Lack Of Association Between The TNF-a -308 (G/A) Genetic Polymorphism And Fascial Spaces Abscess Due To Odontogenic Infection

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Abstract— Odontogenic infection is quite a common case, that can spread into facial spaces, and may result in morbidity and mortality. Consequently, cytokines such as tumor necrosis factor alpha (*TNF-α*) probably take part in the development of odontogenic infection. TNF-αoverproduction or inappropriate expression can lead to a variety of pathological conditions including fascial spaces abscess due to odontogenic infection. The objective of this study is to evaluate whether there is an association between *TNF-α* -308 (G/A) rs1800629 genetic polymorphism and fascial spaces abscess due to odontogenic infection.

This study was case control design using samples from 130 subjects that include 51 fascial spaces abscess subjects and 79 healthy control subjects.DNA was extracted from venous blood and the segment of *TNF-α*-308 (G/A) rs1800629 gene in promoter region were amplified by PCR technique, then sequencing method were evaluated.

The study results indicated that *TNF-α*-308 (G/A) rs1800629 gene was identified as substitution of base G into A. Among all, there were no significant differences between A allele and G allele in both subjects. The odds ratio (OR) of A mutant allele was 1,322 (p>0,05) and the frequency of heterozygous mutant GA genotype was not associated with the increased risk of fascial spaces abscess due to odontogenic infection (OR=1,609, p>0,05).

In conclusion, the presence of *TNF-α* -308 (G/A) genetic polymorphism is not associated with facial spaces abscess due to odontogenic infection.

Keywords: *TNF-α*-308 (G/A) rs1800629 gene, facial spaces abscess, odontogenic infection.

Introduction

Tumor necrosis factor -alpha (TNF-α) is a proinflammatory cytokine, which is mainly produced by macrophages and involved in systemic inflammation that stimulate acute phase reaction.[1] TNF-α act as potent immune-inflammation mediator that promotes bone resorption by activating osteoclast maturation and plays an important role in the immune system during inflammation, cell proliferation, differentiation and apoptosis.[2,3,4,5]The *TNF-α* gene is located in the short arm of chromosome 6p21, in the class III region of the major histocompatibility complex (MHC),where genetic alterations in the *TNF-α* locus are known to be involved directly in TNF-α production,[1] and consists of four exons and three introns.[4,6,7,8,9] Several polymorphisms have been identified in *TNF-α* promoter, one of them was located at nucleotide position -308 which causes substitution of guanine (G) to adenosine (A).[8,9,10,11]Polymorphism in the promoter region may cause alteration in the functional role of its gene because this is where regulation of gene transcription takes place. So, it will influence *TNF-α*promoter activity and to be associated with enhance TNF-α production in vitro and in vivo.[8,12,13]

TNF-α-308 (G/A) rs1800629 has been identified as an effective marker for neonatal sepsis diagnosis,[1,5] but the functional role of this gene caused by this polymorphism in inflammatory situations such as sepsis or trauma still remain unclear.[5]It has been reported that the A allele is correlated with TNF-α serum concentrations caused by this allele was strongly associated with proinflammatory and apoptosis.[5] The A allele of this polymorphism also significantly enhances TNF-α production, which is associated with several diseases[14,15] including several cases of infection. Infection in the head and neck is a common presentation in maxillofacial surgery, the majority being odontogenic in origin.[16,17] The particular source of infection can rapidly spread through the anatomical of fascial spaces of the head and neck through the path of least resistance in a predictable pattern. In severe cases, this can compromise the airway, necessitating surgical airway management and develop into sepsis. Significant morbidity has also been reported by spread to other anatomical regions or tissues such as fascial spaces that include spaces of buccal, canine, infratemporal, submental, submandibular, sublingual, masseteric, retropharyngeal and parapharyngeal.

TNF-α-308 (G/A) rs1800629 has been studied to be associated with periapical lesion,[18] which is also the mainly source of odontogenic infection spreading so we considered

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that fascial space abscess due to odontogenic infection are possibly linked with gene susceptibility such as this polymorphism. This polymorphism may affect gene expression levels and protein production or functions[19]so it may influence inflammatory cytokine secretion and regulate inflammatory responses.[20,21,22] The frequency of genetic polymorphism may vary considerably among distinct ethnic groups, so that the application of such markers for diagnosis and prognosis of fascial spaces abscess due to odontogenic infection should be examined in different populations.[23,25] This study determined whether there is an association between *TNF-α*-308 (G/A) rs1800629 genetic polymorphism and fascial spaces abscess due to odontogenic infection.

Material and methods

Materials

All participants were from Deuteromalay subrace as the majority of the race in Indonesia and only those individuals determined to be diagnosed as fascial spaces abscess due to odontogenic infection were included in this study. Although differences in age, gender and socioeconomic status were observed, the distribution of alleles and genotypes associated with those differences were not included for the analysis because we were only focusing on genetical factor in general of fascial spaces infection due to odontogenic infection cases. All of the patients were obtained from Hasan Sadikin Hospital in Bandung and Gunung Jati Hospital in Cirebon Indonesia. The study was approved by the Ethics Committee of Hasan Sadikin Hospital in Bandung Indonesia. In totally, all individuals including 130 subjects: 51 subjects with fascial spaces abscess due to odontogenic infection and 79 as healthy control subjects, based on molecular epidemiology with case control study which is done in Molecular Biology Laboratory, *Unit penelitian Kesehatan* (UPK) Faculty of Medicine Universitas Padjadjaran/ Hasan Sadikin Hospital in Bandung.

Genotyping

DNA isolation.DNA was isolated from venous blood of each subjects using DNA isolation kit from Phamacia. Venous blood samples were collected with informed consent then DNA was extracted and the segment of TNF-α -308 (G/A) rs1800629 waspolymerase chain reaction (PCR)-amplified.

PCR. PCR was performed by using the primers of forward : 5'-AGGCAATAGGTTTTGAGGGCCAT-3' and reverse : 5'-GAGCGTCTGCTGGCTGGGTG-3'.[25]The PCR program included a step of 95^{0}C for 10 minute, followed by 38 cycles of 94^{0}C for 1 minute, 62^{0}C for 1 minute and 72^{0}C for 1 minute. The placement strategy for the primers for the gene segment and the location of TNF-α -308 (G/A) rs1800629 gene variant can be seen in figure 1.

TGGTCCCCAAAAGAAATGG**AGGCAATAGGTTTTGAGGGGCAT**G**G**GGACGGGGTTCAGCCTCCAGGGTCCTACACACAAATCAGTCAGTGGCCCAGAAGACCCCCCTCGGAATCGGAGCAGGGAGGATGGGGAGTGTGAGGGGTATCCTTGATGCTTGTGTGTCCCCAACTTTCCAAATCCCCGCCCCCGCGATGGAGAAGAAACCGAGACAGAAGGTGCAGGGCCCACTACCGCTTCCTCCAGATGAGCTCATGGGTTTCTCCACCAAGGAAGTTTTCCGCTGGTTGAATGATTCTTTCCCCGCCCTCCTCTCGCCCCAGGGACATATAAAGGCAGTTGTTGGCA**CACCCAGCCAGCAGACGCTCCCTCAGCAAGGACAGCAGAGGACCAGCTAAGAGGGAGAGAAGCAACTACAGACCCCCCCTGAAAACAACCCTCAGACGCCACATCCCCTGACAAGCTGCCAGGCAGGTTCT**

Figure 1. The placement for the primers in the segment to be amplified (underline letters) and the location of *TNF-α* -308 (G/A) rs1800629 shown by red letter.[26]

DNA Sequencing. DNA sequencing covering *TNF-α*rs1800629 was performed by using dideoxy Sanger method. From the sequencing result from whole samples, all nucleotide in those segment compared with normal nucleotide in gene bank by using sequence alignment program from BioEdit.[26] The polymorphism is the form of substitution of base G into Ato create 3 genotypes : GG (normal genotype), GA (heterozygous genotype) and AA (homozygous mutant genotype).

Statistical method. Statistical analysis which was used to determine significantly of differences from sequence variants frequency among fascial spaces abscess due to odontogenic infection subject and control subject was χ^2. The odds ratio (OR) was used to determine a risk factor of fascial spaces abscess due to odontogenic infection.

Results

The characteristic of fascial spaces involved in this abscess due to odontogenic infection can be seen in figure 2.Submandibular space was the most affected site (figure 2). From all cases of fascial spaces abscess due to odontogenic infection in this study, there were only three patients were diagnosed as sepsis.

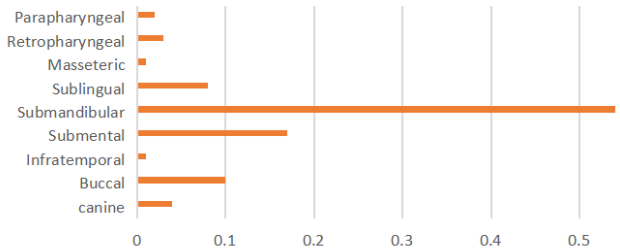

Figure 2. Characteristic of fascial spaces involved in the abscess due to odontogenic infection. Submandibular space is the most affected site in this disease (54%), followed by submental space (17%), buccal space (10%), sublingual space (8%), canine (4%), retropharyngeal space (3%), parapharyngeal (2%), infratemporal and masseteric space (1%)

The initial PCR product showed DNA band of 346 base pairs (bp) (Figure 3).

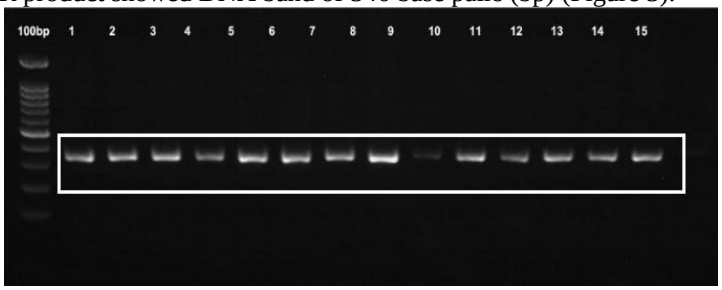

Figure 3. Initial PCR product of TNF-α -308 (G/A) rs1800629segment
Line 1-15. Initial PCR product in 346 bp

After obtaining the initial PCR products of 346 bp, samples were then analyzed by dideoxy Sanger method of sequencing. The sequencing result from all subjects shows GG,GA and AA genotypes (Figure 4).

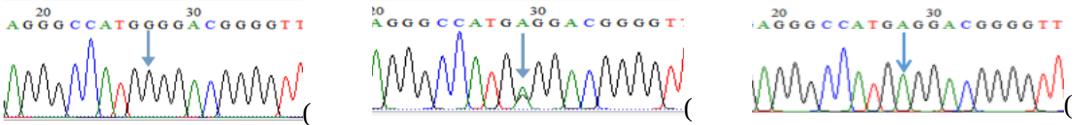

Figure 4. Sequencing result reveal the location of *TNF-α* -308 (G/A) rs1800629. (A) Homozygous normal of GG genotype , (B) Heterozygous mutant of GA genotype and (C) Homozygous mutant of AA genotype

Statistical analysis through all the subjects was done to compare allelic frequency of G normal allele and A mutant allele and also to compare genotype frequency of GG genotype, GA genotype and AA genotype, in between fascial spaces abscess due to odontogenic infection subjects and control subjects, by using χ^2 analysis (Table 1 - 2).

G: guanine (normal allele); A: adenine (mutant allele)

Table 1 Allelic frequency of nucleotide G and A from *TNF-α* -308 (G/A) rs1800629 in fascial spaces abscess due to odontogenic infection subjects compared with normal subjects

Allele	Subjects		χ^2	p	Odds Ratio	CI 95%
	Facial spaces abscess n(%)	Normal n(%)				
G	97(95,10)	151(95,57)	0,000	1,000	-	-
A	5(4,90)	7(4,43)	0,014	0,905	1,322	0,382 - 4,583

Table 2 Genotype frequency of nucleotide G and A from *TNF-α* -308 (G/A) rs1800629 in fascial spaces abscess due to odontogenic infection subjects compared with normal subjects

GG: homozygous normal genotype; GA: heterozygous mutant genotype

Genotype	Subjects		χ^2	p	Odds Ratio	CI 95%
	Facial spaces abscess n(%)	Normal n(%)				
GG	46(90,20)	73(92,41)	0,014	0,905	0,756	0,218 - 2,620
GA	5(9,80)	5(6,33)	0,151	0,697	1,609	0,441 - 5,862
AA	-	1(1,26)	0,000	1,000	-	-

Discussion

Fascial spaces abscess due to odontogenic infection are considered to be complex disease and complex diseases are typically polygenic.[24] Disease modifying genes associated with susceptibility and severity of fascial spaces abscess due to odontogenic infection have not been proposed yet, so very little is known about which genes may be involved in this disease and also as we have not found such previous study which also analyze this polymorphism associated with fascial spaces abscess due to odontogenic infection in other populations so there were still no data to compare about this finding.TNF-α is considered one of the main cytokines related to inflammation and immune processes, and operates in various parts of the body. Among the various of polymorphisms of *TNF-α*, the *TNF-α* -308 (G/A) rs1800629 has been shown to directly affect the expression of TNF-α.[2] The rare A allele was significantly related to greater production of TNF-α in vitro[7] and may influence transcriptional activity in vivo[8,27,28,29] and has been associated with increased risk for various non-related infectious and inflammatory diseases including priodontitis.[24]The -308 A allele is also considered to be the marker of disease susceptibility due to the upregulatory effect of this rare allele on the cytokine production.[4]The AA genotype in the *TNF-α* -308 (G/A) rs1800629 polymorphism has been significantly associated with increased morbidity and mortality in sepsis, malaria, chronic obstructive pulmonary disease, leishmaniasis, systemic lupus erythematosus, autoimmune type 1 hepatitis, and other diseases mediated by immune system.[30] The current study investigated the frequency of the *TNF-α* -308 (G/A) rs1800629 alleles and genotypes of fascial spaces abscess due to odontogenic infection in Indonesians. It is a polymorphism in promoter region that contain so-called *cis*-acting elements that represent short sequence motifs that specific for binding of DNA-binding proteins nuclear factor. Polymorphism in these sequences may cause altered binding of *trans*-acting elements, with the consequence of decreased or increased gene transcription, depending on the activating or supressing effects of a particular nuclear factor. Our results showed that there was no signicant differences of rare A allele between fascial spaces abscess due to odontogenic infection in patient subjects and healthy subjects (Table 1), and also there was only one heterozygous mutant of AA genotype that has been found in healthy subject (Table 2). Based on previous study, there was low frequency of homozygous mutant of AA genotype and it was about 5% in Caucasian population[8] so, the role of A allele in this polymorphism is still can not be elucidated associated with some diseases including fascial space abscess due to odontogenic infection. Usually, the *TNF-α* -308 (G/A) rs1800629 allele is considered to be the marker of sepsis susceptibility due to the upregulatory effect of this allele on the cytokine production.[24,25] Fascial spaces abscess due to odontogenic infection can lead to sepsis condition but the role of *TNF-α* -308 (G/A) rs1800629 in susceptibility of sepsis caused by odontogenic infection has not been identified yet. The question of which genotype is clearly associated with a high proinflammatory response in the clinical situation of severe-gram negative infection and severe sepsis cannot yet be answered.

Odontogenic infection may caused by periapical pathoses of pulpal origin which develope in response to microbial irritants in the root canal systems. Bacterial cell wall components reacts with monocytes, macrophages, other cells of the immune system, as well as with fibroblast, leading to the production of proinflammatory cytokine, such as IL-1α, IL-1ß, TNF-α, IL-6, and IL-8.[18,31] Most of odontogenic infection is caused by periapical lesions and there have been some studies showed that the inflammatory response in the persisting apical lesion protects the host from further microbial invasion[32,33,34] and TNF-α was detected in periapical lesions.[35] TNF-α has also been reported in human apical periodontitis and root canal exudate, which reinforces the hypothesis that this cytokine is involved in pulp and periapical pathogenesis, including the concomitant bone loss.[7,36,37,38] As the periapical infection is the most common form of odontogenic infection, so the pathogenesis of odontogenic infection is polymicrobial, consisting of various facultative and a mix of aerobic and anaerobic bacteria. The dominant isolates are strictly anaerobic gram-negative rods (e.g Fusobacterium species, Prevotella species, and many others) and gram-positive cocci (e.g Peptostreptococci, Viridans streptococci and many others).[39]From odontogenic infection in this study, abscess formation occurs when these bacteria and their toxic products enter the peri-apical tissue through the apical foramen and induce acute inflammation and pus formation. This pus formation spreads into the maxillofacial spaces in proximity with the roots of these teeth.[40] In this study, the most common space involved was the submandibular space (Figure 2) and this space has been reported as the most commonly involved in fascial spaces abscess due to odontogenic infection[40] that could be associated with the posterior mandible teeth to be affected mostly.

TNF-α is secreted by macrophages, lymphocytes and monocytes mostly as a result of the presence of bacterial lipopolysaccharides (LPS) that are part of gram-negative bacterial membrane[7] and gram-positive bacteria,[9,41] LPS is also the main trigger for TNF-α production and LPS stimulation studies showed that TNF-α production was significantly higher among A alleles.[9] In odontogenic infection, as because the dominant isolates are strictly anaerobic gram-negative rods where the LPS is produced so the exact role of TNF-α should be exist in real but when associated with -308 *TNF-α* polymorphism in fascial spaces abscess due to odontogenic infection there was still contradictory result due to this current study.The biological functions of the TNF-α are varied and complex, where on one hand it confers disease resistance and on other causes pathological complications. Indeed, TNF-α plays contradictory role which may be related to genetic polymorphisms in the genes regulating its product and effect. In other case, based on previous study, *TNF-α* -308 A allele may increased levels of circulating TNF-α.[2] In the acute situation, as in fascial space infection, local

production of TNF-α is clearly beneficial. It increases the expression of adhesion molecules on the vascular endothelium to allow immune cells, in particular neutrophils and macrophages, to translocate to sites of tissue damage and infection.[42] Furthermore, TNF-α activates phagocytes to engulf and clear infectious agent and cellular debris. High levels of circulating TNF-α are associated with toxic shock induced by bacterial endotoxins[43] and derangements of metabolism in surgery or trauma patients may be related to the cachetic properties of this cytokine. The induction of interleukin-1 and interleukin-6 production stimulated by TNF-α leads to elevated temperature, sleepiness and the release of glucocorticoids.[44] These may be short-term value in combating certain infections, but their long-term effects are likely to be detrimental.

Although our study showed that there was to report an association between the genetic *TNF-α* -308 (G/A) rs1800629 polymorphism and fascial spaces abscess due to odontogenic infection, the other meta-analysis clearly suggested that the *TNF-α* -308 (G/A) rs1800629 polymorphism is involved in the apical priodontitis[7] that could be main factor in the pathogenesis of odontogenic infection. Meta-analysis is a useful statistical tool to pool data from individual studies, increasing statistical power and precision of effect estimates. Lack of association between alleles and genotypes in this study may be due to small sample size, so larger sample size and the well designed further studies about *TNF-α* and fascial spaces abscess due to odontogenic infection is necessary. Comparing the result with TNF-α expression or another systemic conditions will also be necessary.

Conclusion

The presence of *TNF-α* -308 (G/A) rs1800629 genetic polymorphism is not associated with facial spaces abscess due to odontogenic infection. Because of the small sample size, the conclusion was insufficient to understand the pathogenesis and association between *TNF-α* -308 (G/A) rs1800629 genetic polymorphism and facial spaces abscess due to odontogenic infection.

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