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The Importance of Interferon Gamma in Confirming Infection with *Salmonella Entericaserovartyphi*

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Abstract

Background: Typhoid fever, which is caused by *Salmonella entericaserovartyphi*, affects more than 20 million people and kills nearly 200,000 people annually, mostly in developing countries, as the germ is transmitted through food or water contaminated with it, and current treatments and vaccines are completely inadequate To control the disease, Interferon gamma (IFN- γ) plays a pivotal role in the host's defense against *S. typhi*. Natural killer cells (NK) overproduce it in response to cellular signals secreted by some immune cells. Therefore, the overexpression of IFN- γ Stimulates these cells to kill *S. typhi* inside them, thus giving an indication of its presence inside the host, and therefore, increasing its secretion will give an indication of its importance in diagnosis.

Methods: A group of patients infected with typhoid was used and the *S. typhi* strain was isolated from them, then the levels of IFN-γ were measured in the infected patients, in comparison with the control group that was done using a group of healthy people.

Results: The results showed a significant increase in the levels of IFN-γ for infected with typhoid compared to the control group.

Conclusion: These results show the importance of IFN-γ in confirming the infection of typhoid, as *S. typhi* is facultative intracellular with taking into account some criteria in understanding its role in the life of *S. typhi*.

Keywords: IFN-γ, *S. typhi*, SPI, Typhoid fever

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Introduction

Salmonella is an important bacterial genus that causes one of the most common forms of food poisoning worldwide. Throughout history, the type S. entericaserovartyphi has caused many outbreaks of typhoid fever, and even today there are many pathological characteristics that are unknown to it [1], clinical signs are usually What begins 12 to 36 hours after eating food or water contaminated with *S. typhi*, whose symptoms begin with a rise in temperature during the incubation period, which extends from 5-21 days, as the temperature is a distinctive sign of the disease, and is found in more than 80% of people affected by it [2].

S. typhi is distinguished from the rest of the Salmonella strains as well as from the rest of the other genera of the intestinal family by containing its own structures, as it possesses Vi-Ag virulent antigen, which increases its virulence, in addition to its ability to secrete a distinctive toxin, and because humans are the only host The known species of *S. typhi* is difficult to study [3].

Salmonella is a very successful intestinal pathogen, because it has advanced strategies to counter most of the host's immune defense mechanisms during different stages of the disease [4], and it is a facultative intracellular pathogen that can invade different types of host cells, such as microfold cells, Macrophages. , epithelial cells, and since they are facultative anaerobic, they have the ability to infect various conditions [5], the arrival of *S. typhi* to the liver during infection portends a future danger, as it can easily move to the gallbladder, and the clinical signs of it gradually diminish, and the patient switches from the acute condition to the chronic carrier and thus the spread of the germ significantly, so it is necessary to take treatment and accurately diagnose it to avoid its risks [6].

Many of the virulence factors of Salmonella such as adhesion, invasion and toxin genes congregate in specific regions of the chromosome in the form of strands of genes known as Salmonella pathogenicity islands (SPI), and are highly conserved among the different Salmonella serotypes. SPI in Salmonella is a unique and distinctive marker, so host invasion and intracellular replication are hallmarks of Salmonella disease through the control of SPI [7].

IFN-γ is putatively produced by NK cells in response to IL-12 and IL-18, and mediates the regulation of nitric oxide synthase (NOS) that macrophages depend on as one of the antibacterial mechanisms. Most types of host cells, and leads to the transcription of more than 600 genes have an important role in cellular defense against intracellular pathogens [8].

IFN- plays a pivotal role in host defense against Salmonella, and perhaps its most important function in salmonella infection is its ability to activate macrophages to kill intracellular bacteria. Expression of IFN- has long been considered restricted to T-cells and NK cells. It is becoming clear that IFN-γ production can also occur in other cell types, including murine dendritic cells and human macrophages [9].

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Materials and Methods

Chemicals and instruments

Chemicals and media: Xylose-Lysine deoxycholate agar, Salmonella-Shigella agar, kligler iron agar, Human IFN-gamma ELISA Kit, MacConkey Agar, Typhoid IgG/IgM Test-Cassette Kit

Instruments: Incubator, Autoclave, Vitek 2 compact, Hood,Refrigerator, ELISA, Balance

Methods

Bacterial isolation:A portion of stool of patients with typhoid fever was taken with a sterile swab cotton swab and transplanted directly onto MacConkey Agar medium and X.L.D. Agar, and incubated at 37°C for 24 hours, three replicates were grown for each sample.

After observing the growth, the colonies whose centers are black and not fermented to lactose sugar in X.L.D medium were selected. Agar, while colorless and not fermenting to lactose in MacConkey Agar medium, and replanted on S.S. Agar to ensure its characteristics and to obtain pure and single colonies, and after observing the phenotypic characteristics of the colonies, all isolates were confirmed down to the strain using the phytic system [10].

Diagnosis by serological methods:A portion of the patients' blood was taken, to obtain the serum, and the serum was used in the typhoid rapid analysis, which is a lateral flow chromatographic immunoassay, which consists of a cassette case containing a test strip containing H antigen and O antigen conjugated to the form of HO conjugated, These antigens are compatible with *S. typhi* and S. paratyphi antibodies in human serum or plasma.

Measurement of IFN-γ levels: The working tool (K0331121) was used by the Al-Shkairate establishment (Jordan), the method of working recommended by the manufacturer is followed by the method of Aderka*et al*. (1992) [11].

The ELISA technique was used, which is based on the principle of Sandwich-ELISA, as the small ELISA plate available in this test kit was previously coated with an antibody specific to human interferon gamma, and standard solutions or samples were added to the etching of the ELISA microplate and combined with the antibody. The specific, antibodies were added and incubated, and the free components were washed, then a solution of the base material was added to each hole, the holes containing the interferon gamma and the antibodies appeared in blue, the enzyme and the base material reaction was terminated by adding the stopping solution, as the color turned yellow, then The optical density is measured by spectrophotometry at a wavelength of 450 nm, as the value of the optical density is proportional to the concentration of human gamma interferon.

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Statistical Analysis

The search results were analyzed using one-way analysis of variance and identified specific differences between groups using the Duncan test at probability level ($p \leq 0.05$)[12].

Results and discussion

The results of in vitro culture of 26 patients with typhoid fever out of a total of 180 showed 14.4% positive infection with *S. typhi*, 30 people were used and data were taken from them as a control group, MaCconkey Agar medium is differential and selective for Gram-negative bacteria, while XLD Agar medium and SS Agar is highly selective and designed to inhibit the growth of most types of E. coli, as they contain Sodium Thiosulfate, which allows Salmonella to evolve from among the clinical species, and the results regarding shape and culture are similar to those reported by Collee *et al*. [13].

Accurate diagnosis of typhoid fever at an early stage is important, and this importance comes not only in terms of diagnosing the pathogen, but also in identifying people who may be carriers of it, so they have an effective role in the outbreak of acute typhoid fever in the community in which they live. Typhoid IgG / IgM rapid There were significant significant differences at the probability level of P-Value = 0.005 as shown in Table 1, as 134 patients and a percentage of 74.4% showed a positive result for antibody IgM + only, and this indicates a recent infection, and it was Among them, 26 infected showed a positive result for the stool culture of *S. typhi*, while the rest of the test groups were negative for the stool culture, while they were positive for IgG + antibody by 4.4%, which indicates a previous infection with the disease, and by 21.2% positive for IgM + and IgG + together, which indicates an old and recent infection or an old and unrecovered infection, while the control group was negative for both IgM- and IgG-, as the living body and after the immune response to the pathogen begins to secrete IgM antibody whose concentration gradually decreases inside the body, and from Then The body begins to secrete IgG, the concentration of which remains for several months.

Table 1: Results Typhoid IgG / IgM Rapid Test.

NO.	Typhoid Test Categories Rapid IgG/IgM	The patients		Number and ratio of patients positive for *S. typhi*		control group	
		number	ratio%	number	ratio%	number	ratio%
1	IgG - and IgM +	74.4%	134	100%	26	0	0
2	IgG + and IgM –	4.4%	8	0	0	0	0
3	IgG + and IgM +	21.2%	38	0	0	0	0

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4	IgG - and IgM -	0	0	0	0	100%	30
	Total	100%	180	100%	26	100%	30

This test is one of the important serological tests used in laboratories, and it is of great importance in identifying patients with typhoid fever. When the control group in our study was negative for the test at 100%, it gives high sensitivity in confirming the absence of infection for people who have symptoms similar to typhoid fever.

The sensitivity of the test to positivity is limited. The results of the study showed that only 26 patients, 14.4% of those infected with typhoid, had the result of laboratory culture positive for *S. typhi*. These results are consistent with the findings of Farhan *et al*. (2018) in Diyala Governorate, The infection rate was 13.3% and it was shown that serological methods are less effective in the laboratory diagnosis of typhoid fever, as well as the infection rate was 15% in the results of Hassouni*et al*. (2017) in Babil Governorate, who indicated that the test is good for differentiating between typhoid fever disease and other diseases with signs Clinical similarities [14, 15].

The reason for the low sensitivity of this test may be attributed to the usual exposure of individuals in the community to Salmonella antigens through contaminated food and water. Due to the cumulative presence of these antibodies in the circulation, Salmonella infection may also lead to a broad, polyclonal stimulation of the immune response, resulting in not only specific antibodies to it, but also antibodies to a interacting group or other groups of antigens. (O) and (H) for it, and this may explain the multifaceted picture of serological results that can often be encountered during the serological diagnostic evaluation of salmonella infection [15], as Saleh and Mahmud (2013) mentioned that Iraq is an endemic area of salmonella, so most of the population in Continuous exposure to them with high standards for antibodies in their sera [17].

The laboratory culture remains one of the best and most accurate methods used in diagnosing salmonella, and the sensitivity of the test varies according to the skill of the technical person and the type of sample used in laboratory culture. Blood is less sensitive to *S. typhi* than stool. In this study we could not isolate it from blood, and the reason is The basis for this is that its presence in it is only during the first week of infection, as is the case with the traditional methods of diagnosing it, and giving antibiotics, because their numbers are few in the blood [18].

The study included 42 serum samples from different categories of patients in addition to the control group, and the results showed significant differences at the probability level P-Value = 0.008, where the average level of IFN-γ was significantly high in patients with typhoid infection Recent and positive results for laboratory culture of *S. typhi* as it reached 36.39 pg/ml, while the rest of the groups results were within the limits of the control group, as shown in Table 2.

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Table 2:Serum levels of IFN-γ concentration in groups of subjects.

NO.	studied categories	Number of people infected per class n(%)	IFN-γ . concentration Picogram/milliliter (pg/ml) Standard deviation ± Mean
1	TMM	6 (14.3%)	36.39 a ± 4.66
2	TM	24 (57.1%)	24.19 b ± 4.56
3	TMG	8 (.119%)	25.11 b ± 3.35
4	control group	4 (9.5%)	21.50 b ± 2.54
	Total	42(100%)	** P-Value = 0.008

TMM: patients with typhoid, recent and confirmed infection with *S. typhi*, TM: patients with typhoid with recent and negative infection with laboratory culture, TMG: patients with typhoid with previous and negative infection with laboratory culture, **= Similar letters mean no significant differences

The results of the current study are a good example of the importance of IFN-γ in people with typhoid, since IFN-γ is restricted to viruses as it is obligate intracellular, it can be considered of diagnostic importance for Salmonella because it is facultative intracellular facultative, and this importance also comes from the urgent need For a rapid diagnostic test to confirm the infection of typhoid without the need to return to the laboratory culture because it is delayed by 3-4 days and requires high skill in work, which will double the infection in patients in both cases. Typhoid patients TM group with a high IFN-γ level and people with a low level close to the control group, while most of the people in the TMG group had a low level of IFN-γ concentration, and since the TMM group had a high concentration of IFN-γ, this gives great evidence that the proportionality There is a direct correlation between typhoid infection and the presence of salmonella in infected persons, and our results agree with what was found by Sani *et al.* (2020) in a study conducted in Nigeria, where the concentration of IFN-γ in the control group reached ≤ 15 pg/ml, and by comparing its results, it was mentioned that the proportion was directly proportional between typhoid infection and the level of IFN-γ, which was higher than the normal level in people with typhoid confirmed with laboratory culture [19].

Aburesha and Seger (2019) showed in a detailed study conducted in Iraq on the importance of IFN-γ in patients with typhoid, that its level was higher in the acute phase of infection compared to the control group, and the most important result was its higher level in the chronic phase More From the acute stage, which gives a logical explanation for the importance of IFN-γ in detecting

the pathogen inside cells, as well as in diagnosing *S. typhi* carriers as they are more dangerous to society and contribute significantly to its spread [20].

Studies on the importance of IFN-γ in confirming typhoid fever remain limited and recent, and need more data and results about it, because it is non-specific, and it is generally secreted against all intracellular pathogens, as well as other functions, as it contributes to T-cell differentiation. Type T helper cells, and has a role in intracellular iron regulation [21].

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