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Microbiology

Clinical and Laboratory Characteristics in the Severe Form of Spotted Fever among Patients Admitted to Al-Thawra Hospital in El-Beida City-Libya

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Abstract **Original Research Article**

This work was carried out to investigate the changes in haematological and some physiological parameters among patients who diagnosis with symptoms related to spotted fever in El-Beida City, Libya. This study carried out in the 7 consecutive months (May-November 2018). Over all total number 129 were divided into two groups (adults and children), number 61 males and 30 females for adult, while children's number was 22 males and 16 female. From all patients, blood samples have been taking for multiple tests (these tests including complete blood count, liver function and kidney function). RBC count was statistically different compared to control group in children and adults subjects. Most RBC parameters including were significantly decreased in patients (adults and children/ male and female) compared to control subjects. Only level of mean corpuscular hemoglobin concentration (MCHC) was significantly increased in all patients. Results of WBC were in normal range for couple genders increased slightly without significantly changes except on percentage of lymphocytes that increased in adult's patients not in children. Results of platelets including mean platelets number (PLT), the volume occupied by platelets in the blood (PCT) and mean platelet volume (MPV) were significantly decreased in both female and male (adults and children). Mean levels of enzymes of liver in the serum of the female and male (adults and children) were significantly increased in patient's subjects. Kidney function parameters (creatinine and urea) were found more variable in children than in adults in term of statistically significant. Similar pattern of changable were noticed with the mean concentration of sodium, potassium, and chloride (children was more significantly than adults). The predisposing factors for severity were young age. Understanding these haematological/biochemical test are very helpful. Because, early clinical presentations of spotted fever infection include fever, headache, myalgia, and malaise and are difficult to distinguish from other infectious and non-infectious diseases.

Keywords: Clinical, laboratory characteristics, spotted fever El-Beida City and Libya.

INTRODUCTION

Spotted fever group Rickettsiae and Ehrlichiae are obligate intracellular gram-negative bacteria associated with arthropods, mainly ticks. While feeding, ticks can transmit these microorganisms to humans and animals [1]. Rocky Mountain spotted fever (RMSF) is the most lethal and most frequently reported Rickettsial illness in many centuries [2]. It is widely distributed throughout the eastern, south-central, and mid-western United States, corresponding to the distribution of the American dog tick and the Rocky Mountain wood tick, the expected tick vectors [3]. Mediterranean spotted fever (MSF) is a tick-borne disease caused by *Rickettsia conorii* that transmitted to humans by the brown dog tick *Rhipicephalus sanguineus*. It was first described a century ago as a disease that caused high fever and spots [4, 5]. Initial signs and symptoms of the couple (RMSF/MSF) include sudden onset of fever, headache, and muscle pain, followed by development of rash. The disease can be difficult to diagnose in the early stages, and without prompt and appropriate treatment it can be fatal [2]. MSF is endemic to the Mediterranean area, including northern Africa and southern Europe. Some cases were described in Algeria, Malta, Cyprus, Slovenia, Croatia, Kenya, Somalia, South Africa, and in

areas surrounding the Black Sea. Spotted fever cases have been confirmed as MSF by the use of molecular tools in Portugal, Italy, Malta, Greece, Croatia, Spain, France and South Africa [5]. In fact, an increasing number of regions have been reporting MSF cases, serologic techniques cannot distinguish among different rickettsiae species of the spotted group. Consequently, all rickettsioses with spotted fever group antibodies were considered to have MSF in countries where this disease was endemic. Early clinical descriptions that relied only on serologic test results were likely to include infections related to multiple rickettsial species and were probably not describing a unique entity [4]. Therefore, spotted fever is an emerging or a re-emerging disease in some countries. Although spotted fever is prevalent in the Middle East and Mediterranean, no reports for the prevalence of disease or presence of tick-borne pathogens are available or any studies on the epidemiology of this disease practically in Northeast of Libya in the mountain of Al-Jebel Al-Akhdar (The Al-Jebel Al-Akhdar is a heavily forested, fertile upland area). Due to presence of tick, *Rickettsia*, symptoms related to spotted fever during seasonal activities of the tick and lack of studies on this disease in the Eastern region of Libya. The objective of this study was aimed to find out correlation between prevalence of hospitalized patients, serological tests and pomological factors such as presence of tick-borne pathogens. This would be an important factor for the health authorities to consider in Northeast of Libya.

METHODOLOGY

The study protocol was reviewed and approved by Bioethics Committee at Biotechnology Research Center (BEC-BTRC) with Ref No: BEC-BTRC 04-2017. Inclusion criteria involve: patients agreement participation in the study. Patients experiencing flu-like symptoms with spotted fever after confirmed or suspected tick bite were included in the study (adult and children). Blood samples were collected from each of the 129 patients seeking medical attention from May to November 2018 at Al-Thawra Hospital in El-Beida City. This Hospital was only Center that received the majority of patients in the littoral and outskirt regions which have a population of more than 500,000 inhabitants. Data on the patient demographic characteristics, including their age, gender, area of residence, and related to animals were gathered from patient files. All tests were performed by automatic blood cell analyzer (XP-300 Automated Hematology Analyzer, Sysmex American, Inc [6, 7]. Liver function parameters were estimated in all subjects by a commercially available test kits method on automatic analyser from Biotechnologies, Germany with the manufacturer's instructions strictly adhered to using spectrophotometers (Humalyzer Junior). Kidney function was determined using ready kit from Archem Diagnostic's industry. And electrolytes were measured using an automatic electrolyte analyzer (EasyLyte from Medicacorp, USA).

STATISTICAL ANALYSIS

Data from patient was compared with data of normal subjects. Statistical analysis was carried out in Minitab software; statistical significance was assessed using two samples T- test analysis. After detection normal distribution to the data and appropriate $P < 0.05$ consider significant [8].

RESULTS

This study carried out in the 7 consecutive months (May-November 2018). A total number of patients who diagnosed with symptoms related to spotted fever in Al-Thawra Hospital among months of study were found 129. The number of patients for previous four years was raged from 90-135 each year (data from statistical department in hospital). Initial symptoms include sudden onset of fever, headache, chills, malaise, and myalgia. Other early symptoms might include nausea or vomiting, abdominal pain, anorexia, and photophobia. A rash typically appears 2–4 days after the onset of fever (Figure 1). Total number 129 patients (61 males, 30 females for adults and 22 males and 16 females for children) were enrolled in this study. In children subjects, highest prevalence was noted in age group 4-6 years (21.05%) followed with age groups 0-3 and 7-9 years with 13.16% for males. While, highest prevalence was noted in age group 0-3 years (18.42%) followed with age groups 4-6 years with 13.16% for females. Lowest prevalence was observed in the age group of 12-15 years for couple genders (Figure 2). In adults, highest prevalence was noted in age group 21-30 years (15.47%) followed with age groups 31-40 and 41-50 years with 14.37 and 13.28 % for males. While, highest prevalence was noted in age group 16.20 years (8.88%) followed with age groups 21-40 years with 6.68% for females. Lowest prevalence was observed in the age group of >60 years for couple genders (Figure 3). Values derived from complete blood counts (CBC), including differential cell counts were recorded for each patient and analysed comparison to healthy subjects and shown in Tables 1-5. RBC count was not statistically different compared to control group in children and adults. Most RBC parameters including HGB concentrations, percent haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and red blood cell distribution width (RDW) were significantly decreased in patients (adults and children/ males and females) compared to control subjects. Only level of mean corpuscular haemoglobin concentration (MCHC) was significantly increased in all patients. Similar result in term of significance was obtained with white blood cell parameters in couple categories. Results of WBC were in normal range for couple genders increased slightly without significantly changes except on percentage of lymphocytes that increased in adult's patients not in children. Results of platelets including mean platelets number (PLT), the volume occupied by platelets in the

blood (PCT) and mean platelet volume (MPV) were significantly decreased in both females and males (adults and children). Mean levels of AST, ALT, ALP and total bilirubine in the serum of the females and males (adults and children) were significantly increased in patients. Kidney function parameters (creatinine and urea) were found more effective in children than in adults in term of statistically significant. Moreover, both adults and children were found to have high level of creatinine and low level of urea compared to control subjects. Similar pattern of effective were noticed with the mean concentration of sodium, potassium, and chloride (children was more significantly than adults).

Fig-1: A rash typically appears 2–4 days after the onset of fever in some cases

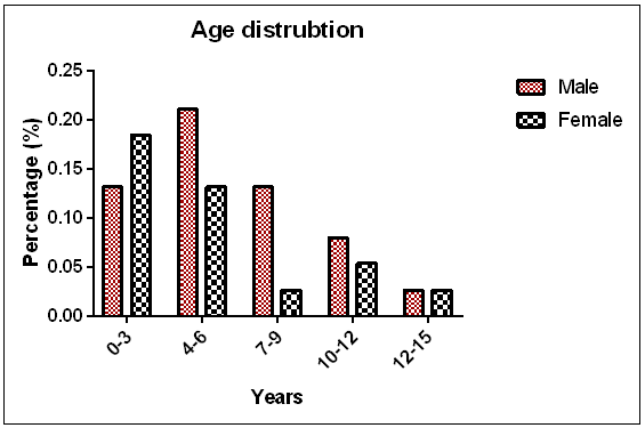

Fig-1: Age distribution for children

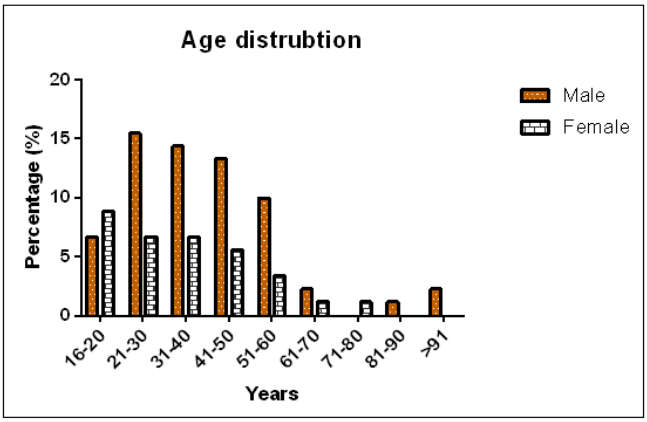

Fig-3: Age distribution for adults

Table-1: Mean values of RBC, HGB, HCT, MCV, MCH, MCHC and RDW in females and males (Children and adults)

Parameters	Female (Children)		Male (Children)	
	Control Mean± SEM	Patient Mean± SEM	Control Mean± SEM	Patient Mean± SEM
RBC(× 10^6/µl)	4.434± 0.14	4.863± 0.14	4.252±0.23	4.669±0.11
HGB (g/dl)	13.19±0.57	11.87±0.24*	13.85±0.43	11.80±0.37**
HCT (%)	38.10±1.2	35.11±0.86*	37.15±0.89	33.69±0.79*
MCV(fL)	86.14±1.4	75.91±0.99**	85.13±1.7	74.10±0.76**
MCH (pg)	29.31±0.68	27.60±0.36*	28.97±0.73	27.01±0.46*
MCHC(g/dL)	33.971±0.35	38.54±0.83*	35.35±.065	36.34±0.50*
RDW (%)	14.93±0.67	13.481±0.17*	15.07±0.62	12.67±0.34*
	Female (adults)		Male (adults)	
RBC (×10^6/µl)	4.13±0.15	4.34±0.11	4.84±0.13	4.231±0.11**
HGB (g/dl)	12.67±0.35	11.27±0.30**	14.08±0.33	13.24±0.23*
HCT %	39.44±0.47	30.37±0.78**	40.28±0.76	33.38±0.53**
MCV (fL)	87.65±1.0	72.30±0.93**	87.01±1.0	80.42±0.92**
MCH (pg)	29.91±0.42	26.76±0.51**	29.53±0.48	31.95±0.55**
MCHC (g/dL)	34.86±0.28	37.15±0.56**	34.77±0.22	39.94±0.28**
RDW (%)	13.52±0.31	14.35±0.25*	13.80±0.22	13.62±0.16

Data are expressed as mean ± SEM of each gender. Within each row for male or female separately, means with different superscript (*, ** or ***) were significantly different at p<0.05 within same raw and same gender. Where means without superscripts mean that there is no significant difference (p>0.05).

Table-2: Mean values of WBC, Lymph, Mid and Gran in females and males (Children and adults)

Parameters	Female (Children)		Male (Children)	
	Control Mean± SEM	Patient Mean± SEM	Control Mean± SEM	Patient Mean± SEM
WBC (×10^3/µl)	9.89±0.61	9.19±0.61	7.75±2.0.92	7.65±0.63
Lymph%	2.68±0.36	3.40±0.30	2.200±0.17	2.36±0.27
Mid%	0.88±0.091	0.86±0.88	0.633±0.11	0.414±0.44
Gran%	6.13±0.60	5.32±0.36	4.75±0.93	4.57±0.34
	Female (adults)		Male (adults)	
WBC (×10^3/µl)	7.37±0.39	6.48±0.50	8.53±0.46	8.76±0.46
Lymph %	2.31±0.20	5.43±1.2*	2.94± 0.40	2.15± 0.18*
Mid %	0.529± 0.070	1.060± 0.11*	0.757± 0.099	0.907± 0.322
Gran %	4.59± 0.33	5.38± 0.37	5.04± 0.33	5.82± 0.28

Data are expressed as mean ± SEM of each gender. Within each row for male or female separately, means with different superscript (*, ** or ***) were significantly different at p<0.05 within same raw and same gender. Where means without superscripts mean that there is no significant difference (p>0.05).

Table-3: Mean values of PLT, MPV, PDW and PCT in females and males (Children and adults)

Parameters	Female (Children)		Male (Children)	
	Control Mean± SEM	Patient Mean± SEM	Control Mean± SEM	Patient Mean± SEM
PLT(10^3/µl)	264.4±13	190.4±13***	270.5±9.2	180.0±16***
MPV (fL)	8.69±0.68	8.10±0.16	9.083±0.36	8.132±0.21
PDW (%)	14.01±0.48	14.27±0.76	15.03±0.61	15.22±0.51
PCT %	0.209±0.015	0.155±0.009*	0.217±0.011	0.156±0.013**
	Female (adults)		Male (adults)	
PLT (10^3/µl)	269±13	150 ±11**	256±15	156±11**
MPV(fL)	9.46±0.29	8.40±0.13**	9.45±1.15	8.96±0.12*
PDW (%)	13.04±0.58	15.57±20**	14.86±0.42	15.83±0.12*
PCT (%)	0.218±0.008	0.142±0.006**	0.195±0.012	0.169±0.007*

Data are expressed as mean ± SEM of each gender. Within each row for male or female separately, means with different superscript (*, ** or ***) were significantly different at p<0.05 within same raw and same gender. Where means without superscripts mean that there is no significant difference (p>0.05).

Table-4: Mean values of AST, ALT, Total Bilirubine and ALP in females and males (Children and adults)

Parameters	Female (Children)		Male (Children)	
	Control Mean± SEM	Patient Mean± SEM	Control Mean± SEM	Patient Mean± SEM
AST(U/L)	21.9±3.4	56.0±9.4*	21.9±3.4	34.0±4.0*
ALT(U/L)	27.6±3.6	45.2±8.4	27.50±3.1	38.3±7.5
Total Bilirubine (mg/dl)	1.81±0.48	0.762±0.13	0.762±0.13	1.086±0.31
ALP(U/L)	143.8±18	413 ±47**	138.2±15	220.3±31*
	Female (adults)		Male (adults)	
AST (U/L)	36.09±2.4	69.1±16*	37.2±2.6	76.8±11**
ALT (U/L)	31.33±2.8	56.8±13	36.6±3.1	71.8 ±13 *
Total Bilirubine (mg/dl)	0.68±0.069	1.13±0.44	0.59±0.050	0.98±0.18
ALP (U/L)	190.7±19	225±34	166.1±9.8	194±20

Data are expressed as mean ± SEM of each gender. Within each row for male or female separately, means with different superscript (*, ** or ***) were significantly different at p<0.05 within same raw and same gender. Where means without superscripts mean that there is no significant difference (p>0.05).

Table-5: Mean values of creatinine and urea in females and males (Children and adults)

Parameters	Female (Children)		Male (Children)	
	Control Mean± SEM	Patient Mean± SEM	Control Mean± SEM	Patient Mean± SEM
Creatinine (µg/dl)	0.537 ± 0.11	1.133 ± 0.21*	0.537 ± 0.11	0.986 ± 0.088**
Urea (mg/dl)	27.9 ±3.1	18.00 ± 1.2**	27.9 ± 3.1	23.06 ± 1.3*
	Female (adults)		Male (adults)	
Creatinine (µg/dl)	0.76±0.063	1.94±1.2	0.77±0.027	1.15±0.11**
Urea (mg/dl)	27.42 ±2.5	21.09±2.2	29.27±1.6	34.1±7.2

Data are expressed as mean ± SEM of each gender. Within each row for male or female separately, means with different superscript (*, ** or ***) were significantly different at p<0.05 within same raw and same gender. Where means without superscripts mean that there is no significant difference (p>0.05).

Table-6: Mean values of Na, K and Cl in females and males (Children and adults)

Parameters	Female (Children)		Male (Children)	
	Control Mean± SEM	Patient Mean± SEM	Control Mean± SEM	Patient Mean± SEM
Na (mmol/l)	137.93±1.3	133.57±1.3*	137.93±1.3	134.02±1.0*
K (mmol/l)	4.257±0.19	4.485±0.15	4.257±0.19	4.117±0.25
Cl (mmol/l)	100.21±1.3	106.23±1.5*	100.21±1.3	101.00±2.5
	Female (adults)		Male (adults)	
Na (mmol/l)	142.00±2.1	131.3±1.6**	142.05±2.1	130.13± 1.5**
K (mmol/l)	4.29± 0.14	3.76± 0.19*	4.29± 0.14	5.53±1.7
Cl (mmol/l)	107.18±0.87	103.89±1.6	107.18± 0.87	92.3± 5.7*

Data are expressed as mean ± SEM of each gender. Within each row for male or female separately, means with different superscript (*, ** or ***) were significantly different at p<0.05 within same raw and same gender. Where means without superscripts mean that there is no significant difference (p>0.05).

DISCUSSION

Most studies of humans with tick-borne diseases have been analyses of the clinical, serologic, or pathologic features of cases collected retrospectively from the records of large medical centers or public health laboratories. Thus studies provide a dependable large scale seroprevalence about RMSF in Brazil, Croatia, France, Spain and Zimbabwe [9-14]. MSF was considered for 70 years a benign disease when compared with RMSF. In fact, because of the lack of medical interest in MSF, its real severity was long ignored. Although the mortality rate was evaluated to be from 1% to 3% in the early reports in the literature, the first description of a highly severe form of MSF was published in the early 1980s [15]. Recently, study reported that MSF is at least as severe as RMSF and has a mortality rate as high as 32.3%, which occurred in Portugal in 1997 [16].

Due to lack study about presence of spotted fever in Libya for common and in Northeast as

particular. Therefore this study was designed to evaluated febrile patients with a recent tick bite and utilized haematological and biochemical laboratory techniques to confirm the diagnosis and draw whole picture for this diseases which became epidemiology at same seasons in El-Beida City and surrounding areas.

The epidemiologic and clinical characteristics of the 129 patients described in this study were strongly compatible with spotted fever. All patients resided in areas where ticks were present, most of them were resided in farms, other of them been close to animal in some point or had picnic in open area. All patients were became ill in mid-spring (May) to early an autumn (November) (2018), and presented with symptoms characterized by myalgias, vomiting, and/or rash. Unfortunately, the specific tests, which used to detect anti-*Rickettsia*l antibody, it is not available in main hospital. For this reasons healthcare was used obvious clinical symptoms to give treatment with haematological and biochemistry analysis. Other important reasons, most patients with spotted fever had difficulty in diagnostic level of anti-*Rickettsia*l antibody titers until the second week of disease.

In this study symptoms of diseases were similar to early signs and symptoms of RMSF/MSF can be non-specific and include fever, headache, myalgia and fatigue. The clinical symptoms and signs were similar to ones presented in other studies [17-20]. Rash occurs in 90% of cases and usually appears 2-4 days after onset of fever (Figure 1), however most patients present for health care before its appearance. For adults and children with MSF, rash frequently occurs earlier in children than in adults [21] and is eventually observed in approximately 90% of children. Rash typically begins on the wrists and ankles and spreads to the palms, soles and much of the body[22]. Geographical distribution for all subjects in our study was from twenty different locations around El-Beida City. These locations were farms and place where animals found (sheep, cows and dogs) and presence of tick was very common. The monthly distribution of cases shows that the apparition of the disease parallels the maximal activity of the immature stages of tick (May-November).

This study reported here data on the sex and age distribution in patients. The sex ratio is elevated and males are more frequently diagnosed as having spotted fever than females and this results agree with previous study [23]. Reports from France [24], Greece [18] and Romania [20] also estimated high proportion of male gender. In contrast, Kuloglu *et al.* [19] found slight prevalence of females. The sex ratio is influenced by local characteristics regarding agricultural work, livestock farming and housekeeping. Moreover, this could be correlated with greater exposure factors as previously described because the majority of these cases are of urban origin. It was previously found that living in a rural area and working in farms were causes of overexposure in males.

In term of age, the cases per age groups were corrected according to demographic data, this study found that young age (under ten years) and older ages (over 50 years) at risk factors [25-27]. This could be refer to physiological condition such illness or as week in immune system. However, the highest prevalence with range age from 20-50 years found in our study was similar to that described in Minas Gerais among cases reported from 2000 to 2008[28].

A study in sub-Saharan Africa also reported a higher seropositivity *to Rickettsia africae* in individuals aged 36-45 years, which was linked to behavior or occupational exposures and leisure activities [29].

Our data show that the sequential changes in many haematological and biochemical parameters in young and adult patients, although less pronounced, mimic those of potentially more severe after ten day if they had not treatment. In this study, most patients infected with symptoms had normal leukocyte counts and lower platelet counts than control subjects. Obvious result that was very significantly, changes in all platelets parameters (PLT, MPV, PDW and PCT). Main results falls in total platelet counts, with nadir levels during the first week of illness. This result is common in MSF caused by *R. conorii* and RMSF caused by *R. rickettsia* [15, 24, 30, 31]. Thrombocytopenia, which commonly evolves in hospitalized cases of MSF and RMSF [32, 24], is postulated to be caused by adherence to infected endothelial cells [33], or immune-mediated consumption. Intracellular replication of *Rickettsia rickettsii*, the etiological agent of RMSF, directly induces lethal injury to host endothelial cells causing pathophysiological changes including thrombosis, hemorrhage, and vasculitis. This disease is characterized by severe vascular lesions attributed to direct microbial replication-induced damage to endothelial cells [34, 35].

Other commonly measured haematological parameters, such as RBC, HGB, HCT, MCV, MCH, MCHC and RDW, are apparently affected in both adults and children. This is in contrast to MSF and RMSF, where severe cases frequently develop anemia, hyponatremia and high sedimentation rates especially during the later stages of the disease [36, 15, 32, 37], 31]. Elevated serum liver enzymes (AST, ALT, Total Bilirubine and ALP), that detected in adults and children showed rise statistically significant only for ALT and AST. In the early phase of the RMSF, serum biochemical analyses usually reveal high hepatic enzyme activities [30, 31]. The underlying pathophysiologic liver process is probably non-specific. Hyperbilirubinemia is uncommon and liver biopsies in cases of MSF usually only reveal scattered foci of hepatocellular necrosis with mononuclear infiltrations,

or, occasionally, granulomas without giant cells or epitheloid cells [38]. Furthermore, the elevations of AST and ALT may also have extrahepatic sources, as both enzymes are secreted in vitro by human endothelial cells infected by *R. conorii.*

Other measured biological parameters, serum creatinine and urea are apparently increased significantly particularly in children. The alteration of renal function has been frequently described in patients affected by MSF. Different pathogenic mechanisms can impair the kidney during MSF including hypovolaemia and shock and a toxic effect of *R.conoriion glomeruli* [39, 37]. In the later phase of the disease, high BUN and creatinine concentrations indicate renal failure [36, 30, 31]. However, this is in contrast to MSF and RMSF, where severe cases frequently develop impaired renal function, especially during the later stages of the disease [15, 32].

The results of the effect of fever on the electrolytes (sodium, potassium, and chloride) are showed decrease in level of Na and an increase in level of Cl. Significant Na loss was observed in the experimental infestation of dogs with *Rhipicephalus sanguineus* (Brown Dog Tick)[16]. Our results agree with another study (in term of increasing and decreasing) that measured the level of electrolytes in samples obtained at selected points in the course of the MSF [40].

CONCLUSION

In conclusion, spotted fever is an overall benign disease but underlying complications may cause difficulties for the treatment and may impair the outcome in all different ages and gender. Further prospective studies of patients with tick bite and fever are needed to confirm and expand upon our findings. For example, it would be useful to know the incidence of *Rickettsia*l infection in patients without a history of tick bite that are found to have fever and thrombocytopenia during the spring and summer months in Northeast regions in Libya.

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Bioethics Committee at Biotechnology Research Center (BEC-BTRC)

Approval Letter

Ref No; BEC-BTRC 07-2018

Dear applicant, ***Dr.*** *Marfoua Salah*

Referring to your request for ethical approval for the research project entitled
(Spot Fever on El-Beyda City, Libya: Correlation between prevalence of Hospitalized patients, Serological test and epidemiological factors).
The bioethics committee at BTRC is pleased to inform you that your proposal has met the standard of bioethics, and have given it's ethical approval for your project for 12 months. It is important to follow the guidelines for bioethics, compliance with the following:

1. Proceed with project according to the study proposal plan.
2. Ensure safe disposal of the samples after the completion of the research study, or to be stored in a safe place.

This approval was given for research purpose under the law obligations

Dr. Nabil Enattah

Chairman of Bioethics Committee

Biotechnology Research Center General Director of BTRC

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