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THE EXPRESSION OF TRANSFORMING GROWTH FACTOR BETA-1 AND INTERLEUKIN 6 ON HUMAN PROSTATE; PROSTATE HYPERPLASIA AND PROSTATE CANCER

Afdal[1], Eryati Darwin[2], Yanwirasti[3], Rizal Hamid[4] & Alimuddin Tofrizal[5]

[1]Research Scholar, Biomedic Doctoral Programme, Faculty of Medicine, Andalas University, Indonesia

[2]Department of Histology, Faculty of Medicine, Andalas University, Indonesia

[3]Department of Anatomy, Faculty of Medicine, Andalas University, Indonesia

[4]Department of Surgery, Faculty of Medicine, University of Indonesia, Indonesia

[5]Department of Pathological Anatomy, Faculty of Medicine, Andalas University, Indonesia

ABSTRACT

Purpose: *Prostate hyperplasia and prostate cancer are two of the most common pathological condition of prostate to be found on male. Both of these diseases share a common pathogenesis involving inflammation of prostatic tissues. Chronic inflammation will induce the release of cytokines, followed by cell's injury and tissue's damage. One of the cytokines that plays role in prostate pathology is IL-6. The inflammation will also induce the releases of anti-inflamatory cytokines such as TGF-β. This study aims to analyze the expression of IL-6 and TGF-β1, in prostate hyperplasia and prostate cancer.*

Material and Methods: *This is an observational study, using paraffin embedded tissue samples of prostate hyperplasia and prostate cancer. Samples were obtained from the laboratory of Pathological Anatomy, Faculty Of Medicine, Andalas University, Padang, Indonesia. Immunohistochmistry was performed to detect the cytokine expression, and a semiqunatitaves measurement according to Immunoreactives score (IRS) was performed for evaluation. For the TGF-β1, the stromal expression were also analized by measurement of stromal stained area. The correlation of cytokine expression to Gleason index score were also analized in prostate cancer.*

Results: *This study found that TGFβ-1 was detected both in stromal component as well as epithelial. With the stromal being dominant site of expression. The stromal TGFβ-1 expression were of significantly higher in prostate hyperplasia compares to prostate cancer ($p<0,05$), while the epithelial expression of TGFβ-1 were not found to be significantly different. IL-6 were mostlyexpressed intracytoplasmic in epithelial. The IL-6 expression were significantly higher in prostate cancer compared to hyperplasia. However there were no significant correlation to found between IL-6 expression to the Gleason Score among prostate cancers.*

Conclusion: *In summary this study reveals that there were differences in expression of both TGFβ-1 and IL-6 between prostate hyperplasia and prostate cancer tissue by immunohistochemistry.*

KEYWORDS: *Prostate Hyperpalsia, Prostate Cancer, IL-6, Tgfβ-1, Gleason Index*

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INTRODUCTION

The benign prostate hyperplasia, and prostate cancer are the most common pathological condition to be found on male's urothelial system. Epidemiological study showed that the incidence of these disease were increasing annually, worldwide[1]. Prostate hyperplasia is the most common benign neoplasm on elderly male. Histopathological study on autopsy reports showed that the prevalence of prostate hyperplasia were found up to 50% on males between 50-60 years of age, and it increases to over 80% in the 70 years group of ages [1-5]. In the other hand, prostate cancer is the most common non skin cancer to be found on male, world wide. This disease serve as the second most killer cancer among males in United states and Europe. [2,6]

Prostate hyperplasia and prostate cancer shares a similar pathogenesis, in which both are related to hormones and inflammation[7-9]. Despite of having different predilection, both diseases are known as a chronic diseases that have an early initiation, followed by a slow progression course, until it shows clinical symptom [2,7]. In the recent five years, it has been revealed that chronic inflammation of prostate tissue is one of the risk factor for both prostate hyperplasia and prostate cancer. [2,3,7,10,11,12]

Although the pathogenesis are still unclear, nowadays there are many studies showed the relations of chronic inflammation to both prostate hyperplasia and prostate cancer [3,4,9,13]. Recently the treatment of BPH and prostate cancer were also start to incorporate the prevention and treatment of chronic inflammation as an integrated treatment [10,14-16].

Chronic inflammation will induces the release of cytokine and other inflammatory factors, both from inflammatory cells as well as the hypoxic prostate epithelium[1,17]. These cytokine will interact with stromal cells and causes further tissue damage [2,17]. The inflammation will induce infiltrations of T cells, B cells and macrophages[8,19]. These inflammatory cells will induce the release of the proinflammatory IL-2, IL-8 and IL-6 from epithelial as well as stromal cells[20]. In return, the increasing of proinflammatory cytokines will induce the production and secretion of anti inflammatory cytokine such as TGF β, and FGF. The complex interaction of those cytokines will eventually affect the function of prostate gland[19-23].

TGF-β is known as a controlling factor of tumor progressiveness. This cytokine has biphasic role in carcinogenesis. On the early phase of cancer, it serves as a tumor suppressor agent by inhibiting cell proliferation[26,27]. However, on the later stage, it functions as a tumor promoter, in which, it will induces the cellular changes related to tumor cells invasion [26-28]

IL-6 is a cytokine that involves in the malignancies process, and could serves as a factor that inhibit the apoptosis of tumor cells as well as inducing angiogenesis[27,30,32,33]. Pace et al., (2011) showed that the IL-6 was found to be significantly higher on patient with prostate cancer compared to prostate hyperplasia[31].

The present study aims to analize the different expression of cytokines that involves in the pathogenesis of BPH ad prostate cancer, especially TGF-β1 and IL-6 in prostate tissues.

MATERIALS AND METHOD

The present study used formalin fixed paraffin embedded tissues from prostate lesion obtained from surgical procedure. 40 samples of previously diagnosed as histologically, [31] as well as 40 samples of prostate cancers were used in this study. The tissues were obtained from laboratory of pathological anatomy, Faculty Of Medicine, Andalas University.

Padang, Indonesia. Immunohistochemistry were used to investigate the expression of cytokines using avidin-biotin complex method. Following primary antibody were used; Rabbit polyclonal anti human TGF-β1, Bioss, 0086R with dillution of 1:200, and Rabbit polyclonal anti human IL-6. Bioss, 07-82R, with dillution 1:100. The goat anti rabbit Igg,Vector laboratories, of dilution 1:200 was used as secondary antibodyand the 3-3' diaminobenzidine (DAB), Dojindo Laboratories, was applied as chromogen.

Method

The immunohistochemistry were performed as suggested in the primary antibody datasheet accordingly. The cellular expression of the cytokines were performed by a semi quantitative system according to Immunoreactive score (IRS). Both proportion and intensiti of sellular staining were measured. The final IRS score is the multiplication of proportion score to intensity score, which ranging between 0 to 12. In this present study the IRS score of 0 to 4 were considered as "low IRS score" and the score above 4 is treated as "high IRS score".

The stromal staining of TGF-β1were also analyzed by measuring the proportion of stained area using the ImageJ software, (ImageJ 1.49v software, National Institute of Health, Bethesda, MD, USA). For the samples of prostate cancer, the correlation of cytokine expression to the Gleason score were also analyzed. The prostate cancers samples were grouped either as "high Gleason score" (score 8-10) or "low Gleason score" (score 7 or below). Chi-square were used for statistical analysis. And the Shapiro-Wilk test were performed as a test of normality

Results

Prior to data analysis the distribution of data were assess for normality test. Since each of groups contain less than 50 samples the normality test were performed with Shapiro-Wilk test, as shown in table 1.

Table 1: Normality Test for Independent Variables

Variables	Group	Shapiro-Wilk		
		Statistic	df	Sig.
Tgf β	Prostate hyperplasia	,852	40	,000
	Prostate cancer	,743	40	,000
Il 6	Prostate hyperplasia	,955	40	,109
	Prostate cancer	,914	40	,005

Table 1 shows the distribution of variables. None of the variables shows a normal distribution (p <, 0,05). Therefore, the test was continued by transforming the data and a repeating normality test was performed, as shown in the table. 2

Table 2: Post-Transformation Normality Test

Variables (Log)	Group	Shapiro-Wilk		
		Statistic	df	Sig.
LOGTGFβ	Prostate hyperplasia	,899	23	,024
	Prostate cancer	,946	24	,223
LOGIL6	Prostate hyperplasia	,799	23	,000
	Prostate cancer	,868	24	,005

Post-transformation normality test showed that only TGF-β on prostate cancer data shows normal distribution. Therefore to investigate the correlation between independent and dependent variables, the statistical analysis will be done using non-parametric Mann Whitney test. The value of each variable will be presented by the average value and standard of deviation.

The Epithelial Expression of TGF-B1 on Prostate Lesions

Immunohistochemistry shows that TGF-β1 were expressed both in epithelial component as well as stromal component (Fig. 1). However, the majority of the staining with strong signal intensities were to be found in the stromal area. The epithelial expression of TGF-β1 can be found in prostatic epithelial both in prostate hyperplasia as well as prostate cancer (Fig. 1a,b). Most of the staining of the TGF-β1 in prostate hyperplasia showing of low expression in IRS score (97,5%). The prostate cancer also mostly showed low TGF-β1 epithelial expression (87,5%). (table 3). The average IRS values of TGF-β1 epithelial expression are slightly lower in prostate Hyperplasia (IRS score 0,7) compared to the prostate cancer group (IRS score 1,3). However, the differences are statistically non significant. Interestingly there are 5 samples of prostate cancers that showed high epithelial TGF-β1 expression as can be seen in table 3 and figure 1b.

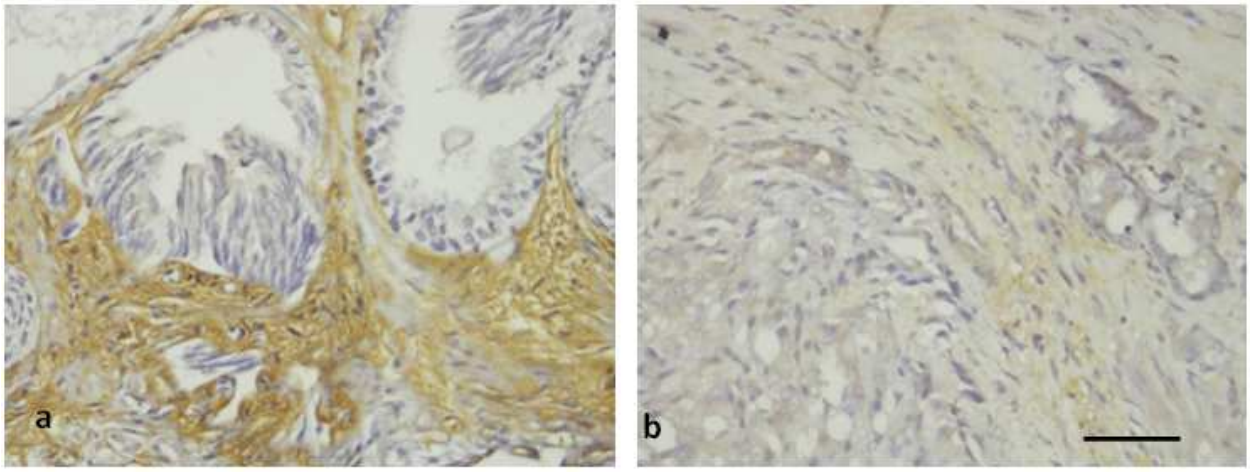

Figure 1: The Expression of TGF B-1 by Immuno Histo Chemistry on Human Prostate Tissue. Prostate Hyperplasia (A) and Prostate Cancer (B). TGF B-1 are mostly Expressed on Stromal Component Prostate Hyperplasia (A). The Stromal Staining is Greatly Reduced in Prostate Cancer, However Cancer Epithelial can Show TGF B-1 Staining in Some Cases (B) Immunoperoxidase of Rabbit Anti Human TGF B-1. Scale Bar; 100µm.

Table 3: The Epithelial Expression of TGF-B1 on Prostate Hyperplasia and Prostate Cancer Tissue

Group	n	Epithelial TGF β-1 (IRS Score)		p
		Low (%)	High (%)	
Prostate hyperplasia	40	39 (97,5%)	1 (2,5%)	0,201
Prostate carsinoma	40	35 (87,5%)	5 (12,5%)	

Table 3. shows the expression of epithelial TGF β-1 on the prostate hyperplasia and prostate cancer. Both groups shows mostly low IRS scores. The carcinoma groups contain slightly higher IRS score, however, there are no significant differences to be found between groups (p=0,21)

The Stromal Expression of TGF-β1 on Prostate Lesions

The immunohistochemistry staining of TGF-β1 showed a distinct pattern between hyperplasia and prostatic cancer. Most of the prostate hyperplasia showed a high stromal staining intensities surrounding the gland (Fig 1a, 2a). In the other hand, prostate cancer shows a low expression of TGF β-1 in their the stromal area (Fig 1b, 2c). The measurement

of stromal staining was done by ImageJ software by selecting the brown staining area, converting the image into a black and white image and measured the stained area.

The average stromal area of TGF β-1 in prostate hyperplasia is 15,3% and is significantly higher compares to that of the prostate cancer 4,5%. The stromal expression of TGF β-1 can be seen in table 4

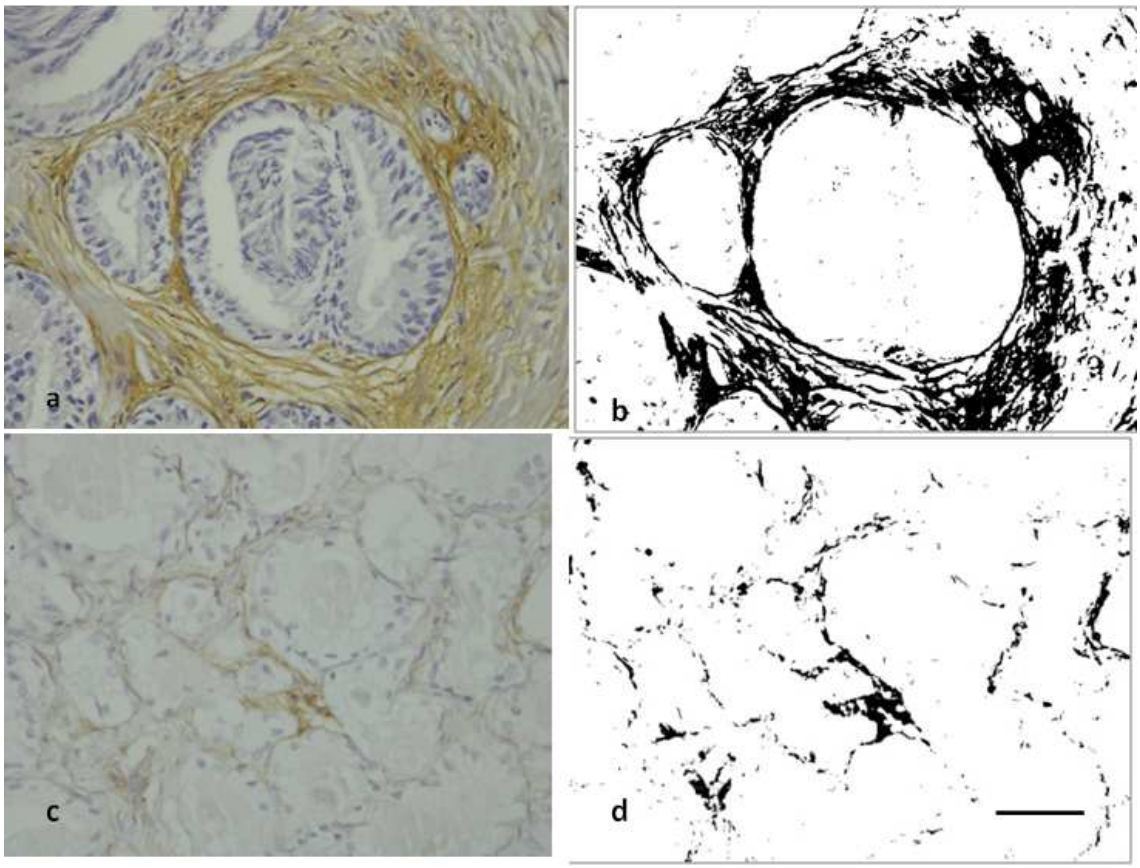

Figure 2: The Expression of TGF B-1 by Immunohistochemistry on Human Prostate Tissue. Prostate Hyperplasia (A) and Prostate Cancer (B). The Stomal Area is Measured by Imagej Software by Isolate the Brown Stained Area and Measuring the Proportion of Stined Area. There are Higher Proportion of Stained Area in Prostate Hyperplasia (B) Compared to Prostate Cancer (D). Immunoperoxidase of Rabbit Anti Human TGF B-1. Scale Bar; 100µm.

Table 4: The Stromal Expression of TGF-β1 on Prostate Hyperplasia and Prostate Cancer Tissue

Group	n	TGF β-1 (% Area)		p
		Average Value	St-Deviation	
Prostate hyperplasia	40	15,32	8,74	0,00
Prostate carsinoma	40	4,47	4,84	

Table 4 shows the expression of stromal TGF β-1 on prostate hyperplasia and prostate cancer. There is a significantly higher expression of TGF β-1 on hyperplasia prostate, compared to prostate cancers. ($p < 0,05$).

The Expression of IL- 6 on Prostate Lesions and its Correlation to the Gleason Score in Prostate Cancer

Immunohistochemistry shows that **IL- 6** were expressed in epithelial component both in hyperplasia prostate and prostate cancer. The staining can also be detected in stromal area but in a faintly low intensity (Fig. 3). Majority (62,5%) of prostate hyperplasia showed low expression of IL-6 in IRS score, in contrast majority (85%) of samples in carcinoma group showed a stronger expression, as can be seen in table 5.

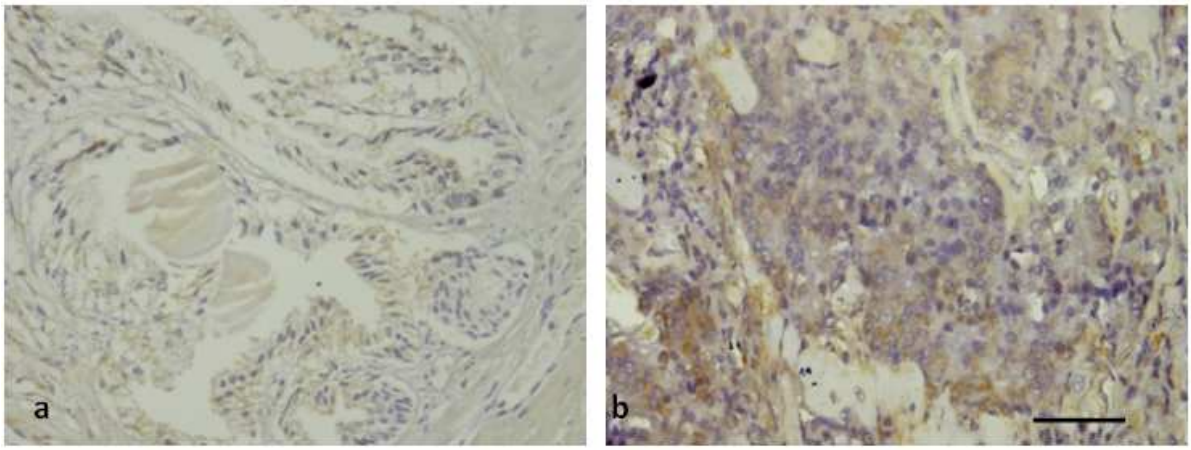

Figure 3: The Expression of IL-6 by Immunohistochemistry on Human Prostate Tissue. Prostate Hyperplasia (A) and Prostate Cancer (B). IL-6 is Expressed on Low Intensity on Prostate Hyperpasia (A). The Expression is Higher in Prostate Cancer Cells (B) Immunoperoxidase of Rabbit Anty Human IL-6. Scale Bar; 100µm.

Table 5: The Expression of IL-6 on Prostate Hyperpalsia and Prostate Cancer Tissue

Group	n	IL-6 (IRS Score)		p
		Low	High	
Prostate hyperplasia	40	25 (62,5%)	15(37,5%)	0,001
Prostate carsinoma	40	6 (15%)	34 (85%)	

Table 5 shows the expression of IL-6 on prostate hyperplasia and prostate cancer. There is a significantly higher expression of IL-6 on prostate cancers compared to hyperplasia prostate ($p < 0,05$).

When the prostate cancer was grouped into "high" and "low" Gleason score index group, both groups showed high IL-6 IRS score, there is no significant correlation between IL-6 expression to the Gleason index score in prostate cancer, as can be seen in table 6.

Table 6: The Correlation of Gleason Score to the Expression of IL-6 on Prostate Carcinoma

Gleason Score	n	IL-6 IRS score (%)		p
		Low	High	
Low	40	1 (7,1%)	13(92,9%)	0,399
High	40	5 (19,2%)	21 (80,8%)	

DISCUSSIONS

The Expression of TGF-B1 on Prostate Lesions; Prostate Hyperplasia and Prostate Cancer

Chronic inflammation plays a significant role in the initiation and progression to the wide spectrum of pathogenesis in prostate lesions. The inflammation will attract the infiltrations of B cells, T Cells as well as macrophages. These immune cells will increase the secretion of proinflammatory cytokine IL2, IL-6 adn IL-8 both on the epithelial tissue or from stromas. In response to the increases of proinflammatory cytokines, the epithelial and stromal cells will produce anti-inflammatory cytokines such as TGF-β1 and FGF which in turn will affect the normal function of prostate glands

In this present study, we found that TGF-β1 was highly expressed in hyperplasia prostate tissue in the extracellular matrix surrounding the glands. The expression of TGF-β1 was significantly different compared to prostate cancer. this result suggesting that TGF β1 play an important role in prostate hyperplasia pathogenesis It has been known that TGF-β1, act as a modulator of other protein such as bFGF 2, synthesis of extracellular matrix and angiogenesis via

VEGF. This interaction plays roles in the pathogenesis of hyperplasia prostate.

TGF-β is a potent mitotic factor for fibroblast as well as other mesenchymalcells [23,24]. It also regulates the synthesis of extracellular matrix and induces the secretion of fibrogenic bFGF-2. TGF β expression act as chemoattractant towards fibroblast, which plays roles in the early process of fibrosis on[25].

TGF-β stimulates fibroblast transformation into myofibroblast and smooth muscle cells through induction of extracellular matrixes. Other studies showed the role of TGF-β1 in fibrosis. Zuhirman (2014) shows the increases of TGF-β1 in chronic ureteral obstruction[33]. Untergasser et al., (2005) showed the increases of TGF-β1 stimulates the collagens synthesis by stromal cells on prostate hyperplasia, as well as the transformation of fibroblast into myofibroblast.

In Contrast to prostatic hyperplasia, our present study shows that there are decreases in stromal TGF-β1expression in prostate cancer. Interestingly we also found the increases of cytoplasmic expression of TGF-β1 in some samples. There were variations of the stromal TGF-β1 expression level among prostate cancer in our study, and the average values of stained area proportion were of 4%. Most prostate cancers in the present study show negative cytoplasmic staining of TGF-β1, 19 samples showed positive staining but only on a low level (IRS score 1). TGF-β1 was known to have a biphasic role in carcinogenesis and has been well known as a factor of tumor progression in the early phase TGF-β act as a tumor suppressor by inhibiting cell proliferation, and induces cells differentiation as well as apoptosis. However, in the late stage, TGF β1 loses its tumor suppressing-function and act as a tumor promoter. in the present study, most of our samples showed a low expression of TGF β1 in cancer cells, suggesting a decreased synthesis or secretion of this protein in prostate cancer. The low expression might have roles in the pathogenesis of prostate cancers. Loss of TGF-β1 function with low expression was also reported in some cancers such as breast, ovarium, oesophagus, and head and neck cancer.

However, in the late stages of carcinogenesis TGF-β1 can play an opposite function as a tumor growth promoter. It is suggested that some tumor are actively secreted TGF-β1 in the late stages of carcinogenesis. The TGF-β1 in these tumor plays a complex role related to angiogenesis, immune cells suppression, cellular transformation related to invasiveness and metastatic ability, as well as mediating interactions of tumor cells and extracellular matrix. Interestingly in our present study, we found 5 samples with higher expression of TGF-β1 (IRS score 4). The high TGF β1expression on some cancer cells raises the question whether these differences related to the biological behavior of tumor cells, the tendencies to metastasize or other clinical outcomes. Further study is required to answers these questions in prostate cancers.

The Expression of IL-6 on Prostate Lesions; Prostate Hyperplasia and Prostate Cancer

Chronic inflammation plays a significant roles in the initiation and progression of prostate lesion. Inflammation were believed to have strong correlation to prostatitis, prostatic hyperplasia and prostate cancer.

Inflammation will invite T cells, B cells and macrophages to the prostate glandular structures and stroma. After the initiation process, the dendritic cells will be activated, and maintained the T cells responses within prostate gland, this will cause a chronic and progressive pathological process that will eventually facilitate the progression of prostate hyperplasia or prostate cancer.

Immune cells infiltration will increases the secretion of pro-inflammatory cytokine such as IL-2, IL-6, and IL-8. The activation of various cytokines will disrupt the balance of cell proliferation an d apoptosis.

IL-6 is produced by various type of cells including macrophages endothelial, and lymphocytes. IL-6 expression and can be detected both intracellular within cells cytoplasm as well on extracellular matrix. Higher expression of IL-6 were detected on prostate cancer group with strong intensities. In the other hand most of prostate hyperplasia showed a weak expression of IL-6.

Our present study showed a similar results with some other study. Engelhard et al., (2014) found that the expression of IL-6 in prostate cancer are significantly higher compared to prostate hyperplasia. We also observed variation of IL-6 expression among prostate cancer, 7 of the samples exhibit a high IL-6 expression of IRS score 9 or above. The strong expression in some of prostate cancer samples raises question whether this finding has any relation to the biological behaviour of cancer cells. Duscharla et al., (2017) reported that a high serum level of IL-6 is related to the bone metastasis of prostate cancer.

IL-6 secreted by imun cells infiltrate will be captured by IL-6-R, this will activates JAK, STAT3 and MAPK pathway, these in turn will induce cell proliferation through androgen receptor inductionangiogenesis and facilitates metastasis. IL-6 are also known to induce intraprostatic testosterone through activation of steroidogenic enzymesIliopoulos et al., (2009) suggested the correlation between inflammation, IL-6 activation, STAT1, PI3K, and NFkB in the pathogenesis of prostate cancer. Other study were also confirm that the increase of IL-6 were correlated to the prognosis and showed a negative relation to survival rate. Based on the above results the present study support the theory that IL-6 plays a role significant role in the pathogenesis and progression of prostate cancer

However, in the present study the expression of IL-6 were not correlated to the Gleason score significantly. Our study employed immunohistochemistry to detect the expression of IL-6 in prostate tissues. The nature of IL-6 as a soluble cytokine could sometimes be difficult to be measured quantitatively by immunohistochemistry. Further study is required to investigate the relation of IL-6 to the Gleason score quantitatively, using a different and more sensitives method.

In summary the present study reveals the difference expression of TGF-β1 and IL-6 between prostate hyperplasia and prostate cancer. The TGF-β1were highly expressed in stromal component of prostate hyperpasia, compared to proste cancer. in the other hand the IL-6 showed a higher expression in prostate cancer cells compare to prostate hyperpalsia. Further study is required to investigate the function of each cytokines in the pathogenesis of prostate lesion.

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