Diagnostic of Brucellosis infection using fluorescence spectroscopy

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In the current study, we have diagnosed Brucellosis infection in two patients on the basis of the results from fluorescence emission spectra, excitation spectra and stoke shift spectra (SSS) of blood serum from the patients. A good discrimination is found in the spectral features of the Brucellosis infected patients as compared to normal blood serum samples. The reported preliminary study clearly demonstrates the proof of concept from samples of Brucellosis acquired from the known cases of the patients of Brucellosis. Both the Brucellosis patients can be diagnosed on the basis of the relative intensity of fluorescence biomarkers like tryptophan, tyrosine, collagen elastin, flavin found in their Serum samples.

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1. Introduction

Brucellosis a multisystem febrile illness and is among the commonest zoonotic infections all over the world [1]. Apart from causing a debilitating disease in humans it is associated with a considerable economic burden because of the time lost by patients in their day to day activities [2]. In addition brucellosis is one of the major factors contributing to losses in animal production [3]. It is caused by Gram-negative bacteria Brucella spp. and humans contract infection by direct contact with infected animals, consumption of dairy products and/or through inhalation [4]. The main clinical sign of brucellosis in humans is undulant fever but this sign is, however, common to several other diseases.

Diagnosing brucellosis on clinical grounds is fraught with problems because of diverse and nonspecific nature of clinical manifestations. Isolation of bacteria from patient samples is the gold standard for a definite diagnosis which is however frequently unsuccessful and the laboratory diagnosis of brucellosis is usually made by serological tests [5, 6]. Despite advances in the serological detection of Brucella infection a reliable and timely detection of Brucella infection still remains a diagnostic challenge [7]. Fluorescence spectroscopy is one of a rapid technique to analyze materials [8, 9].

In the present investigation, we have applied fluorescence spectroscopy for rapid diagnosis of Brucella infection in humans, but it can be applied on animals as well. In this study, we have selected two sets of normal and Brucella infection samples. The fluorescence emission spectra, excitation spectra and stokes shift spectra (SSS) of these two sets of normal and Brucella infection samples are recorded and Brucella infection is diagnosed on the basis of the relative intensity of bio fluorophores like tryptophan, tyrosine, collagen elastin, flavin etc. The experimental results of the study provide significant discriminatory spectral features between two sets of normal and Brucella infection samples.

2. Experimental

Instrument

The instrument used in this study was JASCO spectrofluorometer (FP-8200) which is capable of taking excitation, emission, and stokes shift spectra. We used an excitation and emission slit width of 5 nm and a scan speed of 100 nm/min. Light of a specified wavelength with a spectral width of 5 nm falls on the sample in quartz cuvette.

Serum Sample

Three milliliters of venous blood was collected and was allowed to clot. Serum was extracted by centrifugation at 3000 rpm at room temperature. Brucella antibody titer was estimated by tube agglutination test using stained brucella suspensions (Remel Europe Ltd. Dartford, Kent DA2 6PT, UK) in accordance with the instructions of the manufacturers. Briefly, serum sample was diluted to 1:20 and serial doubling dilutions up to 1:1280 were made in 5 ml tubes. To each tube one drop of Brucella suspension was added and the contents of each tube were incubated at 37°C in a water bath for 24 hours before reading the result.

3. Result and discussion

We present here two sets of normal serum sample and Brucella infection samples. They exhibit distinct discriminating spectral features between samples of normal serum and Brucella infection samples. In order to

identify Brucella infection, Emission, Excitation and Stokes Fluoresence spectra are compared with the corresponding spectra of normal blood serum samples. Spectra of Patient-1 are depicted in Figs. 1, 2, 3 and of Patient-2 in Figs. 4, 5, 6. Fig.1 is the fluorescence emission spectra of normal and Brucella infection samples. For comparison we have plotted these spectra together. We can see a peak at 340 nm due to Amino acid, Tryptophan. In Brucella infection sample its intensity is very high and in normal serum spectrum the peak has very low intensity. The spectra show that Brucella infection increases quantity of Amino acid in the patient.

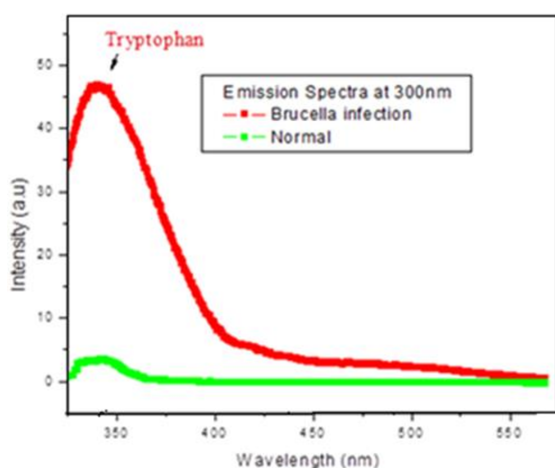

Fig. 1. Emission Spectra of Brucella infection and Normal serum samples excited at 300nm (Patient-1).

Fig. 2 is excitation spectra excited at 360 nm for normal serum and Brucella infection samples which show that there is a very prominent peak at 310 nm due to elastin which is absent in normal serum spectrum. Elastin is a protein primarily composed of the amino acids glycine, valine, alanine, and proline. Just as collagen, it is produced by the connective tissue cells called fibroblasts. This protein is the most important for maintaining youthful skin. Increase of Elastin in the Brucella infected patient might be due to increase of amino acid (Tryptophan) which is shown in Fig. 1.

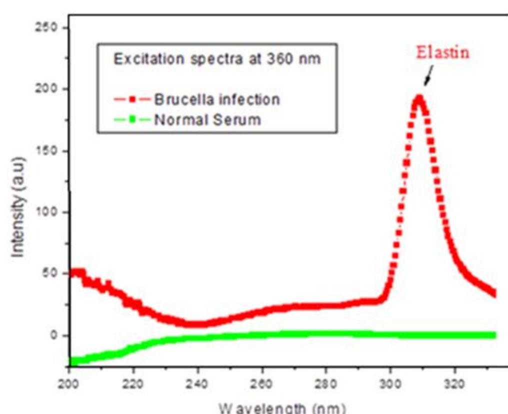

Fig. 2. Excitation Spectra of Brucella infection and normal serum samples excited at 360nm (Patient-1).

Stokes Shift Spectra is depicted in Fig. 3. It shows one peak at 430 nm which is due to Flavin, and other two peaks at 330 nm and 520 which might be due to nicotinamide adenine dinucleotide (NADH) and bilirubin respectively. All these peaks are absent in normal spectra.

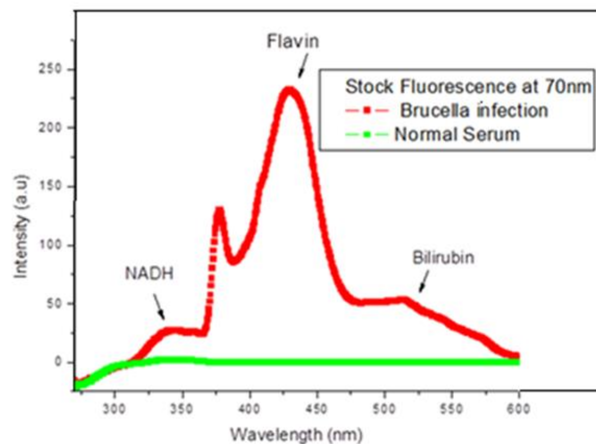

Fig. 3. Stock Shift Spectra of Brucella infection and Normal serum (Patient-1).

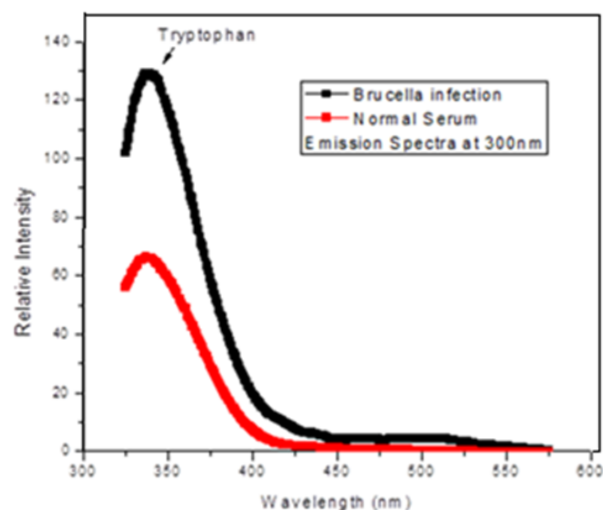

Fig. 4. Emission Spectra of Brucella infection and Normal serum samples excited at 300nm (Patient-2).

For the second set of normal and Brucella infection sample (patient-2) the fluorescence emission spectra shows almost similar behavior as in case of patient-1. Tryptophan peak at 340 nm increases two fold as compared to normal blood serum (Fig. 4). In the case of fluorescence excitation spectra for normal and Brucella infection sample shown in Fig. 5, there is a peak at 310 nm due to elastin which is missing in normal spectra. This is again similar result as in the case of patient-1. The reason for the tremendous increase in Elastin needs to be investigated.

Stokes Shift Spectra of patient-2 is depicted in Fig. 6. This spectra is bit different than the SSS of patient-2. It shows exsitance of Flavin, NADH and Bilirubin but the

corresponding peaks have different intensities. The reason for the difference in intensities might be due to level os stage of the Brucella infection and also because of differet health condition of the patients. The presence of flavin peak indicate excessive riboflavin metabolism in Brucellosis which is also verified by the exiting method.

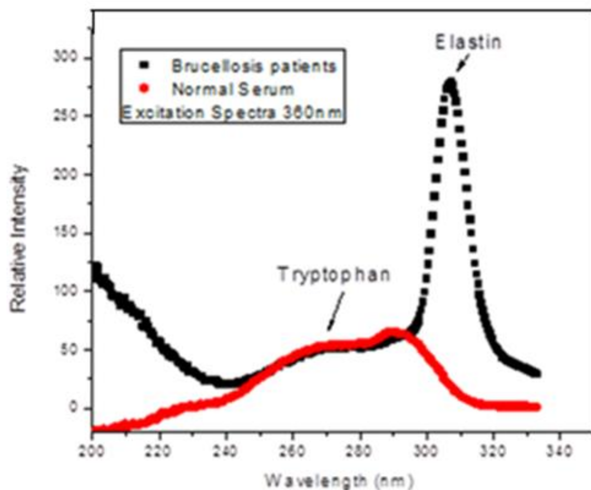

Fig. 5. Excitation Spectra of Brucella infection and Normal serum samples excited at 360nm (Patient-2).

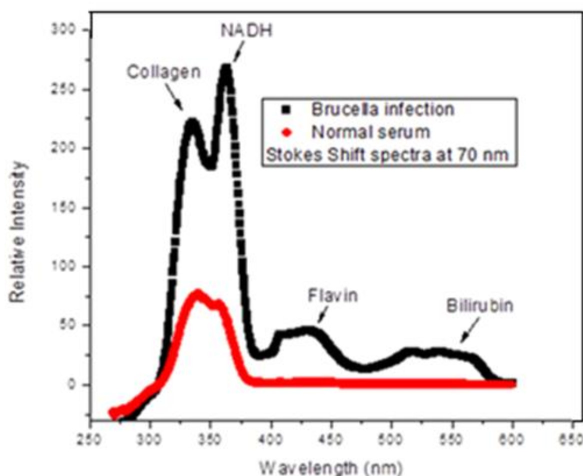

Fig. 6. Stock Shift Spectra of Brucella infection and Normal serum (Patient-2).

The titer of Brucella specific antibody detected was 1:160. No organism was isolated from a concurrent blood sample collected for blood culture. Prior estimation of Brucella specific antibodies two months back yielded a titer of 1:80 and the Brucella organisms were also isolated by blood culture.

The flavin peak observed in the present study indicates excessive riboflavin metabolism in Brucellosis. After gaining access to the intracellular compartment Brucella resides within a membrane-bound vacuole which is fuses with endosomes, lysosomes eventually finding its way to the endoplasmic reticulum (10-11). The replication of Brucella in the intracellular compartment characteristically occurs in locations deprived of nutrients such as amino acids and vitamins under low oxygen tension [13]. Riboflavin (vitamin B2) a precursor of flavin coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) which participate in flavin metabolism and other crucial processes such as energy metabolism, RedOx reactions, detoxification and biosynthesis [14]. FMN has been shown to exist in Brucella and its deficiency induces cytochrome bd a terminal oxidase that works in microaerophilic conditions that Brucella thrives in the intracellular compartment [15]. In addition the existence of an atypical riboflavin metabolism has recently been described in Brucella [16, 17]. It is therefore possible that flavin metabolism in Brucella may help the organism to survive the oxidative stress, lack of nutrients and miroaerophilc conditions and the flavins may be playing a crucial role in these processes thus contributing to bacterial virulence. Moreover flavin metabolism has rarely been described as a virulence factor. The role of flavin metabolism in Brucella was recently investigated in an animal study. Among the two bacterial lumazine synthases (LS) isoenzymes RibH1 and RibH2 evaluated in the study it was found that whereas the presence of at least one of the LS iso-enzymes was necessary for the survival of *Brucella abortus,* RibH2 and not RibH1 was essential for the intracellular replication of Brucella [18]. Collectively these observations highlight the importance of flavin biosynthetic pathway as a potential target for a novel chemotherapeutic approach in Brucella infection.

In other words, we have found one-to-one correspondence between the two techniques first the optical diagnosis and the existing method for diagnosing Brucella.

The present preliminary investigation is based on the limited set of samples of known disease condition. Extensive work with large number of samples should be tried to diagnose Brucella which in future becomes a supplement to the existing methods.

4. Conclusion

We believe this novel method of optical diagnosis of Brucella infection using emission spectroscopy has the potential for diagnosing Brucella disease. This method is clean and rapid. Further studies are underway on larger numbers to better define the use of this technology.

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