Study of Single Nucleotide Polymorphism of Interleukin 1 B in Pediatric Patients with Health Care Associated Sepsis

By

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Abstract

Background: Health care associated sepsis in children is a global health problem. Predisposing to this infection may include single nucleotide polymorphism (SNP) in interleukin 1 β gene (IL1 β).

Objective: the aim was to study the association of L-1β +3954 C>T polymorphism in children with health care-associated sepsis.

Method: The study is a retrograde case-control study that included ninety two children with hospital-acquired sepsis and ninety two children as control group. Blood samples from included children were subjected to blood culture for identification of sepsis and SNP study of IL1 β by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP).

Results: In the study of L-1β +3954, C>T polymorphism comparison was performed between patients and minty two children with cross-match age and sex. The CC genotype and TT genotype had a statistically significant increase in the control subjects (64.1% and 10.9% respectively) compared to patients (51.09%, 3.3% respectively=0.032). While CT genotype had a statistically significant increase in patients (45.7%, and compared to the control subjects (25%, P=0.032). Moreover, CT plus TT genotypes had a statistically significant increase in patients

(48.9%) compared to control subjects (32.6%, P=0.024) with a statistical increase of CC genotype in the control subjects (67.4%) compared to the patients (51.1%, P=0.023).

The present study highlights the role of IL-1β +3954 C>T polymorphism in sepsis in children. There was association between CT genotype and development of sepsis in children.

Keywords: Sepsis, IL-1β +3954 C>T polymorphism, Children, Blood culture.

Introduction

Sepsis in children represents a major health problem despite the development of standardized protocols of treatment, infection control guidelines, and intensive care support techniques (1).

The pathogenesis of sepsis depends upon the virulence factors of invading organisms besides the release of cytokines, complement activation, and genetic host factors related to susceptibility to infections (2). The immune responses to the invading pathogen involve the localization of the organism and the repair of the affected organs. The process includes the activation of circulating and fixed phagocytic cells, alongside with generation of pro-inflammatory and anti-inflammatory mediators secreted by macrophages, which have been triggered and activated by the bacterial invasion of tissue (3).

The polymorphism of the genes involved in the immune response and in the coagulation pathways may be associated with the susceptibility or the severity of sepsis in children (1).

The single nucleotide polymorphism (SNP) in the genes encoding cell membrane receptors, inflammatory cytokines, angiotensin I-converting enzyme, plasminogen activator inhibitor, and caspase-12 have been implicated in the predisposition to

sepsis (4,5). However, the protective genotype pattern to the infections can be associated with exaggerated immune responses leading to delirious outcomes with septic shock. Therefore, the squeal of an altered host response may have competing effects in population-based studies (6).

Among the cytokines involved in the immune reaction to sepsis, the Interleukin-1 (IL-1) family with two agonists (IL-1α and IL-1β) and one antagonist (IL-1 receptor antagonist: IL-1ra) (7, 8). The enormous production of IL-1 leads to shock, multi-organ system failure, and death in patients and animals with sepsis, systemic inflammatory response syndrome, and septic shock (8). The coding genes of IL-1A, IL-1B and IL-1RN are located in chromosome 2 within the cluster of human major histocompatibility complex in the q13-21(9).

There are five SNPs in IL-1 genes studied in the relation to sepsis risk: one SNP at promoter position −889 in the IL-1A gene, two SNPs at promoter position −511 and −31, and one SNP in exon 5 at position +3954 of the IL-1B gene and a variable number of tandem repeats (VNTR) of 86-bp sequence in intron 2 of IL-1RN gene (10).

There are several studies about the associations between IL-1 polymorphisms and sepsis risk (11, 12). Previous studies from Egypt included SNP of IL1 b in children with sickle cell disease (13). However, to the best of our knowledge, there are no reports about the association of SNP in IL1 β +3954 C>T with sepsis in children from Egypt.

Therefore, the aim was to study the association of L-1β +3954 C>T polymorphism in children with health care-associated sepsis.

Material and Method

The study is a retrograde case-control study that included children with hospital-acquired sepsis recruited from Mansoura University Children's Hospital, Egypt from January 2020 to March 2022. The patients were children with hospital-

acquired sepsis with at least one positive blood culture. In addition, healthy children were included as control group. The study was approved by the ethical committee of Mansoura Faculty of Medicine (R.22.10.1920), Egypt. The parents of the children signed a written consent and the study is performed according to Helsinki guidelines.

Blood sample

Seven milliliters of blood samples were obtained from each child under a complete aseptic technique. The blood sample was divided between blood culture bottle Bact/ alert for blood culture (Biomeriex-USA) and between vacutainer tube with ethylene diamine tetra-acetic acid (EDTA) for polymerase chain reaction with restriction fragment length polymorphism (PCR-RFLP) for detection of SNP at IL1 β +3954 C>T (rs1143634).

Blood Culture and Bacterial Identification

The blood culture was performed by Bact/alet ped ((biomerieux- USA) and positive blood culture bottles were subjected to subculture on blood agar and McConkey agar plates Bbiomerieux- USA). The colonies were identified by Gram stain and biochemical identification by automated VITEK® 2(Biomeriex-USA).

PCR-RFLP for IL-1β +3954 C/T (rs1143634)

DNA Extraction

DNA was extracted from blood on EDTA by the use of QIAamp DNA Blood Mini (Qiagen- Germany) according to the manufacturer's instruction. The extracted DNA was kept frozen at -20°C till the time of the amplification.

PCR-RFLP

The extracted DNA was used for the PCR using the primers F-TC AGG TGT CCT CGA AGA AAT CAA A, R-GCT TTT TTG CTG TGA GTC CCG. The amplification procedures were denaturation at 95 °C for

2 minutes then 35 cycles were applied consisting of denaturation at 95 °C for 1 minute, annealing at 57 °C for 1 minute, extension at72 °C for 1 minute, and a final extension step at 72 °C for 5 minutes.

The amplified products were incubated with the restriction enzyme using 10 units of restriction endonucleases Taq 1 (New England Biolabs) and incubated at 37°C for 16 hours. Amplified products and restriction fragments were run on 2% agarose gel and stained with ethidium bromide. TT genotype (182 bp); lane 3, 5, and 6: CC genotype (97 and 85 bp); lane 7: CT genotype (182, 97, and 85 bp); lane 4: 50 bp DNA ladder (13).

Statistical Analysis

The data of the study was analyzed by the use of SPPS 22. The qualitative data was expressed as numbers and percentages. The comparison will be performed by chi-square and P will be considered significant if it was < 0.05. The age was expressed as median, minimum, and maximum as nonparametric data. The comparison of numerical data will be performed by one Way-ANOVA test and P will be considered significant if < 0.05.

Result

The study included ninety-two children with hospital-acquired sepsis. They were fifty-three males (57.6%) and thirty-nine females (42.4%) with an age range from one month up to 168.0 months. The common underlying condition was pneumonia (31.3%), prematurity (22.8%), and surgery (17.4%). The presence of peripheral venous catheter was reported in 59.8%, and central venous catheter and urinary catheter were present in 41.3% for each. The common clinical signs of sepsis were hypothermia (47.5%), fever (44.5%), and hypotension (39.1%). The commonly isolated organisms from the blood culture were *Staphylococcus aureus* (62.0%), and *Klebsiella species* (23.9%), table 1.

Table (1): The demographic, clinical, and microbiological findings of the studied patients

Sex	
Male (No.-%)	**53 57.6**
Female(No.-%)	**39 42.4**
Age (months)	
Minimum	**1.00**
Maximum	**168.0**
Median	**2.00**
Underlying etiology (No.-%)	
Infection	**8 8.7**
Congenital anomalies	**13 14.1**
Surgery	**16 17.4**
Prematurity	**21 22.8**
Pneumonia	**29 31.3**
Cardiac diseases	**2 2.2**
Hepatic diseases	**3 3.3**
Central venous catheter(No.-%)	**38 41.3**
Peripheral venous catheter(No.-%)	**55 59.8**
Urinary catheter(No.-%)	**38 41.3**
Fever(No.-%)	**41 44.5**
Hypothermia(No.-%)	**44 47.5**
Hypotension(No.-%)	**36 39.1**
Chills(No.-%)	**7 7.6**
Metabolic acidosis (No.-%)	**11 12.00**
Isolated bacterial pathogens (No.-%)	
Staphylococcus aureus	**57 62**
Klebsiella species	**22 23.9**
Pseudomonas aeruginosa	**8 8.7**
Enterobacter species	**4 4.3**
Escherichia coli	**1 1.1**

In the study of L-1β +3954, C>T polymorphism comparison was performed between patients and minty two children with cross-match age and sex. The CC genotype and TT genotype had a statistically significant increase in the control subjects (64.1% and 10.9% respectively) compared to patients (51.09%, 3.3% respectively=0.032). While CT genotype had a statistically significant increase in

patients (45.7%, and compared to the control subjects (25%, P=0.032). Moreover, CT plus TT genotypes had a statistically significant increase in patients (48.9%) compared to control subjects (32.6%, P=0.024) with a statistical increase of CC genotype in the control subjects (67.4%) compared to the patients (51.1%, P=0.023), table 2.

Table (2): The genotype of L-1β +3954 C>T polymorphism in patients compared to control.

	Control		Patients		P
CC	59	64.1	47	51.09	P=0.005
CT	23	25	42	45.7	
TT	10	10.9	3	3.3	
CC	59	64.1	47	51.1	P=0.05
CT+TT	33	35.9	45	48.9	

In the study of the association of the genotype of **L-1β +3954 C>T polymorphism in the patients and different demographic and clinical findings, there was statistically** insignificant difference between patients with CC+TT genotypes and patients with CT genotype as regards clinical signs of sepsis, fever (P=0.9), hypothermia (P=0.06), hypotension (P=0.057), table 3.

Table (3): The association between the genotype of L-1β +3954 C>T polymorphism in the patients and different demographic and clinical findings

	CC+TT (n=50) No. %	CT (n=42) No. %	P	OR	95%CI
Sex					
Male	**28 56**	**25 59.5**	**0.7**	**0.9**	**0.34-1.99**
Female	**22 44**	**17 40.5**			
Age			**0.052**		
Minimum	**1.00**	**1.00**			
Maximum	**166.0**	**144.0**			
Peripheral Intravenous catheter	**28 56**	**27 62.3**	**0.14**	**0.71**	**0.30-1.65**
Central venous catheter	**23 46**	**15 35.7**	**0.32**	**1.5**	**0.66-3.56**
Urinary catheter	**25 50**	**13 30.9**	**0.07**	**2.2**	**0.95-5.25**
Underlying clinical conditions			**0.11**		
Infection					
Congenital anomalies	**5 10**	**3 7.1**			
Surgery	**8 16**	**5 11.9**			
Prematurity					
Pneumonia	**8 16**	**8 19.04**			
Cardiac diseases	**6 12**	**15 35.7**			
Hepatic diseases	**19 38**	**10 23.8**			
	1 2	**1 2.4**			
	3 6	**0**			
Fever	**22 44**	**19 45.2**	**0.9**	**0.95**	**0.42-2.2**
Hypothermia	**19 38**	**25 59.5**	**0.06**	**0.42**	**0.18-0.97**
Metabolic acidosis	**4 8**	**7 16.7**	**0.2**	**0.43**	**0.12-1.6**
Hypotension	**24 48**	**12 28.6**	**0.057**	**2.3**	**0.96-5.5**

Discussion

Hospital acquired sepsis in children is a global health problem that may have rapid progress and grave outcome (14).

The development of sepsis can be attributed to various infections such as pneumonia, cholangitis and abscess and associated with predisposing factors such as prematurity. In the present study, the common underlying condition was pneumonia (31.3%), prematurity (22.8%), and surgery (17.4%). Previous study on the associated infection in pediatric patients with sepsis, reported respiratory tract infections as a common underlying infection in 19/65 (29.2%), infections in

digestive tract in 21/65 (32.3%) and intracranial infections in 10/65 (15.4%) (14). The difference in the underlying etiology of hospital acquired sepsis in children depends upon the health care services supported by different hospitals.

The common pathogens isolated from blood culture in the present study were *Staphylococcus aureus* and *Klebsiella species.* This finding was online with previous study in community acquired sepsis in young infants in sub-Saharan Africa with *Staphylococcus aureus* and *Klebsiella pneumonia* as predominating isolated bacteria from blood cultures (15). Also, this finding was reported in systemic review from the same region (16). These finding may suggest some sort of association of pathogens with geographical regions (15).

In the present study, the common clinical signs of sepsis were hypothermia, fever and hypotension. These clinical signs are recognized as alarming signs of sepsis in children beside the presence of suspected infections (17, 18). There must be incorporation of screen process of sepsis to the triage to identify patients with high risk to develop sepsis (19). The development of an electronic health record process decrease the time needed for rapid diagnosis screen (20).

The sepsis is associated with inflammatory response mediated by pro-inflammatory cytokines such as IL-1β (21[1]. The polymorphism of IL11ß gene with the replacement of thymine at the position of 3954 (C3954T) may lead to profound increase in IL1 ß levels (22). The data about this hypothesis in pediatric sepsis are limited.

In the present study, the CT genotype had a statistically significant increase in patients and compared to the control subjects with significant increase in CC and TT genotypes in control subjects compared to pediatric patients with sepsis. Contrary to this finding previous studies revealed no association between IL-1β +3954 C>T polymorphism and the risk of sepsis (23-28). However, previous meta-

analysis study confirmed found that the IL-1β +3954 C>T polymorphism reduced the risk of sepsis [29]. The Stratified analysis gives a clue about the protective effect of SNP toward the development of sepsis in Caucasians population [29]. Moreover, a previous study in a Han Chinese population supported that CT genotype of IL-1β +3954 predispose to sepsis (30).

The difference of the findings between the studies may reflect the difference in the risk of exposure to sepsis, the ethnic differences between studied population and the differences in the studied sample size.

There was statistically insignificant difference between patients with genotype CC+TT and patients with CT genotype as regard clinical signs such as fever, hypothermia and hypotension. This finding may reflect the roles of other cytokines in the clinical signs of sepsis.

The present study had limitations such as the interaction between other genes and the gene environment were not studied and the mechanism of action of SNP of IL-1β +3954 in protection or predisposing to sepsis.

Conclusion

The study highlights the role of IL-1β +3954 C>T polymorphism in sepsis in children. There was association between CT genotype and development of sepsis in children.

Ethical Approval and Consent to participate

The study was approved by Mansoura Faculty of Medicine ethical committee, Egypt (R.22.10.1920). Informed written consent was taken from the parents each child. The study was performed according to Helsinki guidelines.

Consent for publication

All authors approve the publication

Author Contributions

Abdelrahman Eid Mahmoud Mossad design of the study, recruitment of the participant, draft of the article. Maysaa El Sayed Zaki design of the study, laboratory work, data analysis,

recruitment of the participant, draft of the article. Dina Mohamed Abdel- Hady design of the study, recruitment of the participant, draft of the article. Emad Ibrahim Mohammed Elmasry design of the study, laboratory work, data analysis, recruitment of the participant, draft of the article. Ahmed Gomaa Ahmed El Sayed design of the study, laboratory work, data analysis, recruitment of the participant, draft of the article

Availability of supporting data

https://data.mendeley.com/drafts/3h7gzkb8d7

Competing interests

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