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Effect of iron overload on prevalence of common bacterial infection in thalassemia patients

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Abstract:

BACKGROUND: Thalassemia is the most common human monogenic disease in the world, associated with several consequences, including recurrent infections, many studies have demonstrated a high prevalence of infection among individuals with thalassemia accounting for about 10% of cases.

OBJECTIVE: This study aimed to detect the effect of serum iron imbalance on the prevalence of infection in thalassemia patients.

MATERIALS AND METHODS: This research was conducted in the Thalassemia Teaching Department at Al Karama Hospital, between March and October 2023. The case–control study was conducted by comparing 60 people with thalassemia with 60 other people who were not thalassemic but suffering from fever and similar symptoms. Through examining urine, sputum, blood, and skin swab samples, samples with a positive result for the bacteria were identified, their sensitivity to antibiotics was examined, and these results were compared with the control group. *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*, three types of the most common bacteria were chosen for testing due to the limited resources.

RESULTS: Control groups of 20 males and 30 females with a mean age of 20 ± 4.5 years compared to 60 nonthalassemia febrile patients classified into 34 males and 26 females with the same age range as control groups. It was discovered that the majority of thalassemia patients are infected with *E. coli* bacteria. It also depends in its infection rate on an imbalance in the level of iron in the blood, whether it is increased, as in thalassemia, or decreased in cases of iron deficiency anemia that *E. coli* bacteria is the type that most infects thalassemia patients.

CONCLUSION: Common species of bacteria responsible for infection in thalassemia patients represented by *E. coli*, *K. pneumoniae*, and *Staphylococcus aureus* associated with serum iron level changes highlighting the need for additional research into the impact of iron imbalance on infections. The study found that imipenem and meropenem had a profound effect on the vast majority of bacterial species involved in thalassemia.

Keywords:

Bacterial infection, culture and sensitivity, serum iron, thalassemia

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Introduction

Thalassemia is a group of hereditary anemia caused by mutations in the hemoglobin (Hb) gene clusters that slow down the production of one or more of the globin chain subunits of the Hbs tetramer. They are the most common human monogenic disease in the world.[1] Although thalassemia has been found in people of every race and culture, it is especially prevalent in a large swath of the world spanning from the southern tip of Africa through the Mediterranean and the Middle East and Arabian Peninsula to the Indian

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subcontinent, India, and the rest of South and Southeast Asia.[2]

The prevalence of beta-thalassemia carrying exceeds 1.1%.[3] The prognosis of the condition is enhanced by the administration of regular blood transfusions and iron-chelating therapy[4] that life expectancy of individuals with homozygous β-thalassemia has significantly increased from a mere decade to approximately 30.[5,6] Thalassemia is associated with several consequences, including recurrent infections, many studies have demonstrated a high prevalence of infection among individuals with thalassemia accounting for about 10% of cases.[7-11] The primary causes of infection have been attributed to blood transfusions, specifically involving hepatitis B and C viruses, hepatitis G virus, cytomegalovirus,[12,13] human immunodeficiency virus,[14] and malaria.[15]

Bacterial infections observed in individuals with thalassemia were pneumonia, biliary tract infection, soft-tissue infection, septicemia, and liver abscesses. The bacteria most commonly isolated in these cases included *Klebsiella pneumoniae*, *Escherichia coli*, *Streptococcus pneumoniae*, Salmonella typhi, *Yersinia enterocolitica*, and other Gram-negative bacteria[8-10] usually lead to a number of unusual complications.

Up to 40% of all healthcare-associated infections are caused by urinary tract infections (UTIs), and the prevalence of UTIs varies by population. *E. coli*, the most common pathogen responsible for UTIs, has been shown to thrive in iron-rich environments, whereas clinical research has not yet confirmed a definitive link between iron burden and infection risk. However, it is evident that iron overload makes a number of other microorganisms more dangerous.

The reasons why people with thalassemia are more likely to get sick from infections are not well understood. Anemia, reticuloendothelial system dysfunction due to iron and hemolyzed erythroblasts, iron overload, and altered immune response are all possible causes, as are splenectomy, transfusion-related infections, and iron chelation[16] The aim of this research is to ascertain the rate of serious bacterial infections among individuals with thalassemia and the correlation between iron levels and their variations in blood serum and the prevalence of bacterial infection.

Materials and Methods

Study design

A case–control research was conducted at the Al Karama Teaching Center of Thalassemia in Baghdad, Iraq. Between March and October 2023, a study was conducted with a group of 60 febrile patients with thalassemia. This group was compared to a control group of 60 febrile patients without thalassemia.

Inclusion and exclusion

The age range of the participants in both groups was between 10 and 40 years, with complaint of signs and symptoms of infection, especially fever. The current inquiry involved the collection and analysis of urine, blood, sputum, and stool samples to determine the presence of bacterial growth.

The patients sought medical consultation either at the outpatient clinic or the emergency unit of the hospital. The inclusion criteria for this study consist of B-thalassemic patients, specifically those with B-thalassemia major or intermedia, who have a fever exceeding 37.4°C. These patients have not used antibiotics in the 3 days before the study and do not have any conditions that would increase their risk of UTI, such as obstructive uropathy or diabetes mellitus. The study excludes patients with other hemoglobinopathy.

All participants, including both patients and the control group, underwent blood examination. The blood testing device used to do a complete blood count or hemogram is called a hematology analyzer. The most accurate test for determining the body's iron levels is the ferritin test. The iron status profile includes a ferritin test. There is a clear relationship between the body's total iron storage and ferritin levels. A general urine examination and urine culture/subheading title in examination. The urine samples were obtained using the midstream urine technique and stored in sterile universal containers. The samples were evaluated within 15 min of collection. Swabs were used to collect discharge from the pharynx, nasal cavity, sputum, stool, and other diseases. Then, inoculate the clinical samples into media that would enrich, differentiate, and selectively grow the organisms of interest.

Pyuria was deemed positive if there were more than 5 pus cell/m^3, and a pure colony count of 105/mL or more was regarded as indicative of a positive culture. The other media for different bacteria include deoxycholate citrate agar for intestinal pathogens, mannitol salt agar for staph bacteria, MacConkey agar, blood agar, eosin-methylene blue agar (EMB) agar for Gram-negative bacteria, cholate agar, and xylose-Lysine deoxycholate agar for growth of salmonella and shigella.

After 24 h, the plates were examined for signs of bacterial development. Gram staining was used to determine the isolates' morphologies.

Catalase, coagulase, IMViC, urease test, oxidase test, O/F test, nitrate reduction test, triple sugar iron agar test, CAMP test, PYRase test, bile solubility test, sugar fermentation test, and many more biochemical tests were performed to confirm the organism.

The Kirby–Bauer diffusion method was used to determine the antibiotic susceptibility pattern of bacteria on Mueller–Hinton agar, which involves plating bacteria on a solid growth medium and then adding antibiotic wafers (shown as white disks) to the plate. Overnight bacterial growth in the presence of the antibiotic was shown to be inhibited by the presence of clear media around the disks. As the antibiotic diffuses more into the media, the concentration of the antibiotic diminishes. As a result, the clear bacteria-free zone around the disk containing that antibiotic grows in size as the bacteria become more sensitive to it (free to use; courtesy of Wikipedia). Standard antibiotics were utilized in the study,[17] and the inhibition zone was measured and compared with the clinical standard manufacturer guideline.

Ethical consideration

The Iraqi Council of Medical Specializations Review Ethics Committee approved this study, and each patient provided written informed consent before they were enrolled in the research.

Statistical analysis

The statistical analysis was performed using the Chi-square test (EPI info V3.5, Microsoft comp., USA), the Statistical Package of Social Sciences Stati (SPSS, IMB company, USA), and data presented as mean, standard deviation, and range and presented by frequency and percentage.

Results

In this study, 60 febrile thalassemic patients were classified into 20 males and 30 females with a mean age of 20 ± 4.5 years compared to 60 nonthalassemia febrile patients classified into 34 males and 26 females with the same age range as control groups.

Table 1 presents the hematological assessment conducted on male and female patients with β-thalassemia major (βTM), in comparison to the control group. The hematological parameters exhibited signs of deterioration.

The occurrence of statuses was observed in both male and female individuals among our βTM patients. The male βTM patients exhibited severe anemia with a mean hemoglobin level of 7.8 ± 0.4 g/dL, whereas the female βTM patients had a mean hemoglobin level of 7.2 ± 1.4 g/dL. In addition, both males and females with βTM presented with significant iron overload, with mean serum ferritin levels of 6124.4 ± 3290 ng/mL and 6040.2 ± 3872.0 ng/mL, respectively.

After comprehensive assessment was conducted, encompassing the examination of the chest, abdomen, tonsils, meningeal signs, and skin.

The clinical diagnosis of UTI was established in individuals with signs and symptoms of UTI such as abdominal pain, dysuria (painful urination), and increased frequency of urination. The assessment of individuals for indications and manifestations of respiratory distress, as well as the utilization of chest X-ray for individuals who are thought to have pneumonia, is important diagnostic measures. Historical analysis and physical examination were conducted, accompanied by several diagnostic tests, to confirm or rule out alternative diagnoses such as hepatitis, measles, mumps, otitis media, and other potential conditions. The final result of the diagnosis is in Table 2 with the number of patients.

As shown in Table 3, there are 34 (56.6%) of infected thalassemia patients had positive cultures whereas only 12 (20%) positive cultures were reveald in the control group. Through culturing various samples, we notice that the most common bacteria infecting thalassemia patients is *E. coli* especially in the urine sample. The percentage of different types of bacteria responsible for various infections in thalassemia patients is mentioned in Table 3.

Table 1: Haematological and age mean value evaluation in thalassemic patients and control groups

Parameter	Male			Female		
	Cases (*n*=20)	Control (*n*=26l)	*P*	Cases (*n*=34)	Control (*n*=40)	*P*
Age	20±5.5	34±8		20±2.2	20±2.2	
CBC						
WBC ×10^9/L	36.4±26.8	7.5±1.5	<0.001	40±24.7	7.1±1.2	<0.001
Hb (g/dL)	7.8±0.4	13.9±2.7	<0.001	7.2±1.4	12.6±2.8	<0.001
PLT ×10^9/L	603.6±208.6	261.0±37.2	<0.001	571.2±253.8	270.1±41.6	<0.001
Ferritin (ng/mL)	6124.4±3290	84.7±28.1	<0.001	6040.2±3872.0	40.1±36.2	<0.001

CBC=Complete blood count, WBC=White blood cell, Hb=Hemoglobin, PLT=Platelet

Due to the constraints of limited resources and budgets, I have chosen the most widespread bacterium for my research in order to conduct an antibiotic susceptibility test. The organism is *Escherichia coli, K. pneumonia* and *Staphylococcus aureus*. The number of positive results for was 16 and 9, respectively.

Discussion

Significant numbers of Iraqi people in Baghdad city carry thalassemia variations of the HHB gene, making thalassemia a serious public health issue in Iraq.

Prevalence estimates for the thalassemia gene in the Baghdad city range from 2.6% to 4.3%, according to prior studies.[18]

In this case–control study, the primary objective was to ascertain the prevalence of bacterial infections among individuals with thalassemia major who were receiving treatment at Al-Karama Teaching Hospital/thalassemia center in comparison to the control group of the same age group and complaints from symptoms almost similar to that in thalassemia patients and who visit the hospital for treatment of other diseases. The basic clinical characters are detailed in Table 1. Both male and female patients with βTM exhibited deteriorated hematological profiles that severe anemia and iron overload were reported in both male and female in corresponding the control group. These results are consistent with all research on hematological value in thalassemia patients. Statistical analysis revealed that there were no statistically significant differences ($P > 0.05$) observed between males and females This result is consistent with what was reported in the Abdulomohsin MM *et al*.[19]

Out of a total of 120 patients of both groups visiting the hospital due to fever and symptoms of other organ infections, which were diagnosed by the consultant physician, as mentioned in Table 2. We note that UTI represents most of the infections in compared to the control group, thalassemic individuals have a significantly higher incidence of UTIs (14% vs. 5%, $P = 0001$). This is an approach to the study in Thailand by Wanachiwanawin[11] the second-most common infection is pneumonia as 10% incidence and this result is lower than that of previous research[12,20] and may be due to the widespread use of pneumococcal vaccines which became widely available, the number of cases of pneumococcus disease dropped significantly.[21] Furthermore, as a result of the large number of viral lung infections that were considered nonspecific infections.

Infections are a common problem for thalassemia patients., with a significant proportion (more than 56%) experiencing infections/replaced by/Thalassemia patients frequently encounter infections, with a substantial percentage (about 56%) experiencing such illnesses. In the current investigation, a total of 60 febrile thalassemia patients were examined, with 66% being females and 34% being men.

The present study has assessed that a majority of thalassemic individuals, specifically 56.6%, exhibited positive blood cultures compared with the control group of 20%. In a separate study conducted by Wang *et al*., in 2003, it was observed that 22.5% of individuals with thalassemia experienced episodes of bacterial infection.[12]

The most common pathogens responsible for infection in thalassemia were *E. coli* (35.4%), *S. aureus* (17.6%), and Klebsiella (17.6%).

Table 2: Common diseases diagnosed in both groups

Causes of fever	Thalassemia patients, *n* (%)	Control patients, *n* (%)
Pneumonia	6 (10)	1 (1.6)
UTI	12 (20)	7 (11.6)
Gastroenteritis	3 (5)	13 (21.7)
Tonsillitis	1 (1.6)	18 (30)
PUO	20 (30.3)	10 (16.6)
Nonspecific infection	10 (20)	8 (13.3)
Abscess	8 (13.3)	3 (5)

UTI=Urinary tract infection, PUO=Pyrexia of unknown origin

Table 3: Microorganisms causing different types of bacterial infection in both groups

Bacteria names	Percentage of positive cultures	
	Thalassemia patients (*n*=34), *n* (%)	Control patients (*n*=12), *n* (%)
Escherichia coli	12 (35.4)	4 (33.3)
Klebsiella pneumoniae	6 (17.6)	3 (25)
Streptococcus pneumoniae	3 (8.8)	0
Staphylococcus aureus	6 (17.6)	1 (8.3)
Pseudomonas aeruginosa	3 (8.8)	1 (8.3)
Enterococcus	2 (5.9)	2 (16.8)
Streptococcus pyogenes	2 (5.9)	1 (8.3)

In a related study conducted in Belgium, *E. coli* was reported to be the most common cause of Gram-negative bacteremia, whereas *S. aureus* was the most common cause of Gram-positive bacteremia among hemodialysis patients.[22] Half of the pathogens in thalassemia infections in Thailand were Gram-negative bacilli such as *E. coli* and *K. pneumoniae*. In this research, *E. coli* species proved to be the most prevalent pathogens. Previous research involving thalassemia patients from Asia confirms that klebsiella.

There are three predisposing factors that can increase the infection rate in individuals with thalassemia: the chronic nature of the disease, splenectomy, and the use of deferoxamine as an iron chelator.[23] Another study conducted in Saudi Arabia found that this particular risk factor had no significant impact on the prevalence of infection in individuals with thalassemia major, Al-Zahrani *et al*.[24] among the investigation that was performed on the control group and was not mentioned in the results, there were more than half of the cases of positive *E. coli* culture had iron deficiency anemia, perhaps the reason for this is that iron deficiency also leads to an increase in infections, this result revealed that iron imbalance either overload or deficiency enhances *E. coli* infection but this need farther study to prove it, Prol *et al*.[25] where several study explaining the effect of high serum iron on bacterial growth, Hereditary hemochromatosis and thalassemia are two examples of iron overload illnesses that leave patients extremely vulnerable to bacterial and fungal infections.[26-28]

Most important details that focused on, is the sensitivity of Bacteria for different types of common antibiotics used in Iraq.

We clearly notice the sensitivity of all types of bacteria that appeared in the culture to epimenem and meropenem, While the sensitivity of this bacteria to other antibiotics varies to varying degrees [as mentioned in Table 4, and also in Figure 1].

The most commonly isolated bacteria was *E.coli* most often seen in urine samples (34.7%). It was highly resistant to augmentin and third generation cephalosporins and exhibited great sensitivity to imipenem and meropenem. The second most common bacteria found in cultured samples was *K. pneumonia*, which is the most prevalent cause of pneumonia in children and young adults, (20% of tests) Which had nearly similar antibiotic sensitivity result of *E. coli*, except for doxycycline, which revealed extreme sensitivity. Similar findings were reported by Liao *et al*.[29] and Tabar *et al*.[30]

S. aureus positive culture in about 15% of results, especially from skin samples where about 5% of this growth comes from skin sample. The findings matched those of a community-based study conducted in rural Erbil;[31] bacteria most frequently susceptible to imipenem, meropenem, ciprofloxacin and nalidixic acid, less sensitive to third cephalosporin generation and rapidly evolves resistance to amikacin and doxycycline.

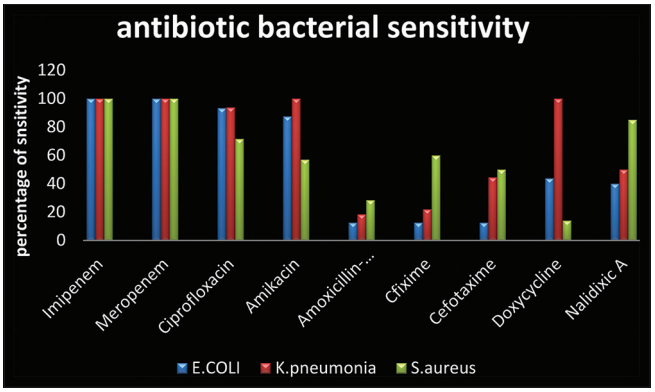

Figure 1: Bacterial susceptibility to different antibiotics

Table 4: Antibiotic sensitivity and resistance pattern of *Escherichia coli, Klebsiella pneumoniae*, and *Staphylococcus aureus* isolated from different sites of thalassemia patients

Antibiotics	*Escherichia coli*		*Klebsiella pneumoniae*		*Staphylococcus aureus*	
	Sensitivity	Resistence	Sensitivity	Resistence	Sensitivity	Resistence
Imipenem	100	0	100	0	100	0
Meropenem	100	0	100	0	100	0
Ciprofloxacin	93.75	6.25	93.75	6.25	71.5	28.5
Amikacin	87.5	12.5	100	0	57	43
Amoxicillin-clavulanic A	12.5	87.5	18.5	81.25	28.5	71.5
Cefixime	12.5	87.5	22	78	60	40
Cefotaxime	12.5	87.5	44.4	55.6	50	50
Doxycycline	43.75	56.25	100	0	14	86
Nalidixic A	40	60	50	50	85	15

Conclusion

The study's findings are consistent with those of other studies looking at the link between elevated iron levels and an increased risk of bacterial infections, but it also shows that low iron levels often accompany UTIs, highlighting the need for additional research into the impact of iron imbalance on infections. Found that imipenem and meropenem had a profound effect on the vast majority of bacterial species involved in thalassemia.

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Conflicts of interest
There are no conflicts of interest.

References

1. Cappellini MD, Porter JB, Viprakasit V, Taher AT. A paradigm shift on beta-thalassaemia treatment: How will we manage this old disease with new therapies? Blood Rev 2018;32:300-11.
2. Weatherall DJ. The evolving spectrum of the epidemiology of thalassemia. Hematol Oncol Clin North Am 2018;32:165-75.
3. Lin KH, Lin LL, Su S, Lin KS. The incidence of b-thalassemia trait in Taiwan. Int J Pediatr Oncol 1994;1:383-8.
4. Borgna-Pignatti C, Rugolotto S, De Stefano P, Piga A, Di Gregorio F, Gamberini MR, *et al.* Survival and disease complications in thalassemia major. Ann N Y Acad Sci 1998;850:227-31.
5. Forget BG. Thalassemia syndrome. In: Hoffman R, Benz EJ, Shattil SJ, Furie B, Cohen HJ, Silberstein LE, editors. Hematology: Basic Principles and Practice. 3rd ed. Orlando, FL: Churchill Livingstone Inc.; 2000. p. 486-506.
6. Hong GR, Beharman RE. Hemoglobin disorder. In: Nelson Text Book of Pediatrics. 6th ed. Nashville, USA: HarperCollins Christian Publishing; 2000. p. 1484-5.
7. Aswapokee P, Aswapokee N, Fucharoen S, Sukroongreung S, Wasi P. Severe infection in thalassemia: A prospective study. Birth Defects Orig Artic Ser 1987;23:521-6.
8. Chung BH, Ha SY, Chan GC, Chiang A, Lee TL, Ho HK, *et al. Klebsiella* infection in patients with thalassemia. Clin Infect Dis 2003;36:575-9.
9. Issaragrisil S, Wanachiwanawin W, Bhuripanyo K, Benjasuratwong Y, Piankijagum A, Wasi P. Infection in thalassemia: A retrospective study of 1,018 patients with beta-thalassemia/Hb E disease. Birth Defects Orig Artic Ser 1987;23:505-11.
10. Peng CT, Tsai CH, Wang JH, Chiu CF, Chow KC. Bacterial infection in patients with transfusion-dependent beta-thalassemia in central Taiwan. Acta Paediatr Taiwan 2000;41:318-21.
11. Wanachiwanawin W. Infections in E-beta thalassemia. J Pediatr Hematol Oncol 2000;22:581-7.
12. Wang SC, Lin KH, Chern JP, Lu MY, Jou ST, Lin DT, *et al.* Severe bacterial infection in transfusion-dependent patients with thalassemia major. Clin Infect Dis 2003;37:984-8.
13. Singh H, Pradhan M, Singh RL, Phadke S, Naik SR, Aggarwal R, *et al.* High frequency of hepatitis B virus infection in patients with beta-thalassemia receiving multiple transfusions. Vox Sang 2003;84:292-9.
14. Schiliro G, Romeo MA, Di Gregorio F, Sciacca A, Musumeci S, Russo G. Infections and Cooley disease. Birth Defects Orig Artic Ser 1987;23:487-93.
15. Choudhury NJ, Dubey ML, Jolly JG, Kalra A, Mahajan RC, Ganguly NK. Post-transfusion malaria in thalassaemia patients. Blut 1990;61:314-6.
16. Vento S, Cainelli F, Cesario F. Infections and thalassaemia. Lancet Infect Dis 2006;6:226-33.
17. Collee JG, Mackie TJ, McCartney JE. Mackie and McCartney Practical Medical Microbiology. 14th ed. New York: Churchill Livingstone, An Imprint of Elsevier Publications; 1996.
18. Hassan IY, Khalid JK, Nasir A, Helmi F. Thalassaemia genes in Baghdad, Iraq. East Mediterr Health J 1996;2:315-9F.
19. AL-Zubaidi MM, Alkhtaua KJ. Assessment of serum ferritin levels in thalassemia and non-thalassemia patients presented with anemia. Iraqi J Haematol 2015;4:119-125.
20. Rahav G, Volach V, Shapiro M, Rund D, Rachmilewitz EA, Goldfarb A. Severe infections in thalassaemic patients: prevalence and predisposing factors. Br J Haematol 2006;133:667-74.
21. Chen CH, Su LH, Li HC, Hsu MH, Wu TL, Chen CL, *et al.* Evaluation of the impact of 13-valent pneumococcal conjugate vaccine immunization in children by surveillance of culture-confirmed pneumococcal disease: A prospective clinical microbiological study. Vaccine 2019;37:5147-52.
22. Tielemans CL, Lenclud CM, Wens R, Collart FE, Dratwa M. Critical role of iron overload in the increased susceptibility of haemodialysis patients to bacterial infections. Beneficial effects of desferrioxamine. Nephrol Dial Transplant 1989;4:883-7.
23. Yapp AR, Lindeman R, Gilroy N, Gao Z, Macintyre CR. Infection outcomes in splenectomized patients with hemoglobinopathies in Australia. Int J Infect Dis 2009;13:696-700.
24. Al-Zahrani F, Algethmi FA, Makin MA, Barayan NA, Hilal RM, Alnakhli SM. Prevalence and risk factors of severe bacterial infections in thalassemia patients. Curr Pediatr Res 2020;24:264-9.
25. Prol B, Selva A, Patton P, Chen Z, Bird V. Increased prevalence of urinary tract infection and catheter associated urinary tract infection in disorders characterized by iron dysregulation. Am Urol Assoc Educ Res 2021;206:e493.
26. Weinberg ED. Iron and infection. Microbiol Rev 1978;42:45-66.
27. Weinberg ED. Iron withholding: A defense against infection and neoplasia. Physiol Rev 1984;64:65-102.
28. Cartwright GE. The anemia associated with chronic infection. Science 1946;103:72-3.
29. Liao K, Chen Y, Wang M, Guo P, Yang Q, Ni Y, *et al.* Molecular characteristics of extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* causing intra-abdominal infections from 9 tertiary hospitals in China. Diagn Microbiol Infect Dis 2017;87:45-8.
30. Tabar MM, Mirkalantari S, Amoli RI. Detection of ctx-M gene in ESBL-producing *E. coli* strains isolated from urinary tract infection in Semnan, Iran. Electron Physician 2016;8:2686-90.
31. Kerim SI, Hasan KH. Cutaneous manifestation among patients with β-thalassemia major. Iraqi J Hematol 2014;3:98-107.