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Cross-sectional Study

Clinical profile and biochemical abnormalities in brucellosis: A cross-sectional study

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ABSTRACT

Introduction: Brucellosis is the commonest zoonotic disease worldwide and a common public health problem in Nepal. Because of the highly variable clinical presentation and non-specific manifestations, it remains a big challenge for clinicians from developing countries. Brucellosis has a tropism for the reticuloendothelial system, the liver is frequently involved. There is a paucity of data about the laboratory and clinical findings of human Brucellosis from Nepal. To address this knowledge gap, we conducted this study to find out the clinical profile and biochemical abnormalities of patients with brucellosis at a tertiary-care teaching hospital in western Nepal.
Methods: A cross-sectional study was carried out at Gandaki Medical College Teaching Hospital, Pokhara, Nepal. All patients admitted to the in-patient department of our hospital with probable or definitive diagnoses of brucellosis were included. We excluded those who did not consent to their participation in our study, those who were under 18 years of age, and those who had deranged liver function due to other pre-existing illnesses. Descriptive statistics were used to analyze the data in terms of demography, clinical manifestations, and laboratory parameters.
Results: There was a total of 40 confirmed cases of Brucellosis (age: 18–66 years) during the study period. More than half (55%, n = 22) of the study participants were males and most of them lived in a rural setting (77.5%, n = 31). Most of them (70%, n = 28) gave history of ingestion of high-risk food. The commonest clinical findings were fever with/out chills (90%, n = 36) followed by nausea/vomiting (72.5%, n = 29), headache (40%, n = 16) and malaise (37.5, n = 15). Liver function was deranged in a majority of the patients, the common parameters being Alkaline phosphatase in 96% (n = 38) cases, followed by SGOT (62.5%, n = 25), leukocytosis (57.5%, n = 23), total bilirubin (52.5%, n = 21) and SGPT (37.5%, n = 15). Characteristic increment (more than two folds of the upper limit of normal) was observed for alkaline phosphatase.
Conclusion: The reticuloendothelial system is frequently involved in brucellosis. Notable changes were observed in liver function and hematological parameters in a majority of the participants in our study. These findings highlight the need for the implementation of effective control programs to address this problem in the Nepalese context.

1. Introduction

1.1. Background

Brucellosis is the commonest zoonotic disease globally caused by an intracellular bacterium of the genus *Brucella*. It is a major public health problem in resource-poor countries. Human brucellosis occurs due to ingestion of infected animal products or contact with its tissue or fluids. Annually, more than 500,000 cases of human brucellosis occur globally [1]. Incidence of human Brucellosis is increasing in Eastern

Mediterranean countries such as Sri Lanka, India, China, Pakistan, Mongolia, and Nepal [2]. This disease is especially prevalent in communities and countries based on an agrarian economy, where agriculture and livestock rearing are common. The lower incidence of Brucellosis in endemic areas such as Nepal denotes either the absence or low level of disease surveillance and monitoring [2].

1.2. Pathogenesis

Clinical manifestations of human brucellosis are highly variable, ranging from an asymptomatic infection- causing subclinical increase in serum transaminase level to debilitating conditions producing multisystem illness [3]. The disease has a predilection for the reticuloendothelial system involving the liver, spleen, and lymph nodes. The liver is the largest of the three and is frequently involved [4]. Because of the highly variable clinical presentation and non-specific manifestations, it is usually misdiagnosed and remains a big challenge for clinicians from developing countries. This situation is further aggravated by the lack of epidemiologic surveillance as well as the unavailability of proper diagnostic tests in these countries.

1.3. Scientific rationale of the study

In Nepal, brucellosis is reported to be endemic [5]. However, there is a substantial lack of evidence in the literature about the demographics, clinical spectrum, and laboratory findings of human brucellosis. To address this knowledge gap, we conducted this study to find out the clinical and biochemical abnormalities of patients with brucellosis at a tertiary-care teaching hospital in western Nepal.

1.4. Objective

The purpose of this study was to identify the demographic features, clinical manifestations, and patterns of laboratory tests profile of the patients with brucellosis at our center.

2. Materials and methods

2.1. Registration

The manuscript has been reported in line with the STROCSS criteria [6]. It has also been registered with Research Registry at http://www.researchregistry.com/on April 10, 2022, and the unique identifying number is researchregistry7798.

2.2. Study design and setting

A descriptive cross-sectional study was carried out from January 2020 through February 2022 at Gandaki Medical College Teaching Hospital, Pokhara, Nepal.

2.3. Inclusion and exclusion criteria

All patients admitted to the in-patient department of our hospital with probable or definitive diagnoses of brucellosis were included.

Exclusion criteria included those who did not consent to their participation in our study, those who were under 18 years of age, and those who had deranged liver function due to other pre-existing illnesses.

2.4. Diagnosis of brucellosis

Suspected patients underwent serological testing with a rapid chromatographic immunoassay for the qualitative detection of Brucella antigen. This flow assay is a qualitative, membrane-based immunoassay for the detection of Brucella antigen in human serum. The membrane is pre-coated with mouse anti-Brucella antibody. The antigen in the serum if present reacts with the dye-conjugate which has been pre-coated with anti-brucella on the conjugate pad. The mixture then migrates upward on the membrane by capillary action and reacts with mouse anti-brucella antibody on the membrane in the test line region. The absence of the colored lines in the test line region indicates that the specimen does not contain the brucella antigen. To serve as a procedure control, a colored line will always appear in the control line region indicating that the proper volume of serum has been added and membrane wicking has occurred (Acro Biotech Inc., Rancho Cucamonga, California, USA). The diagnosis was confirmed by IgM and IgG sandwich ELISA tests using a commercial kit (Bio-Techne India Pvt Ltd, Pune, India). Concurrently, we did LFT and CBC in those suspected cases. LFT parameters were measured by a fully automatic analyzer, BA-A-280 using the principle as per the manufacturer's manual and CBC was done by Auto Hematology Analyzer, HEMA-D6051 using Flow Cytometry (FCM) principle (Bioevopeak Inc., Seattle, Washington, USA) according to the manufacturer's instruction.

2.5. Statistical methods

The data collected was entered into MS-Excel. After data cleaning, it was imported and analyzed by using SPSS (Statistical Package for Social Sciences) 21. Descriptive statistics were used to analyze the data in terms of demography, clinical manifestations, and laboratory parameters. To check if the data was normally distributed, the Kolmogorov Smirnov test was used. The data followed a normal distribution. So, statistical tests for parametric data were used.

3. Results

3.1. Study sample

During our study period, there were 732 patients with clinical suspicion of brucellosis whose samples were sent to the laboratory for confirmatory diagnosis. A total of 40 patients with confirmed brucellosis were included in the final analysis.

3.2. Demographic characteristics

The demographic and general characteristics of our study population are shown in Table 1. Slightly more than half (55%, n = 22) of the study participants were males and most of them lived in a rural setting (77.5%, n = 31). The patient's ages ranged from 18 to 66 years with a mean age of 49.67 years. Most of the study participants were from the high-risk profession- shepherds and farmers (47.5%, n = 19), shopkeepers

Table 1
Demographic profile among brucellosis patients.

Demographic variables	Frequency (%) (N = 40)
Gender	
Male	22 (55)
Female	18 (45)
Age	
<30	6 (15)
30–60	21 (52.5)
>60	13 (32.5)
Occupation	
Shepherds/Farmers	19 (47.5)
Shopkeepers	10 (25)
Veterinary personnel	6 (15)
Others	5 (12.5)
Ingestion of high-risk food	
Yes	28 (70)
No	12 (30)
Place of stay	
Rural	31 (77.5)
Urban	9 (22.5)

(25%, n = 10), and veterinary personnel (15%, n = 6). Most of them (70%, n = 28) gave a history of ingestion of high-risk food such as unpasteurized milk and dairy products.

3.3. Clinical features

The most consistent clinical findings (Table 2) were fever with/out chills (90%, n = 36) followed by nausea/vomiting (72.5%, n = 29), headache (40%, n = 16) and malaise (37.5, n = 15). Abdominal pain was present in a quarter of the study participants (n = 10) and arthralgia was complained by 20% (n = 8) of the study participants.

3.4. Frequency of laboratory derangement

Table 3 shows the prevalence of abnormal laboratory parameters in our study participants. Alkaline phosphatase (ALP) was deranged in 96% (n = 38) cases. This was followed by deranged SGOT (62.5%, n = 25), leukocytosis (57.5%, n = 23), total bilirubin (52.5%, n = 21) and SGPT (37.5%, n = 15).

3.5. Laboratory profile abnormalities

The derangement of laboratory parameters in our study is summarized in Table 4. Total bilirubin was slightly elevated (1.7 ± 1.2 mg/dL; normal range: 0.3–1.2 mg/dL). Characteristic increment of ALP- more than two folds of the upper limit of normal was seen overall (297 ± 124; normal range: 30–120 IU/L). SGOT and SGPT were also elevated. Leukocytosis, albeit mild, was also found.

4. Discussion

4.1. Background

Brucellosis is a zoonotic disease transmitted to humans by direct or indirect contact with infected animals. It is the most common zoonotic disease globally and is an important public health problem in low and middle-income countries, including Nepal [7]. There are very few papers from Nepal that have tried to explore this disease and our study may be significant to fill in this knowledge gap.

4.2. Demographics

In our study, brucellosis is slightly more prevalent amongst males and it is those who live in rural areas, who are more affected by this condition. This is consistent with findings from other studies from Nepal as well as from other countries [2,8]. Nepal is a primarily agrarian-based economy and the majority of people live in rural areas. Agriculture and livestock farming is the principal occupation of the majority of the Nepalese population and this explains why zoonotic diseases like brucellosis are common in rural areas [9]. Moreover, males are principally involved in shepherding and rearing animals and this may explain why it is more common in males in our setting. Brucellosis usually affects people in the third to fifth decade of life, and our study is also reflective of this finding [10].

Table 2
Reported clinical manifestations in patients with Brucellosis.

Clinical features	Frequency (%)
Fever ± chills	36 (90)
Gastrointestinal upset (Nausea/Vomiting)	29 (72.5)
Headache	16 (40)
Malaise	15 (37.5)
Arthralgia	8 (20)
Abdominal pain	10 (25)

Table 3
Frequency of deranged laboratory parameters among brucellosis patients.

Abnormal laboratory parameters	Frequency (%) (N = 40)
Total Bilirubin	21 (52.5)
SGPT	15 (37.5)
SGOT	25 (62.5)
ALP	38 (95)
WBC	23 (57.5)
Hemoglobin	19 (47.5)

Table 4
Laboratory abnormalities among the brucellosis patients.

Laboratory parameters (Normal range)	Mean ± SD
Total Bilirubin (0.3–1.2 mg/dL)	1.7 ± 1.2
SGPT (5–40 IU/L)	63 ± 59
SGOT (5–40 IU/L)	73 ± 64
ALP (30–120 IU/L)	297 ± 124
Total protein (6–8.3 g/dl)	6.9 ± 0.5
Albumin (3.4–5.4 g/dl)	3.9 ± 0.5
Globulin (2–3.9 g/dl)	3.0 ± 0.3
WBC (4,000–11,000 cells/mm^3)	12,576 ± 4,544
Hemoglobin (12–15 g/dL)	11.7 ± 1.64

4.3. Disease transmission

Brucellosis is transmitted from animals to humans by ingestion of food products such as unpasteurized milk and dairy products, and contact with infected tissue or fluids [2]. In our study, we found out that more than two-thirds of the study participants had a history of ingestion of high-risk food. Brucellosis is increasingly seen in individuals who prefer untreated products. The majority of the Nepalese population in rural areas are still unaware of food processing techniques like pasteurization and consume animal products directly. Many cultural practices in Nepal, for example, the handling of animal dung and consumption of raw blood of animals on a festive scale may also be the reason why brucellosis is seemingly higher in our setting.

4.4. Clinical manifestations

Regarding the clinical manifestations, the most common findings in our study were fever, gastrointestinal upset, headache, malaise, joint pain, and abdominal pain. Brucellosis has a tropism for the reticuloendothelial system, including the liver and spleen but can virtually affect any organ system and can have very variable presentations [11,12].

4.5. Lab findings

Hematological abnormalities are a frequent finding in brucellosis. Anemia, leucopenia or leukocytosis, thrombocytopenia or thrombocytosis, and elevated ESR were detected in patients with brucellosis in many previous studies [13,14]. In our study, anemia, and leukocytosis were found in 47.5% and 57.5% of the participants; their values being 11.7 ± 1.64 g/dL and 12,576 ± 4,544 cells/mm^3 respectively. Anemia in brucellosis may be explained by the alterations in iron metabolism secondary to chronic infection, hypersplenism, bleeding, or autoimmune hemolysis [8]. We did not, however, encounter patients with leucopenia and thrombocytopenia. Different studies have reported different hematological abnormalities and this may be because of differences in the reference range and cut-off values of these parameters, geographical location, severity and duration of infection, co-morbidities, and the timing of collection of specimens for testing during the diseased period [15].

The liver, being one of the major reticuloendothelial organs, is commonly involved in brucellosis and the liver function may be deranged in more than 50% of the patient with active disease [16].

Brucella has been found in liver tissue even in cases without any laboratory liver function changes [4]. In our study, a significant elevation of ALP was found in more than 95% of the study participants, the average value being 297 IU/L. Transaminases (SGPT and SGOT) were deranged in 37.5% and 62.5% of the patients, their mean values being 63 and 73 IU/L respectively. Latent jaundice was seen in more than half the study participants, the serum total bilirubin level being 1.7 ± 1.2 mg/dL. Clinical jaundice was, however, not commonly appreciated. The magnitude of change in liver function tests in brucellosis is variable. However, most studies have reported modest alterations in transaminases and ALP levels [4,8,11,17]. The ALP level in our study was found to be more than two-fold the upper limit of normal, which is reportedly higher than in many other studies [17,18]. This may be explained by the fact that most Nepalese adults, especially in rural areas, produce and consume locally prepared alcoholic beverages. Alcoholism is widespread and culturally accepted in many Nepalese communities. It may also be because of the difference in the biochemical estimation of ALP in different settings and the use of different cut-off values of ALP across studies.

4.6. Limitations

Though our study is important in the Nepalese setting, it has quite a few limitations. Our study is a single-center study and may not accurately reflect the trend in brucellosis incidence. Because of the low sample size, the results may not be generalizable to other parts of the country or across different clinical settings. We didn't follow up with the participants and have not recorded clinical outcomes, which may be an important limitation. We suggest future studies address these concerns and be carried out across multiple centers for a considerable period.

5. Conclusion

Though Brucellosis is an endemic zoonosis in Nepal, there is a paucity of clinical and laboratory data about human brucellosis. Notable changes were observed in liver function and hematological parameters in a majority of the participants in our study. Extensive research and implementation of effective control programs seem imperative.

Ethical approval

Written informed consent was obtained from the study participants. Ethical clearance was taken from the Institutional Review Committee of Gandaki Medical College (Ref. 73/078/079).

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None.

Author contribution

Sanjeeb Shrestha (SS) and Mitesh Karn (MK) were involved in the conceptualization of the study. Sanjeeb Shrestha (SS), Sandip Pahari (SP), and Sanjib Mani Regmi (SR) were involved in the data analysis. All authors (SS, MK, SP, SR, AN) were involved in the design of the study, data collection, literature review, writing and editing of the manuscript, and approved the final version of the manuscript.

Declaration of competing interest

No conflicts of interest.

Registration of research studies

1. Name of the registry: Research Registry (http://www.researchregistry.com).

2. Unique Identifying number or registration ID: researchregistry7798.

3. Hyperlink to your specific registration (must be publicly accessible and will be checked): https://www.researchregistry.com/browse-the-registry#home/registrationdetails/6252443ee90953001e762fcb/

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Consent

All the participants were informed about the study and its objectives during the time of data collection.

Provenance and peer review

Not commissioned, externally peer-reviewed.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.amsu.2022.103922.

References

[1] M. Bosilkovski, M. Dimzova, K. Grozdanovski, Natural history of brucellosis in an endemic region in different time periods, Acta Clin. Croat. 48 (1) (2009) 41–46.

[2] A.K. Prasad, Brucellosis in Nepal - a potential threat to public health professionals, Curr Heal Sci J 42 (4) (2016) 396, https://doi.org/10.12865/CHSJ.42.04.10.

[3] B. Mantur, S. Amarnath, R. Shinde, Review of clinical and laboratory features of human brucellosis, Indian J. Med. Microbiol. 25 (3) (2007) 188–202, https://doi.org/10.4103/0255-0857.34758.

[4] N. Akritidis, M. Tzivras, I. Delladetsima, S. Stefanaki, H.M. Moutsopoulos, G. Pappas, The liver in brucellosis, Clin. Gastroenterol. Hepatol. 5 (9) (2007) 1109–1112, https://doi.org/10.1016/J.CGH.2006.08.010.

[5] C. Knox, L. Gillies, D.D. Joshi, Veterinary public health in the Nepal Himalaya, Can. Vet. J. 41 (11) (2000) 879. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1476451/. (Accessed 29 March 2022).

[6] G. Mathew, R. Agha, J. Albrecht, et al., Strocss 2021: strengthening the reporting of cohort, cross-sectional and case-control studies in surgery, Int. J. Surg. 96 (2021), 106165, https://doi.org/10.1016/J.IJSU.2021.106165.

[7] G. Pappas, N. Akritidis, M. Bosilkovski, E. Tsianos, Brucellosis. *N Engl J Med.* 352 (22) (2005), https://doi.org/10.1056/NEJMRA050570.

[8] H.R. Najari, M.O. Sigaroudi, M. Rastgoo, Clinical manifestations and laboratory findings of brucellosis at Boo-Ali-siena hospital of Ghazvin province of Iran, Jundishapur J Heal Sci 10 (1) (2018), https://doi.org/10.5812/JJHS.14948.

[9] M. Karn, M. Sharma, Climate change, natural calamities and the triple burden of disease, Nat. Clim. Change 11 (10) (2021) 796–797, https://doi.org/10.1038/s41558-021-01164-w.

[10] K.S. Hassan, H. Schuster, A. Al-Rawahi, A. Balkhair, Clinical presentations of brucellosis over a four-year period at sultan Qaboos university hospital and armed forces hospital, Muscat, Oman, Sultan Qaboos Univ Med J 21 (2) (2021) e282–e288, https://doi.org/10.18295/SQUMJ.2021.21.02.018.

[11] S. Mugahi, R. Nashibi, S.M. Alavi, S. Gharkholu, K. Najafi, Epidemiological features, clinical manifestation and laboratory findings of patients with brucellosis, Arch Clin Infect Dis 9 (1) (2014), https://doi.org/10.5812/ARCHCID.17270.

[12] M.P. Franco, M. Mulder, R.H. Gilman, H.L. Smits, Human brucellosis, Lancet Infect. Dis. 7 (12) (2007) 775–786, https://doi.org/10.1016/S1473-3099(07)70286-4.

[13] S. Guler, O.F. Kokoglu, H. Ucmak, M. Gul, S. Ozden, F. Ozkan, Human brucellosis in Turkey: different clinical presentations, J Infect Dev Ctries 8 (5) (2014) 581–588, https://doi.org/10.3855/JIDC.3510.

[14] M.R. Hasanjani Roushan, M. Mohrez, S.M. Smailnejad Gangi, M.J. Soleimani Amiri, M. Hajiahmadi, Epidemiological features and clinical manifestations in 469 adult patients with brucellosis in Babol, Northern Iran, Epidemiol. Infect. 132 (6) (2004) 1109–1114, https://doi.org/10.1017/S0950268804002833.

[15] B. Aygen, M. Doğanay, B. Sümerkan, O. Yildiz, Ü. Kayabaş, Clinical manifestations, complications and treatment of brucellosis: a retrospective evaluation of 480

patients, Med. Maladies Infect. 32 (9) (2002) 485–493, https://doi.org/10.1016/S0399-077X(02)00403-1.

[16] J.D. Colmenero, J.M. Reguera, F. Martos, et al., Complications associated with Brucella melitensis infection: a study of 530 cases, Medicine (Baltim.) 75 (4) (1996) 195–211, https://doi.org/10.1097/00005792-199607000-00003.

[17] O.F. Kokoglu, S. Hosoglu, M.F. Geyik, et al., Clinical and laboratory features of brucellosis in two university hospitals in Southeast Turkey, Trop. Doct. 36 (1) (2006) 49–51, https://doi.org/10.1258/004947506775598752.

[18] W. Jiang, J. Chen, Q. Li, et al., Epidemiological characteristics, clinical manifestations and laboratory findings in 850 patients with brucellosis in Heilongjiang Province, China, BMC Infect. Dis. 19 (1) (2019) 1–6, https://doi.org/10.1186/S12879-019-4081-5/TABLES/3.