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Relationship of Bactericidal Permeability Increasing Protein (*BPI*) Polymorphysm rs1341023, rs5743507, Tumor Necrosis Factor Alpha (*TNF-α*) rs361525, rs1800629 with Neonatal Sepsis

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Abstract

BACKGROUND: Neonatal sepsis is a problem in the field of child health because the incidence is increasing every year and often ends in death.

AIM: This study aimed to investigate the relationship between TNF-α and BPI gene polymorphisms with neonatal sepsis.

METHODS: PCR and sequencing examinations were performed on 60 DNA samples consisting of 30 samples of neonatal sepsis and 30 samples of non neonatal sepsis. Furthermore, data in the form of DNA mutation tables were statistically processed by univariate, bivariate, and multivariate analysis. It is considered meaningful if $p < 0.05$.

RESULT: The results showed that *BPI* rs1341023, rs5743507 and *TNF-α* rs361525, rs1800629 only *BPI* rs1341023 and *TNF-α* rs1800629 were mutated. Of the two genes, only *TNF-α* rs1800629 had a significant association with neonatal sepsis ($p < 0.05$).

CONCLUSION: This study proved that the *TNF-α* rs1800629 mutation was the most important polymorphism in the occurrence of neonatal sepsis ($p < 0.05$).

Introduction

Neonatal sepsis is a problem in the field of child health because the incidence is increasing every year and often ends in death. Globally, every year 3 million neonates suffer from sepsis [1]. The incidence of sepsis has more than doubled over the past 10 years [2]. It is known that three out of every ten deaths due to neonatal sepsis are caused by resistant pathogens [3].

Bactericidal permeability increasing (*BPI)* gene is one of the antimicrobial proteins/peptides (APP) which functions to defend the host against bacterial pathogens. *BPI* has specific antimicrobial and anti-infective activity against Gram-negative bacteria by increasing membrane permeability, opsonizing bacteria to increase phagocytic uptake and neutralizing LPS endotoxic activity. *BPI* is also excreted in the pulmonary mucosal epithelium and in intestinal epithelial cells [4]. Tumor necrosis factor-α (*TNF-α*) is one of the most important pro-inflammatory cytokines, plays an important role in the pathogenesis of this acute inflammatory response (acute phase reaction), and is involved in systematic inflammation.

Based on this preliminary study, it is known that there is no research regarding the relationship of *BPI* gene polymorphisms rs1341023, rs5743507 and *TNF-α* rs361525, rs1800629 with sepsis neonatorum. In this study, the gene polymorphisms associated with the incidence of neonatal sepsis will be analyzed so that they can assist clinicians in making a diagnosis and predicting prognosis and possible therapy.

Material and Methods

Sample

DNA samples were collected from two groups, namely, 30 infants with neonatal sepsis and 30 infants without neonatal sepsis who met the inclusion and exclusion criteria.

DNA isolation

The primary data in this study were genomic DNA from neonatorum sepsis infants from the previous studies [5]. DNA samples were previously extracted using the Invitrogen Purelink Genomic DNA Mini Kit (procedure according to the manufacturer's instruction manual) and stored according to standards at −80°C in the Biomedical Laboratory, Faculty of Medicine, Andalas University.

PCR and electrophoresis

After it was found that the DNA quality was still good and the number of samples was sufficient, the primer construction was carried out. After the primer was obtained, a PCR examination was performed. The PCR results were directly electrophoresed using 2% agarose gel, then visualized using GelDoc (Bio Rad-USA).

Statistical analysis

The data obtained from the results of the examination are in the form of a DNA mutation table. Furthermore, the data were statistically processed with univariate analysis and bivariate with Chi-square, and followed by binary logistic multivariate analysis to determine which gene polymorphisms most played a role in the incidence of neonatal sepsis. Analysis of research data was carried out at a 95% CI level of confidence (α = 0.05), if the results obtained were p < 0.05, there was a significant relationship.

Results

The characteristics of the research subjects are shown in Table 1.

Based on Table 1, it is known that male infants experience more neonatal sepsis than female infants, namely, 21 (60.0%) compared to 9 (36.0%). Furthermore, infants with birth weight 2500 g experienced more sepsis (53.1%) than infants weighing > 2500 g (46.4%). It is also known that 75% of infants with sepsis had an APGAR score 3. In addition, sepsis was more common in deliveries assisted by other health workers than doctors, namely, 19 (57.6%) compared to 11 infants (40.7%). From the characteristics of the research subjects, there was no significant difference in the parameters of gender, birth weight, APGAR score, and birth attendant with neonatal sepsis (p>0.05). The results of the sequencing and bioinformatics were processed with SPSS 25 and the results were obtained as shown in Table 2.

Table 1: Characteristics of research subjects

Variable	Neonatal				Total		p
	Sepsis (n = 30)		No sepsis (n = 30)				
	F	%	f	%	f	%	
Age							
Male	21	60.0	14	40.0	35	100	0.116
Female	9	36.0	16	64.0	25	100	
BBL							
≤ 2500 g	17	53.1	15	46.9	32	100	0.796
> 2500 g	13	46.4	15	53.6	28	100	
Score of APGAR							
≤ 3	3	75.0	1	25.0	4	100	0.612
> 3	27	48.2	29	51.8	56	100	
Childbirth helper							
Other health workers	19	57.6	14	42.4	33	100	0.299
Doctor	11	40.7	16	59.3	27	100	

Table 2: Polymorphisms of *BPI* Genes rs1341023, rs5743507 and *TNF-α* rs361525, rs1800629

Variable	Alel	f	%
BPI rs1341023 C>T	TT (mutant)	24	40.0
	CT (mutant homozygous)	30	50.0
	CC (wild type)	6	10.0
BPI rs5743507 G>C	CC (mutant)	0	0
	GC (mutant homozygous)	0	0
	GG (wild type)	60	100
TNF-α rs1800629 G>A	AA (mutant)	0	0
	GA (mutant homozygous)	12	20
	GG (wild type)	48	80
TNF-α rs361525 G>A	AA (mutant)	0	0
	GA (mutant homozygous)	0	0
	GG (wild type)	60	100

Based on Table 2, it is known that only the *BPI* rs1341023 and *TNF-α* rs1800629 genes experienced polymorphisms, namely, 54 and 12 samples, respectively. Meanwhile, *BPI* rs5743507 and *TNF-α* rs361525 did not experience polymorphism. The relationship between *BPI* gene polymorphisms rs1341023, rs5743507 and *TNF-α* rs361525, rs1800629 is shown in Table 3.

Table 3: Relationship of *BPI* gene polymorphisms rs1341023, rs5743507 and *TNF-α* rs361525, rs1800629 with neonatal sepsis

Variable	Group	Groups						p
		No sepsis		Sepsis		Total		
		f	%	f	%	f	%	
Mutation of *BPI*	*Wild type*	5	83.33	1	16.67	6	100	0.227
rs1341023 C>T	Mutant	25	46.30	29	53.70	54	100	
Mutation of *BPI*	*Wild type*	30	50.0	30	50.0	60	100	-
rs5743507 G>C	Mutant	0	0	0	0	0	-	
Mutation of *TNF-α*	*Wild type*	30	50.0	30	50.0	60	100	-
rs361525 G>A	Mutant	0	0	0	0	0	-	
Mutation of *TNF-α*	*Wild type*	28	58.33	20	41.67	48	100	0.024
rs1800629 G>A	Mutant	2	16.7	10	83.3	12	100	

Based on Table 3, the *BPI* gene rs1341023 showed that the percentage of neonatal sepsis was higher in the mutant than the wild type, namely, 53.7% compared to 16.67%. Statistically, the difference was not significant (p>0.05). No mutants were found in either the neonatal sepsis group or those without neonatal sepsis in the *BPI* rs5743507 and *TNF-α* rs361525 genes. Meanwhile, in the *TNF-α* gene rs1800629, the percentage of neonatal sepsis was higher in the mutant than the wild type, namely, 83.3% compared to 41.67%. Statistically, the difference was significant (p<0.05). To determine the gene polymorphisms that most play a role in neonatal sepsis, a logistic binary multivariate analysis was carried out as shown in Table 4.

Based on Table 4, the *TNF-α* gene rs1800629 has p value <0.05. This proves that the *TNF-α* rs1800629 polymorphism is the most important polymorphism in the occurrence of neonatal sepsis.

Table 4: Results of logistic binary multivariate analysis

Variable	B	S.E.	Wald	df	Sig.
Step 1[a]					
BPI rs1341023	−0.509	0.428	1.412	1	0.235
TNF-α rs1800629	1.982	0.838	5.589	1	0.018
Constant	−1.514	1.147	1.741	1	0.187
Step 2[a]					
TNF-α rs1800629	1.946	0.828	5.522	1	0.019
Constant	−2.282	0.971	5.525	1	0.019

Discussion

The existence of polymorphisms that influence the occurrence of neonatal sepsis and severe infection has been discussed in several literature reviews and scientific journals. It was stated that genes associated with neonatal sepsis include tumor necrosis factor (*TNF-α* and *TNF-β/LTA*), tumor necrosis factor receptor (*TNFR*), interleukin-10, interleukin-6, interleukin-1β, toll like receptor (TLR), interleukin-1 receptor antagonist gene (*IL-IRN*), heat shock protein (*HSP*), and *BPI* [6], [7].

In this study, it was found that only the *BPI* rs1341023 and *TNF-α* rs1800629 genes had polymorphisms, namely, 54 and 12 samples, respectively. Meanwhile, *BPI* rs5743507 and *TNF-α* rs361525 did not have mutations. From the table, it is known that the *TNF-α* rs1800629 mutation has a strong relationship with neonatal sepsis ($p < 0.05$). Various *in vitro* and *in vivo* experimental studies (using natural and recombinant forms of *BPI*) have shown that *BPI* plays an important role in the host immune response. *BPI* is bactericidal against Gram-negative bacteria. *BPI* binds to LPS on the outer membrane of Gram-negative bacteria leading to increased permeability and cell death. *BPI* which is part of APP has specific antimicrobial and anti-infective activity. Although the ability of PMNs to release *BPI* in neonates is still unknown and quite, a number of studies have been carried out [4]. This study found that *BPI* rs1341023 had a higher percentage of neonatal sepsis in the mutant than the wild type, namely, 53.7% compared to 16.67% although statistically the difference was not significant ($p > 0.05$).

The findings of our study are in line with those of Esposito *et al.,* (2014) who examined *BPIs* of rs4358188, rs1341023, rs5743507, and rs2232478. They found that *BPI* rs4358188 and rs1341023 were associated with neonatal sepsis ($p < 0.05$), whereas *BPIs*, rs5743507 and rs2232578 were not significantly associated [7]. However, our findings differ from those of Abu-Maziad *et al*., (2010) who investigated the *BPIs* of rs4358188, rs1341023, rs5743507, and rs2232478 who did not find any association between these three SNPs [8]. This difference is due to differences in the characteristics of the subject and ethnicity of the study population. It can also be caused by the small sample size.

TNF-α plays an important role in systemic inflammation in releasing other cytokines and has a direct functional effect on septic shock. *TNF-α* plasma levels correlate with death from sepsis. *TNF-α* is involved in septic immunodepression through increased apoptosis [9], [10]. From Table 3, it is known that the *TNF-α* rs1800629 mutation has a strong relationship with neonatal sepsis ($p < 0.05$).

Although the pathogenesis of sepsis is still not fully understood, an exaggerated pro-inflammatory response has been established as a fundamental component of severe sepsis. The proinflammatory cytokine *TNF-α* is an important component of the host immune response to infection and has been widely reported as an important mediator in severe sepsis and septic shock. High circulating levels of *TNF-α* correlate with poor outcome in septic patients. *TNF-α* is encoded by an adjacent gene locus in the middle or Class III region of the human major histocompatibility complex (MHC), between HLA Classes I and II genes on the short arm of chromosome 6. Several SNPs in the TNF promoter region include rs1800629, rs361525, and rs1800630 thought to affect *TNF-α* and have therefore been identified as a candidate variant that might influence susceptibility and/or outcome to severe sepsis and infectious disease [7], [11], [12].

Various studies have tried to evaluate the possibility that genetic variability of cytokines can lead to differences in immune responses that impact the susceptibility and severity of sepsis. Some of them suggest a role for *TNF-α* in generating and promoting the inflammatory response in systemic infections. In particular, rs1800629 has been the focus of many investigations in sepsis [9]. This is in line with our finding that the *TNF-α* rs1800629 mutation is the gene that most plays a role in the incidence of neonatal sepsis ($p < 0.05$).

Although many studies have identified an association of rs1800629 with the risk or outcome of sepsis, other studies have not replicated the association. A study of *TNF-α* rs1800629 in premature infants in Germany showed no significant results as well as low birth weight infants in Hungary [13]. This inconsistency may be due to the small sample size and ethnic differences of the subjects studied.

Ultimately, the pathophysiology of neonatal sepsis is a complex and multifactorial process. Many factors influence neonatal sepsis besides genetic polymorphisms, including the virulence of the etiologic organism, the length of time between disease onset and initiation of treatment, appropriate monitoring, and management of the underlying disease or condition. Environmental factors, both types and sources of pathogens, found in other countries compared to Indonesia which is located in the tropics can also lead to differences in research results [13].

Conclusion

Based on the results of the study, it can be concluded that the *BPI* rs1341023 and *TNF-α* rs1800629 genes were mutated. Meanwhile, *BPI* rs5743507 and *TNF-α* rs361525 did not have mutations. From the multivariate analysis of logistic binaries, it was found that the *TNF-α* rs1800629 mutation was the polymorphism that most played a role in the occurrence of neonatal sepsis ($p < 0.05$).

Ethics of Study

This research has received ethical consideration and approval from the Research Ethics Committee Team of the Faculty of Medicine, Andalas University with registration number 419/UN.16.2/KEP-FK/2021.

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