Typhoid fever in Karbala Governorate, an epidemiological study

Emtenaa Mohammed Hashem AL- Araji*;Ali Mansoor Jasim Al-Ameri**;

*Kerbala University, College of Medicine

** Lecturer, Kerbala University, College of Medicine, Microbiology Dept.

Abstract

Background: Typhoid fever is a common systemic illness caused primarily by infection with *Salmonella typhi*. It could be diagnosed depending on classic clinical features supported by serologic and bacteriologic demonstration. It is well known that this disease is characterized by sharp seasonal variation in addition to many other factors affecting its prevalence in the population. These factors may include age, residence, psychosocial status and certain environmental conditions.

Study design and objective: This is a cross sectional epidemiological study. It was done during August, 2008 through July, 2009 in Karbala. It aims to isolate and identify *Salmonella typhi* from blood samples collected from clinically suspected cases to predict the diagnostic value of the clinical suspicion versus the bacteriologic investigation including isolation and characterization of the main causative pathogen, *Salmonella typhi*. Also it aims to determine the effect of age, gender, educational level and seasonal variation on the frequency distribution of typhoid fever during the study period.

Method: Blood samples were collected from 718clinically suspected patients (355 males and 363 females) who attended Al-Hussein Teaching Hospital and Pediatric teaching Hospital in Karbala with signs and symptoms of typhoid fever (proven by specialist physician/pediatrician). Blood was cultured in enriched, selective and differential media to isolate the causative agent, *Salmonella typhi* and was confirmed by some specific biochemical tests.

Results: Of the 718 blood samples, only379 (~52.8%) gave positive blood culture for *Salmonella typhi* proven by specific biochemical tests. The percentage of infected patients according to gender showed non-significant difference (males ~45.8% compared with females ~54.2%). Similarly, when incidence compared in terms of standard of education, it was (46.4% among educated versus 53.6% uneducated). Additionally, frequency distribution of infection in terms of different age groups showed significantly higher infection rates in age groups 5-14 and 15- 45 years than others (recording 28.21% and 46.58%, respectively).Regarding seasonal variations, the infection rates were recorded to be peaked in June recording 19.22% from the annual infection rates, while the lowest rate was recorded in February (1.81%) indicating a highly significant difference (p-value <0.01).

Conclusion: According to the study data, it was concluded that only about half the clinically suspected cases of typhoid fever give positive blood culture results for the main causative pathogen, *Salmonella typhi.* Also, it was shown that there is no significant effect of gender and education standard on rate of infection with typhoid fever. However, seasonal variation and age significantly affect this rate.

Key words: Typhoid fever, *Salmonella typhi*, epidemiology, bacteriologic diagnosis, clinical suspicion.

الخلاصة

مقدمة: تعد حمى التايفوئيد من الامراض الخطرة و الشائعة والتي تسببها العدوى بجرثومة السالمونيلا التيفية كمسبب رئيس للمرض. يمكن تشخيص المرض اعتمادا على الاعراض و العلامات السريرية الكلاسيكية مدعومة بالفحوصاتالبكتريولوجية والكيميائية الحيوية الاخرى. ومن المعلوم أن هذا المرض يتميز بتغير نسب الاصابة به مع تغير الظروف المناخية الموسمية فضلا عن العوامل الاخرى المؤثرة على نسبة حدوث المرض. وقد تشمل هذه العوامل العمر, السكن, الحالة النفسية وعوامل بيئية أخرى.

نوع الدراسة و الهدف منها: أجريت هذه الدراسة المسحية المقطعية في محافظة كربلاء خلال الفترة من آب 2008 الى تموز 2009. تهدف الدراسة الى عزل وتحديد البكتريا المسببة الرئيسية للمرض (السالمونيلا التيفية) من نماذج الدم المأخوذة من المشتبه سريريا بأصابتهم بالعدوى وذلك لغرض أختبار القيمة التشخيصية للاعراض السريرية مقابل الطرق البكتريولوجية متمثلة بعزل وتشخيص المسبب المرضي الرئيسي الا وهو السالمونيلا التيفية. وكذلك تهدف الى تحديد تأثيرالجنس و العمر والمستوى التعليمي أضافة الى التغيرات المناخية على معدل الاصابة بعدوى حمى التايفوئيد خلال فترة الدراسة.

طريقة اجراء الدراسة: تم جمع عينات الدم من 718 مريضا مشتبه سريريا بأصابتهم بالمرض(355 من الذكور و 363 من الاناث) وممن راجعوا مستشفى الحسين "ع" التعليمي ومستشفى الاطفال التعليمي في كربلاء والذين يشكون من اعراض وعلامات المرض بحسب استشارة الطبيب المختص. تم استنبات عينات الدم في اوساط زرعية أغنائية ثم أنتقائية و ثالثة تفريقية وذلك لغرض عزل البكتريا المسببة الرئيسية للعدوى (السالمونيلا التيفية). ومن ثم أجريت بعض الأختبارات الكيموحيوية للتأكد من التشخيص.

النتائج: من مجموع 718 نموذج دم, فقط 379 (52.8%) منها كانت نتيجة الحضن موجبة حيث تم عزل البكتريا منها. ونسبة الاصابة من حيث جنس المرضى كانت لدى الذكور 174 (45.8%) مقارنة بالاناث 205 (54.2%) مسجلة فرقا غير معنوي احصائيا. وكذلك الحال عند مقارنة نسبة الاصابة اعتمادا على مدى المستوى التعليمي حيث كانت (46.4% لدى المتعلمين مقابل 53.6% لغير المتعلمين). بالأضافة الى ذلك فأن نسبة الاصابة في الفئات العمرية المختلفة بينت ان اعلى نسبة قد سجلت للفئتين العمريتين 5-14 سنة و15-45 سنة (28.21% و 46.58% على التوالي). مبدية اختلافا معنويا عن بقية الفئات العمرية. أما بالنسبة للأختلافات الموسمية فقد سجلت أعلى نسب الأصابة خلال أشهر الصيف و الخريف حيث بلغت ذروتها خلال حزيران مسجلة 19.22% من مجموع الاصابات خلال سنة, أما أوطأ النسب فقد سجلت خلال شهر شباط بالغة 1.81%. أشارت هذه البيانات الى وجود اختلاف احصائي معنوي في نسب الأصابة بحمى التيفوئيد الأحصائية أقل من 0،01.(pمن حيث اختلاف أشهر السنة وقد بلغت قيمة

الاستنتاج: وفقا لنتائج الدراسة فأنه فقط حوالي نصف المرضى المشكوك سريريا بأصابتهم بحمى التيفوئيد قد أعطوا نتائج زرع دم ايجابية للمسبب الرئيسي (السالمونيلا التيفية). أضافة الى ذلك فقد بينت النتائج انه ليست هناك تأثيرات معنوية لجنس المريض او مستواه التعليمي على نسبة التعرض للاصابة بالعدوى. و بالرغم من ذلك فقد تبين أن عمر المريض و الأختلافات الموسمية على مدار السنة لها تأثير أحصائي معنوي على نسبة الأصابة بالمرض

Introduction

Typhoid fever, also known as enteric fever, is a potentially fatal multisystemic illness caused primarily by *Salmonella typhi*.It affects roughly 21.6 million people (incidence of 3.6 per 1,000 populations) and kills an estimated 200,000 people every year.[1]It has been documented thatfood sources such as eggs, poultry and meat products comprise major risk of transmission of enteric fever to humans. However, typhoid fever canlargely be passed via fecal-oral route through contact with active, convalescent cases or chronic carriers[2,3]. The lack of specificity of the clinical spectrum together with the difficulty of achieving a definitive bacteriologic or serologic diagnosis, impede making early and accurate diagnosis of typhoid fever[4,5]. However the bacteriologic identification, namely blood culture, is still the mainstay of the diagnosis.[6]

Herein, we try to predict the diagnostic value of the clinical suspicion versus the bacteriologic investigation including isolation and characterization of the main causative pathogen, *Salmonella typhi*. In addition, it is an attempt to determine the effect of some epidemiologic factors including age, gender, education standardand seasonal variations on the occurrence of typhoid fever.

Materials and method

Patients' selection and sample collection: Seven hundred and eighteen patients (355

males and 363 females) who presented with signs and symptoms suggesting typhoid fever including fever for more than one week, malaise, relative bradycardia, abdominal pain and tenderness were included into the study. Patients with clinical features suggesting other cause(s) for fever were excluded. From each patient, a 5-ml venous blood sample was collected during febrile period.

Bacteriological Diagnosis:

Blood culture: The blood was immediately dispersed into a tube with 10 ml brain heart infusion broth for enrichment. Thereafter, 1 ml of the growth wasdispersed into blood agar-containing Petri dish and incubated for 24 hrs at 37 ^{0}C and selected colonies wereinoculated into Mc Choncky's agar as a selective medium for 24 hrs at 37 ^{0}C. Then, selected colonies were inoculated into salmonella-shigella agar as a differential medium for 24-48 hrs at 37 ^{0}C[7]. The growth was then kept in the refrigerator for further biochemical tests.

Biochemical tests:

The isolated bacteria were able to grow at 45^{0}C. Furthermore, in triple sugar iron test: the bacteria can turn the broth color into top red, bottom yellow and middle black color after 24 hrs incubation. Also it can coagulate human plasma after 5 hrs incubation (Coagulase test +ve). The citrate utilization, methyl red and oxidase tests were also +ve. The bacteria were shown to be urase negative, unable to produce indole and gave –ve gelatin liquefaction test[3].

Statistical analysis:

Results were analyzed statistically using Chi square test and F-testto find out any significant difference in the infection rates with the bacterium *S. typhi* in regards to differences in gender, age groups, education levels and seasonal variations. The representative confidence interval was 95% and any test with p-value < 0.05 was regarded statistically significant [8,9].

Results

Of the 718 blood samples from the clinically suspected patients included in this study (355 males and 363 females), only 379 (□52.8%) gave positive blood culture for *Salmonellatyphi* proven by specific biochemical tests. The percentage of infected patients according to gender were in males 174 (~45.8%) compared with females 205 (~54.2%) recording statistically non-significant difference as indicated by table(1).

Table 1.The percentage of infected patients with typhoid fever according to gender:

Gender	(+) / %	(-) / %	Total Number/ %	X_2 (P)
Male	174 (45.8%)	181 (53.4%)	355(49.5%)	X_2 = 0.640
Female	205 (54.2%)	158 (46.6%)	363 (50.5%)	P= (0.424)
Total	379 (52.8%)	339 (47.2%)	718 (100.00%)	

Similarly, when incidence compared in terms of standard of education, it was (46.4% among educated versus 53.6% uneducated) with insignificant differences as shown in table (2).

Table 2. The percentage of infected patients with typhoid fever compared in terms of standard of education

Gender	Educated (+) Number / %	Uneducated (+) Number / %	Total(+) Number/ %	X_2 (P)
Male	77 (20.49%)	97 (25.83%)	174 (46.3%)	
Female	99 (25.68%)	106 (27.99%)	205 (53.7%)	X_2= 0.218
Total	176 (46.4%)	203 (53.6%)	379 (100%)	P = 0.641

Additionally, frequency distribution of infection in different age groups was as as shown in table (3). The age groups represented by those falling between (5-14) and those between (15 -45) years showed significantly higher infection rates than others (p-value < 0.05).

Table 3. The percentage of infected patients with typhoid fever in different age groups:

Age group (years)	Number (+)/ %	X_2 (P)
Below 1	16 (4.14%)	
1-4	43 (11.50%)	
5-14	107 (28.21%)	X_2 (df, 4) = 10.653
15-45	176 (46.58%)	P = 0.031
Above 45	37 (9.70%)	
Total	379 (100%)	

Regarding seasonal variations, the highest infection rates were recorded during months of summer and autumn peaking in June recording 19.22% from the annual infection rates, while the lowest rate was recorded in February (1.81%) as shown in figure (1). These data indicated a significant difference in term of frequency of the infection in different months of the year with p-value <0.01.

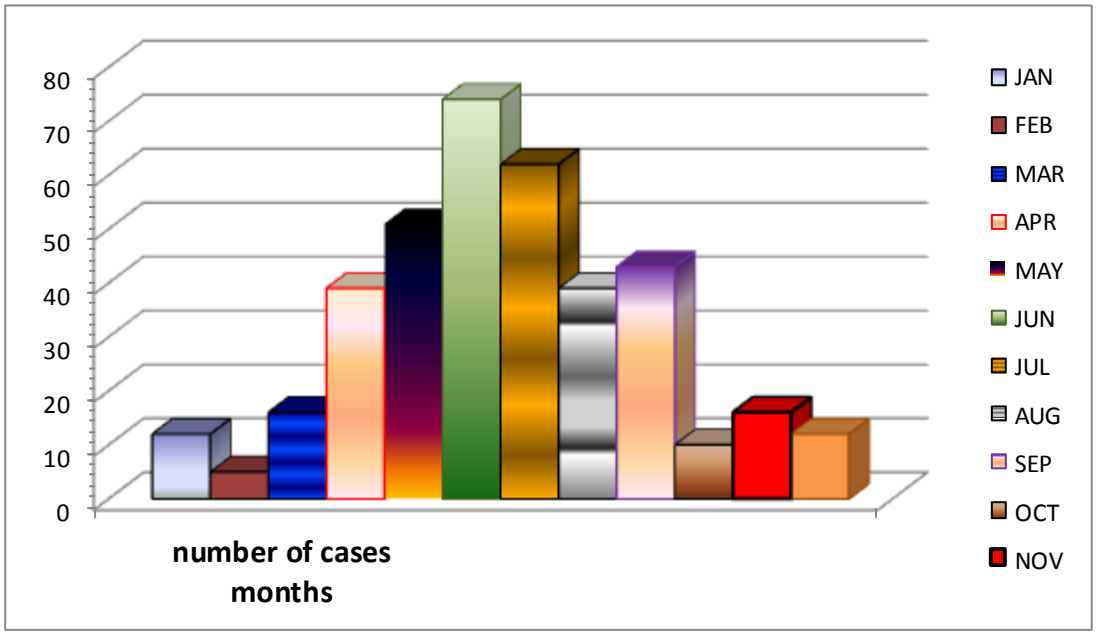

Figure 1. The number of infected patients with typhoid fever distributed according to months

Discussion

The study data regarding the factors that affect the incidence of typhoid fever are consistent with many researchers. Here, the peak incidence was shown to be in june (with high temperature) and this is consistent with the suggestion that the variation in incidence may in part be related to high temperature [10,11]. Tempratureis an important factor aids in the severity and frequency of typhoid fever, via favoring the growth of *Salmonella* during transfers and food

conservation.Another important factor that could regulate incidence of typhoid fever isstress factor that plays a significant role in the events of this disease[12].To address this concern, the peakincidenceof the disease in theage groups (15-45 years) and secondly (5-14 years) may be explained, in part, by the more stress beard by these ages.The results of the current study was comparable with the results of a study by Sobhani *et al.,* (2004) where the infection rates in both age groups are close[13].The percentage of infection in this study for the age group (less than one year) was (4.14%), attributable risk to this percentage is high compared with a previous study in Karbala: (2.3%)[14]. This increase may be attributable to the increased use of bottle-feeding over breast feeding with its draw backs including lower immunity and risk of cross contamination during milk preparation and others. The incidence of typhoid in the age group(1-4 years old) was shown to be (11.5%) and this may be contributed to the possible risk of contamination due to poor child's or parents' hygiene. It has been suggested that the true incidence among very young children and infants is thought to be higher than the expected one. This may be explained by the fact that the presentations in these age groups may be atypical, ranging from a mild febrile illness to severe convulsions, and the *S typhi* infection may go unrecognized. This may contribute to conflicting reports in the literature that this group has either a very high or a very low rate of morbidity and mortality [15]. The study data are inconsistent with previous finding that the age range considered to be at greatest risk was 5-25 years [16]. However this has been questioned in a study from a private laboratory in Bangladesh, which found that the 57% of S. typhi isolates were in children less than 5 years of age and 27% less than 2 years [17]. Furthermore, other study found that the incidence is highest in children and young adults between 5 and 19 years old.[18]. This fact may have significant implications on vaccination policies and these age-variations could be contributed to geographical and/or environmental conditions in addition to factors related to technical and statistical bias. The incidence in the age group (>45 years) was (9.7%) and we can attribute typhoid infection in this age group to the fact that there is generally limited immune response against the invading pathogen. Gender variation was shown to be non significant and our results are comparable to a previous study in USA which recorded that fifty-four percent of typhoid fever cases reported between 1999 and 2006 involved males, the rest being females[19]. Regarding the level of education, it was shown here that it has non significant effect on the disease occurance. However, this finding is inconsistent with two hospital based case-control studies from Vietnam which found that risk of infection was related to lack of education beside recent contact with an infected person, and drinking untreated water[20,21]. Blood cultures are the standard method of diagnosing typhoid fever, being positive in 60% to 80% of cases [6]. However, some researchers have stated that bone marrow is the most sensitive source (80-95%) and positive blood cultures (60-80%) are facilitated by increasing the volume sampled[22].The results of the current methods used in bacteriologic isolation have been close with the results of some studies including the study of Sharma *et al.,* (2003) where the blood culture positivity was (49.2%); and in Nepal, the study of Jog *et al.,* (2008) blood culture was positive in (52.6%)of the total clinically suspected patients[23,24]. Nevertheless, these data are incompatible with the study of Parry *et al.,* (2002)[6] and the reasons could be attributed to the variance and the differences in samples' size examined, in the diagnostic methods used and also may be caused by the difference(s)in the type and/or method of using culture media in addition to variable laboratory conditions.

Conclusion

According to the study data, it is concluded that clinical suspicion alone is not enough to diagnose typhoid fever, because only about half the clinically suspected cases of typhoid fever gave positive blood culture results for the causative pathogen, *Salmonella typhi*. Also, it was shown that there is no significant effect of gender and education standard on rate of infection with typhoid fever (p value > 0.05). However, seasonal variation significantly affect it with highest infection rates during summer and autumn peaking in June; and that lowest rates were recorded in February. In addition, the effect of patients' age significantly affects the rate of infection with peak rates affecting those aging (15-45) yrs. And second most affected age group was (5-14) yrs., while the lowest rate was in young infants below one year old (p-value < 0.01).

Recommendation

Further studies with larger sample size and involving larger geographical areas are recommended to predict the exact prevalence and incidence rates of typhoid fever in Iraq. More advanced future studies are recommended to evaluate the routine serologic test (Widal test) by comparison to blood culture and/or molecular technique such as PCR to determine the exact diagnostic value regarding the sensitivity and specificity rates of such serologic test.

References

1. Crump JA, Luby SP, Mintz ED. (2004). The global burden of typhoid fever. *Bull World Health Organ.*;82(5):346-53. [Medline].
2. Maijala, R. and Ranta, J. (2003). AQuantitative Risk Assessment on Salmonella in Broler Production in Finland . National Veterinary and Food Research Institute, *EELA*, Finland.
3. Kayser F , Medical Microbiology © 2005 Thieme, chapter 2; Bacteriology, pp. 283-89.
4. Vollaard AM, Ali S, Widjaja S, Asten HA, Visser LG, Surjadi C et al. (2005). Identification of typhoid fever and paratyphoid fever cases at presentation in outpatient clinics in Jakarta, Indonesia.Transactions of the Royal Society of Tropical Medicine & Hygiene; 99(6):440-450. *(222 kb)*
5. Ngwu BA, Agbo JA, Ngwu BA, Agbo JA. (2003). Typhoid fever: clinical diagnosis versus laboratory confirmation. Nigerian Journal of Medicine: Journal of the National Association of Resident Doctors of Nigeria; 12(4):187-192. *(16 kb)Abstract only*
6. Parry CM, Hien TT, Dougan G, White NJ, Farrar JJ. (2002).Typhoid fever. *N Engl J Med.*; 347(22):1770-82.
7. Old, D, Collee J. C, Fraser, Y A, Marm B.P.and SimmonsA.Macki and Mccartney Practical Medical Microbiology(1996). 4th ed , produced by lengman singapore publisher LTD ,pp. 385-404.
8. Belle V G,. Fisher D L, Patrick J. Heagerty, Thomas Lumley: Biostatistics.A Methodology for the Health Sciences, Second Edition, Copywrite © 2004 JOHN WILEY & SONS, INC., chapter 11, p 444.
9. Newman S C: Biostatistical Methods in Epidemiology, Fifth Edition, Copywrite © 2001 JOHN WILEY & SONS, INC., Chapter 1, p 8.
10. Latimer H.K.(1999). Quantitive Microbial Risk Assessment for Human Salmonella Associated with consumption of Raw shell Eggs. *PhD.Dissertation* .chapel Hill, NC.
11. Vollaard, A.M. , Ali S. , VanAstan H.A.,SuwandhiW. , Visser L.G. , and Surjadi C.(2004). Risk Factors for Typhoid and paratyphi in Jakarta,

Indonosia .*The Journal of American Medical Assocition* , vol.291 No.21.

12. Ammar M A. (2003). Bacterial isolation and serological diagnosis of Salmonella infection in cattle and rabbits. Master thesis. College of Veterinary Medicine. University of Mosul. Iraq. 89 pages.
13. Sobhani A. , Shodjai H. and Javanbakht S.(2004) Drug Resistance Pattern in Isolated Bacteria From Blood Cultures . *Acta Medica Iranica*, 42(1): 46-49.
14. Kubaisi A H, Hatem R , Caesar A. (2007). Effect of water extracts of the gourd against the parasite Giardia lamblia in the province of Karbala / Iraq. Journal of Karbala, 5 (2) June: 180-81.

    Dutta TK, Beeresha, Ghotekar LH(2001). Atypical manifestations of typhoid fever. *J Postgrad Med*. Oct-Dec;47(4):248-51.
15. Typhoid fever, reviewed byBrian Ostrow MD, CS(C), http://www.SurgeryinAfrica.com; DOE: 7, 24, 2011.
16. Saha SK, Baqui AH, Hanif M, Darmstadt GL, Ruhulamin M, Nagatake T et al. (2001).Typhoid fever in Bangladesh: implications for vaccination policy. Pediatric Infectious Disease Journal; 20(5):521-524.
17. "Typhoid Fever". World Health Organization,http://www.who.int/vaccine_research/diseases/diarrhoeal/en/index7.html. Retrieved 2007-08-28.DOE: 23,7,2011.
18. Lynch MF, Blanton EM, Bulens S, Polyak C, Vojdani J, Stevenson J. (2009).Typhoid fever in the United States, 1999-2006. *JAMA*.;302(8):859-65.
19. Tran HH, Bjune G, Nguyen BM, Rottingen JA, Grais RF, Guerin PJ et al. (2005). Risk factors associated with typhoid fever in Son La province, northern Vietnam. Transactions of the Royal Society of Tropical Medicine & Hygiene; 99(11):819-826.
20. Luxemburger C, Chau MC, Mai NL, Wain J, Tran TH, Simpson JA et al. (2001). Risk factors for typhoid fever in the Mekong delta, southern Viet Nam: a case-control study. Transactions of the Royal Society of Tropical Medicine & Hygiene; 95(1):19-23.
21. Wain J, Pham VB, Ha V, Nguyen NM, To SD, Walsh AL et al. (2001)Quantitation of bacteria in bone marrow from patients with typhoid fever: relationship between counts and clinical features. Journal of Clinical Microbiology; 39(4):1571-1576.
22. Sharma, N. ; Koju, R.; Karmacharya, B. ;Tamang, M.D. ; Makaju, R.; Nepali , N. ; Shrestha, P. ; Adhikari , D. (2003) Typhoid fever in Dhulikhel hospital, Nepal. *Kathmandu University Medical Journal* Vol. 2, No. 3, Issue 7, 188-192.
23. Jog, S. ; Soman , R. ; Singhal, T. ; Rodrigues, C. ; Mehta, A. ; Dastur, F.(2008). Enteric Fever in Mumbai – Clinical Profile, Sensitivity Patternsand Response to Antimicrobials.*JAPI.*Vol.(56) : 237-240.