Prevalence of malaria infection in some localities of Fayoum governorate
Thesis
Submitted for fulfillment for Master Degree of Medical Parasitology

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Abstract

The present work was carried on 600 cases, 500 from household cases from El-Khaldia and Abo-Shanab villages, Abshoy District, Fayoum governorate and 100 selected cases from Fayoum Fever Hospital in order to find prevalence of malaria infection in some localities of Fayoum governorate. Diagnosis of malaria was done by thin and thick blood film for all cases in addition to malaria RDT applied to the 100 selected cases. Three cases were diagnosed by malaria RDT; while one case was positive by thick blood film. All positive cases were imported from Sudan.

Key Words: malaria, RDT, blood film, Fayoum, imported.
<table>
<thead>
<tr>
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<th>Description</th>
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<tbody>
<tr>
<td>ACC</td>
<td>Automated cell counter</td>
</tr>
<tr>
<td>AIDS</td>
<td>Acquired Immunodeficiency Syndrome</td>
</tr>
<tr>
<td>An.multicolor</td>
<td>Anopheles multicolor</td>
</tr>
<tr>
<td>DBL</td>
<td>Duffy binding like</td>
</tr>
<tr>
<td>DLL</td>
<td>depolarized laser light</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-Linked Immunosorbent Assay</td>
</tr>
<tr>
<td>FCM</td>
<td>Flow cytometer</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
</tr>
<tr>
<td>HRP-II</td>
<td>histidine-rich protein II</td>
</tr>
<tr>
<td>IFA</td>
<td>Immunofluorescence antibody testing</td>
</tr>
<tr>
<td>IFU</td>
<td>Instruction for use</td>
</tr>
<tr>
<td>IRMA</td>
<td>Immunoradiometric assay</td>
</tr>
<tr>
<td>LAMP</td>
<td>loop-mediated isothermal amplification</td>
</tr>
<tr>
<td>LDH</td>
<td>lactate dehydrogenase</td>
</tr>
<tr>
<td>LDMS</td>
<td>laser adsorption mass spectrometry</td>
</tr>
<tr>
<td>LED</td>
<td>light emitting diode</td>
</tr>
<tr>
<td>ml</td>
<td>Milliliter</td>
</tr>
<tr>
<td>MOHP</td>
<td>Ministry of Health and Population</td>
</tr>
<tr>
<td>P. falciparum</td>
<td>Plasmodium falciparum</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PfEMP1</td>
<td>plasmodium falciparum erythrocyte membrane protein 1</td>
</tr>
<tr>
<td>pLDH</td>
<td>pan-malaria lactate dehydrogenase</td>
</tr>
<tr>
<td>QBC</td>
<td>Quantitative buffy coat</td>
</tr>
<tr>
<td>RDT</td>
<td>Rapid diagnostic test</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>UN</td>
<td>United Nation</td>
</tr>
<tr>
<td>UNMIS</td>
<td>United Nation Missions In Sudan</td>
</tr>
<tr>
<td>VCS</td>
<td>volume, conductivity, and scatter</td>
</tr>
<tr>
<td>WBC(s)</td>
<td>White blood cell (s)</td>
</tr>
<tr>
<td>WHO</td>
<td>The World Health Organization</td>
</tr>
<tr>
<td>μm</td>
<td>A micrometre or micron</td>
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<td>18</td>
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<td>Clinical signs detected in the selected cases</td>
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</table>
Introduction

Forty-one percent of the world's population lives in areas where malaria is transmitted (e.g., parts of Africa, Asia, the Middle East, Central and South America) (WHO, 2002). About 3.3 billion people -half of the world's population- are at risk of malaria leading to about 250 million malaria cases and nearly one million deaths every year. People living in the poorest countries are the most vulnerable (WHO, 2009).

About Ninety-percent of all malaria deaths in the world today occur in Africa South of the Sahara. This is because the majority of infections in Africa are caused by *Plasmodium falciparum*, which is considered as the most dangerous of the four human malaria parasites. The vector of *falciparum* malaria (*Anopheles gambiae*) is widely spread in Africa and considered as the most difficult vector to control. An estimated one million people in Africa die from malaria each year and most of these are children under 5 years old (Daoud, 2003).

Malaria control in Egypt achieved a considerable progress in the last few decades due to widespread indoor residual spraying (IRS) with long-lasting insecticides and introduction of artemisinin combination therapy with artemether-lumefantrine (Coartem©). These procedures led to a decrease in malaria caseload from about 85000 cases in 1960 to 5400 cases in 1970, with a preponderance of *P. vivax* cases (MOHP, 2006). After application of intensive control measures, only 4 indigenous cases caused by *falciparum* were reported in 1997 (WHO, 2006). There were few annual imported malaria cases from 1998-2003 (Dawoud, 2003). In 2005, Ministry of health in Egypt reported 23 cases of imported malaria from Sierra Leon and Sudan (WHO, 2006).

It has been shown that malaria infection increased with the decrease of socioeconomic level of families, educational level of examined individuals and among unemployed or students. The infection increased among those living in
muddy or bad constructed houses near the breeding places of mosquitoes. Also, it decreased significantly among individuals who owned animal sheds (Dahesh et al., 2009).

However, there are many factors which may contribute to re-emergence of the disease in Egypt. Such factors include infection of local *Anopheline* mosquitoes by imported cases, continuous movement of populations between Aswan governorate and Sudan as well as the influx of large populations from Africa and Asia to Egyptian governorates for educational and religious purposes. Another risk factor is the environmental change brought about by water-sources development projects as Toshka and El Salam Canals (Hassan et al., 2003).
The present work aimed to:

1- Study the prevalence of malaria in some localities in Fayoum governorate; in addition to study the demographic criteria of the examined population incorporated in this study.

2- Determine the likelihood of acquisition of malaria infection in this area using thin and thick blood film, in addition to malaria pf/pan one step rapid test to detect plasmodium antigen in blood samples.
Review of Literature

Malaria prevalence in the world

The global human population has grown geometrically during the 20th century from approximately 1 to 6 billion. These demographics have important implications for the percentage of the human population exposed to all-cause malaria risk through time. The percentage of the global population at risk has decreased from 77% at the turn of the 20th century to a low of 46% in 1994. This figure increased to 48% in 2002 due to population growth in an unchanged geographic distribution. In absolute terms the numbers of people at risk has increased consistently from 0.9 to 3 billion over the same period (1900-2002). At the turn of the 21st century, it is estimated that 48% of the global population remain exposed to the risk of malaria, a situation that has deteriorated since the early 1990s and a figure substantially higher than the 40% widely cited (Hay et al., 2004).

Almost 300 million clinical cases of malaria occur worldwide each year and over a million people die. Almost 90% of these deaths occur in sub-Saharan Africa, where young children are the most affected. Malaria is directly responsible for one in five childhood deaths in Africa and indirectly contributes to illness and deaths from respiratory infections, diarrhoeal disease and malnutrition (World Health Report, 1999).

According to WHO malaria report in 2011, the number of reported cases of malaria decreased more than 50% in 35 of the 53 countries ongoing transmission between 2000 and 2010 while decreased 25%-50% in the other 4 countries. In 2010, the Europe region reported only 176 indigenous cases. The number of cases continued to fall least in countries with the highest incidence rates, indicating that greater attention should be
given to countries which harbour most of malaria burden outside Africa (WHO, 2011).

There were 8 countries in the pre-eliminating stage of malaria control in 2011 and 9 countries are implementing elimination programmes nationwide.

A further 8 countries including Bahamas, Egypt, Georgia, Iraq, Jamica, Oman, Russian federation and Syria have interrupted transmission and are in the prevention of reintroduction phase (WHO, 2011).

An estimated 3.3 billion people were at risk of malaria in 2010, 2.1 billion were at low risk (<1 reported case per 1000 population), 94% of whom were living in geographic regions other than the WHO African region. The remaining 1.2 billion were at high risk (>1 reported case per 1000 population) and were living mostly in WHO African region (47%) and South East Asia (37%) (WHO, 2011). Approximately 81%, or 174 million cases, were in Africa and 13% in South East Asian region. There were an estimated 655000 malaria deaths in 2010, of which 91% were in Africa. Approximately 86% of malaria deaths occurred in children under 5 years of age (WHO, 2011).
Malaria situation in Egypt

The present distribution of malaria cases in Egypt as reported by the Ministry of Health is demonstrated in table (1).


<table>
<thead>
<tr>
<th>Year</th>
<th>No.of slides</th>
<th>No.of</th>
<th>+ve</th>
<th>% Of malaria in the examined slides</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Plasmodium</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Plasmodium</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Falciparum</td>
</tr>
<tr>
<td>1960</td>
<td>400000</td>
<td>85201</td>
<td>83205</td>
<td>1996</td>
</tr>
<tr>
<td>1965</td>
<td>674044</td>
<td>7997</td>
<td>7853</td>
<td>144</td>
</tr>
<tr>
<td>1970</td>
<td>609329</td>
<td>5394</td>
<td>5241</td>
<td>153</td>
</tr>
<tr>
<td>1975</td>
<td>1399101</td>
<td>1805</td>
<td>1759</td>
<td>46</td>
</tr>
<tr>
<td>1980</td>
<td>1332541</td>
<td>374</td>
<td>370</td>
<td>4</td>
</tr>
<tr>
<td>1985</td>
<td>1180900</td>
<td>72</td>
<td>53</td>
<td>19</td>
</tr>
<tr>
<td>1990</td>
<td>1145251</td>
<td>71</td>
<td>2</td>
<td>69</td>
</tr>
<tr>
<td>1995</td>
<td>1139859</td>
<td>313</td>
<td>15</td>
<td>298</td>
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</tr>
<tr>
<td>2003</td>
<td>1041767</td>
<td>0</td>
<td>0</td>
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</tbody>
</table>

The last focus of malaria in Egypt was in Fayoum which became free from transmission of malaria since 1998 and Egypt was certificated as free of malaria. There were few annual imported malaria cases since the year 1998.

As regards malaria situation in Egypt from the period 2004-2010; All the detected cases were imported as shown in Table (2) (WHO, 2012).
Review of Literature

Table (2): Malaria in Egypt from 2004-2010 (WHO, 2012)

<table>
<thead>
<tr>
<th>Year</th>
<th>Suspected</th>
<th>Examined microscopically</th>
<th>Confirmed microscopically</th>
<th>Imported cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>2005</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>2006</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>2007</td>
<td>0</td>
<td>3402</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2008</td>
<td>0</td>
<td>4880</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2009</td>
<td>4</td>
<td>1344</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>2010</td>
<td>5</td>
<td>64294</td>
<td>5</td>
<td>5</td>
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</tbody>
</table>

Malaria situation in Fayoum governorate:

Fayoum governorate is considered as a large agricultural area. It lies 90 Km south-east of Cairo. It is composed of six districts, Fayoum, Sinnuris, Ebshawy, Youssef-Elsdek, Tamiya and Itsa.

The main problem in malaria transmission in Fayoum governorate is the high level of subsoil water leading to formation of many swamps and pools creating suitable environmental conditions for *Anopheline* vectors (Harb, 1994). Also, the favourable meteorological conditions, mainly optimum temperature and relative humidity leading to the extension of the transmission season to 8 months a year from the end of March to the end of November (Bassiouny, 1996).

Two main *Anopheline* vectors in Fayoum governorate were responsible for the transmission of malaria; *Anopheles sergenti* and *Anopheles pharoensis* (Shehata et al., 1989).
Table (3) Recorded indigenous malaria cases in Fayoum governorate (1971-2004) (Bassiouny, 2001).

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of malaria cases</th>
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<tbody>
<tr>
<td></td>
<td>P. falciparum</td>
</tr>
<tr>
<td>1971</td>
<td>208</td>
</tr>
<tr>
<td>1972</td>
<td>264</td>
</tr>
<tr>
<td>1973</td>
<td>8</td>
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<td>1974</td>
<td>6</td>
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<td>1975</td>
<td>175</td>
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<td>1976</td>
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<td>0</td>
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</tbody>
</table>

The early eradication of *P. vivax* first before *P. falciparum* was due to higher sensitivity of *P. vivax* than *P. falciparum* to chloroquine (Bassiouny, 2001).
Review of Literature

In Fayoum governorate, it seems that malaria control achieved significant progress when widespread indoor residual spraying (IRS) with long-lasting insecticides and introduction of artemisinin combination therapy with artemether-lumefantrine (Coartem©) and this led to disappearance of clinical cases of indigenous malaria and interruption of malaria transmission (MOHP, 2006).

The researchers in the Military Fever Hospital, Egypt diagnosed thirty six patients as having malarial disease. Twenty of them were recruited from Peace Keeping Mission Forces in Africa and sixteen cases presented with prolonged fever coming from different locations in Egypt. Their results showed that 12.5% of them were from Fayoum governorate. The diagnosis was by the use of bone marrow smears as they were negative by peripheral blood examination (El-Bahnasawy et al., 2010).

Imported malaria, 2001–2010

Imported malaria refers to infections acquired outside and brought into a national territory. The character of imported malaria and the problems it possess for countries in the prevention of reintroduction and malaria-free stages has changed over the period 2001–2010. Factors influencing the change include the reduction of malaria incidence in tourist destinations, an increase in the number of countries recently classified as malaria-free and new patterns of travel and international migration prior to year 2000, the importation of malaria into non-endemic countries as “traveller’s malaria” was primarily a matter for foreign tourists returning home after visiting endemic areas (WHO, 2012).

Since 2000, the problem has grown and changed in at least four ways: (i) in non-endemic countries with large and relatively affluent immigrant populations (e.g. countries in North America and Western Europe), immigrants returning home to endemic areas to visit friends and relatives
have become a high-risk group among travellers; (ii) non endemic countries take refugees from malaria-endemic areas; (iii) malaria cases are imported with returning members of national armed forces and UN peacekeeping forces; and (iv) malaria infections are often brought into countries by temporary migrant workers (WHO, 2012).

Imported malaria was reported by 91 countries between 2001 and 2010; the largest total numbers of cases were in the United States of America (12701) in the Region of the Americas, the United Arab Emirates (20 452) in the Eastern Mediterranean Region, France (48 580) and the United Kingdom (17 063) in the European Region and Australia (3355) in the Western Pacific Region. Between 2001 and 2010, 45 countries in the European Region reported a striking and consistent decline in imported malaria cases and deaths, for reasons that have not yet been investigated (WHO, 2012).

Critical for malaria control is whether imported cases lead to local outbreaks of malaria, transmitted by indigenous Anopheline mosquitoes. The risk can be high, for example when temporary agricultural workers infected with malaria are recruited for harvesting during the malaria transmission season. However, while malaria outbreaks are commonly documented, they are less frequently investigated to understand the precise circumstances of the outbreak and to identify the local vectors. In the European Region, local transmission from imported cases has been reported in Republic of Moldova (2003), Ukraine (2003), France (2006, 2008–2010), Italy (2007), Greece (2009–2010) and Spain (2010). In all these instances, local outbreaks were limited to fewer than 10 cases (WHO, 2012).

In the Region of the Americas, the United States of America reported an outbreak of eight cases of *P. vivax* in Palm Beach County, Florida, in 2003, probably originating from a single infected person. Immigration was
the cause of a large outbreak of *P. falciparum* malaria that occurred in Jamaica between September 2006 and December 2009, in which there were 406 confirmed cases. In the Bahamas, 19 *P. falciparum* cases were identified on the island of Great Exuma between May and June 2006, apparently brought to the island by Haitian immigrants. These outbreaks in the Americas were contained by a swift reaction from public health authorities (WHO, 2012).

In other parts of the world: three cases arising from local *P. falciparum* transmission were reported in Singapore in 2003; Oman, which interrupted transmission in 2004, has experienced several subsequent outbreaks of *P. vivax* and *P. falciparum* brought in by migrant workers from the Indian subcontinent; and Morocco, certified malaria-free in 2007, recorded two cases of “airport malaria” in 2009 (WHO, 2011).

Other countries which eliminated malaria many years ago, including the Maldives, Mauritius and Tunisia, continued to invest effort in preventing the reintroduction of malaria. For the growing number of countries progressing to the prevention of reintroduction and malaria-free stages, the nature of malaria control will change, moving towards outbreak preparedness, surveillance and rapid response and studies of malaria risk and receptivity (WHO, 2011).

The Imported Malaria cases in Egypt were recorded by Dawoud (2003). Blood samples were taken from ship passengers travelling between Aswanwady Half , sent to fever hospitals , examined for malaria parasites. The reported data are shown in table (4).
Table (4): Recorded Imported Malaria cases 1998-2004 in Egypt (Dawad,2003).

<table>
<thead>
<tr>
<th>Year</th>
<th>No of examined slides</th>
<th>Positive</th>
<th>P.falciparum</th>
<th>P.vivax</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998</td>
<td>32403</td>
<td>24</td>
<td>23</td>
<td>1</td>
</tr>
<tr>
<td>1999</td>
<td>28992</td>
<td>38</td>
<td>37</td>
<td>1</td>
</tr>
<tr>
<td>2000</td>
<td>26581</td>
<td>17</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>2001</td>
<td>26341</td>
<td>9</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>2002</td>
<td>25785</td>
<td>9</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>2003</td>
<td>23813</td>
<td>45</td>
<td>44</td>
<td>1</td>
</tr>
</tbody>
</table>

3.2- Taxonomy

Phylum: Apicomplexa

Class: Aconoidasida

Order: Haemosporida

Family: Plasmodiidae

Genus: Plasmodium

Species: falciparum, ovale, vivax and malariae. (Marchiafava & Celli, 1885).

Plasmodium knowlesi. P. knowlesi is one of the species of Plasmodium most recently identified as an agent of human malaria (Cox-Singh et al., 2008).
3. Life cycle of malaria:

3.3.1-Pre-erythrocytic development

The small motile *Plasmodium* sporozoites are injected by the feeding female *Anopheline* during the phase of probing (Rosenberg *et al.* 1990). After injection, sporozoites enter the circulation and rapidly target the hepatic parenchymal cells and begin a phase of asexual reproduction. This stage lasts on average between 5.5 (*P. falciparum*) and 15 days (*P. malariae*) before the hepatic schizont ruptures to release merozoites into the bloodstream (Smith *et al.*, 2004). In *P. vivax* and *P. ovale* infections a proportion of the intrahepatic parasites do not develop, but instead they rest inert as sleeping forms or ‘hypnozoites’ causing the relapses which characterize infections with these two species. During the hepatic phase of development, asexual multiplication takes place and many thousands of merozoites are released from each ruptured infected hepatocyte. However, as only a few liver cells are infected, this phase is asymptomatic for the human host (Smith *et al.*, 2008).

3.3.2-Asexual blood-stage development

The merozoites liberated from ruptured hepatocytes invade red cells rapidly. The process of invasion involves attachment of the merozoite to the erythrocyte surface, orientation so that the apical complex (which protrudes slightly from one end of the merozoite and contains the rhoptries, the micronemes and dense granules) abuts the red cell. Interiorization takes place by a wriggling or boring motion inside a vacuole composed of the invaginated erythrocyte membrane. Inside the erythrocyte, the parasite lies within the erythrocyte cytosol enveloped by its own plasma membrane and a surrounding parasitophorous vacuolar membrane. The attachment of the merozoite to the red cell is mediated by the attachment of one or more of a
family of erythrocyte binding proteins, localized to the micronemes of the merozoite apical complex, to a specific erythrocyte receptor. In *P. vivax* this is related to the Duffy blood group antigen Fya or Fyb (Miller *et al.*, 1976).

After approximately 12–14 h of development, *P. falciparum* parasites begin to exhibit a high molecular weight strain-specific variant antigen, *Plasmodium falciparum* erythrocyte membrane protein 1 (*PfEMPI*) on the exterior surface of the infected red cell which mediates attachment of the infected erythrocyte to vascular endothelium (Leech *et al.*, 1984).

Approximately 36 h after merozoite invasion (or 54 h in *P. malariae*), repeated nuclear division takes place to form a schizont. As the red cell ruptures, from 6 to 36 merozoites are released destroying the remnants of the red cell. The released merozoites rapidly re-invade other red cells and start a new asexual cycle. The process of gametocytogony takes about 7–10 days in *P. falciparum*. Thus, there is an interval of 1 week between the peaks of asexual and sexual stage parasitaemia in acute falciparum malaria. In contrast, *P. vivax* begins gametocytogenesis immediately and the process of gametocytogony in the blood stage infection takes only 4 days. Symptomatic *P. vivax* infections are therefore more likely to present with patent gametocytaemia and was able to transmit infection to mosquitoes before treatment than acute *P. falciparum* infections (Leech *et al.*, 1984).

### 3.3.3- Sexual stages and development in the mosquito

Following ingestion in the blood meal of a biting female *Anopheline* mosquito, the motile male microgametes separate and seek the female macrogamete. Within 24 h the enlarging zygote becomes motile and forms the ookinete which penetrates the wall of the mosquito mid-gut (stomach) where it encysts as an oocyst. (Ghosh *et al.*, 2000).
The oocyst finally bursts to liberate myriads of sporozoites into the coelomic cavity of the mosquito. The sporozoites then migrate to the salivary glands to await inoculation into the next human host during feeding. The development of the parasite in the mosquito is termed sporogony, and takes between 8 and 35 days depending on the ambient temperature and species of parasite and mosquito. The longevity of the mosquito is a critical factor in determining its vectorial capacity (Ghosh et al., 2000).

3.4-Methods of transmission:

1. From female *anopheline* mosquitoes to humans: Malaria infection in humans is initiated with the bite of an infectious female mosquito, which injects sporozoites of *Plasmodium* species into the circulation. These sporozoites rapidly bind and invade liver cells and undergo rapid multiplication, leading to the release of thousands of infective merozoites (Raether et al. 1989).

2. From humans to *anopheline* mosquitoes: The journey of *Plasmodium* within the mosquito begins as the mosquito ingests gametocytes with the blood of an infected host (Ghosh et al., 2003).

3. From humans to humans: *Plasmodium* is sometimes transmitted by means other than the bite of a mosquito. The blood cycle may be initiated by blood transfusion, by malaria therapy of certain paralytic disease, by syringe-passed infection among drug addicts, or, rarely, by congenital infection (Schmidt and Roberts, 1985).

3.5-Anopheline vector

Malaria is transmitted only via *Anopheline* mosquitoes. In principle, reducing or eliminating mosquito populations should stop disease transmission. In practice, this approach is difficult to implement, especially in sub-Saharan Africa, where mosquitoes can easily grow in environments
such as small pools of water, which are extremely difficult to manage or target with insecticides. Insecticide campaigns might reduce mosquito populations temporarily, but leave a largely intact biological niche, where mosquitoes can continue to thrive (Ghosh et al., 2003).

*Anopheles gambiae* is the principal mosquito vector of malaria in Africa (Land, 2003).

*Anopheles multicolor*: *Plasmodium* infection rates determined by enzyme-linked immunosorbent assay (ELISA) were compared for *Anopheles sergentii* and *An. multicolor* Cambouliu in Siwa Oasis, Egypt, an area with low-level *Plasmodium vivax* transmission, and in Bahariya and Farafra, two other Egyptian oases which appear to be free of malaria. Initial testing indicated that 4.4% (23 of 518) and 0.8% (4 of 518) of the *An. sergentii* were positive for *P. vivax* and *P. falciparum*, respectively, and that 1.4% (1 of 71) of the *An. multicolor* was positive for *P. falciparum* (Kenawy et al., 1990).

*Anopheles pharoensis*: *Plasmodium vivax* and *P.falciparum* epidemiology were studied for parasitological and entomological samples collected during the period 1989 and 1990, respectively, from Gambella, South West Ethiopia. Of the total population examined (n = 1091), 147 (13.5%) were found to be positive for malaria parasites. Prevalence rates among males and females were 13.8% and 13.1%, respectively. The mosquito species responsible for malaria transmission were the indoor-resting *A. gambiae* and *A. pharoensis*. The parasite infection rates of these species were 0.76% and 0.46% and they were found to be the exclusive vectors of *P. falciparum* and *P. vivax*, respectively (Nigatu et al., 1992).

*Anopheles sergenti*: Detection and identification of malaria sporozoites is usually done by two immunoassay: immunoradiometric assay
IRMA) and the enzyme-linked immunosorbent assay (ELISA) using the species-specific monoclonal antibodies is routinely performed. A field study analyzed (573) anopheline mosquitoes of A. sergenti (463), A. pharoensis (81) and A. multicolor (29) collected from Siwa-oases and Fayoum governorate (two known active malaria foci in Egypt), for detection of *P. falciparum* and *P. vivax* sporozoites. *P. falciparum* sporozoites were detected by both IRMA and ELISA tests in two *A. sergenti* mosquitoes (one from Siwa 1/389 = (0.26%) and one from Fayoum governorate 1/74 = (1.35%)). No *P. vivax* sporozoites were detected. This finding is important in explaining the malaria transmission and provides first incrimination of *An. sergenti* as the responsible vector of malaria in Siwa-oasis, Egypt (Shehata et al., 1989).

**3.6-Clinical picture of malaria**

**3.6.1-Uncomplicated malaria**

Incubation period ranges from 9 days in *P. falciparum* to 30 days in *P. malariae* infections. As far as the degree of previous protection possessed by the infected subject is concerned, it is known that effective immunity prolongs incubation period and reduces level of parasitemia and clinical manifestations. Low asymptomatic parasitemia may persist in migrants from endemic areas long after their arrival in the host country (Harinasuta and Bunnang, 1988). Delayed clinical presentation of *P. falciparum* has been described as long as 2, 4 or even 8 years (Szmitko et al., 2008) after subjects have left malaria-endemic areas. Prolonged incubation period may also be caused by the use of antimalarial drugs that, although ineffective, may impact on the parasite multiplication rate (D’Ortenzio et al., 2008).

The clinical manifestations of malaria are dependent on the previous immune status of the host. In areas where endemicity of *P. falciparum* malaria is stable, severe malaria most commonly occurs in children up to 5
years of age, while is less common in older children and adults because of the acquisition of partial immunity. In areas of lower endemicity, the age distribution of severe malaria is less well defined and may also occur in adult semi-immune (Cook et al., 2009).

The first symptoms of malaria, common to all the different malaria species, are nonspecific and mimic a flu-like syndrome. The hallmark of malaria is fever. Up to two days before the onset of fever, prodromal symptoms, such as malaise, anorexia, lassitude, dizziness, with a desire to stretch limbs and yawn, headache, backache in the lumbar and sacroiliac region, myalgias, nausea, vomiting and a sense of chillness may be experienced (Warrell, 1993).

In *P. vivax* and *P. ovale* infection, if left untreated, asexual cycles become synchronous after 5 to 7 days causing periodic febrile paroxysms. The classical malaria paroxysm presents three stages: a cold stage, followed by a hot stage with a terminal sweating stage. The cold stage is typically characterized by a sudden onset with a feeling of extreme coldness. The subject may shiver and his or her teeth may chatter. In virtue of an intense peripheral vasoconstriction phenomenon, the skin is cold, dry, pale, cyanosed and sometimes goose-pimpled. (Cook et al., 2009).

In *P. falciparum* malaria, the onset of fever occurs few days after prodromal symptoms started during the last days of the incubation period (normal range 9-14 days). At first, fever is irregular, but usually occurs daily. It may be intermittent or continuous, and shows no sign of periodicity until the illness has continued for a week or more. The symptoms present in the prodromal phase continue and increase configuring a flu-like syndrome. Anorexia, dyspepsia, epigastric discomfort, nausea, vomiting and watery diarrhoea are frequent and may be misdiagnosed as a gastrointestinal infection. Herpes labialis may be present. A dry cough and an increase in the respiration
rate may be observed, arising the suspect of an acute respiratory infection. When periodic febrile paroxysms occur, they may be daily (quotidian), every third day (tertian) or at about 36-hour intervals (subtertian) (Taylor and Strickland, 2003).

*P. malariae* causes the mildest and most persistent form of malaria infection after an incubation period that is never less than 18 days, but that may be up to 30-40 days, prodromal symptoms resembling those of *vivax* malaria precedes the onset of fever. The clinical picture of the primary attack is similar to that of vivax malaria. The onset is often insidious, but febrile paroxysms, often occurring in the late afternoon, show well synchronized schizogony from an early stage and are typically separated by intervals of 72 hours (quartan malaria) (Harinasuta and Bunnang, 1988).

Left untreated, the acute attack is self limiting but may last for several months before spontaneous remission occurs. Severe complications of *P. malariae* infection are rarely observed. However, recrudescences may occur, more frequently during the first year and then at longer intervals, even after 30-50 years. *P. malariae* has no hypnozoite form, so recrudescences arise from persisting blood stage. Asymptomatic *P. malariae* parasitaemia in blood donors may cause transfusion malaria (Harinasuta and Bunnang, 1988).

*P. malariae* infection is associated with development of a nephrotic syndrome. *P. Malariae* parasitemia is common in children, but not in adults. Transient clinical remissions with period of asymptomatic proteinuria are frequent but progressive deterioration and development of renal failure often occurs within 3 to 5 years (Taylor and Strickland, 2003).

*Knowlesi* malaria (a simian parasite) is the most common locally acquired human malaria in Malaysian Borneo (~70% of malaria cases) (Daneshvar *et al.*, 2009). with the disease also reported from other countries of Southern and
Eastern Asia. (Baird, 2009). On the basis of clinical features, it is not possible to distinguish *knowlesi* malaria from *vivax* or *falciparum* malaria (Daneshvar et al., 2009). The development of hyperparasitemia and other complications are fairly common (Baird, 2009).
4-Diagnosis of malaria infection

4.1- Microscopic diagnosis using stained thin and thick peripheral blood smears (PBS):

Malaria is conventionally diagnosed by microscopic examination of stained blood films using Giemsa, Wright's, or Field's stains (Warhurst and Williams, 1996). This method has changed very little since Laverran's original discovery of the malaria parasite, and improvements in staining techniques by Romanowsky in the late 1,800s. More than a century later, microscopic detection and identification of *Plasmodium* species in Giemsa-stained thick blood films (for screening of malaria parasite), and thin blood films (for species' confirmation) remains the gold standard for laboratory diagnosis (Bharti et al., 2006). The wide acceptance of this technique by laboratories all around the world can be attributed to its simplicity, low cost, its ability to identify the presence of parasites, the infecting species, and assess parasite density—all parameters useful for the management of malaria. Recently, a study showed that conventional malaria microscopic diagnosis at primary healthcare facilities in Tanzania could reduce the prescription of antimalarial drugs, and also appeared to improve the appropriate management of non-malarial fevers (Ngasala et al., 2008). However, the staining and interpretation processes are labor intensive, time consuming, and require considerable expertise and trained healthcare workers, particularly for identifying species accurately at low parasitemia or in mixed malarial infections.

The most important shortcoming of microscopic examination is its relatively low sensitivity, particularly at low parasite levels. Although the expert microscopist can detect up to 5 parasites/μl, the average microscopist detects only 50-100 parasites/μl (Payne, 1988). This has probably resulted in underestimating malaria infection rates, especially cases with low
parasitemia and asymptomatic malaria. The ability to maintain required levels of in malaria diagnostics expertise is problematic, especially in remote medical centers in countries where the disease is rarely seen (Ohrt et al., 2002). Microscopy is laborious and ill-suited for high-throughput use, and species determination at low parasite density is still challenging. Therefore, in remote rural settings, e.g. peripheral medical clinics with no electricity and no health-facility resources, microscopy is often unavailable (Erdman et al., 2008).

Concerning diagnosis, the identification of *P. knowlesi* infection by using microscopy only is difficult because it is very similar to *P. malariae* (Lee et al., 2009). Polymerase Chain Reaction (PCR) is currently the method of choice to obtain a certain diagnosis (Cox-Singh et al., 2008).

**4.2-Quantitative Buffy Coat technique:**

The Quantitative buffy coat (QBC) technique was designed to enhance microscopic detection of parasites and simplify malaria diagnosis (Clendennen et al., 1995). This method involves staining parasite deoxyribonucleic acid (DNA) in micro-hematocrit tubes with fluorescent dyes, e.g. acridine orange, and its subsequent detection by epi-fluorescent microscopy. Briefly, finger-prick blood is collected in a hematocrit tube containing acridine orange and anticoagulant. The tube is centrifuged at 12,000 gram for 5 min and immediately examined using an epi-fluorescent microscope (Chotivanich et al., 2006). Parasite nuclei fluoresces bright green, while cytoplasm appears yellow-orange. The QBC technique has been shown to be a rapid and sensitive test for malaria diagnosing in numerous laboratories settings (Bhandari et al., 2008). While it enhances sensitivity for *P. falciparum*, it reduces sensitivity for non-falciparum species and decreases specificity due to staining of leukocyte DNA (Moody et al., 2002).
Recently, it has been shown that acridine orange is the preferred diagnostic method (over light microscopy and immunochromatographic tests) in the context of epidemiologic studies in asymptomatic populations in endemic areas, probably because of increased sensitivity at low parasitemia (Ochola LB et al., 2006). Although the QBC technique is simple, reliable, it requires specialized instrumentation and it is more costly than conventional light microscopy, also it is poor at determining species and numbers of parasites (Tangpukdee et al., 2009).

4.3-Rapid diagnostic tests (RDTs):

Since the World Health Organization (WHO) recognized the urgent need for new, simple, quick, accurate and cost-effective diagnostic tests for determining the presence of malaria parasites to overcome the deficiencies of light microscopy, numerous new malaria-diagnostic techniques have been developed (WHO, 1996). This, in turn, has led to an increase in the use of RDTs for malaria, which are fast and easy to perform, and do not require electricity or specific equipment (Bell et al., 2006).

Currently, 86 malaria RDTs are available from 28 different manufacturers (WHO, 2008). Unlike conventional microscopic diagnosis by staining thin and thick peripheral blood smears, and QBC technique, RDTs are all based on the same principle and detect malaria antigen in blood flowing along a membrane containing specific anti-malaria antibodies; they do not require laboratory equipment. Most tests target a P. falciparum-specific protein, e.g. histidine-rich protein II (HRP-II) or lactate dehydrogenase (LDH). Some tests detect P. falciparum specific and pan-specific antigens (aldolase or pan-malaria pLDH) and distinguish non-P. Falciparum infections from mixed malaria infections. Although most RDT products are suitable for P. falciparum malaria diagnosis, some also claim that they can effectively and rapidly diagnose P. vivax malaria (Lee et al.,
Recently, a new RDT method has been developed for detecting *P. knowlesi* (McCutchan *et al.*, 2008).

RDTs provide an opportunity to extend the benefits of parasite-based diagnosis of malaria beyond the confines of light microscopy, with potentially significant advantages in the management of febrile illnesses in remote malaria-endemic areas. RDT performance for diagnosis of malaria has been reported as excellent (Doderer *et al.*, 2007). However, some reports from remote malaria-endemic areas have shown wide variations in sensitivity (Murray *et al.*, 2008). Murray and co-authors recently discussed the reliability of RDTs in an "update on rapid diagnostic testing for malaria in their research (Murray *et al.*, 2008).

Overall, RDTs appears a highly valuable, rapid malaria-diagnostic tool for healthcare workers; however it must be used in conjunction with other methods to confirm the results, characterize infection and monitor treatment. In malaria-endemic areas where no light microscopy facility exists that may benefit from RDTs, improvements are required for ease of use, sensitivity for non-falciparum infection, stability, and affordability. The WHO is now developing guidelines to ensure lot-to-lot quality control, which is essential for the community's confidence in this new diagnostic tool (WHO, 2008). As the simplicity and reliability of RDTs have been improved for use in rural endemic areas, RDT diagnosis in non-endemic regions is becoming more feasible, which may reduce time-to-treatment for cases of imported malaria (Erdman *et al.*, 2008).
4.4-Serological tests

Diagnosis of malaria using serological methods is usually based on the detection of antibodies against asexual blood stage malaria parasites. Immunofluorescence antibody testing (IFA) has been a reliable serologic test for malaria in recent decades (She et al., 2007). The literature clearly illustrates the reliability of IFA, so that it was usually regarded as the gold standard for malarial serology testing (Doderer et al., 2007). IFA is useful in epidemiological surveys, for screening potential blood donors, and occasionally for providing evidence of recent infection in non-immunes. Until recently, it was a validated method for detecting *Plasmodium*-specific antibodies in various blood bank units, which was useful for screening prospective blood donors, so avoiding transfusion-transmitted malaria (Mungai et al., 1978).

In France, for example, IFA is used as part of a targeted screening strategy, combined with a donor questionnaire (Oh et al., 2008). The principle of IFA is that, following infection with any *Plasmodium* species; specific antibodies are produced within 2 weeks of initial infection, and persist for 3-6 months after parasite clearance. IFA uses specific antigen or crude antigen prepared on a slide, coated and kept at -30°C until used, and quantifies both IgG and IgM antibodies in patient serum samples. Titers > 1 : 20 are usually deemed positive, and < 1 : 20 unconfirmed. Titers > 1 : 200 can be classified as recent infections (Chotivanich et al., 2008).

In conclusion, IFA is simple and sensitive, but time-consuming. It cannot be automated, which limits the number of sera that can be studied daily. It also requires fluorescence microscopy and trained technicians; readings can be influenced by the level of training of the technician, particularly for serum samples with low antibody titers. Moreover, the lack of IFA reagent standardization makes it impractical for routine use in blood-
transfusion centers and for harmonizing inter-laboratory results (Tangpukdee 
et al., 2009).

4.5-Molecular diagnostic methods

4.5.1-PCR technique:

PCR-based techniques are a recent development in the molecular diagnosis of malaria and have proven to be one of the most specific and sensitive diagnostic methods, particularly for malaria cases with low parasitemia or mixed infection (Morassin et al., 2002). The PCR technique continues to be used extensively to confirm malaria infection, follow-up therapeutic response, and identify drug resistance (Chotivanich et al., 2008). It was found to be more sensitive than QBC and some RDTs (Makler et al., 1998). Regarding the gold standard method for malaria diagnosis, PCR has shown higher sensitivity and specificity than conventional microscopic examination of stained peripheral blood smears, and now seems to be the best method for malaria diagnosis (Morassin et al., 2002).

PCR can detect as few as 1-5 parasites/µl of blood (≤ 0.0001% of infected red blood cells) compared with around 50-100 parasites/µl of blood by microscopy or RDT. Moreover, PCR can help detect drug-resistant parasites, mixed infections, and may be automated to process large numbers of samples (Hawkes et al., 2007). Some modified PCR methods are proving reliable as nested PCR, real-time PCR, and reverse transcription PCR, and appear to be useful second-line techniques when the results of traditional diagnostic methods are unclear for patients presenting with signs and symptoms of malaria. They also allow accurate species determination (Hawkes and Kain, 2007).

Recently, the PCR method has become widely accepted for identifying \textit{P. knowlesi} infections (Cox-Singh et al., 2008). Although PCR
appears to have overcome the two major problems of malaria diagnosis—sensitivity and specificity—the utility of PCR is limited by complex methodologies, high cost, and the need for specially trained technicians. PCR, therefore, is not routinely implemented in developing countries because of the complexity of the testing and the lack of resources to perform these tests adequately and routinely. Quality control and equipment maintenance are also essential for the PCR technique, so that it may not be suitable for malaria diagnosis in remote rural areas or even in routine clinical diagnostic settings (Hanscheid et al., 2008).

**4.5.2-Loop mediated isothermal amplification (LAMP) technique:**

It detects 18s ribosome RNA gene of *P. falciparum*. Observations suggest that LAMP is more reliable and useful for routine screening for malaria parasites in regions where vector-borne diseases, such as malaria, are endemic. LAMP appears to be easy, sensitive, quick and lower in cost than PCR. However, reagents require cold storage, and further clinical trials are needed to validate the feasibility and clinical utility of LAMP (Erdman et al., 2008).

**4.5.3-Microarrays:**

Publication of the *Plasmodium* genome offers many malaria-diagnostic opportunities (Doolan et al., 2008). The principle of the microarrays technique parallels traditional Southern hybridization. Hybridization of labeled targets divided from nucleic acids in the test sample to probes on the array enables the probing of multiple gene targets in a single experiment. Ideally, this technique would be miniaturized and automated for point-of-care diagnostics (Holland et al., 2005). A pan-microbial oligonucleotide microarray has been developed for infectious disease diagnosis and has identified *P. falciparum* accurately in clinical specimens (Palacios et al., 2007). This diagnostic technique, however, is still in the early stages of development (Erdman et al., 2008).
4.5.4- Flow cytometry (FCM) assay:

Flow cytometry has been used for malaria diagnosis (Izumiyama et al., 2009). Briefly, the principle of this technique is based on detection of hemozoin, which is produced when the intra-erythrocytic malaria parasites digest host hemoglobin and crystallize the released toxic heme into hemozoin in the acidic food vacuole. Hemozoin within phagocytotes can be detected by depolarization of laser light, as cells pass through a flow-cytometer channel. This method may provide a sensitivity of 49-98%, and a specificity of 82-97%, for malarial diagnosis (Padial et al., 2005), and is potentially useful for diagnosing clinically unsuspected malaria. The disadvantages are its labor intensiveness, the need for trained technicians, costly diagnostic equipment, and that false-positives may occur with other bacterial or viral infections. Therefore, this method should be considered a screening tool for malaria.

4.5.5- Automated blood cell counters (ACC):

An ACC is a practical tool for malaria diagnosis, with 3 reported approaches. The first approach used a Cell-Dyn® 3500 apparatus to detect malaria pigment (hemozoin) in monocytes, and showed a sensitivity of 95% and specificity of 88%, compared with the gold-standard blood smear. The second method also used a Cell-Dyn® 3500, and analyzed depolarized laser light (DLL) to detect malaria infection, with an overall sensitivity of 72% and specificity of 96%. The third technique used a Beckman Coulter ACC to detect increases in activated monocytes by volume, conductivity, and scatter (VCS), with 98% sensitivity and 94% specificity. Although promising, none of the 3 techniques is routinely available in the clinical laboratory; further studies are required to improve and validate the instrument and its software. These methods show a promising accuracy for
detection of malaria parasite so that they would become available and routine malaria diagnostic method (Briggs et al., 2006).

4.5.6-Mass spectrophotometry:

A novel method for in vitro detection of malaria parasites, with a sensitivity of 10 parasites/µl of blood, has been reported recently. It comprises a protocol for cleanup of whole blood samples, followed by direct ultraviolet laser desorption mass spectrometry (LDMS). For malaria diagnosis, the principle of LDMS is to identify a specific biomarker in clinical samples. In malaria, heme from hemozoin is the parasite-specific biomarker of interest. LDMS is rapid, high throughput, and automated. Compared with the microscopic method, which requires a skilled microscopist and up to 30-60 min to examine each peripheral blood smear, LDMS can analyze a sample in < 1 min However, the remote rural areas without electricity are inhospitable for existing high-tech mass spectrometers. Future improvements in equipment and techniques should make this method more practicable (Scholl et al., 2004).

4.6-Culturing:

The methodological breakthrough for culturing the asexual intraerythrocytic stages of Plasmodium falciparum parasites published over 35 years ago by Trager and Jensen (1976) is still used in most malaria laboratories today (Butcher, 1979 and Schuster, 2002).

These basic culturing procedures have been essential to almost all molecular, genomic, and immunological and biochemical studies of malaria over the last 30 years and has been critical to the development of much-needed drugs and vaccines. The procedure for maintaining P.falciparum in vitro generally involves growing the parasites in static cultures in the presence of human erythrocytes at low O₂ conditions (& Hurd et al., 2003).
One drawback of growing *P. falciparum* using static tissue culture flask methods is the low yield of parasite material for the study of native parasite molecules or organelles (Radfar *et al.*, 2009).

Cultures of 5 ml (in T25 flasks) to ml (in T150 flasks) can be routinely maintained at a 4–5% haematocrit and up to 5–10% parasitaemia. Further bulking-up of parasite cultures requires establishing multiple flasks, which becomes time consuming and labour intensive since each flask requires at least one or two daily media changes, depending on parasitaemia levels (Hurd *et al.*, 2003). There have been few endeavours to develop large-scale cultures of malaria parasites (Hurd *et al.*, 2003). Most notable was the deep 8 L cultures of *P. falciparum* in large 15 Litre vessels whereby cells were kept in suspension by stirring.

However, medium needs to be replenished by continuous flow and the assemblage and dismantling of this large-scale apparatus was cumbersome (Moloney *et al.*, 1990). More recently, hollow-fibre capillary bioreactors have been used to obtain asynchronous cultures of *P. falciparum* with high parasitaemia, although these systems are of relatively low volume (Li *et al.*, 2003).

Radfar *et al.*, (2009) reported a detailed stepwise protocol for the production of synchronous parasite cultures at high parasitaemia. This method used static cultures maintained at low haematocrit (0.8–1.5%) to seed additional flasks to reach parasitaemia as high as 60%.

Other reliable malaria-diagnostic tests have been developed and introduced and some tests are commercially available, for example, enzyme linked immunosorbent assay (ELISA)/enzyme immunoassay (EIA), latex agglutination assay and post-mortem organ diagnosis (investigating malaria parasites in tissue autopsy, e.g. liver and spleen, kidney and brain) have also been described. However, parasite culture, molecular techniques,
serology techniques and pathological diagnostic techniques, although sometimes useful in research laboratories, but are not practical or appropriate for the routine clinical diagnosis of malaria (Sachanonta et al., 2008).
Materials and methods

Study population

The present study was conducted on a total of 600 cases from Fayoum governorate during a period of 13 months from March 2013 to March 2014. Out of these 600 cases; 500 cases were collected randomly from inhabitants of Abo – Shanab and EL-Khaldia villages of Abshoy District. The remaining 100 cases were selected from Fayoum Fever Hospital and were presenting with symptoms suggestive of malaria as headache, fever or darkening of urine.

Sampling

Blood samples were collected from all cases included in the study. Samples were collected in sterile syringes labeled with patient name and date of collection.

Thin and thick blood films were immediately prepared and stained. The remaining whole blood samples were transferred to clean sterile dry tubes containing EDTA. Blood specimens were stored at 2°C-8°C for up to 3 days or at -20°C for longer storage.

Plan of work

All cases included in the present study were subjected to the following:

1. History taking, symptoms and clinical examination.
2. Laboratory examination (blood film) for detection of different malarial stages.
3. Immunological methods: by malaria pf/pan one step rapid test for detection of plasmodium antigens in blood samples was performed only for 100 blood samples obtained from 100 selected cases in Fayoum Fever Hospital.
1- History taking and clinical examination.

Clinical data were obtained from each case in the present study according to a clinical sheet that included the following items:

- Personal history: name, age, sex and residence.
- History of travelling abroad.
- Present history (symptoms): rigors, fever, sweating, headache and darkening of urine.
- History of intake anti-malaria drug.
- General examination for pallor and jaundice.
- Abdominal examination for detection of splenomegaly.

2- Laboratory examination

(I) Giemsa staining preparation: (Clendennen et al., 1995).

Reagents:

1. **Giemsa Stain**: Concentrated liquid stock.
2. **Absolute Methanol**: acetone-free.
3. **Phosphate Buffer Solution pH 7.2 (Giemsa buffer)**: used for dilution of Giemsa stock.

Preparation of Phosphate Buffer Solution (Giemsa buffer):

Phosphate buffer solution (67 mmol / L, pH 7.2) was prepared according to Hawkey and Lewis (2004) as follow:

1. **Solution A**: was prepared by adding 9.5 grams of disodium hydrogen phosphate (anhydrous salt) (Oxford Lab., India) to 1 liter of distilled water to obtain 67 mmol / L Na$_2$HPO$_4$. 

2. **Solution B:** was prepared by dissolving 9.2 grams of sodium dihydrogen phosphate (Oxford Lab., India) in 1 liter of distilled water to obtain 67 mmol/L NaH$_2$PO$_4$.

3. **Phosphate Buffer Solution** was prepared by mixing 72 ml of solution A with 28 ml of solution B and 900 ml of distilled water.

   Working solution was prepared by adding 1 part of stock Giemsa solution to 20 parts of phosphate buffer (pH 7.2). The prepared buffer should be clear with no precipitates.

Blood samples were collected from all cases included in the present study and examined (Chotivanich *et al.*, 2006).

1. A clean glass slide was prepared.
2. Date, name and age of patient was written on slide.
3. The patient's finger was cleaned with 70% ethyl alcohol and allowed to dry.
4. The side of fingertip was picked with a sharp sterile lancet to obtain blood drops.

**(II) Preparation of thin blood film:** (Cheesbrough, 1999).

A- The smooth edge of a spreader slide was placed in a drop of blood.

B- The angle between slide and spreader was adjusted to 45°.

C- The blood was smeared with a swift and steady sweep along the surface.

D- The film was allowed to air-dry and was fixed with absolute methanol.

E- The sample was stained with diluted Giemsa (1:20, vol/vol) for 20 min.

F- The sample was washed by briefly dipping the slide in and out of a jar of buffered water.
G- The slide was then allowed to air-dry in a vertical position and examined under a light microscope X100.

(III) Preparation of thick blood film: (Salako et al., 1999)

A- A blood spot was stirred in a circular motion with the corner of the slide.
B- Blood spot was allowed to dry without fixative.
C- The spot was stained with diluted Giemsa (1 : 20, vol/vol) for 20 min.
D- The slide was washed by placing the film in buffered water for 3 Min.
E- The slide was allowed to air-dry in a vertical position and was examined using a light microscope X1000.

3- Immunological methods:

Detection of *Plasmodium falciparum* and non-*falciparum* Plasmodium antigens was done using the commercially available malaria pf/pan one step rapid test[Abon Biopharm (Hangzhou) co., Ltd, China].

The Malaria (Pf/Pan) One Step Rapid Test is a lateral flow chromatographic immunoassay for the simultaneous detection and differentiation of antigens of Plasmodium species in human blood samples or serum samples.

Test principle (Cooke et al., 1999)

The Malaria (Pf/Pan) One Step Rapid Test is a lateral flow chromatographic immunoassay. The lysis buffer contains a detergent that lyses the red blood cells and releases various *Plasmodium* antigens, which migrate by capillary action across the strip held in the cassette. If plasmodium histidine rich protein-II (pHRP-II) is present in the specimen, it will bind to the pHRP II-gold conjugates. The immunocomplex is then captured on the membrane by the pre-coated anti-pHRP-II antibodies, forming a burgundy colored Pf band, indicating a *plasmodium falciparum* positive test result.
If pLDH (plasma lactate dehydrogenase) is present in the specimen, it will bind to the pLDH gold conjugates. The immunocomplex is then captured on the membrane by the pre-coated anti pLDH antibody, forming a burgundy colored band, indicating a *Plasmodium* positive test result. In the absence of pf band, a positive test result for any of the other three *Plasmodia* can be recommended.

**Reagents**

The *Malaria (Pf/Pan) One Step Rapid Test* comprises the following:

1) A burgundy colored conjugate pad containing mouse anti- pHRP-II antibody conjugated with colloid gold (pHRP II-gold conjugates) and mouse anti-pLDH antibody conjugated with colloid gold (pLDH-gold conjugates)

2) A nitrocellulose membrane strip containing two test bands (pf and pan bands) and a control band (C band). Pf band is pre-coated with monoclonal anti-pLDH antibody and polyclonal anti-pHRP-II antibodies by which the infection with *Plasmodia falciparum* can be detected, the pan band is precoated with monoclonal anti-pLDH antibody and polyclonal anti-pHRP-II antibodies by which the infection with *Plasmodium vivax, Plasmodium ovale* or *Plasmodium malariae* can be detected. While the control band (C band) is coated with goat anti-mouse IgG.

3) Pipette dropper

4) Desiccant

5) Buffer

6) Package Inser

7) Timer

8) Lancing device for whole blood test.
**Methods** (According to the manufacturer instructions)

The specimen and test components were brought to room temperature before use. then the following steps were done:

1- The specimen was mixed well prior to assay.

2- The pouch was opened at the notch and device was removed. the test device was Placed on a clean, flat surface.

3- The device was labeled with specimen’s ID number

4- The mini plastic dropper was filled with 10 µL of blood specimen

5- The dropper was hold vertically, the entire specimen was dispensed into the center of the sample well -1(w1) making sure that there were no air bubbles.

6- Three drops (about 100-150 µL) of Lysis Buffer were added to W2

7- After five minutes, 1 full drop of buffer was added to W1.

8- The results were read after 15 minutes

**Interpretation of results**

- Presence of C band was indicator of validity of the test.

- Positive test for *Plasmodium falciparum* infection was indicated by development of pf band In addition to C band.

- Positive test for *Plasmodium vivax, Plasmodium ovale* or *Plasmodium malariae* was Indicated by development of pan band in addition to C band.

- Negative test for all species was indicated by absence of pf and pan bands in addition to the presence of C band.
Figure (1): Malaria rapid test device positive for *Plasmodium falciparum*.

Figure (2): Malaria rapid test device negative for all *Plasmodium* species.
Materials and methods

Figure (3): Malaria p.f / pan rapid test device.
RESULTS

The present study was conducted on a total of 600 cases from Fayoum governorate during a period of 13 months from March 2013 to March 2014. Out of these 600 cases, 500 cases were randomly from inhabitants of Abo–Shanab and EL-Khaldia villages of Abshoy District in a random manner. The remaining 100 were selected from Fayoum Fever Hospital and were presenting with symptoms suggestive of malaria as headache, fever or darkening of urine. The data collection lasted for six months from June 2013 to December 2013.

The examined populations were subjected to complete history taking and detailed general examination. Blood samples were collected from all cases included in the study and were subjected to thin and thick blood films in addition to malaria pf/pan one step rapid test aiming at detection of malaria antigens.

All data were collected and statistically analysed and presented as follow:

I. Demographic criteria of the examined population.
II. Clinical history.
III. Clinical examination.
IV. Results of thin and thick blood films.
V. Results of malaria pf/pan one step rapid test.
Demographic criteria of examined populations:

Age distribution

The examined population's ages ranged from 1 to 90 year. The mean (average) age for all 600 cases participated in the study was 23.73±17.89 years old.

The mean (average) age of household cases was 23.30 ± 17.70. Two hundreds and thirteen of them (42.6%) were below eighteen years old and 287 (58.4%) were above eighteen years old as shown in table (5) and Figure (4). As regard 100 selected cases it was found that ages ranged from 1 to 83 years with mean of 25.89±18.70 years old. Twenty nine of selected cases (29%) were below eighteen years old and 71 of them (71%) were above eighteen years old as shown in figure (5) and table (6).

Table (5): Age distribution of household cases.

<table>
<thead>
<tr>
<th>Age</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Child</td>
<td>213</td>
<td>42.6%</td>
</tr>
<tr>
<td>Adult</td>
<td>287</td>
<td>58.4%</td>
</tr>
<tr>
<td><strong>Total examined populations</strong></td>
<td><strong>500</strong></td>
<td><strong>100 %</strong></td>
</tr>
</tbody>
</table>

Figure (4): Age groups of the household cases
RESULTS

Table (6): Age distribution of selected cases.

<table>
<thead>
<tr>
<th>Age</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Child</td>
<td>29</td>
<td>29%</td>
</tr>
<tr>
<td>Adult</td>
<td>71</td>
<td>71%</td>
</tr>
<tr>
<td>Total examined</td>
<td>100</td>
<td>100%</td>
</tr>
<tr>
<td>populations</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure (5): Age groups of the selected cases

Gender distribution:

Among the household cases, 160 (32%) were males and 340 were females (68%) as shown in table (7) and figure (6). While among the 100 selected cases, 16 of them (16%) were males and 84 (84%) were females as shown in figure (7) and table (8).
RESULTS

Table (7): Gender distribution of household cases.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>male</td>
<td>160</td>
<td>32%</td>
</tr>
<tr>
<td>female</td>
<td>340</td>
<td>68%</td>
</tr>
<tr>
<td>Total examined populations</td>
<td>500</td>
<td>100 %</td>
</tr>
</tbody>
</table>

Figure (6): Gender distribution of household cases.

Table (8): Gender distribution of the selected cases.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>16</td>
<td>16%</td>
</tr>
<tr>
<td>Female</td>
<td>84</td>
<td>84%</td>
</tr>
<tr>
<td>Total examined populations</td>
<td>100</td>
<td>100 %</td>
</tr>
</tbody>
</table>
History of travelling to malaria endemic area:

Out of 500 household cases, 80 cases (16%) gave a history of travel to Sudan as shown in table (9). Nine persons (9%) of the selected cases from Fayoum Fever Hospital gave a history of travel to Sudan as shown in figure (8).

Table (9): Number and percentage of household cases with history of travel to Sudan.

<table>
<thead>
<tr>
<th>Travelling abroad</th>
<th>Frequency (Total= 500)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>80</td>
<td>16%</td>
</tr>
<tr>
<td>No</td>
<td>420</td>
<td>84%</td>
</tr>
<tr>
<td>Total examined population</td>
<td>500</td>
<td>100 %</td>
</tr>
</tbody>
</table>
RESULTS

Figure (8): History of travelling abroad in the selected cases

**History of intake anti-malaria drug:**

Out of 500 household cases, 120 cases (24%) gave a history of intake anti-malaria drug as shown in table (10).

Chloroquine was given to 100 cases (83%), 15 cases received Coartem (12%) and 5 cases (4%) received Larum as shown in table (11) and Figure (9). Regarding the 100 selected cases, only 3 cases (3%) received Coartem. These data are demonstrated in figure (10).

Table (10): History of intake of anti-malaria Drug in household cases

<table>
<thead>
<tr>
<th>Intake of anti- malaria drug</th>
<th>Frequency (Total= 500)</th>
<th>Percent.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>120</td>
<td>24%</td>
</tr>
<tr>
<td>No</td>
<td>380</td>
<td>76%</td>
</tr>
<tr>
<td>Total examined patients</td>
<td>500</td>
<td>100%</td>
</tr>
</tbody>
</table>
Table (11): Frequency of intake of anti-malaria drug in household cases

<table>
<thead>
<tr>
<th>Name of the drug</th>
<th>Frequency (Total= 120)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroquine</td>
<td>100</td>
<td>83%</td>
</tr>
<tr>
<td>Coartem</td>
<td>15</td>
<td>12%</td>
</tr>
<tr>
<td>Larum</td>
<td>5</td>
<td>4%</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>100%</td>
</tr>
</tbody>
</table>

Figure (9): Drug intake in the household cases.
III-Symptoms detected by history taking and clinical examination of the examined population incorporated in the study.

Out of 500 population, 416 persons (83.2%) had elevated body temperature, 80 persons (16%) had rigors, 47 persons (9.4%) had sweating, 18 persons (3.6%) had darkening of urine with no cerebral coma (0%) as shown in figure (11). As regard the 100 selected cases, 99 (99%) had elevated body temperature, 19 persons (19%) had rigors, 12 persons (12%) had sweating, 4 persons (3.6%) had darkening of urine; while cerebral coma was absent (0%) as shown in figure (12).
RESULTS

Figure (11): Clinical symptoms detected in the household cases

Figure (12): clinical symptoms detected in the selected cases.
RESULTS

Signs detected by clinical examination of populations incorporated in the study:

Out of 500 population 43 persons (8.6%) had splenomegaly, 33 person (6.6%) had hepatomegaly and 329 persons (65.9%) had pallor as shown in table (12) and figure (13).

As regard 100 selected cases, it was found that 12 persons (12%) had splenomegaly, 11 person (11%) had hepatomegaly and 80 persons (80%) had pallor as shown in figure (14) and table (13).

Table (12): Clinical signs detected in household cases

<table>
<thead>
<tr>
<th>Clinical signs</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Splenomegaly</td>
<td>43</td>
<td>8.6%</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>33</td>
<td>6.6%</td>
</tr>
<tr>
<td>Pallor</td>
<td>329</td>
<td>65.9%</td>
</tr>
</tbody>
</table>

Figure (13): Clinical signs detected in household case

Figure (14): Clinical signs detected in selected cases
Table (13): Clinical signs detected in the selected cases

<table>
<thead>
<tr>
<th>Clinical signs</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Splenomegaly</td>
<td>12</td>
<td>12%</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>11</td>
<td>11%</td>
</tr>
<tr>
<td>Pallor</td>
<td>80</td>
<td>80%</td>
</tr>
<tr>
<td>Total of populations</td>
<td>80</td>
<td>80%</td>
</tr>
<tr>
<td>have clinical signs</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure (14): Clinical signs in the selected cases
Results of microscopic examination of blood films

Blood samples were taken from all cases in the study. Thick and thin blood films were prepared, stained with Giemsa and examined under oil immersion lens at magnification of 1000 X. Microscopic examination of blood films of the 500 cases revealed absence of *Plasmodium* stages in all cases (100%). Ring stage of *Plasmodium falciparum* detected by thick blood film of one of the 100 selected cases as shown in figure (15).

Figure (15): Thick blood film showing ring stage of *Plasmodium falciparum* (magnification X1000).
Results of immunological test (One step rapid test):

Blood samples were obtained from 100 selected cases from Fayoum Fever Hospital and were tested using malaria (Pf/Pan) One Step Rapid Test to detect *Plasmodium falciparum* antigen and other *Plasmodium* species. Three cases (3%) were positive for *Plasmodium falciparum* antigen as shown in figure (18) figure (20), but were negative for all species as shown in figure (19).

![Bar chart showing 97% negative and 3% positive results](image)

Figure (16): malaria (Pf/Pan) One Step Rapid Test applied for 100 selected cases
Figure (17): Malaria rapid test negative for all *Plasmodium* species.

Figure (18): Malaria rapid test positive for *Plasmodium falciparum*. 
RESULTS

Distribution of positive cases as regard different characteristics (history and clinical examination) (N=3)

Regarding demographic criteria of positive cases, it was found that the mean (average) age was 32.7 ±11.2 and all cases were males. Regarding travelling abroad, it was found that they came from Sudan after one visit. Regarding clinical symptoms of positive cases it was found that all three cases (100%) presented with fever, rigors and sweating with absence of darkening of urine, cerebral coma, hepatomegaly and splenomegaly. All these are shown in table (14) and figure (21).

Table (14): Distribution of positive cases as regard different characteristics (history and clinical examination) (N=3)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>32.7</td>
<td>11.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender</td>
<td>3</td>
<td>100.0</td>
</tr>
<tr>
<td>Fever</td>
<td>3</td>
<td>100.0</td>
</tr>
<tr>
<td>Rigor</td>
<td>3</td>
<td>100.0</td>
</tr>
<tr>
<td>Sweating</td>
<td>3</td>
<td>100.0</td>
</tr>
<tr>
<td>Dark urine</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Coma</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Hepatomegally</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Splenomegally</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>History of drug taking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coartem for 4 weeks</td>
<td>3</td>
<td>100%</td>
</tr>
</tbody>
</table>
Statistical Analysis

- Data was collected, coded, translated to English to facilitate data manipulation and double entered into Microsoft Excel and data analysis was performed using SPSS software version 22 under windows 7.
- Simple descriptive analysis in the form of numbers and percentages for qualitative data, and arithmetic means as central tendency measurement, standard deviations as measure of dispersion for quantitative parametric data was done.
Discussion

About 3.3 billion people; half of the world's population are at risk of malaria leading to about 250 million malaria cases and nearly one million deaths every year. People living in the poorest countries are the most vulnerable (WHO, 2009). Forty-one percent of the world's population lives in areas where malaria is transmitted (e.g., parts of Africa, Asia, the Middle East, Central and South America) (WHO, 2002).

The present study was conducted on a total of 600 cases from Fayoum governorate during a period of 13 months from March 2013 to March 2014. Out of these 600 cases, 500 cases were selected from inhabitants of Abo – Shanab and EL-Khaldia villages of Abshoy District in a random sampling. The remaining 100 were selected from Fayoum Fever Hospital and were chosen from those having symptoms suggestive of malaria as headache, fever or darkening of urine.

All cases included in the present study were subjected to history taking, clinical examination and laboratory examination (thin and thick blood film) for detection of different malaria stages. Also, immunological method was done by malaria pf/pan one step rapid test for detection of Plasmodium antigens in blood samples collected from 100 selected cases in Fayoum Fever Hospital.

Blood samples were collected from all cases included in the study. Samples were collected in sterile syringes labeled with patient name and date of collection. For laboratory examination of samples thin and thick blood films were immediately prepared and stained. The remaining whole blood samples were transferred to clean sterile dry tubes containing EDTA. Blood specimens were stored at 2°C-8°C for up to 3 days or at -20°C for longer storage.

Regarding age and gender, the examined cases ranged from 1 to 90 years with average age of 23.73±17.89 years old. One hundred and sixty of participants (32%) were males and three hundred and forty (68%) were females in the
household cases and as regard 100 selected cases, Sixteen (16%) were males and eighty four (84%) were females.

As regard gender and age of exposure to malaria; Rahman et al., (1993) showed that males above 18 years old were more exposed to malaria than females and those less than 18 years old were less exposed.

Canada statistics 2013 analysis indicated that the rate of imported Plasmodium infection in males was twice that of females(Nelder et al., 2013). Also, predominance of men in imported malaria is well represented, for example Western Australia, 80% of all cases are men (Baas, 2006); Amsterdam, the Netherlands(69%)(Smith, 2008).

Risk of infection is often greater in men because they are more likely to migrate to malaria-endemic areas for agricultural or mining work and more likely to sleep outdoors during peak biting times of vector (Reuben, 1993).

Out of 500 household cases, 80 (16%) gave history of travel to malaria endemic areas in addition to 9 cases of selected cases from Fayoum Fever Hospital. All these cases gave history of travel to Sudan. Malaria appears to have been caught by travel of persons to endemic country (Sudan) for working as in the present study, all the three positive cases detected in the present work gave a history of traveling to Sudan and this was in accordance with the Malaria Control Program reports that published on WHO website in 2008. In this report, 23 cases of malaria were discovered in Egypt in 2005, but they were imported from Sierraleon and Sudan. They came immediately from the quarantine so no period of relapse.

In contrast, the researchers in the Military Fever Hospital, Egypt diagnosed thirty six patients as having malarial disease. Twenty of them were recruited from Peace Keeping Mission Forces in Africa and sixteen cases presented with prolonged fever coming from different locations in Egypt. Their results showed that two locally acquired cases were malaria positive. The detection of these two
locally acquired cases in this study was diagnosed by the use of bone marrow smears as they were negative by peripheral blood examination and that El-Gabal El-Ahmar area (Cairo) was the most extensively infected region (37.4%). El-Sharkia and El-Fayoum Governorates were next in order (18.7%) and (12.5%). (El-Bahnasawy et al.,2010)

Malaria is an important threat not only for autochthonous populations, but also for non-immune individuals travelling or working in malaria endemic areas. According to the 2011 international travel and health book; approximately 125 million international travellers visit malaria-endemic countries yearly and over 10,000 cases are reported having malaria after returning home (WHO, 2011).

The incidence of imported malaria cases among UK travellers visiting West Africa varied from 52 to 196 cases/1,000 traveller per year between 2003 and 2006 (Behrens et al.,2008). In a cohort of the French general population, followed from 1994 to 1998, the incidence of malaria imported from endemic areas was 178 cases per 1,000 travellers per year (Malvay et al., 2006).

In the French Armed Forces, the annual incidence rate was 14 per 1,000 people per year in 2006. Amongst French soldiers who served in Ivory Coast between 1998 and 2007, the annual malaria incidence rate ranged from 37 to 388 cases per 1,000 people per years (Gaeten et al., 2013).

Non-immune travellers should be protected from malaria by chemoprophylaxis and prophylactic measures against mosquito bites (including insecticide-impregnated bed nets, repellents and insecticide-treated long-sleeved clothes and pants). In malaria-endemic areas, the use of most of these prophylactic measures is mandatory for non-immune employees of most major international groups and soldiers.

However, the effectiveness of these measures is limited by the lack of compliance (Sagui et al.,2011) even among military personnel (Michel et al.,2007) and even if
the chemoprophylaxis is adapted to the chemosusceptibility of *Plasmodium falciparum* (Henry, 2007).

The increased number of malaria cases, occurring among traveller populations, has been frequently attributed to behavioral factors. Machault et al., (2008) stated that the lack of compliance with protective measures was identified as the second most important factor that determined the malaria incidence rate among non-immune travellers after environmental factors.

In Fayoum Governorate, it seems that malaria control achieved significant progress when widespread indoor residual spraying (IRS) with long-lasting insecticides and introduction of artemisinin combination therapy with artemether-lumefantrine (Coartem®) and this led to absence of clinical cases of endogenous malaria and interruption of malaria transmission (MOHP, 2006).

As regard the symptoms of malaria, out of 500 household populations, four hundreds and sixteen persons (83.2%) had elevated body temperature, eighty person (16%) had rigors, forty seven persons (9.4%) had sweating, eighteen persons (3.6%) had darkening of urine and none with cerebral coma (0%).

As regard the 100 selected cases, ninety-nine (99%) had elevated body temperature, 19 person (19%) had rigors, twelve persons (12%) had sweating, four persons (4%) had darkening of urine and none with cerebral coma (0%).

Malaria paroxysms are defined by intense chills, fever and sweating caused by new merozoites burst from the erythrocytes and infect more cells (Sadanand, 2010).

The study was in line with (Genton and D’ Acremont, 2001) who said that in general, the majority of patients experience fever (>92%), chills (79%), headaches (70%), and diaphoresis (64%).

In the present study, fever was a constant symptom in malaria positive patients and this was in accordance with the universal screening symptom for malaria in
research studies (D’Acremont et al., 2010). In Fayoum, where the present study was carried out, local clinical officers state that they must inquire about fever to capture all cases of malaria.

On the contrary; Murray et al., (2007) stated that fever is not always a feature of malaria, and signs may be unusual if prophylaxis has been given, and is partly effective. Also; Sakaria et al., (2013) stated that the classic presentation of malaria with paroxysms of fever is seen only in 50-70% of the patients. In addition, Abdel-Wahab et al., (2012) studied the use of fever alone as a presumptive prompt for anti-malarial treatment would result in a huge over-treatment burden.

Bejon et al., (2010) reported that the clinical signs of malaria may be nonspecific and parasitaemia accompanied by clinical symptoms consistent with malaria does not necessarily imply clinical malaria especially in endemic areas.

In addition, Yaw et al.,(2014) stated that in non-endemic areas, peripheral parasitaemia accompanied by fever could be used to define clinical malaria. In endemic areas this is not so since over 60% of individuals could always have asymptomatic parasitaemia. Illnesses such as typhoid fever, which companying fever, could be confuse with clinical malaria because of accompanying parasitaemia.

Out of 500 population forty three persons (8.6%) had splenomegaly, thirty three person (6.6%) had hepatomegaly and three hundred and twenty-nine persons (65.9%) had pallor and as regard 100 selected cases, it was found that twelve persons (12%) had splenomegaly , eleven person (11%) had hepatomegaly and eighty persons (80%) had pallor.

There was absence of hepatomegaly , splenomegaly , jaundice and cerebral coma in the three positive cases, which had been reported in the literatures as common signs associated with a complication of falciparum malaria (WHO,2010) and
Discussion

this was in contrast to El-Bahnasawy et al., (2010). Their study was conducted in Military Fever Hospital, Egypt where they found that cerebral coma occurred and one patient died. This difference in the apparent complication maybe due to early diagnosis and treatment by the use of multi-drug regimen (Coartem) in our study in contrast to the monotherapy based one such as Chloroquine or Mefloquine.

Of particular interest was the high percentage of pallor (80%) among the selected cases but this was not due to malaria as pallor was absent in the three positive cases. Pallor is most probably due to anemia due to low socioeconomic levels.

The lack of classical severe malaria syndromes in malarial patients has important implications for the surveillance of malaria, considering that many healthcare facilities with limited resources throughout sub-Saharan Africa often rely on clinical presentation and the ascertainment of coma, seizures and renal failure for severe malaria diagnosis and management decisions in adults (Reyburn et al., 2004). The data confirm the emphasis of WHO that reliance on clinical features alone for the diagnosis of severe malaria is inaccurate (WHO, 2010).

As regard intake of chemoprophylaxis regimen in the 500 random population, it was found that 105 (17%) of cases had a history of intake of chemoprophylaxis regimen in the form of Chloroquine in 100 cases (95%), and Larum (Mefloquine hydrochloride) 5 cases (5%). Because of its long half-life, mefloquine is the preferred chemoprophylactic agent against *Plasmodium falciparum* malaria in UNMIS. However, its side effects, which include vomiting, dizziness, syncope, extra systoles, tinnitus, and emotional problems, cause some people refuse or cease mefloquine.

In the present study, some of population (3%) received antimalarial drug; Coartem (artemether-lumefantrine ) as a therapeutic agent and this was in line with WHO
guidelines that documented that artemisinin derivatives should not be used as monotherapies for the treatment of uncomplicated malaria because this can promote resistance to this critically important class of antimalarial agents (WHO, 2011). However, in a randomized single-blinded clinical trial, dihydroartemisinin-piperaquine was found to be superior to artemether-lumefantrine for reducing the risk of recurrent parasitemia and gametocytemia. It also fostered better hemoglobin recovery in the treatment of uncomplicated *Plasmodium falciparum* malaria (Kamya et al., 2007). In contrary to this, El-Bahnasawy et al.,(2010) said that monotherapy such chloroquine or mefloquine is drug of choice for local cases.

Malaria diagnosis has for a long time, and particularly at community level, depended on clinical diagnosis. However, this is unreliable due to the non-specific nature of signs and symptoms of malaria leading to over-diagnosis and over-treatment (Reyburn, 2010). Misdiagnosis can lead to inappropriate or delayed treatment that has been implicated in malaria-associated deaths in developed countries (Kain and Keystone,199).

Our results showed that the differences in detection rates of microscopy and ABON PLUS (RDT) test are 0.16%, and 3% respectively. On the other hand, reported studies from different countries of South Asia: Sri Lanka (Fernando et al., 2004), Pakistan (Iqbal et al., 2003) and Thailand. (Pattanasin et al.,2003) that demonstrated 38%, 42%, 53% malaria positive cases were diagnosed among studied groups using microscopy and RDTs. Also;Abdel-Wahab et al.,(2012) showed that the differences in detection rates of microscopy, RDT test (45%, 42.5% respectively) in 120 clinically suspected cases.

Species differentiation in the positive samples by blood film examination showed presence of *P.falciparum* in travellers coming from Sudan and this coincides with reports on the high levels of *P. falciparum* disease activity in Sudan.
Accurate identification of malaria parasites to the species level is imperative so that the patient receives appropriate therapy, particularly when the patient has relapsing malaria caused by *P. vivax* and *P. ovale* It is also important because of the severe morbidity and mortality associated with *P. falciparum* and growing resistance to antimalarial therapy. Furthermore, it is vital to obtain follow-up specimens from malaria-positive patients to monitor therapy outcome and detect drug failure (Palmer *et al.*, 2003).

Self-diagnosis of febrile illness with reliable malaria RDTs could accelerate early therapy with the standby treatment, preventing complications and death, or avoid unnecessary use of antimalarial (Magill, 2006).

In the present study, the three cases were positive for *Plasmodium falciparum* by malaria pf/pan one step rapid test in contrast to the thick smears which gave one positive result.

In general, the screening of Giemsa stains (GS) by light microscopy is still considered the gold standard (Jonkman, 1995). Microscopy is the preferred diagnostic test for patients with severe febrile illness. This method is cheap and simple but labor intensive and time consuming and requires well-trained personnel (Reyburn, 2004), particularly for the detection of low levels of parasites (Gilles, 1993). In resource-poor areas, microscopic diagnosis has been shown to be insensitive and nonspecific, especially when parasitemias are low or mixed infections are present (Amexo, 2004, ). Because sensitivity of RDTs is low when the parasite density <100/μl, the diagnosis of afebrile or atypical malaria with low parasitemia mainly depended on microscopy (Moody, 2002). The sensitivity of thick-film microscopy is 10 to 30 parasites/μl of blood (Gilles, 1993). Therefore, lack of personnel experience in microscopic diagnosis of malaria may bring the possibility of high false negative rates in the microscopy test.
In the present study, positive RDT with negative blood films may be explained by treatment that clears parasitaemia with persistent of antigenaemia. (WHO, 2004). Other possible reasons include persistence of antigens due to sequestration of malaria parasites from peripheral blood (Reyburn, 2006), incomplete treatment, delayed clearance of circulating antigen (free or in antigen-antibody complexes), and cross reaction with non- \textit{falciparum} malaria, rheumatoid factor or heterophile antibodies (Moody and Chiodini, 2002).

Thus, the slightly better RDT performance in our study is most likely due to two facts. First, the study population consisted of non-immune, returning Sudan travelers who typically have higher parasitemia levels than other studies’ populations in malaria endemic areas. Second, we compared the RDT to single blood smears performed under “real world” conditions (rather than the gold standard three tests read by expert malariologists) and finally hemolysis of the blood during transport.

On the contrary, the successful implementation of RDT has been bedeviled by poor product performance, inadequate methods to determine the quality of products (Bell et al., 2006). Another group described the limitations of RDTs as having: the inability to diagnose non \textit{falciparum} malaria, variable heat stability and safety risks related to blood sampling (especially HIV and hepatitis B). Also of equal concern is that negative RDT results are often ignored and patients are treated anyway (Christopher et al., 2008).

Therefore, it is important that the instruction for use (IFU) clearly mentions that reading test results should be performed within the time specified in the IFU and any test line becoming visible beyond the recommended reading time should be ignored and this occurs clearly For One Step even reading a few minutes too late resulted in some false positive \textit{P. falciparum} test lines observed by the second observer and explaining the low interobserver agreement.
False negative results of RDTs have been attributed to possible genetic heterogeneity of HRP2 or LDH expression, deletion or mutation of HRP2 or LDH gene, presence of blocking antibodies, or immune-complex formation and also inability of Optimal test to detect parasitaemia levels below 100 parasites/μl of blood (Chaijaroenkul, 2011).

However, limitations should be considered in this study. The present study used a limited and selected number of samples, precluding calculation of predictive values and providing wide confidence intervals for non-\textit{falciparum} results. Besides, the present study has not used a collection of samples comprising all four human \textit{Plasmodium} species at different parasite densities for providing relevant data on diagnostic accuracy.
SUMMARY AND CONCLUSION

Malaria infection is a life threatening protozoan infection and is associated with several adverse health outcomes. Almost 300 million clinical cases of malaria occur worldwide each year and over a million people die. Almost 90% of these deaths occur in sub-Saharan Africa, where young children are the most affected. Malaria is directly responsible for one in five childhood deaths in Africa and indirectly contributes to illness and deaths from respiratory infections, diarrhoeal disease and malnutrition. Fayoum governorate had the last focus of malaria which has been eradicated from Egypt since 1998 according to WHO records.

The present study was designed to study the prevalence of malaria in some localities in Fayoum governorate; in addition to study the demographic criteria of the examined population incorporated in this study and to determine the likelihood of acquisition of malaria infection in this area using thin and thick blood film, in addition to malaria pf/pan one step rapid test to detect plasmodium antigen in blood samples.

Blood samples were collected randomly from 500 inhabitants of Abo—Shanab and EL-Khaldia villages of Abshoy district and 100 selected cases from Fayoum Fever Hospital in Fayoum governorate, Egypt during a period of 13 months from March 2013 to March 2014. Thin and thick blood films were immediately prepared and stained.

All cases included in the present study were subjected to history taking, clinical examination, laboratory examination (blood film) for detection of different malaria stages and immunological method by malaria pf/pan one step rapid test for detection of plasmodium antigens in blood samples was carried out only to the 100 selected cases in Fayoum Fever Hospital.

In the current work, the examined populations ages ranged from 1 to 90 years. The mean (average) age for population participated in the study was 23.73±17.89 years old.
As regard the 500 household cases, 213 of participants (42.6%) were below eighteen years old and 287 of them (58.4%) were above eighteen years old, 160 of them (32%) were males and 340 were females (68%). As regard the 100 selected cases it was found that ages ranged from 1 to 83 years with mean (average) age 25.89±16.43 years old. Twenty nine of them (29%) were below eighteen years old and seventy one (71%) was above eighteen years old. Sixteen (16%) were males and eighty four (84%) were females.

History of travelling abroad to malaria endemic area showed that eighty populations (16%) have been traveled and as regard the 100 selected cases it was found that nine case (9%) have been travelled to Sudan.

History of intake anti-malaria Drug showed that one hundred and twenty populations (24%) had a history of intake antimalaria drug out of one hundred and twenty persons received malaria drug, 100 person (84%) received chloroquine, 5 persons (4%) received larum and 15 persons (12%) received coartem and as regard 100 selected cases it was found that 3 populations (3%) received Coartem.

Symptoms detected by history taking and clinical examination showed that out of the 500 household cases, it was found that 416 persons (83.2%) had elevated body temperature, 80 person (16%) had rigors, 47 persons (9.4%) had sweating, 18 persons (3.6%) had darkening of urine with no cerebral coma (0%). Regarding the 100 selected cases, 99 (99%) had elevated body temperature, 19 person (19%) had rigors, 12 persons (12%) had sweating, 4 persons (4%) had darkening of urine; while cerebral coma was absent (0%).

Signs detected by clinical examination among the household cases showed that it was found that 43 persons (8.6%) had splenomegaly, 33 person (6.6%) had hepatomegaly and 329 persons (65.9%) had pallor. As regard the 100 selected cases, it was found that 12 persons (12% have splenomegaly, 11 person (11%) had hepatomegaly and three 380 persons (80%) had pallor.

Demographic criteria of the three positive cases showed that the mean (average) age was 32.7 ±11.2 and all cases were males. Regarding clinical symptoms of positive cases it was found that all three cases (100%)
presented with fever, rigors and sweating with absence of darkening of urine, cerebral coma, hepatomegaly and splenomegaly.

Microscopic examination of thin and thick blood films obtained from the 500 house hold cases showed absence of *Plasmodium* stages while microscopic examination showed ring stage of *Plasmodium falciparum* in one case in the 100 selected cases. Using the malaria (pf/pan) rapid step test among the 100 selected cases, it was found that three cases detected as positive for *Plasmodium falciparum* antigen. None of the cases are positive for other plasmodium species.

The results of the present study pointed to the prevalence of malaria infection in some localities of Fayoum governorate and this was among workers returning from Sudan. Also, RDT of malaria gave higher detection rate (3%) in comparison to thick and thin blood films results which showed lower detection (0.16%) for diagnosis of malaria.

In the present study, positive RDT with negative blood films may be explained by treatment that clears parasitaemia with persistent of antigenaemia. Other possible reasons include persistence of antigens due to sequestration of malaria parasites from peripheral blood, incomplete treatment, delayed clearance of circulating antigen (free or in antigen-antibody complexes), and cross reaction with non-*falciparum* malaria, rheumatoid factor or heterophile antibodies.
Malaria control programme should be strengthened along South Egypt and Fayoum governorate and Oases to prevent reintroduction of malaria.

Combination of RDT and blood film examination is recommended for accurate laboratory diagnosis of *Plasmodium* parasite. Thus, proper treatment and control of infection can be achieved.

PCR is needed to evaluate validity of ABON PLUS test in diagnosis of malaria.

Non-immune travellers should be protected from malaria by chemoprophylaxis and prophylactic measures against mosquito bites (including insecticide-impregnated bed nets, repellents and insecticide-treated long-sleeved clothes and pants).

Attention must be paid to prevention of mosquito vector of malaria in suspected areas.
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الملخص العربي

يعتبر مرض الملاريا من الأمراض الطفيلية التي تهدد حياة الأنسان، ولها عواقب صحية وخيمة، فهناك ما يقارب من 300 مليون حالة مصابة بهذا المرض غالبيتهم في أفريقيا حيث يُمكن الأطفال أكثر عرضة فيه. ومسؤل عن وفاة حالة في كل 5 حالات وفاة ولقد صمتت هذه الدراسة لمعرفة مدى انتشار الإصابة بمرض الملاريا في بعض مناطق محافظة الفيوم، وكذلك معرفة الصفات الديموغرافية لأشخاص الدراسة كما تم استخدام الفحص المجهرى لجميع حالات الدراسة واختبار وجود الخطوة السريع (الأبوين بس) للكشف عن انتخابات البلازموديوم في عينات الدم للمنطقة. من بين هذه الطريقة من مستشفى حميات الفيروس والمشتبه فيها الإصابة بالملاريا إكلينيكياً.

وقد تم فحص 600 شخص من بينهم 500 شخص اخذوا بطريقة عشوائية من قريتي أبو شنب والخالدية بالإضافة إلى 100 شخص اخذوا بطريقة منتسقة من مستشفى حميات الفيروس والمشتبه فيها الإصابة بالملاريا إكلينيكياً وتتراوح اعمار أشخاص الدراسة من 1 - 90 سنة، بتنوع عمر 35، 30 وعمر 17 ووجد من خلال الاستقصاء أن ما أقرب من 89 شخص (15%) قد سافروا إلى السودان وهي دولة موبوءة بالمملاريا ووجد أيضاً أن 120 شخص (24%) قد تناولوا أدوية مضادة للمملاريا منهم 100 شخص تناولوا الكلوروكوين (98%)، 15 (12%) تناولوا الكورتيم 5 أشخاص (4%) تناولوا البيرل، بينما تناول 3 أشخاص (3%) الكورتيم من المائة حالة.

والثانية لمستشفى حميات الفيروس.

وتبين من خلال الفحص الإكلينيكى أن ما يقرب من 43 شخص (8%) يعانون من تضخم بالحذاء، 33 شخص (6%) يعانون من تضخم بالكبد 309 شخص (51%) يعانون من شحوب الوجه بين أشخاص الدراسة المنزلية بينما وجد أن ما يقرب من 12 شخص (12%) يعانون من تضخم بالحوامل، 11 شخص (11%) يعانون من تضخم بالكبد، 80 شخص (80%) يعانون من شحوب الوجه بين أشخاص الدراسة المنتقاة من الحميات.

وتبين من خلال الفحص الإكلينيكى أن ما يقرب من 606 شخص (83%) يعانون من ارتفاع درجة الحرارة و 80 شخص (16%) يعانون من الرعى و 47% شخص يعانون من العرق مع غياب المضاعفات مثل الغلوية المخية بين أشخاص الدراسة المنزلية، و تبين من خلال الفحص الإكلينيكى أن ما يقرب من 99 شخص (99%) يعانون من ارتفاع درجة الحرارة و 19 (19%) يعانون من الرعى و 12 شخص (12%) يعانون من العرق في أشخاص الدراسة المنتقاة من الحميات.
من خلال الفحص المجهرى باستخدام الفحص المجهرى وذلك لجميع حالات الدراسة واختبار وحيد الخطوة السريع الذي يكشف الانتييجينات التي تنتجها طفيليات الملاريا أظهرت النتائج إصابة ثلاث حالات بالملاريا باستخدام اختبار وحيد الخطوة السريع (الآبون بلس) بمعدل (3%) ، وحالة واحدة منهم باستخدام الفحص المجهرى بمعدل (16.1%) ، وكانت الملاريا نتيجة الأصابة بطفيل الأفاطير، وكانت كل الحالات وافدة من السودان، ويعتبر هذا الفارق إلى أن العلاج قد أزال الطفيلي مع بقاء الانتييجينات أو انحصار الطفيلي من الدورة الطرفية أو نتيجة تفاعله مع معامل الروماتويد هو الجسم الهيتيروفيلية.
دراسة انتشار الإصابة بمرض الملاريا في بعض مناطق محافظة الفيوم

رسالة توطئة للحصول على درجة الماجستير في علم الطفاليات الطبية

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