PIGF, placental growth factor; RANTES, regulated on activation, normal T-expressed, and presumably secreted; SCF, stem cell factor; SDF-1α, stromal cell-derived factor 1α; TNF-α/β, tumor necrosis factor-alpha/beta; VEGF, vascular endothelial growth factor. * Insufficient number of data points. *P* value by ANOVA post hoc test[a] and Spearman Rank correlation test[b].

Immune mediators with a *P* value < 0.05 signify their potential as diagnostic markers during the acute phase of the infection.

Table 4.4.1.2. Correlation analysis between DENV parameters (viral load, NS1, IgG & IgM) during acute DENV infection.

	Viral load (PFU/mL)	IgM	IgG	NS1
Viral load (PFU/mL		-	-	-
NS1	0.06	-	-	-
IgM	0.06	-	-	**0.001**
IgG	0.10	2.63	-	1.53

The correlations between DENV parameters were identified and assessed according to *P* value. The association between the viral load (PFU/mL) and (NS1, IgM & IgG) were tested by Kruskal-Wallis test, the correlations between NS1, IgM, & IgG were tested using Fisher/Chisquare test. The corresponded *P* value identified and reported in the table.

P value < 0.05 indicates significant association.

4.4.2. *N. meningitidis* serotypes (W135, A, B, X) association with differential immune mediators

In my cohort group, there are 4 *N. meningitidis* serotypes identified in acute meningococcal meningitis infection. The immune mediators stimulated in response to the different *N. meningitidis* strains were identified. Analyses of significant differences between the cytokines concentrations produced in response to each serotype during acute meningococcal meningitis infection were assessed and reflected in (Table 4.4.2.1). IL-1α, IL-10 and IL-5 concentrations stimulated in response to W153 *N. meningitidis* strain were significantly different compared to serotype A stimulated concentrations (*P* value < 0.05). Concentration of IL-2 was significantly different after stimulation with W135 strain, compared to serotype A stimulated concentration (*P* value < 0.05). In comparison between serotype A and serotype X, the produced IL-2 concentrations were significantly different (*P* value < 0.05).

Table 4.4.2.1. *P* values for immune-mediators concentrations in correlation with *N. meningitidis* serotypes (W135, A, B, X) during acute meningococcal meningitis infection.

I. mediator	serotype	serotype	*P* val-adj	I.mediator	serotype	serotype	*P* val-adj
BDNF	W135	A	0.88	IL-2	W135	A	**0.02**
BDNF	W135	X	0.88	IL-2	W135	X	0.74
BDNF	A	X	0.88	IL-2	A	X	**0.02**
Eotaxin	W135	B	0.97	IL-21	W135	A	0.05
Eotaxin	W135	A	0.97	IL-22	W135	X	0.08
Eotaxin	W135	X	0.97	IL-23	W135	A	**0.01**
Eotaxin	B	A	0.97	IL-27	W135	A	0.77
Eotaxin	B	X	0.97	IL-27	W135	X	0.77
Eotaxin	A	X	0.97	IL-27	A	X	0.77
FGF-2	W135	A	0.93	IL-4	W135	A	0.48
GRO-α	W135	A	0.50	IL-5	W135	A	**0.04**
HGF	W135	B	0.86	IL-6	W135	B	0.92
HGF	W135	A	0.86	IL-6	W135	A	0.92
HGF	W135	X	0.86	IL-6	W135	X	0.92
HGF	B	A	0.86	IL-6	B	A	0.92
HGF	B	X	0.86	IL-6	A	X	0.92
HGF	A	X	0.86	IL-6	A	X	0.93
IFN-γ	W135	B	0.52	IL-7	A	W135	0.25
IFN-γ	W135	A	0.91	IL-7	A	X	0.34
IFN-γ	B	A	0.52	IL-7	W135	X	0.27
IL-1α	W135	A	**0.02**	IL-8	W135	A	0.72
IL-1β	W135	B	0.85	IL-8	W135	X	0.72
IL-1β	W135	A	0.48	IL-8	A	X	0.77
IL-1β	B	A	0.84	IP-10	W135	B	0.26
IL-1RA	W135	B	0.84	IP-10	W135	A	0.79
IL-1RA	W135	A	0.84	IP-10	W135	X	0.79
IL-1RA	W135	X	0.84	IP-10	B	A	0.79
IL-1RA	B	A	0.84	IP-10	B	X	0.99
IL-1RA	B	X	0.84	IP-10	A	X	0.79
IL-1RA	A	X	0.84	LIF	W135	A	0.09
IL-10	W135	A	**0.02**	LIF	W135	X	0.88
IL-12p70	W135	A	0.88	LIF	A	X	0.66
IL-13	W135	A	0.66	MCP-1	A	x	0.95
IL-15	W135	A	0.61	MCP-1	W135	B	0.95
IL-18	W135	A	0.94	MCP-1	W135	A	0.95
MIP-1α	W135	B	0.99	MCP-1	W135	x	0.95
MIP-1α	W135	A	0.63	MCP-1	B	A	0.95
MIP-1α	W135	x	0.06	MCP-1	B	x	0.95
MIP-1α	B	A	0.99	PDGF-BB	W135	A	0.37
MIP-1α	B	x	0.99	PIGF-1	W135	A	0.06
MIP-1α	A	x	0.99	RANTES	W135	B	0.98
MIP-1β	W135	B	0.45	RANRES	W135	A	0.98
MIP-1β	W135	A	0.93	RANTES	W135	x	0.98
MIP-1β	B	A	0.45	RANTES	B	A	0.98
SCF	W135	A	0.57	RANTES	B	x	0.98
SDF-1α	W135	B	0.87	RANTES	A	x	0.98
SDF-1α	W135	A	0.87	VEGF-B	W135	A	0.68
SDF-1α	W135	x	0.87	VEGF-D	W135	A	0.82
SDF-1α	B	A	0.87	bNGF	W135	A	0.93
SDF-1α	B	x	0.87	bNGF	W135	X	0.93
SDF-1α	A	x	0.87	bNGF	A	X	0.93

The *P* values for immune-mediators concentrations in comparison to *N. meningitidis* serotypes were identified during acute meningococcal meningitis infection. EGF. GM-CSF, IL-17A, IL-31, IL-9, and TNF-β were not included due to insufficient data. Abbreviations: BDNF, brain-derived neurotrophic factor; bNGF, beta-nerve growth factor; EGF, epidermal growth factor; FGF-2, basic fibroblast growth factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; GRO-α, growth-regulated oncogene alpha; HGF, hepatocyte growth factor; IFN-γ, interferon gamma; IL-1α, interleukin 1 alpha; IP-10, interferon gamma-induced protein 10; LIF, leukemia inhibitory factor; MCP, monocyte chemoattractant protein; MIP-1α/β, macrophage inflammatory protein-1alpha/beta; IL-1RA, interleukin1 receptor antagonist, PDGF-BB, platelets-derived growth factor; PIGF, placental growth factor; RANTES, regulated on activation, normal T-expressed, and presumably secreted; SCF, stem cell factor; SDF-1α, stromal cell-derived factor 1α; TNF-α/β, tumor necrosis factor-alpha/beta; VEGF, vascular endothelial growth factor; I. mediator, immune mediator. *P* value by ANOVA post hoc test.

P value < 0.05 indicates significant association

4.4.3. Plasmodium species, parasitemia level and positive malaria thick blood film association with differential immune mediators

The immune mediators concentrations in response to positive thick blood film samples during acute malaria infection were analyzed for significant correlations, are shown in (Table 4.4.3.1). Only BDNF was significantly associated with positive thick blood film (P value < 0.05). In comparison between the immune mediators concentrations stimulated in response to Plasmodium falciparum versus other unknown plasmodium species during acute malaria infection, only BDNF and IL-15 showed significant differences in stimulated concentrations (*P* value < 0.05) (Table 4.4.3.1). IL-17A and IL-4 were significantly correlated with parasitemia level 1 and 2, respectively in comparison to parasitemia level zero (*P* vale < 0.05 for IL-17A; *P* < 0.01 for IL-14) during acute malaria infection, results are reflected in (Table 4.4.3.1).

Table 4.4.3.1. Immune mediators correlation in compare to (parasitemia levels, plasmodium species and malaria thick blood film) and their corresponded *P* values during acute phase of malaria infection.

I. med	Para. level		*P* val-adj	MAL *spp*	Thick BF	I. med	Para. level		*P* val-adj	MAL *spp*	Thick BF
				P value-adj						P value-adj	
BDNF	2	0	0.33	0.003	0.03	**IL-22**	0	1	0.64	0.80	0.80
BDNF	2	1	0.79			**IL-22**	0	2	0.64		
BDNF	0	1	0.33			**IL-22**	1	2	0.64		
EGF	0	1	0.31	0.16	0.16	**IL-23**	0	1	0.10	0.16	0.16
Eotaxin	2	0	0.46	0.38	0.38	**IL-27**	0	1	0.78	0.99	0.99
Eotaxin	2	1	0.46			**IL-27**	0	2	0.40		
Eotaxin	0	1	0.94			**IL-27**	1	2	0.40		
FGF-2	2	1	0.57	0.06	0.06	**IL-31**	0	1	0.40	0.09	0.09
FGF-2	2	0	0.73			**IL-31**	0	2	0.40		
FGF-2	0	1	0.57			**IL-31**	1	2	0.47		
GM-CSF	0	1	0.88	0.95	0.95	**IL-4**	2	0	0.009	0.06	0.06
GRO-α	2	1	0.34	0.13	0.13	**IL-4**	2	1	0.16		
GRO-α	2	0	0.78			**IL-4**	0	1	0.16		
GRO-α	0	1	0.34			**IL-5**	0	1	0.36	0.36	0.36
HGF	2	1	0.91	0.59	0.59	**IL-6**	2	0	0.60	0.68	0.68
HGF	2	0	0.10			**IL-6**	2	1	0.33		
HGF	0	1	0.10			**IL-6**	0	1	0.33		
IFN-γ	2	0	0.86	0.87	0.78	**IL-7**	2	0	0.46	0.28	0.28
IFN-γ	2	1	0.86			**IL-7**	2	1	0.46		
IFN-γ	0	1	0.86			**IL-7**	0	1	0.46		
IL-1 α	2	0	0.35	0.28	0.28	**IL-8**	2	0	0.43	0.74	0.74
IL-1 α	2	1	0.53			**IL-8**	2	1	0.27		
IL-1α	0	1	0.35			**IL-8**	0	1	0.43		
IL-1 β	2	0	0.95	0.74	0.74	**IL-9**	*	*	*	*	*
IL-1β	2	1	0.95			**IP-10**	2	0	0.86	0.69	0.69
IL-1 β	0	1	0.95			**IP-10**	2	1	0.78		
IL-1 RA	2	0	0.26	0.76	0.76	**IP-10**	0	1	0.78		
IL-1 RA	2	1	0.26			**LIF**	2	0	0.18	0.17	0.17
IL-1 RA	0	1	0.49			**LIF**	2	1	0.22		
IL-10	2	0	0.91	0.40	0.40	**LIF**	0	1	0.47		
IL-10	2	1	0.58			**MCP-1**	2	0	0.66	0.73	0.73
IL-10	0	1	0.58			**MCP-1**	2	1	0.66		
IL-12p70	2	0	0.24	0.27	0.27	**MCP-1**	0	1	0.70		
IL-12p70	2	1	0.35			**MIP-1 α**	2	0	0.27	0.82	0.82
IL-12p70	0	1	0.24			**MIP-1 α**	2	1	0.15		
IL-13	2	0	0.32	0.07	0.07	**MIP-1 α**	0	1	0.27		
IL-13	2	1	0.40			**MIP-1 β**	2	0	0.20	0.61	0.61
IL-13	0	1	0.32			**MIP-1 β**	2	1	0.08		
IL-15	0	1	0.24	0.01	0.10	**MIP-1 β**	0	1	0.20		
IL-15	0	2	0.25			**PDGF-BB**	2	0	0.36	0.07	0.77
IL-15	1	2	0.48			**PDGF-BB**	2	1	0.38		
IL-17A	0	1	0.04	0.06	0.06	**PDGF-BB**	0	1	0.38		
IL-18	2	0	0.97	0.34	0.34	**PIGF-1**	2	0	0.32	0.22	0.22
IL-18	2	1	0.65			**PIGF-1**	2	1	0.71		
IL-18	0	1	0.65			**PIGF-1**	0	1	0.32		
IL-2	0	1	0.42	0.31	0.31	**RANTES**	2	0	0.54	0.73	0.73
IL-2	0	2	0.83			**RANTES**	2	1	0.54		
IL-2	1	2	0.28			**RANTES**	0	1	0.73		
IL-21	2	0	0.36	0.05	0.05	**SCF**	2	0	0.46	0.91	0.91
IL-21	2	1	0.36			**SCF**	2	1	0.46		
IL-21	0	1	0.27			**SCF**	0	1	0.46		
SDF-1 α	2	0	0.28	0.29	0.29	**VEGF-B**	2	0	0.22	0.22	0.22
SDF-1 α	2	1	0.71			**VEGF-B**	2	1	0.43		
SDF-1 α	0	1	0.28			**VEGF-B**	0	1	0.14		
TNF-α	2	0	0.77	0.65	0.65	**VEGF-D**	2	0	0.74	0.97	0.97
TNF-α	2	1	0.77			**VEGF-D**	2	1	0.74		
TNF-α	0	1	0.77			**VEGF-D**	0	1	0.74		
TNF-β	0	2	0.46	0.32	0.32	**bNGF**	0	1	0.28	0.25	0.25
TNF-β	0	1	0.60			**bNGF**	0	2	0.28		
TNFβ	2	1	0.60			**bNGF**	1	2	0.52		

The Immune mediators during the acute phase of malaria infection were identified and compared against the parasitemia level, malaria species type (P. falciparum or unknown species), and thick blood film. Abbreviations: BDNF, brain-derived neurotrophic factor; bNGF, beta-nerve growth factor; EGF, epidermal growth factor; FGF-2, basic fibroblast growth factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; GRO-α, growth-regulated oncogene alpha; HGF, hepatocyte growth factor; IFN-γ, interferon gamma; IL-1α, interleukin 1 alpha; IP-10, interferon gamma-induced protein 10; LIF, leukemia inhibitory factor; MCP, monocyte chemoattractant protein; MIP-1α/β, macrophage inflammatory protein-1alpha/beta; IL-1RA, interleukin1 receptor antagonist, PDGF-BB, platelets-derived growth factor; PIGF, placental growth factor; RANTES, regulated on activation, normal T-expressed, and presumably secreted; SCF, stem cell factor; SDF-1α, stromal cell-derived factor 1α; TNF-α/β, tumor necrosis factor-alpha/beta; VEGF, vascular endothelial growth factor; I.media, immune mediator; Paras. level, parasitemia level; BF, blood film; MAL *spp,* malaria species; *P* val.adj, *P* value-adj, * Insufficient number of data points. *P* value by ANOVA post hoc test.

Immune mediators with *P* value < 0.05 signify their potential as diagnostic markers.

Chapter 5. Discussion

Preface

The re-emergence of infectious diseases in the Arabian Peninsula has increased steadily in the last few years. However, there remains limited specific data about the extent of endemicity, geographic distribution, patterns, and pathogenesis. In my project, I focussed on eight key infectious diseases that are considered more significant at the start of my study. Among these eight infectious diseases, four are vector borne. Over the last 4 years, incidence reports and demographic data were collected and analysed using multi-modal approaches that included routine labotory tests, specific confirmatory investigations, and multiplex bead-arrays to profile immune signatures.

The clinical manifestations represented by each disease will be discussed and correlated with other studies reported from different regions.

5.1. Older age group, male gender, skin rash, joints pain, elevated liver enzymes, MIP-1α and MCP-1 as predictive features of DENV infection

My results in (Chapter 4.1.1 and 4.2.1) indicates that the common age group of presentation was ≥ 45 years, age specific risk of symptomatic disease in primary and secondary DENV infection revealed that advanced ages of adolescents are more likely to develop symptomatic infection than in younger children with the risk of apparent complications reported commonly in younger ages. Apparent symptomatic infection in older ages (≥ 45 years) could be related to the presence of other co-morbidities resulted in complicated clinical course of DENV infection that requires medical care support which may also be due to decreased host's capacity against infections. Other factors involved in age specific susceptibility to infection were DENV strains evolution and serotype specific virulence (Guzman et al., 2002).

Molecular epidemiological studies identified that long-term expansion of DENV epidemics are associated with selection-driven adaptive evolution of DENV strains (Steel et al., 2010). In many southeast Asian countries, demographic transition occurred, increase age was associated with increased risk of DENV infection, as increase age was associated with decrease force of infection that lead to an evident old age symptomatic people (Cummings et al., 2009; Acestor et al., 2012).

In my cohort group, DENV clinical parameters were more predominant with almost all patients reported with skin rash, joints pain, and chills acutely during clinical assessment. Skin rash was not exclusive to acute DENV infection (Alera et al., 2016). DENV infection predictive clinical parameters in all age groups are headache and eye pain (WHO, 2009).

Analyzed biochemical tests in DENV positive patients revealed low lymphocytes and neutrophils percentages, PLT count, and Hb levels. Elevated liver enzymes, high CRP and ESR levels were significantley increased. Many studies reported the same blood parameters changes due to direct virus effect (Zhao-fan et al., 2017). Detected Hb levels after day 6 of DENV infection were lower in secondary infections than in primary ones (Jih-Jin et al., 2017). The immune profile of DENV infection, characterized by increased production of TNF-α, IL-8, and IFN-γ, which mediate the pathogenesis of the disease (Vaughn et al., 2000). The cytokines produced by cultured DENV infected human Dendritic Cells (DC) showed elevated level of IFN-α and TNF-α, absence of significant IL-6 and IL-12 production (Ling-Jun et al., 2001),

these are different from what is detected in my cohort group in which, elevated IFN-γ, IL-6, IL-18, IL-10, IL-1RA, MIP-1α, MCP-1 and IP-10 levels, were reported (Figure 4.3.1.1, 4.3.1.2, 4.3.1.3, and 4.3.1.4). IFN-γ and TNF-α associated with disease severity, TNF-α linked to increase vascular permeability and development of hemorrhagic fever (Junyuan et al., 2018). IL-10 was mentioned in many studies as a predictor of severity in DENV infection and associated with plasma leakage, thrombocytopenia, and elevated liver enzymes (Yeh et al., 2006; Nguyen et al., 2004). IL-10 was more predominantly involved in secondary DENV infection and a distinctive biomarker of death in DHF/DSS (Tang et al., 2010; Chen et al., 2006). MCP-1 was produced in high quantities in response to DENV serotype 2. MCP-1 was produced more in DHF and is associated with humans vascular endothelial cells increased permeability, it was found to be a predictor of poor outcome in DENV infection (Ying-Ray et al., 2006; Rathakrishnan et al., 2012). IP-10 was measured in high quantities in DENV patients with warning signs (Kiran et al., 2018). Inflammatory cytokines identification are important to understand the pathogenesis and future vaccines synthesis.

CHIKV infection reported in this study was demonstrated in two age groups: 15-44 years old and ≥ 45 years old, which agreed with other studies. Clinical parameters of CHIKV infection are markedly overwhelming in this studied cohort group compared to other studies (Fischer and Staples, 2014). All patients were presented with skin rash, joints pain, headache, and chills (Figure 4.2.1.1). Laboratory investigations were found to be approximately within normal levels. PLT count, AST, and ESR levels were not significantly elevated in comparison to the control cases levels. Hb levels, lymphocytes, and neutrophils percentages were significantly lower than the control levels, while the CRP levels were significantly elevated. Observations noticed by other studies reported neutrophils, lymphocytes, and PLT count below the normal levels (Megan et al., 2013).

Interestingly 15 cases of DENV+CHIKV co-infection were detected in this research project with a predominance of male gender of ≥ 45 years old (Table 3.2.3.1). In a surveillance carried on Puerto Rico in which around1000 (916 specimens) were tested for DENV and CHIKV infection by RT-PCR, only one case of DENV+CHIKV co-infection was reported (Sharp et al., 2016). Other studies conducted in Southern Sri Lanka to identify CHIKV infection as a cause of acute

febrile illness, detected CHIKV infection in 3.5%, with no single case of DENV+CHIKV co-infection (Megan et al., 2013, Hapuarachchi et al., 2008).

Thus, it is possible that a new *Ae. spp* could be responsible of the DENV and CHIKV re-emergence in Jizan province, since studies have indicated that new *Ae aegypti* strains could be infected by three arboviruses at the same time (Nuckols et al., 2015). *Ae. aegypti* was the primary vector responsible of DENV+CHIKV co-infection in South-East Asian regions, while in Africa *Ae. Albopictus* was found to be the main vector of transmission. Therefore, co-existence of two *Ae. spp* is very likely as Africa is proximal to Saudi Arabia (Caron et al., 2012).

It was observed that the clinical manifestations of the DENV+CHIKV co-infection were more severe and aggressive than DENV and CHIKV infections separately, it was clearly observed in this study (Figure 4.2.1.1). The DENV+CHIKV co-infection are associated with severe clinical course and poor prognosis. According to other studies mortality and morbidity rates are increased in co-infections compared to those in mono-infections (Bhooshan et al., 2015; Omarjee et al., 2014). Severe clinical manifestations noted in DENV+CHIKV co-infection were most likely related to the pathogenesis mediated by the two arboviruses and the host immune response to it is existence, further studies needed to elaborate more on this.

There is a link between poverty and these Neglected Tropical Diseases (NTD) noticed by many studies. Low socioeconomic status is a major risk factor for DENV+CHIKV co-infection, poor neglected and illegal populations living in bad conditions with poor sanitation, inappropriate garbage collection, interrupted water, electricity supply and still water are also known as contributing factors (Oviedo et al., 2017). Jizan province is a developing area and part of Arab world facing water pollution and scarcity problems, population growth and expansion, immigration, rural urban migration and sanitary problems (Al-Gabbani, 2008). Moreover, Makkah, northwest to Jizan, contains the largest expatriate population living in low-level housing conditions.

The biochemical parameters associated with DENV+CHIKV co-infection are more deranged than in DENV and CHIKV infections separately. Detected PLT count and low Hb levels were the lowest in DENV+CHIKV co-infection than in mono-infection with DENV and CHIKV infections, separately. The liver enzymes were more elevated in DENV+CHIKV co-infection similar to reported results from study in India (Londhey et al., 2016). CRP levels were elevated, neutrophils and lymphocytes percentages

were significantly reduced in DENV+CHIKV co-infection more than in DENV and CHIKV infections. In another research project, leukopenia and lymphopenia were noticed more in DENV infections (D. Nkoghe et al. 2012).

5.2. Older age group, male gender, fever and low Eotaxin, GRO-α, PlGF-1, RANTES, and SDF-1α as predictive features of bacterial infections

The four types of bacterial infections studied in this project were, GAS infections, meningococcal meningitis, brucellosis and leptospirosis. In GAS skin-infected patients, the common age group was found to be mostly ≥ 45 years old males (Table 4.1.1). The median age in my cohort is younger when compared to another study where GAS skin infections were detected in 981 patients, with their median age being 61 years old with a male gender predominance (Anna et al., 2015; Stevens et al., 2014). It was found in many other studies that GAS skin infections are generally more common in older patients, due to increase bacterial virulence and age related reduction of host immunity, it was also noted that higher recurrence rate is significantly associated with pre-existing co-morbidities (Pavlotsky et al., 2004).

Invasive GAS infections are more common in long-term care facilities, as most patients are elderly and suffering from multiple co-morbidities like cardiovascular diseases, diabetes and other underling skin disease such as skin infections caused by *Staphylococcus aureus*. Other factors such as crowding in some nursing homes and presence of GAS infected residents were also considered to be factors affecting this, which lead to an outbreaks and transmission of infections (CDC, 2002), this supports our results of GAS skin infections detection in older ages.

Notably, my results also indicated that GAS skin infections were more common in the summer months, similar to earlier reports where GAS infections peaked during May and June (Yaunhai et al., 2018; Stefaan et al., 2007).

The immune profile produced by GAS infected primary bone marrow derived macrophages were IL-6 and TNF-α. The higher level of IL-6 and TNF-α produced by *emm1* type GAS isolates than any other type. The *emm1* isolate type affect the inflammatory cytokines production more than the invasiveness character of the causative GAS pathogen (Marcia et al., 2014). Released pro-inflammtory cytokines by activated macrophages in innate response to GAS infection involve TNF-α, IL-1β and IL-6 (Rojas et al., 2010). GAS infection induce expression of the negative regulatory protein A20, which in turn reduce the inflammatory mediators production and lead to pathogenic spread of the organism by escaping the immune system (Cuiqing et al., 2018). In this research project, IL-RA, IL-6 and IL-18 was detected in high level in GAS positively diagnosed patients, while IL-7, RANTES, SDF-1α, GRO-

α, and Eotaxin were significantly low in comparison to the control cases levels. This is in contrast to what reported by other studies in which RANTES was constantly detected in high level in patients presented with sepsis, community acquired pneumonia and skin abscess. The same reports are in agreement that RANTES was involved in bacterial infections pathogensis (Cavalcanti et al., 2016). GAS infection resulted in lower chemokines and cytokines production (MCP-1, RANTES, IL-1, TNF-α, and IL-6) than in other bacterial infections such as *E. coli* and *S. aureus* infections, which resulted in bacterial growth and dissemination in the affected patients (Shuhui et al., 2016). Although IFN-γ has a protective role against invasive GAS infections, however it did not show any significance in my studied cohort group (Takayuki et al., 2012).

The mean age of presentation of meningococcal meningitis diagnosed cases in my cohort group of patients was 29.1 with male gender predominance. In Hamad General Hospital, it was found to be 26.4 with also male predominance (Khan et al., 2017). The most common causative pathogens of acute bacterial meningitis are; *Neisseria meningitidis*, *Streptococcus pneumonia* and *Haemophilus influenzae* (Theodoridou et al., 2007). *N. meningitidis* was the isolated causative organism in my study in agreement with a studies conducted in Ethiopia and Greece (Wude et al., 2016; Maria et al., 2009). Almost all patients complained of headache and nausea with small percentage presented with skin rash (Figure 4.2.2.1), other studies showed similar clinical observations (Van de beek et al., 2004).

Meningococcal meningitis detection rate peaks in children below one year and in young adults. During Hajj and Umrah, Muslims enters Saudi Arabia from all over the world in large numbers. Pilgrims overcrowding, hot climate, physical exhaustion and carriers from meningococcal endemic areas, such as those from the African belt, facilitate the disease transmission and international outbreaks occurrence. Serogroup A and W135 are the commonly reported in Saudi Arabia (Saber et al., 2016; Ziad, 2002). Biochemical parameters analyzed showed elevated lymphocytes, neutrophils percentages, and CRP levels (Figure 4.2.2.2). Neither CRP level, nor neutrophils count had a high diagnostic value, as CRP level more than 99 mg/L was of low specificity but high sensitivity to meningococcal disease and abnormal neutrophils counts were noticed in 38% of children in the absence of meningococcal disease (NICE Clinical Guidelines, 2010). CSF culture for identification of meningococcal meningitis causative organism remains the gold standard test for

diagnosis (Brouwer et al., 2010). In this research project ESR, PLT count and AST levels were significantly elevated in meningeococcal meningitis infection. In a study conducted in Spain to compare bacterial meningitis characterstics in cirrhotic and non-cirrhotic patients. The non-cirrhotic cases showed thrombocytopenia percentages of 13.8% and 33.3% were reported in cirrhotic patients. Liver functions impairment were reported in 12.4% of non-cirrhotic cases (Cabellos et al. 2008). Thrombopoietin (TPO) is the main regulater of PLT proliferation and maturation (Kaushansky, 1995). A study reported elevated TPO level during early fulminant meningeococcal septicaemia with low PLT count instead, while in mild to moderate meningococcemia the PLT count remains within normal level and TPO was not detected after 48 hours (Anna et al., 2000).

Identification of meningococcal meningitis immune profile is important in clinical diagnosis, assessment of disease severity, and long-term complications. The detected biomarkers in the serum of positively diagnosed patients were mentioned in (Chapter 4.3.2). Most of other researches analyzed the immune profiles of acute menigococcal meningitis in the CSF, in this project biomarkers detected in serum samples. Analyzed immune profiles detected in the CSF of meningococcal meningitis patients revealed highly elevated MCP-1, MIP-1α, and IL-8 in fulminant meningococcal septicemia, beside it is correlation with plasma libopolysaccrides LPS level, RANTES was significantly low in fulminant meningococcal septicaemia while it is elevated in mild to moderate meningococcal meningitis (Anne-Sophie et al., 2005; Bozza et al., 2007). These results are similar to what was detected in my patients cohort group, as RANTES was significantly low in comparison to the control cases levels, instead there is no detected meningococcal meningitis fulminant case. IL- 6 from CSF samples and IL-12p70 from serum samples correlated significantly with disease severity and long-term complications (Federico et al., 2015). IL-12p70 did not show a significant correlation in my cohort group. However, IL-6 was expressed in high quantities from patients serum collected during the acute phase of the disease.

In my research project, PDGF-BB, PIGF-1, EGF, and BDNF were significantly low compared to the control levels. Other studies showed CSF elevation of VEGF, HGF, and murine model bFGF in bacterial meningitis which did not come in agreement with my findings. In which, increased VEGF was reported in 70% of bacterial meningitis patients. VEGF is highly indicated to be involved in endothelial damage,

cerebral edema, and blood brain barrier (BBB) functional disbalance (Flier et al., 2001). FGF was found to be elevated in severe acute bacterial meningitis and associated with brain tissue distruction, poor outcome as well as neurological complications (Huang et al., 1999). HGF was reported in acute bacterial meningitis in which, neuroprotective role was observed (Nayeri et al., 2000).

In this research project, brucellosis infection was more prominent in adult males (89%). Male predominance can be explained by Saudi Arabia cultural behaviours, which involve tenting in countrysides, milking goats, camels and sheep with bare hands, milk intake without boiling, making of yoghurt on a cylindrical shape containers made of animal's leather following animals slaughtering (Aloufi et al., 2016). Close man-animal relationship is perhaps by far the most logical explanation of Brucellosis male gender predominance.

Fever, joints pain, back pain, and headache were reported as complains in all brucellosis diagnosed patients (Figure 4.2.2.1). A systematic review in 2385 published articles from 1990 to 2010 in which the severity of brucellosis clinical manifestations were assessed, fever was reported in 73%, Joints pain was reported in only 13% (Anna et al., 2012). In other recent study, joints pain manifested by 50% of patients (Jingbo and Xiaotao, 2017).

Musculoskeletal complications of brucellosis are more common in endemic areas, peripheral arthritis, sacroilitis, spondylitis, and osteomyelitis were also reported. Musculoskeletal complications of brucellosis are disabling and sometimes prompt hospital admission. The commonly involved joints are the knees, hips and sacroiliac joints (Bosilkovski et al., 2016). Joints pain, back pain, and fever are brucellosis distinctive clinical manifestations as predominantly mentioned in many studies. Laboratory invstigations conducted showed significantly elevated liver enzymes (Figure 4.2.2.2), acute hepatitis was reported in 3 - 6% of brucellosis positive cases (Humberto et al., 2015). Hb levels and PLT count were significantly below the control cases level, while the ESR and CRP levels were elevated. Many studies reported thrombocytopenia, anemia, and leukopenia in response to brucelosis (Akbayram et al., 2011)). Elevated ESR and CRP levels were reported in (38 - 87%) and (34 - 81%) of patients, respectively (Tanir et al., 2009; Afsharpaiman and Mamishi, 2008).

Leptospirosis infections were only detected in two cases in this research project. The prevalence is high during summer and rainy seasons (Muhammad et al.,

2013; Alton et al., 2009; Ward, 2002). In our findings, the two patients were adult males and reported in July.

One of the possible reasons to explain the low number of burcellosis and leptospirosis detection rate in Saudi Arabia is the excessive use of antibotics without doctor's prescription. Leftover antibiotics from others, patients discontinuing their own treatment, the use of antibiotics directly from the pharmacy without medical prescribtion, direct antibiotics release from the healthcare system by health care personnels. These behaviours are very common in Saudi Arabia (Sharafaldeen et al., 2017). With 2 positively diagnosed samples of leptospirosis, it is therefore very difficult to assess the clinical manifestations frequencies. The common presenting symptoms were fever, headache, nausea, vomiting, and joints pain. Other researchers reported that skin rash was not a common feature in leptospirosis, only noticed in 1 - 3% of cases (Dierks et al., 2018; Padmakumar et al., 2016; Gochenourws et al., 1952).

The wide difference in clinical presentation frequencies could be due to proportion of biased informations during recalling, mis-diagnosis or related to the virulence factors of the detected pathogens. Reported laboratory parameters from only 2 cases showed only significantly elevated liver enzymes (Figure 4.2.2.2), thrombocytopenia and lymphopenia were mentioned in other studies (Nipun et al., 2014; Loic et al., 2016). The small number of leptospirosis patients in my cohort group of patients were not statistically significant.

Leptospirosis associated with low Hb level in other study found to be due to the direct toxic effect of *leptospires*. Anaemia and neutrophil leukocytosis can differentiate uncomplicated leptospirosis from sever one (Craig et al., 2009). There is a significant association between the highest reported neutrophils count and maximally reached creatinine level during illness, which suggest renal function impairment (Loic et al., 2016).

5.3. Thrombocytopenia, elevated liver enzymes and IFN-γ during summer season as predictive features of malaria in non-sickle cell disease patients

In a study conducted in India, most of the patients fall in the age group of 21-40 years old (Jagjit et al., 2013), in agreement to what was noticed in my cohort group (Table 4.1.1). The highest detection rate was in summer months from July to September. The seasonal pattern of malaria detection reached the peak from April to September (Figure 4.1.3.1). Fever was reported in 93.6% and vomiting was a complaint by 36.8% of patients (Francis et al., 2016). Entirely all of the recruited patients in this project were *P. falciparum* positive cases, presented with fever, chills, headache, and nausea (Figure 4.2.3.1). The clinical manifestations of malaria in Saudi Arabia were associated with good outcome and good prognosis, as there was no reported deaths during the study period. The low number of detected malaria cases cauld be explained by the decline in reporting due to the good control efforts in countries nearby to the Kingdom of Saudi Arabia such as Yemen, and other African countries.

The co-existence of malaria species cauld be the reason behind the less severe form of clinical presentations seen in this research study. Living in an endemic area with malaria and repeated malaria infections, results in gradual development of clinical protection against severe malaria. On travelling to non-endemic areas, immunity protection decreased and the risk of developing severe malaria in returning back to malarious areas becomes very high. The duration of protective immunity persistence in individuals departed from an endemic area was not defined (Farnert et al., 2015). Other factors responsible of malaria severity were heamoglobinopathies like sickle cell trait and α-thalassaemia, which provide protection against severe malaria (Phillips et al., 2009; Mackinnon et al., 2005).

Some parts of Saudi Arabia, e.g the eastern part, are known areas of Sickle Cell Disease (SCD) patients which is a common inherited genetic disease among Africans populations (Rana, 2016).

Malaria laboratory parameters showed Hb level of 11.9 - 14.1g/dL and the median PLT count was 90×10^9 /L (Francis et al., 2016), in this research project the Hb level dropped to below 10 g/dL in some cases and PLT count range between 100 – 250×10^9 /L in most of the patients. Elevated liver enzymes, CRP levels, ESR levels, and lymphocytes percentages were significantly associated with malaria infection (Figure 4.2.3.2). There was no detected significant association between

malaria and neutrophils percentages. In another study, neutrophils and monocytes counts were significantly increased in malaria positive patients in comparison to non-malaria cases, while PLT count, WBCs, lymphocytes, eosinophils, RBCs, and Hb levels were decreased (Van Wolfswinkel et al., 2013).

Many researches studied the association between anemia and malaria infection especially in children (Clarke et al., 2008). Host genotype was implicated as a dependent cause of anemia in children during malaria seasons (Atkinson et al., 2008). There was also a significant association between peripheral parasitemia and anemia in primigravidas and multigravidas women in which the severity of parasitemia is directly proportional to the degree of anemia. The number of pregnancies showed no significant correlation (Agan et al., 2010).

PLT count is a sensitive marker for malaria diagnosis in acute febrile illness. Malaria parasite mediated pathological changes include splenomegaly, coagulation disturbances, antibody-mediated PLT destruction, and bone marrow alterations (Faseela et al., 2011). In 100 malaria positive patients, PLT count were assessed and thrombocytopenia was significantly detected in correlation with non-malaria (control group) (Lal et al., 2016).

The hepatic stage of the parasite life cycle, in which sporozoites causes liver congestion, sinusoidal block, and hepatocytes pathological changes has led to the release of liver transaminases in the circulation (Onyesom, 2012). Elevated liver transaminases detected in small number of our patients (Figure 4.2.3.2). In a study conducted in Hajjah, Northwest Yemen significant increase in the liver enzymatic activities of ALT, AST, and ALP were reported (Mohamed Al-Salahy et al., 2016).

The detected immune profile of malaria infection by *P. vivax* and *P. falciparum* species acutely showed elevated IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-17, IFN-γ, TNF-α, MIP-1, and G-CSF. The convalescent phase showed significant increase of the same cytokines in comparison to the acute state except for IL-10 which was detected in the acute phase of disease only, *P. falciparum* infection specifically associated with IL-6, IL-8, IL-17, IFN-α, MIP-1, and G-CSF (Rodrigo et al., 2014). In correlation with my research project IL-6 and IFN-γ were highly elevated, TNF-α was also detected in lower level than the previously mentioned cytokines (Figure 4.3.3.1).

TNF-α was a recognized biomarker of severe malaria infection as identified by many studies. TNF-α production was associated with the development of severe

malaria complications like; cerebral malaria, severe anemia, and acute respiratory distress syndrome (Clark, 2009).

Interestingly, Rodrigo Nunes detected IL-10 during acute malaria infection and noticed it was back to normal level in the convalescent period, in this cohort group IL-10 was also detected in high level among malaria positive cases. IL-10 produced by Th2 cells and monocytes involved in regulating the production of other cytokines and changes the immune response towards Th1 and macrophages (Ho et al., 1995). IL-10 reported to be associated with hyperparasitemia, jaundice, and shock as well as suppression of immune-protection against malaria infection (Angulo and Fresno, 2002; Zhang et al., 2012). IL-1RA and IP-10 were significantly elevated in my cohort group, while EGF, PDGF-BB, and BDNF were decreased compared to the control cases levels. In relation to other studies, elevated IL-1RA and IP-10 levels, decreased PDGF-BB levels were detected from CSF samples of malaria infected childrens. Each one of these cytokines found to be a poor prognostic factor associated with increased mortality in cerebral malaria (Henry et al., 2007).

The differences in biomarkers production in response to malaria infection in endemic areas from non-endemic areas revealed that IFN-γ, IL-10, IL-6, and IL-4 were prognostic factors for malaria infection endemicity in a particular regoin (swapnil et al., 2010).

Release of IFN-γ is associated with malaria infection in endemic areas, which activate the innate immune response. Re-infection with malaria results in optimal production of IFN-γ by memory T-cells which along with other innate immune responses and cellular defense mechanisms can overcome the acute infection effectively with less severe symptoms (D’ Ombrain et al., 2008). These observations supports the good prognosis and the lesser severity in clinical presentations in this research project.

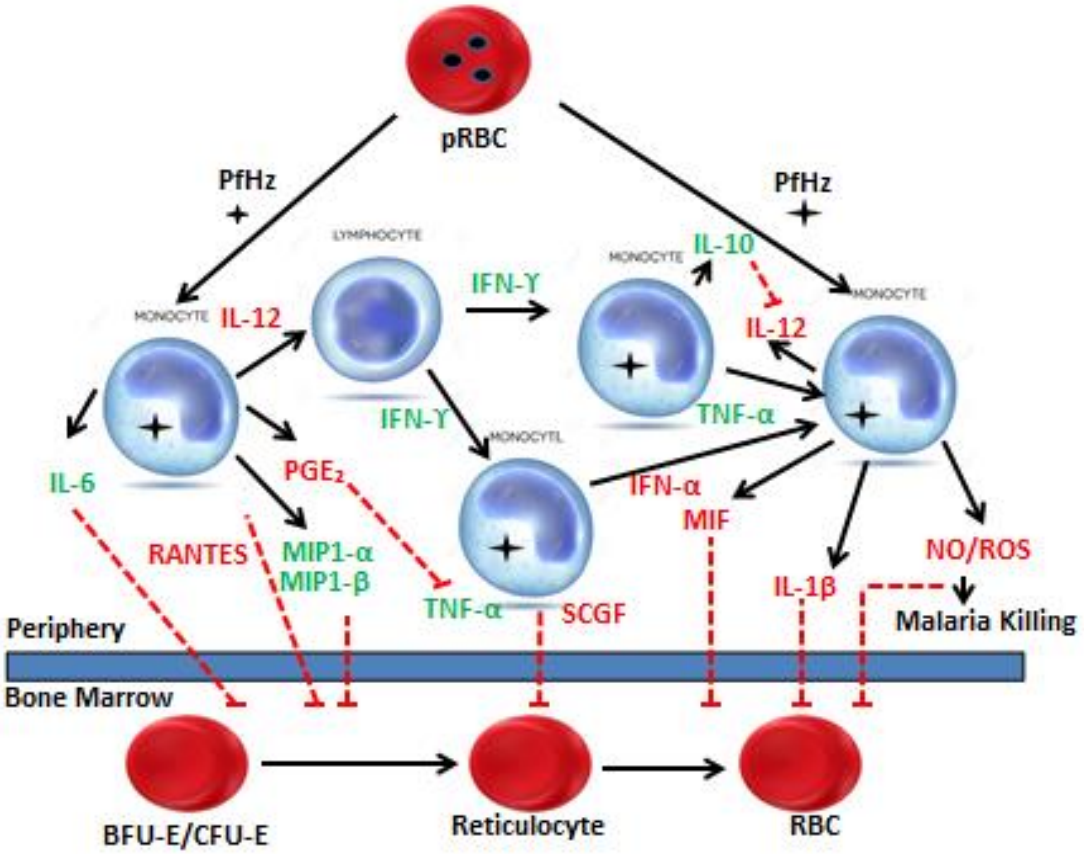

Figure 5.3.1. A reproduced schematic diagram illustrating the innate immune responses in severe malarial anemia (SMA). Based on concomitant measurement of innate inflammatory mediators (using multiplex technologies) it describe how dysregulation in innate inflammatory mediators promotes suppression of erythropoiesis. Central to the model is the fact that phagocytosis of hemozoin (*Pf*Hz) by monocytes is one of the primary causes of altered production of innate inflammatory mediators. Elevated inflammatory mediators are shown in green text, while those that are decreased in children with SMA are shown in red text. Solid lines indicate positive signaling (up-regulation), whereas dashed lines indicate suppression (down-regulation). Children with SMA have decreased levels of IL-12 in response to ingestion of parasitized red blood cells (pRBC) and/or hemozoin by monocytes. Suppression of IL-12 in children with SMA is due to *Pf*Hz-induced IL-10 over-production. Children with SMA have increased circulating levels of TNF-α, IFN-γ, IL-6, MIP-1α, and MIP-1β. Although TNF-α can induce PGE_2 and nitric oxide (NO), these effector molecules are suppressed in children with SMA. Suppression of PGE_2 allows over-production of TNF-α, which is associated with enhanced severity of anemia. In addition, MIF is suppressed in children with falciparum Malaria, an event associated with phagocytosis of *Pf*Hz by monocytes, and enhanced severity of anemia. Circulating levels of IFN-α, IL-1β, RANTES, and SCGF are also decreased in children with SMA. Reduced production of these innate inflammatory mediators, along with increased TNF-α, IL-6, MIP-1α and MIP-β, likely contribute to the development of SMA by suppressing the erythropoietic response. Lastly, although the reduced NO and reactive oxygen species (ROS) generation reported in children with falciparum malaria may promote ineffective parasite killing and, thereby, prolong parasitemia, children with malarial anemia have elevated levels of NO and ROS that can directly inhibit erythropoiesis (Douglas et al., 2011).

5.4. DENV NS1, IgM, IgG, and viral load correlation with Immune mediators in acute DENV infection

DENV NS1 antigen is one of DENV non-structural proteins, it was detected in the serum of DENV infected patients as early as one day from the appearance of symptoms, and remains up to 18 days afterwards. It is specific for DENV and used for diagnostic differentiation from other Flaviviruses. DENV NS1 antigen has a major role in viral replication but the exact mechanism still remains unknown (Morrison and Scholle, 2014; Farah and Frank, 2016). Many studies highlighted the strong association between the higher levels of NS1 antigen and the disease severity (Avirutnan et al., 2006; Hang et al., 2009). NS1 increases the DENV infectivity and alters the cytokines production, which in the presence of different cell types enhance viral production. In my study, the cytokines produced during acute DENV infection were assessed against positive NS1 antigen. Only IL-7 and IL-21 were significantly correlated with the presence of NS1 antigen (P value < 0.05) (Table 4.4.1.1). IL-21 significantly detected in primary and secondary DENV infections, higher concentrations reported in secondary acute DHF fever compared to secondary acute DF infection. Elevated levels of IL-21 during the convalescent phase of primary infection suggest it is protective role by enhancing B cells proliferation and differentiation into plasma cells, immunoglobulin production, and T cells activation (H. Vivanco-Cid et al., 2014). In the acute phase of secondary infection, increased IL-21 level was associated with memory B cells activation by T cells. IL-21 strongly correlated with DENV specific IgG and stimulates the production of both IgG and IgM in acute secondary DENV infection (Kuchen et al., 2007). Many cytokines such as GM-CSF, IL-1, IL-2, IL-7, IL-15, IFN-γ, IL-8, RANTES, MIP-1α, Eotaxin, and IP-10 were stimulated by IL-21 during the acute phase of DHF secondary infections, beside it is role in the activation of different immune cells such as monocytes, macrophages, dendritic cells and T cells. IL-21 receptor is expressed by vascular endothelial cells, but the direct involvement of IL-21 in vascular activation and vascular leakage is unknown (Francis and Denis, 2013; J. Li et al., 2006). IL-7 was significantly increased in patients with severe form of DENV infection compared to mild disease (Fernando et al., 2008).

In a monocyte dendritic cells (mo-DCs) pre-treated with DV1 NS1, IL-6, IP-10, MCP-1, TNF- α, and IL-12A were highly up-regulated in comparison to mo-DCs pre-

treated with heat- inactivated NS1 (HNS1) which support NS1 enhanced viral replication (Farah and Frank, 2016). In another study, DENV NS1 correlated with IL-10 production and the experiment showed monocyte production of IL-10 by NS1 beside it is involvement in T cell apoptosis. These results support the involvement of NS1 in the disease pathogenesis and it is association with sever clinical outcome like DHF (Adikari et al., 2016; Libraty et al., 2002). Anti-NS1 antibodies produced in vaccination by NS1 in mice reduced the mortality of DENF by 50% and decreased the level of viremia (Amorim et al., 2012; Beatty et al., 2015). NS1 is a potential reasonable target for vaccination.

In my study DENV specific IgM positively correlated with IFN-γ, IL-18, SCF, IL-21, IL-22, and EGF with a significant *P* value (*P* value < 0.05) during acute DENV infection (Table 4.4.1.1). IFN-γ and IL-18 are associated with severe DENV infection, such as DENV infection related hemorrhagic manifestations, plasma leakage, and hypotension. IFN-γ plays a major role in the pathogenesis of severe DENV infection and is a good predictor of DENF severity (Priyanka et al., 2018). In DENV2 infected HepG2 cells (human liver cancer cell line) pre-treated with recombinant human IL-22, decrease cell death and IL-6 production was noticed. IL-22 plays a major role in maintaining liver homeostasis during DENV infection (Rodrigo and Bernhard, 2014). Serum IL-21 correlates with DENV specific IgM during the convalescent phase of primary DENV infection (H. Vivanco-Cid et al., 2014).

DENV specific IgG significantly correlated with IFN-γ, GM-CSF, bNGF, FGF-2, and IL-17A (P value < 0.05) in my cohort group of patients during acute DENV infection (Table 4.4.1.1). Some studies reported the presence of high level of IL-17 in patients with severe DENV-2 infection (DHF) (Jain et al., 2013), while other studies reported correlation between IL-17 and mild DENV infection (DENF) (Becquart et al., 2010). The role of IL-17A in the pathogenesis of DENV infection is still unknown. In experimental DENV-2 infection, IL-17A was strongly associated with disease severity beside neutrophils accumulation and activation enhancement (Rodrigo and Bernhard, 2014).

DENV viral load (PFU/mL) is not significantly correlated with any of the 42 cytokines analyzed in my study during acute DENV infection (*P* value > 0.05) (Table 4.4.1.1). In a study conducted in India among 127 symptomatic DENV infected patients, significant negative correlation between viral load (copy number/mL) and IFN-γ level reported (*P* value 0.0001). The low level of IFN-γ in the presence of high

viral load promotes viral replication and associated with severe form of DENV infection during the acute phase of illness (Tithi et al., 2014). In an in vitro study, enhanced viral replication reported in mice deficient IFN-γ with sever manifestations of DENF in form of hematological alterations and hepatic injury (Fagundes et al., 2011). These findings pointed to the importance of IFN-γ in host defense mechanisms against DENV infection. In an experimental model of DENV-2 infection, mice deficient in IL-22 associated with increase viral load and inflammation with sever tissue injury, particularly in the liver (Guabiraba et al., 2013).

NS1 antigenemia or increased viral load in acute DENV infection associated with severe clinical manifestations in endemic areas (Nunes et al., 2016; Allonso et al., 2014). Endothelial hyper-permeability and vascular leakage are associated with NS1 level without the other effect of inflammatory cytokines as a result of it is direct effect on endothelial glycocalyx (Beatty et al., 2015; Puerta-Guardo et al., 2016; Glasner et al., 2017). In my cohort group of patients, a correlation analysis between NS1 antigen, viral load (PFU/mL), IgM, and IgG during acute DENV infection reported no significant association between the viral load and either NS1, IgM or IgG (*P* value > 0.05). In a study conducted in Brazil, NS1 antigenemia is significantly higher in DENV fatal cases compared to non- fatal ones (*P* value = 0.01) but no correlation with viremia. The study reported no differences in NS1 antigenemia between primary and secondary DENV infections (Priscila et al., 2018). In another study, viremia and NS1 antigenemia were higher in primary DENV infection than in secondary DENV infection (Perdomo-Celis et al., 2017). In vitro, DV1 NS1 pre-treatment of mo-DCs (human monocyte-derived dendritic cells) results in early enhancement of DV1 replication without increase in virus production (Farah and Frank, 2016). In 74 adult patients with acute DENV infection, DENV- specific T cell responses have a major protective role, results in mild DENV infection and decrease level of viremia. Semi-quantitative DENV-specific IgM and IgG antibody titers in patients with DHF and DF were not correlated with T cell responses to DENV-NS1, NS5 and NS3, while DEN-specific IgG antibody titer is negatively correlated with viral load in DHF patients (*p* value = 0.03) (Wijeratne et al., 2018). Higher levels of DENV specific IgG were reported in DHF patients during the pre-defervescence phase, responsible of DHF associated viremia and involved in ADE (Antibody-dependant enhancement) response (Guzman et al., 2013). For IgM, higher levels of IgM were reported in DENF than in DHF and in post-defervescence samples than in pre-defervescence.

Low levels of IgM in DHF associated with high level of viral load. IgM antibodies have a neutralizing host defence mechanism against DENV (Puschnik et al., 2013).

Analysis of significant correlation between DENV NS1, IgM, and IgG reported significant association between DENV specific IgM and DENV NS1 in my cohort group of patients during acute DENV infection (*P* value < 0.01) (Table 4.4.1.2). In comparing the level of NS1 concentration between IgM positive and IgM negative samples, the median concentration of NS1 is slightly increased in IgM negative samples (Allonso et al., 2014). These results support the neutralizing role of IgM in acute DENV infection.

5.5 *Neisseria meningitidis* serotypes correlation with immune mediators during acute meningococcal meningitis infection

Many studies reported the immune mediator expressed in meningococcal meningitis and it is association with disease severity. The pathogenesis of meningococcal meningitis is significantly affected by the cytokines produced, *Neisseria meningitidis* serotype virulence, and the genetic background of the patient (Clementien et al. 2001). The cytokines produced in response to meningococcal meningitis infection are different than what produced in pneumococcal meningitis. IL-1β, TNF-α, and IL-6 were the commonly studied immune mediators in bacterial meningitis, and showed no difference in production between meningococcal and pneumococcal meningitis (Coutinho et al, 2013). TNF-α, IL-1β, IL-10, MIP-1α, MIP-1β, and G-CSF were released faster in meningococcal meningitis, while IFN-γ and IL-2 present in higher concentrations in pneumococcal meningitis (Coutinho et al, 2013; Kornelisse et al,1997). IL -4, IL-6, IL-10, and IFN-α were associated with sever meningococcal disease (University of São Paulo School of Medicine, 2011). The immune mediators stimulated in response to different *N. meningitidis* serotypes during acute meningococcal meningitis were not the same. In my cohort groups, there was a significant difference in IL-1α, IL-10, IL-5, and IL-2 concentrations produced in serum in response to W135 serotype compared to the released by serotype A during acute meningococcal meningitis (*P* value < 0.05) (Table 4.4.2.1). The IL-2 concentration released in serum in response to serotype A was significantly different compared to serotype X during acute meningococcal meningitis (*P* value < 0.05). There is a few studies reported the cytokines produced in meningococcal meningitis in response to different *N. meningitidis* serotypes. In in vitro experiments, IL-1β, IL-6, and TNF-α level were significantly up regulated in response to stimulation by B strains compared to hyper-virulent B and C strains (*P* value < 0.01). There was a significant difference in stimulation of IL-10 by serotype B and hyper-virulant B strain (*P* value < 0.05) (M. Holub et al., 2007). These area needs more researches to identify the potential immune mediators that characterize each strain, as it predict the clinical outcome and prognosis of meningococcal meningitis.

5.6. Plasmodium species, parasitemia levels, and positive malaria thick blood film in correlation with immune mediators during acute malaria infection

The plasma level of immune mediators triggered in response to plasmodium species play a major role in malaria pathogenesis and predicts the severity of malaria. In my cohort group of patients, the correlation between the plasmodium species and immune mediators concentrations showed significant association between BDNF, IL-15 and the identified plasmodium species (*P* value < 0.01, *P* value < 0.05, respectively) (Table 4.4.3.1). BNDF was also the only cytokine correlated significantly with positive thick blood film during acute infection (*P* value < 0.05) (Table 4.4.3.1). In experimental cerebral malaria (ECM) in mice, decreased level of BDNF was reported in the most severe cerebral malaria stage which characterized by severe hemi-paralysis and intense weakness. BDNF was significantly associated with cerebral malaria neurological manifestations and involved in cerebral malaria pathogenesis and outcome (Maria et al., 2013). BDNF is a potential marker of severe cerebral malaria and could be a target of treatment. In experimental cerebral malaria (ECM) in mice, prophylactic treatment with IL-15 complexes (IL-15C) prevents the development of cerebral malaria in 70% of mice. The parasitemia level or parasite replications were not changed in response to IL-15C treatment (Kristina et al., 2018). Both BDNF and IL-15 significant detection in my cohort explain their importance as potential marker of severity for BDNF and promising agent of new adjuvant therapy for IL-15. *P. vivax* and *P. falciparum* infected patients were significantly correlated with elevated HuMIF (human MIF), TNF-α, MCP-1, and IL-12 plasma concentrations (*P* value < 0.0001), and significantly correlated with low TGF-β1 concentration. IL-10, TNF-α, and MCP-1 were significantly increased in Pv infected patients compared to Pf (*P* value < 0.01) (Cong Han et al., 2010).

In correlation between the degree of parasitemia and immune mediators activated, IL-17A was found to be significantly associated with parasitemia level during acute malaria infection (*P* value < 0.05). Furthermore, IL-4 was strongly associated with parasitemia level 2 (*P* value < 0.01) (Table 4.4.3.1). Acute malaria infection caused by Pv increase the production of IL-17 by CD4 T cells with increase in number of IL-17 producing CD4 T lymphocytes in peripheral blood, which responsible of the immune pathological response associated with Pv infection (Lilian et al., 2012). In vivo experimental studies in mice reported strong significant

correlation between parasitemia level and IL-17, IL-10, TGF-β immune mediators (*P* value < 0.05), IL-17 was upregulated in response to parasitemia level while IL-10 and TGF-β were down regulated in response to parasite load (Zainabur et al., 2014). Among 110 patients with complicated malaria and 169 patients with uncomplicated malaria, IL-4 was significantly higher in patients with complicated malaria compared to uncomplicated. The parasitemia level was inversely correlated with IL4: IFNγ ratio in complicated malaria patients. IL-4 involved in promoting immunoglobulins release, pro-inflammatory reponse regulation and tissue injury prevention in acute malaria infection. IL4-590 C/T polymorphism responsible of the regulating the balance between IL-4 and IFN-γ, which is determinant of disease clinical features and parasitemia level (Piyatida et al., 2007).

5.7. Diagnostic methods developments, new policies suggestions recommended for future plans

More than 2000 cases were included in this project over around four years. Approximately 80% of the recruited patients who presented to the IDC remained undiagnosed, as their samples were negative for the eight infectious disease studied and they were classified as febrile unknown, also known as Pyrexia of unknown origin (PUO) cases.

This research project highlighted many major health problems such as under diagnosis of acute febrile illnesses and the prevalence of these new re-emergent diseases DENV, CHIKV and co-infection of DENV+CHIKV in the study area. In addition, DENV infection identified in adults ≥ 45 years with less complications points towards new strains evolution that needs further research. The higher prevalence of DENV+CHIKV co-infection in Jizan related to *Aedes* species breeding in this area.

The immune profile detected in Meningococcal meningitis was different from what noticed in other studies. The sever clinical presentations noticed in my detected patients, the pathogenesis and the involved cytokines in the host immune response needs further focused researches.

Malaria positive cases were less severe with less clinical manifestations and good clinical outcome without complications, in contrast to our findings, in which most of the significantly associated cytokines studied were linked to poor prognosis. More light need to be shed in this area.

Unlike other developed countries, the government of Saudi Arabia along with the Ministry of Health (MOH) are oblivious of the disconnection and mis-communications between the MOH and other Ministries. For example, the air-conditioning system used in houses across the kingdom is in my ''humble opinion'' is a major breeding site for both types of mesquites, *Anopheles* and *Aedes spp*. This is because the Ministry of Housing is not willing to block the use of what is so called 'window air-conditions' that keeps dripping water on the ground all day.

In Singapore, the National Environmental Agency (NEA) introduced a multipronged system to reduce and control the wide spread of the DENV and CHIKV which comprise surveillance, control, public education, and research enforcement. No similar approach is in place or even forecasted to be implemented in the near

future in the Kingdom of Saudi Arabia. The level of knowledge and education in regards to vector-borne diseases and its implications was also very limited.

The Saudi government through the MOH should start to implement better measures. Clinicians outreach visits for training and education to reduce the number of misdiagnosed cases, door-to-door campaigns to educate the public to stop in-human behaviors such as what is well known in Saudi as ‘’ treatment by pressing’’ were a patient suffering from jaundice is not allowed to go to the doctor and treated only by pressing a flamed metal in his/her abdomen or arm with the burned area left until it heals, if not infected with a permanent scar. Other exclusive Saudi’s behaviors that may have contributed to the wide spread of vector-borne or zoonotic diseases includes but not limited to, drinking of and showering with camel’s urine.

Visits to educate the publics about the importance of allowing women to go to the hospitals even without a guardian or a chaperone is also crucial. Along with standardized vector control activities in most of the times, it is up to the person himself, the tenant, or the householder to spray any larval or adulticides in his own house. This should be done under the government umbrella and under its direct specialized and professional employees, ideally within a 200 m radius around homes of case-patients with outdoor residual and spatial insecticide treatment and not inside the house only.

Poor diagnostic tools such as diagnosing of diseases based on screening methods only such as ELISA had contributed significantly to the level of undetected cases. Other more specific and sensitive diagnostic tools should be in-place to reduce the number of under-detected cases. This should be combined with ‘‘no case definition only policy’’ with movements towards newer and more advanced diagnostic policies and protocols.

PCR and immune mediator’s profiling used for better and accurate diagnosis of diseases.

As there is a very limited informations regarding infectious diseases in the kingdom, Saudi MOH should allow more doctors and scientists to publish more data regarding newer discovery of emerging and re-emerging diseases such as ZIKV and others. Together with more statistical data about the implications of man-made disasters such as war and the number of refugees moving across the borders of the Kingdom.

Health system deficiencies and shortages regarding infectious diseases early detection strategies in Saudi Arabia necessitate development of predictive statistical models for early warning, specially in Jizan were the province facing increasing challenges such as changes in climatic conditions, surge in population density and growth due to massive constructions and patterns of human settlement in a war zone area.

Finally, the Saudi government shauld change its attitude toward neglected diseases such as DENV, CHIKV, ZIKA, Leptospirosis, and others infectious diseases cited in this research project. There must be a robust and continuous assessment of the direct and indirect economic burden of infectious diseases to invest and build more specialized reference laboratories. Sharing and liasie data with other globally recognized centers for accurate diagnosis of diseases in order to guard against complications and limits the progression of these diseases.

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