Comparison of Serum MMP-9 Value in Spondylitis Tuberculous with Degenerative Spine Disease

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Abstract: Fourteen (14) subjects were divided into 2 groups, with seven (7) subjects of spondylitis tuberculous and 7 subjects of degenerative spine disease in the period from December 2017 to November 2018 who were included in the inclusion criteria included in this study and blood sampling was taken for examination of serum MMP-9 levels. There were significant differences in serum MMP-9 levels between spondylitis tuberculous and degenerative spine diseases with a significance value of 0.002 ($p<0.05$) with low serum MMP-9 levels in the spondylitis tuberculous study group 1857.14 ± 377.96 and mean in the control group 857.14 ± 243.97. There were significant differences in serum MMP-9 levels between spondylitis tuberculous and degenerative spine diseases with a significance value of 0.002 ($p<0.05$) with low serum MMP-9 levels in the spondylitis tuberculous study group 1857.14 ± 377.96 and mean in the control group 857.14 ± 243.97. Patients suffering from spondylitis tuberculous have higher serum MMP-9 levels than patients with degenerative spine disease, although MMP-9 is not a specific marker examination for spondylitis tuberculous, the results of this study can be suggestive into that can help to evaluate enzyme activity in patients with spondylitis tuberculous disease.

1 INTRODUCTION

Tuberculous (TB) is one of the long-known diseases and is still the leading cause of death in the world. The prevalence of TB in Indonesia and other developing countries is quite high. In 2006, new cases in Indonesia amounted to more than 600,000 and most of them were suffered by people in productive age (15–55 years).

About 20% of infections with pulmonary TB will spread out of the lungs (extrapulmonary TB). Eleven percent of extrapulmonary TB is osteoarticular TB, and nearly half of patients suffer from spinal TB infection. Half have lesions in the spine with neurological deficits of 10% - 45% of sufferers.

Spondylitis tuberculous is an infection of the spine caused by Mycobacterium tuberculous (Lindsay et. Al., 1991; Martini and Welch, 2001; Savant and Rajamani, 1997; Tachdjian, 1990). Spondylitis tuberculous results in damage to the body in the form of a defect that causes spinal instability and disruption of surrounding structures (Lindsay, et. Al., 1991; Tachdjian, 1990). The occurrence of infection in spondylitis tuberculous can originate from primary infection (Graham and Kozak, 1993; Martini and Welch, 2001; Savant and Rajamani, 1997), bacteria directly infect the corpus, or secondary infections (Graham and Kozak, 1993; Savant and Rajamani, 1997), namely bacteria spread hematogenously or lymphogens from the location of the primary disease to the spinal cord (Martini and Welch, 2001). Infections that occur in spondylitis tuberculous are generally secondary infections of the lungs, but in some cases are primary infections. Pro-inflammatory and anti-inflammatory cytokines play an important role in the development and control of Mycobacterium tuberculous infection (Patil, et. Al., 2015). It has been demonstrated that cytokine profiles will differ in each degree of disease (Graham and Kozak, 1993; Lindsay, et. Al., 1991).

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Several cytokines have been known to be biomarkers of disease activity in tuberculous infections. Matrix metalloproteinase (MMP) is a zinc-dependent protease, which plays a role in the process of degradation of the extracellular matrix and modulates the inflammatory response by facilitating and inhibiting different cytokines (Salgame, 2011).

In determining the form of treatment for spondylitis tuberculous, it is generally divided into two groups of patients, namely groups of patients accompanied by complications in the way of neurological disorders and groups that are not accompanied by neurological disorders. In patients without neurological complications, medical treatment is the primary choice in treatment, and surgical treatment is only needed in some special cases. But in patients who are accompanied by neurological complications, the combination of medical treatment and surgical management is the most appropriate treatment.

Matrix Metalloproteinase is a zinc-dependent proteinase that has an important role in the degradation and rotation of the extracellular matrix. [4] Since it was first reported, 24 members of MMP have been identified with specificity and function of overlapping substrates.4 Following each substrate and function specification, MMP is divided into six classes: stromelysins, collagenases, matrilysins, gelatinases, membrane-type MMPs, and others.

Two gelatinases, MMP-2, and MMP-9 can reduce original type IV collagen and denaturation of type I collagen (gelatin). Both circulation and resident inflammatory cells can synthesize MMP-9. Experimental studies have provided evidence that MMP-9 levels were significantly higher in bronchoalveolar fluid patients with cavitary active tuberculous, and lung extracts of mice infected with M. tuberculous, compared to the control group. An increased significance of MMP-9 was also observed in tuberculous cerebrospinal fluid (CSF) meningitis patients and also compared with people suffering from viral meningitis, where usually these enzymes are generally not found in cerebrospinal fluid.

Many previous studies have shown that M. tuberculous can stimulate MMP-9 expression in the lungs of infected organisms, but no studies have examined how the expression of MMP-9 in tuberculous spondylitis when compared to the control group. Therefore, researchers are interested in trying to evaluate and compare the expression of MMP-9 in tuberculous spondylitis using serum levels in the blood of patients suffering from tuberculous spondylitis and compared to the control group, in this case, the control group in this study were patients with degenerative diseases of the spine.

2 METHODS

This cross-sectional study was conducted at the Faculty of Medicine, Universitas of North Sumatra / Haji Adam Malik Hospital, North Sumatra, Indonesia for 11 months from December 2017 to November 2018 by taking patient data and examining serum values of MMP-9, 14 subjects met the inclusion criteria.

Patients who met the inclusion criteria recorded age, sex and blood tests to determine serum MMP-9 levels.

The study sample was divided consecutively with equal numbers into two groups, namely: spondylitis tuberculous, degenerative spine disease. Patients were obtained from outpatient or inpatient care diagnosed with spondylitis tuberculous and degenerative spine disease. Patients who were included in the inclusion criteria were taken for blood sampling to examine levels of MMP-9.

The MMP-9 examination in this study used MMP-9 (Matrix Metallo Proteinase 9) ELISA Kit from Fine Test with the catalog number ERB0080, size 48T / 96T and reactivity to humans. The scale value used is 3.125-200ng / ml with sensitivity: <1.875ng / ml. The application of this dosage application is a quantitative detection of MMP-9 in serum, plasma, tissue homogenates, and other biological fluids. Differences in serum MMP-9 values in spondylitis tuberculous with the degenerative disease in the spine were analyzed using the Mann-Whitney test because the data obtained were abnormally distributed. All statistical calculations are carried out using a computer-based statistical program. The study was approved by the Health Research Ethics Committee of the Medical Faculty of the University of North Sumatra / Haji Adam Malik Hospital, and informed consent was obtained from all subjects.

3 RESULTS

Before discussing the results of the study, because this study had no references, it was conducted with a small-scale preliminary study using 10 balanced numbers of research subjects (5 subjects with spondylitis tuberculous, 5 subjects with degenerative spine disease) with women as many as 6 people

(60%) and men as many as 4 people (40%). With a mean and standard deviation of 41.6 ± 18.8 years. While the mean of subjects with spondylitis tuberculous (ST) 1800 ± 447.2 and mean degenerative spine disease (DSD) 800 ± 273.8.

Table 1. Preliminary Study Distribution

Variable	Disease	
	ST	DSD
Gender M/F	1/4	3/2
MMP-9 (nm/ml)	1800 ± 447.2	800 ± 273.8
Mean age	41.6 ± 18.8	
ST, Spondylitis Tuberculous; **DSD,** Degenerative Spine Disease; **n,** Subject		

A total of 14 subjects were studied until the final analysis. Data retrieval and examination of serum MMP-9 values in spondylitis tuberculous (ST) and degenerative spine disease (DSD) were carried out in stages with the initial phase of selecting samples of subjects included in the inclusion criteria. 7 subjects with diagnosed spondylitis tuberculous (ST), 7 subjects with diagnoses of degenerative spine disease (DSD), 8 women (57.1%) and 6 men (42.9%), 13 years youngest research subjects and 73 years old oldest subjects with mean and standard deviations amounting to 44.79 ± 16.98 years. From the results of the analysis, it was found that the mean and standard deviation of serum ST MMP-9 values were 1857.14 ± 377.96 while the mean and standard deviation of serum DSD levels of MMP-9 were 857.14 ± 243.97.

Table 2. Study Subject Distribution

Variable	Disease	
	ST MMP-9 (nm/ml)	DSD MMP-9 (nm/ml)
Patient		
1	2000	1000
2	1000	1000
3	2000	500
4	2000	500
5	2000	1000
6	2000	1000
7	2000	1000
Mean MMP-9 (nm/ml)	1857.14	857.14
SD	± 377.96	± 243.97
p-Value	0.002	
Youngest age	13 year old	43 year old
Oldest age	62 year old	73 year old
Tuberculous drug consumption	9.14 ± 5.29 weeks	0
ST, Spondylitis Tuberculous; **DSD,** Degenerative Spine Disease; **n,** Subject		

From the results of the statistical analysis of the comparison of serum MMP-9 values in tuberculous spondylitis (ST) with degenerative spine disease (DSD), the results were significant that the serum MMP-9 values were higher and this was indicated by a p-value of 0.002 ($p < 0.05$).

4 DISCUSSION

Pott's disease is the most common spinal granulomatous bacterial infection and is the most frequent bone TB. [6] The occurrence of infection in spondylitis tuberculous can originate from primary infection (Graham and Kozak, 1993; Martini and Welch, 2001; Savant and Rajamani, 1997), i.e. bacteria directly infect the corpus, or secondary infections (Graham and Kozak, 1993; Savant and Rajamani, 1997), namely bacteria spread hematogenously or lymphogens from the location of the primary infection to the spinal cord (Martini and Welch, 2001). Infections that occur in spondylitis tuberculous are generally secondary infections of the lungs, but in some cases are primary infections. Pro-inflammatory and anti-inflammatory cytokines play an important role in the development and control of Mycobacterium tuberculous infection (Patil, et. Al., 2015). It has been demonstrated that cytokine profiles will differ in each degree of disease (Graham and Kozak, 1993; Lindsay, et. Al., 1991). Several cytokines have been known to be biomarkers of disease activity in tuberculous infections. Matrix metalloproteinase (MMP) is a zinc-dependent protease, which plays a role in the process
of degradation of the extracellular matrix and modulates the inflammatory response by facilitating and inhibiting different cytokines (Salgame, 2011).

Two gelatinases, MMP-2, and MMP-9 have the ability to reduce original type IV collagen and denaturation of type I collagen (gelatin). Both circulation and resident inflammatory cells have the capacity to synthesize MMP-9. Experimental studies have provided evidence that MMP-9 levels were significantly higher in bronchoalveolar fluid patients with cavitary active tuberculous, and lung extracts of mice infected with M. tuberculous, compared to the control group.

In a study conducted by Hrabec et al., 2002 it was found that serum MMP-9 levels in patients with active pulmonary tuberculous had significantly higher levels of the control group with mean MMP-9 levels in the group with pulmonary tuberculous 1.23 ± 0.43 µU / µL, while the mean MMP-9 level in the

control group was 0.37 ± 0.10 µU / µL and this result was in accordance with the function of MMP-9 which could induce enzymes produced by mononuclear phagocytes and stimulated neutrophils. This finding is in accordance with the study conducted that there was a significant difference in MMP-9 levels in patients with spondylitis tuberculous with a control group used in this group with subjects with degenerative spine disease with mean spondylitis tuberculous patients 1857.14 ± 377.96 while the mean in patients with degenerative spine disease 857.14 ± 243.97 with p-value 0.002 (p <0.05), but in this study there was no comparison of the ratio of white blood cell levels in the two study groups as did the research conducted by Hrabec et al 2002 with this study obtained MMP-9 rate with an increase in white blood cells was significantly higher in the study group with pulmonary tuberculous 0.150 ± 0.054 mU/ml/L while in the control group 0.059 ± 0.023 mU/ml/L. In this study no correlation was found between the duration of consumption of anti-tuberculous drug and an increase in serum MMP-9 levels (p> 0.05) with an average use for tuberculous medicines 9.14 ± 5.29 weeks, although this was not a primary goal in this study, but need to be reviewed, because this assessment only uses 7 research subjects.

5 CONCLUSION

From the results of the comparison of serum MMP-9 values in spondylitis tuberculous with degenerative spine diseases, it was found that the serum MMP-9 values in spondylitis tuberculous gave higher results so that MMP-9 examination could be used as a barometer for the diagnosis of spondylitis tuberculous.

Further studies are needed to find out whether the increase in serum MMP-9 levels in tuberculous spondylitis is also accompanied by an increase in the ratio of white blood cells and further studies are needed to determine whether an increase in serum MMP-9 levels can also be influenced by the duration of use of anti-tuberculous drugs.

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