



Applying the Indirect Immunofluorescent Antibody Test (IFAT) for Detection of Coxiella Burnetii in Chronic Hepatitis Patients and Fever of Unknown Origin Patients from the Village with Direct Contact with Animals in Assiut Governorate

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Abstract

Background: Q fever is a neglected zoonotic disease caused by the bacterium *Coxiella burnetii*. Although hepatitis is a one of the most presentations of Q fever, the attainable influence of chronic hepatitis C virus & hepatitis B virus, the apparent healthy person, and fever of unknown origin (FUO) in Q fever has, to our data, never been investigated. Clinical manifestation of Q fever in humans include acute, chronic to fatigue syndrome. It is a vital issue in regions where Q fever hepatitis and viral hepatitis are prevalent.

Aim and Methods: We tend to conduct a study to research the possible attainable of viral hepatitis in cases of Q fever hepatitis. The serum samples were examined for IgM and IgG antibodies against *C. burnetii* antigen by indirect immunofluorescent antibody test (IFAT). The results showed that 20 patients with hepatitis C virus were studied, of whom 10(50%) IgM and 12 (60%) IgG had Q fever, A total of 40 patients with fever of unknown origin (FUO) studied (Patient suffer from fever, cough, rhinitis, nausea, vomiting (during winter), of whom 17 (42%) IgM and 18(45%) IgG had Q fever. Three samples from patients infected with Hepatitis B virus with kidney dialysis of whom 1(33%) IgM and IgG positive. 10 samples from Apparent healthy persons of whom 3(30%) IgM positive 4(40%) IgG positive (females) one of them suffers from the reproductive disorder and another one suffer from toxic goitre. We concluded that we have a tend to terminate that the data on the medicine of Q fever in Egypt is proscribed with no attention to regulate and hindrance programs. The results of this study boost the data on the prevalence of fever of unknown origin (FUO). Corroding to our knowledge, Q fever influences the replication of hepatitis virus is continues to be not understood and wishes more studies.

Keywords: Immunofluorescent; Chronic Hepatitis; *Coxiella Burnetii*; Goiter; Fluorescence

Abbreviations: IFAT: Immunofluorescent Antibody Test; FUO: Fever of Unknown Origin; BMI: Body mass index; IHCV: Patients infected with hepatitis C Virus.

Patients and Methods

Ethical approval and Consent

The National ethics committee of Assiut University in Assiut province, Egypt, was approved the method of work and the strategy of labor.

Patients

73 cases (20 cases of chronic hepatitis C virus, 40 patients with fever of unknown origin (FUO), 3 with hepatitis B virus with kidney dialysis, and 10 apparent healthy persons). Samples collected from hospitals located in Assiut Governorate in Egypt. Patients who had clinically suffered from hepatitis C virus (age between 49-60 years), hepatitis B virus patients with kidney dialysis (19, 40, 56 years). Patients with fever of unknown origin (FUO) with a history of cough, rhinitis, and flu-like symptoms (age 22-50 years) and apparent healthy persons (14-40 years) were tested for Q fever.

Serologic Assessments of Q Fever were Performed by Using Fluorescence Microscopy Through Indirect Immunofluorescent Antibody Test

Cases of acute Q fever confirmed by serologic examination were enclosed within the study by indirect immunofluorescent antibody test (IFAT). Serologic assessments of Q fever were performed using the indirect immunofluorescence antibody (VIRCELL COXIELLA BURNETII I+II SLIDE) & Enzyme-Linked Immunosorbent assay. Q fever was diagnosed on the idea of either (1) anti-phase II antigen IgG and anti-phase II antigen IgM in a single serum specimen.

Collection of Samples: blood should be collected aseptically using vein puncture techniques by a certified individual. The utilization of sterile or aseptic techniques can preserve the integrity of the specimen. Serum samples are to be refrigerated (2-8°C) upon collection or frozen (-20°C) if the take look at cannot be performed at intervals seven day. Samples must not be repeatedly frozen and thawed, to avoid immune serum globulin concentration decrease, particularly IgM. Do not use hyperlipemic or contaminated sera. Samples containing particles should be clarified by centrifugation. Serum specimens were collected and sent to the Animal reproduction Research institute for serological assessment of

Q fever by indirect immunofluorescent antibody test (IFAT). The methods was performed according to Vaidya V, et al. [1].

Investigations of Hepatitis Patients

Hepatitis patients were examined for the followings parameters: HCV PCR, Platelets count, albumin, prothrombin and alfa feto protein, total lymphocytes, haemoglobin, and BMI (Body mass index).

Results

Twenty cases of chronic hepatitis C virus were identified in a hospital in Assiut governorate. The result of investigation of Hepatitis patients as the followings: HCV PCR (592411 ± 0.666), Platelets count (172.7 ± 86.2), albumin (3.9 ± 0.5), prothrombin (68.6 ± 13.6) and alfa feto protein (16.5 ± 19.8) total lymphocyte (6.14 ± 2.2), haemoglobin (14.1 ± 1.8) and BMI (Body mass index 28.6 ± 4.8) (Table 1). The serum samples were investigated for IgM and IgG antibodies against C. burnetii antigen by indirect immunofluorescent antibody test (IFAT). Results by were a total of 20 patients with hepatitis C virus were studied, of whom 8 (40%) IgM and 12 (60%) IgG Q fever antibodies (Table 2).

Demographic characteristic	Patients with Chronic HCV had Q FEVER infection (n =12)
Male	8
Female	4
Average Age	54.4years \pm 8.8
BMI(body mass index)	28.6 \pm 4.8
Platelet count	172.7 \pm 86.2
Prothrombin	68.6 \pm 13.6
Hemoglobin	14.1 \pm 1.8
Lymphocytes	6.14 \pm 2.2
Albumin	3.9 \pm 0.5
Alfa feto protein	16.5 \pm 19.8
Fibrosis	F2-F4
PCR	592411(high viremia) \pm 0.666
Diabetes mellitus	5 were diabetic
Smoking	2 were smoker

Table 1: Demographic characteristics of symptoms and signs of chronic Hepatitis C virus in patients with Q fever infection. The expression as average \pm standard deviation by using Excel.

A total of 40 patient with fever of unknown origin (FUO) were studied (Patient suffer from fever, cough, rhinitis, nausea, vomiting (during winter), of whom 17 (42%) IgM and 18(45%) IgG had Q fever. These were from the village and had animals in their homes as sheep, goats, and cows

and direct contact with animals (Table 2).

Three samples from patients infected with Hepatitis B virus with kidney dialysis of whom 1(33%) IgM and IgG positive (Table 2).

Patients	No. of serum samples	Percentage of positive samples by IFAT	No. of positive IFAT	
			IgM	IgG
			%	%
IHCV	20	12	8	12
		60%	40%	60%
FUO	40	35	17	18
		87.50%	42%	45%
IHBV & Kidney Dialysis	3	1	1	1
		33%	33%	33%
Apparent healthy persons	10	7	3	4
		70%	30%	40%
Total	73	64	29	35
		87.67%	63%	76%

Table 2: Summary of the result of indirect immunofluorescence (IFAT) of all cases.

Ten samples from apparent healthy persons of whom three (30%)IgM positive, four (40%)IgG positive(females), one of them suffers from reproductive disorder(Habitual abortion), and another one suffers from hypothyroidism due to autoimmune thyroiditis because of high level of

antimicrosomal antibodies(>1300 IU/ML) while normal up to 34IU/ML,TSH thyroid stimulating hormone >100 uIU/ml while normal range 0.27-5.5 uIU/ml and thyroglobulin in serum 0.3 ng/ml while normal range 0.8-84 ng/m(Figure 1 & Table 3).

Patient	Sex	Age	Case History
1	Female	22 y	Reproductive disorder ((Habitual abortion))
2	Female	29y	TSH 2.4(normal), HB 12.7 (normal)
3	Female	14y	Random glucose 70
			TSH >100
			Thyroglobulin 0.3 ng/ml (0.8-84)
			Anti-thyroid microsomal antibodies >1300 (up to 34 IU/ml)
			(Diagnosis: autoimmune thyroiditis due to high level of anti-microsomal antibodies(>1300 IU/ML) while normal up to 34IU/ML

Table 3: Investigation of three Case reports from apparent healthy females with Coxiella Burnetii positive by indirect immunofluorescent test.

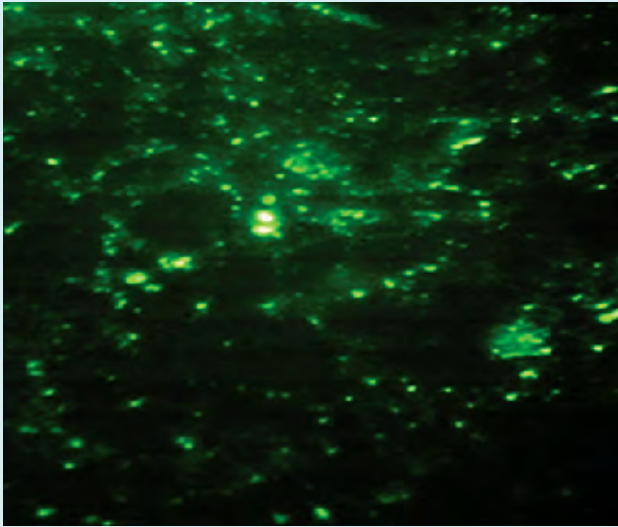


Figure 1: photomicrograph of positive *C. burnetii* IgG antibodies in serum of human samples in hepatitis C patients by indirect immunofluorescence (IFAT) test under fluorescence microscope 400x.

Discussion

Patients with fever of unknown origin (FUO), forty patients of whom 35(87.5%), and twenty chronic hepatitis C patients of whom twelve (60%) by IFA, this result supported by Oh M, et al. [2] who mentioned a 53-year-old man with fever of unknown origin underwent F-18 fluorodeoxy glucose positron emission tomography/computed tomography (F-18 FDG PET/CT) as a workup for a fever of unknown origin. On presentation, he complained of fever, chills, and pain. The F-18 FDG PET/CT scan showed diffusely exaggerated uptake of the liver with delicate hepatomegaly. A liver biopsy then discovered fibrin-ring granulomas typically seen in Q fever. The patient was later serologically diagnosed as having acute Q fever because of the titers for *C. burnetii* IgM and IgG Kobbe, et al. [3] reported Q fever should be suspected in any disease with fever of unknown origin. Clinical manifestations are variable and repeated serological testing is mandatory. In some cases, diagnostic biopsies might help to determine early diagnosis Lefebvre M, et al. [4] told that Clinicians must remember of the potential diagnosis of Q fever, and *C. burnetii* serology may be a helpful diagnostic tool within the investigation of fever of unknown origin with atypical systemic symptoms Lin PH, et al. [5] mentioned that acute Q fever presenting as fever of unknown origin with rapidly progressive hepatic failure in a patient with alcoholism. A 51-year-old electrician, who was a habitual drinker, given with a 2-week history of intermittent high fever, acute hepatomegaly, and quickly progressive jaundice when being

accidentally exposed to dirt from bird nests once he was repairing an electrical instrumentality in Taipei County. Ascites and prolonged clotting factor time were noted at admission. Trans jugular liver biopsy and bone marrow biopsy found multiple little fibrinoid-ring granulomas in liver parenchyma and bone marrow, The diagnosing of acute Q fever was confirmed by high titers of antibodies against *Coxiella burnetii* (phase I IgM 1:160 and IgG 1:2560, phase II IgM > 1:320 and IgG 1:5120) and a four-fold elevation of phase II IgG titer within the paired serum. The expertise of this case shows that the chance of Q fever should not be unmarked in patients who have associates an unexplained febrile illness and severe liver function impairment following exposure to a contaminated atmosphere t in Taiwan.

Twenty chronic viral hepatitis C patients of whom 12(60%) by IFA, this result disagrees with Lai, et al. [6]. A complete of fifty eight patients with acute Q fever hepatitis were studied, of whom sixteen (27.6%) had viral hepatitis (hepatitis B virus infection in twelve and hepatitis C virus infection in 4). Patients this distinction thanks to completely different numbers of samples and incidence of illness in numerous country. It is essential issue in regions wherever Q fever hepatitis, and viral hepatitis, are common. We conducted a study to analyze the potential influence of viral hepatitis in cases of Q fever hepatitis Lai, et al [6]. In Derrick's Derrick E, et al. [7] original description of Q fever in 1937, one of the nine patients had jaundice which urged liver involvement in Q fever. In 1956, Gerstl initial delineate the pathologic findings of a granuloma-like lesion, as determined by biopsy, in their patients with Q fever and liver involvement, subsequently study, the characteristics of pathologic Q fever hepatitis "doughnut" lesions, presenting as granulomatous changes with fibroid rings and clear central spaces became wide delineate by several others Dupont H, et al., Dupont H, et al. and Srigley J, et al. [8-10]. Within the study conducted by Alkan, et al. [11], Q fever occurred in seventeen (13.3%) of 128 patients with infectious hepatitis, and severe headache perceived to be the sole symptom that distinguished between Q fever and infectious hepatitis; but, this distinctive feature was not specific. additionally to the matter of differentiation between Q fever, hepatitis, and infectious hepatitis, there is a requirement for investigations whether or not the clinical manifestations, responses to treatment, and biochemical resolution differed among patients with underlying viral hepatitis. This is often a very important issue, notably in regions wherever viral hepatitis is rife, like Egypt, additionally to the matter of the problem of differentiation between Q fever, hepatitis, and infectious hepatitis.

Ten samples from Apparent healthy persons of whom three (30%)IgM positive, four (40%)IgG positive(females), one amongst them suffer from reproductive disorders with habitual apportion and another one suffers from

autoimmune thyroiditis with hypothyroidism due to high level anti-microsomal antibodies. Thus, it is vital to study the reproductive disorders caused by Q fever, especially with unknown cause of habitual abortion as obstetricians neglect this point now.

Q fever in pregnancy involving thirty eight human cases, incontestable that Q fever in pregnancy was related to high morbidity and mortality, spontaneous abortion occurred in 26% of Q fever-related pregnancies, with 5.3% of pregnancies resulted in intrauterine death, 45% resulted in premature birth and 5.1% reported intrauterine growth retardation Tozer S [12]. In another study by Aitken, et al. [13] of 53 pregnant women with Q fever, it was identified that more than 50% of these mothers developed a Q fever serological profile consistent with chronic Q fever, compared to a 5% conversion rate in the general population; this study additionally highlighted the link between placentitis and obstetric complications. Getting Q fever during pregnancy results in long-term risk of developing chronic Q fever disease, along with adverse outcomes for the unborn child. Despite the many associations between Q fever infections together with adverse outcomes of pregnancy in animals, comparatively very little has been documented regarding the outcomes of pregnancy and Q fever infections in humans. This could result to an absence of awareness of Q fever as a serious human microorganism by obstetricians, leading to an associate underestimates of the incidence of the illness Carcopino X, et al. and Raoult D, et al. [14,15] declared that cases of Q fever have often been reported throughout pregnancy in humans as they reported a case of infection during pregnancy, which was followed by fetal infection and death, a 26-year-old nurse who was eight weeks pregnant associated worked in an intensive care unit bestowed with fever and cough Stein A, et al. [16] declared that Q fever is may be an important reason for morbidity and mortality in pregnancy and In Martigues, Q fever is present in at least once per 540 pregnancies and constitutes the foremost important public health problem associated with intrauterine infections Vaidya V, et al.[1] according that the determined association of *C. burnetii* infection with reproductive disorders in humans and therefore the high rate of excretion of the microorganism, notably within the placenta, reveal that Q fever infection in humans remains underdiagnosed; the prevalence is underestimated and presents a possible public health threat.

Three samples from patients infected with Hepatitis B virus with kidney dialysis of whom 1(33%) IgM and IgG positive. These patients listed below immunocompromised patients, this result supported with Ljutic D, et al. [17] who suggested that Acute Q fever should be thought of as a potential reason of acute kidney failure Vizinho R, et al. [18] mentioned that chronic Q fever has antecedently solely

delineated in two dialysis patients Gwida M, et al. [19] mentioned that there is a requirement for further analysis on the impact of Q fever on each veterinary and public health [20-25].

Conclusion

Q fever could be a neglected zoonosis caused by the microorganism *Coxiella burnetii*. The data on the Epidemiology of Q fever in Egypt is restricted with no attention to manage and prevention programs. To our data, Q fever influences the replication of hepatitis virus continues to be not understood. Although hepatitis is one amongst the most important manifestations of Q fever, the potential influence of viral hepatitis on Q fever has never been investigated in Egypt. We would like to understand the relationship between viral hepatitis and Q fever as each effect the liver and cause liver cirrhosis. Many studies ought to be done to understand the link between Q fever and reproductive disorder in human as immunocompromised persons and pregnant women also are at great risk for the chronic form.

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