Niveles Plasmáticos de Citoquinas Proinflamatorias en Cáncer de Próstata

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Serum levels of proinflammatory cytokines in prostate cancer

Abstract

Introduction: *Prostate cancer has become an important public health problem affecting millions of men worldwide every year. Like other malignant tumors, prostate cancer shows evidence of a strong inflammatory component that is dependent on the release of pro-inflammatory cytokines, which might play a major role in the development and progression of the tumor, helping in its early stage, progression and aggressiveness.*

Aims: *The goal of this study was to determine the relationships between the serum levels of pro-inflammatory cytokines and the different stages of prostate cancer. To this end, sera from patients enrolled by The Laboratory of Metabolic Diseases and Cancer of the Faculty of Pharmacy and Biochemistry at the University Juan Agustín Maza in Argentina, were analyzed through ELISA and their pro-inflammatory cytokines (IL-6, TNF-α and MCP-1) quantified. Patients were first classified into three groups (Control, at Risk, and Cancer subjects) and anthropometric, biochemical and histological parameters of prostate were then determined for all groups.*

Results and Conclusions: *Despite displaying elevated serum concentrations of IL-6 and TNF-α in the Cancer and the Risk groups compared to the Control group, the differences did not reach significance. However, there was a positive correlation between these cytokines only in the Risk and Cancer groups, showing a general inflammatory behavior in these patients.*

The results obtained provide general data about the behavior of pro-inflammatory cytokines in prostate cancer. However, they do not demonstrate a direct correlation between serum levels and neoplastic progression. Nevertheless, these findings do not rule out a possible relationship between prostate cancer and serum levels of pro-inflammatory cytokines.

Keywords: prostate, cancer, cytokines, inflammation.

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Introduction

Prostate cancer is the second most frequently diagnosed cancer in men over 50 years of age and is rapidly becoming a public health problem due to a rise in its incidence, mainly associated to population ageing, and its high mortality (1, 2).
The contribution of chronic inflammation to cancer progression has been well established. It has been estimated that 20% of cancers affecting adult individuals are associated to states of chronic inflammation, which would play a key role in the selection and proliferation of tumor cells (3). Thus, inflammation may affect the pathogenesis of cancer through several mechanisms: i) it can inflict cell and genome damage, (ii) it may activate reparative cell proliferation to replace damaged cells, and (iii) it is associated to the synthesis and release of a series of cytokines that can promote cell replication, angiogenesis and tissue repair in a paracrine manner (4).
Over the last years, the role of inflammation has emerged as an important player in the pathogenesis of prostate cancer, particularly in association to conditions that determine an increase in the local levels of pro-inflammatory cytokines, inflammatory mediators and growth factors with the potential of inducing an uncontrolled cell proliferation response (5). Indirect evidence showing a possible causal association between inflammation and prostate cancer are provided by several epidemiological studies. According to Nakai and Nonomura (2013), most of these studies suggest an inverse relation between the use of non-steroidal anti-inflammatory drugs (NSAIDs) and the risk of prostate cancer (6).
It has been demonstrated that chronic inflammation induces important pre-malignant alterations in the prostate tissue microenvironment. These alterations are thought to be dependent, at least in part, on the production of free radicals (ROS) and the consequent oxidative stress, changes in the cellular composition of the tissue, as well as an increase in cyclooxygenase (COX) activity and nitric (NO) oxide synthesis (7). At the same time, inflammatory infiltrates would be responsible for the secretion of cytokines involved in the autocrine and paracrine regulation of proliferation of both the epithelial and stromal components of the prostatic tissue (8). Taken together, there seems to be a correlation between the amount of inflammatory cytokines released from an inflammatory process and the increased risk of developing prostate cancer (9). For example, interleukin-6 (IL-6), a pleiotropic cytokine that is involved in host immune defense has also been associated to the modulation of the growth and proliferation of various malignant tumors. In this case, prostate cancer cells commonly display an activation of mitogen activated protein kinase (MAPK)- and phosphatidylinositol 3-kinase (PI3-K)-dependent signaling pathways that, in turn, activate the expression of pro-inflammatory genes that favor the development of cancer (10).
Another cytokine involved in the pathogenesis of inflammatory processes as well as in cancer is tumor necrosis factor alpha (TNF-α). TNF-α is a cytokine that is produced and secreted primarily by cells of the immune system after cell injury and mediates various types of cellular response, including proliferation, differentiation, cytotoxicity and apoptosis, and tumor angiogenesis (11, 12). Besides IL-6, TNF-α and monocyte chemotactic protein 1 (MCP-1) plays an important role in the recruitment of circulating monocytes to the tumor microenvironment, where they undergo a transformation and an in situ maturation into tumor-associated macrophages (TAM). This chemokine is also produced by macrophages and adipocytes (13).
In summary, cytokines play an important role in the pathogenesis of cancer and could be used as auxiliary biomarkers to determine progression and the prognosis of patients with this disease (14). The aim of this study was to clarify the importance of determining the levels of pro-inflammatory cytokines as markers of early prognosis and the presence of prostate cancer and its aggressiveness.

Materials and Methods

Patients and sample preparation
A total of 52 male individuals were enrolled for this study. They were divided into 3 groups: a control group formed by healthy volunteers (n=15), a group of patients at risk of developing prostate cancer based on rectal examination (n= 20) and a group of patients already diagnosed with prostate Cancer (n=17).

The collection of blood samples was conducted at the Laboratory of Metabolic Diseases and Cancer, Faculty of Pharmacy and Biochemistry of the University Juan Agustín Maza, Argentina. Serum concentrations of cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, uric acid (expressed in mg/dl), leptin (ng/ml) and prostate specific antigen (PSA, expressed in ng/ml) were determined by spectrophotometry. In addition, the results of histologic analyses of prostate tissue, expressed as Gleason grades, were included for the group of patients with cancer or at risk of developing the disease.

All the individuals enrolled in this study signed an informed consent form, in accordance with the ethical guidelines of the University Juan Agustín Maza. Procedures involving human subjects were likewise revised and approved by the Bioethics Committee of the University Juan Agustín Maza.

Quantification of cytokines

Serum samples provided by from the Laboratory of Metabolic Diseases and Cancer were processed in the Laboratory of Immunology of the University of Talca, Chile. Sandwich ELISA performed on micro-plates was used to quantify serum levels of IL-6 (IL-6 EASIA KIT, Biosource), MCP-1 (Human MCP-1 ELISA, Peprotech) and TNF-α (TNF-α Human ELISA, Invitrogen), essentially as recommended by the kit's manufacturers. Micro-plates were read using the ELx800 reader (Biotek). Values of MCP-1 levels were expressed in ng/ml, those of IL-6 and TNF-α were expressed in pg/ml.

Statistical analysis

Statistical Analyses were conducted using the software Statistical Package for Social Sciences (SPSS) for Windows, version 15.0. Descriptive statistics were used to represent characteristics of the appointed groups. The difference between groups was assessed using ANOVA and Kruskal-Wallis tests, while correlations between variables were determined through Pearson´s coefficient. Results are represented as Media + Standard Deviation (SD). Statistical significances were accepted with a value $p < 0.05$.

Results

Characterization of patients

Table 1 shows the anthropometric characterization of the individuals under study. Of the 52 patients, 15 belong to the control group, 20 individuals were part of the group at high risk of prostate cancer (Risk), and 17 patients belong to the group of individuals already diagnosed with Prostate Cancer. Overall, there was a predominance of obese patients (67.2%) followed by overweight patients (28.8%), showing differences in BMI between the Risk group and Cancer group compared to the Control group. This last group shows a higher BMI compared to the Risk Group and prostate cancer group.

When grouping patients into normal, overweight and obese (based on BMI), no significant differences were detected between groups and the cytokines or chemokines evaluated. Thus, according to these results, the inflammatory state that might be promoted or perpetuated by these pro-inflammatory mediators is not directly associated to the BMI (Table 1).

A histopathological analysis of tumors from patients with cancer revealed a higher proportion of tumors with a Gleason score of 7, characterized by a state of high undifferentiation and high aggressiveness (Table 2). When grouping by Gleason, no significant differences were detected between the various parameters studied as cancer aggressiveness increased.

Analysis of pro-inflammatory cytokines

The average plasma concentrations of TNF-α were 302.5 ± 49.4, 443.8 ± 234.7 and 371.5

± 104.8 for the control, the risk, and the cancer groups, respectively. When compared to the control group, there was an increase in the concentration of this cytokine in the risk group and the cancer group of 74% and 69%, respectively. In the case of IL-6, plasma concentrations were 134.8 ± 89.4; 233.3 ± 148.4; and 226.0 ±179.7, for the control, the risk, and the cancer groups, respectively. Compared to the control group, therefore, there was an increase in 47% and 23% of IL-6 levels in the risk and cancer groups, respectively.

Despite the tendency of TNF-α and IL-6 levels to be higher in the groups of patients at risk of developing prostate cancer and with diagnosed cancer, we fail to demonstrate a significant difference with the control group by ANOVA test, a likely reflection of the elevated dispersion in data per group. Similarly, the nonparametric Kruskal-Wallis test demonstrated no significant differences in the levels of pro-inflammatory cytokines of the different groups (Table 2).

Next, we conducted an analysis to determine the degree of correlation between different parameters (Pearson correlation test). To this end, we focused on parameters specifically related to inflammation and cancer. We found a positive correlation between the levels of TNF-α and IL-6 in the Risk group (0.510; $p = 0.005$) and Cancer group (0.605; $p = 0.023$), while in the control group such correlation between these cytokines does not occur. In addition, there was a significant correlation (positive correlation) in biochemical parameters related to lipid profile (CHOL, LDL, HDL and TGL). We found no correlation between Gleason score or PSA levels and the level of pro-inflammatory cytokines.

Discussion

Inflammation plays an important role in the pathogenesis of cancer, favoring and promoting the development and progression of several types of malignant tumors, including prostate cancer. This association is supported by pathological evidence of neoplastic prostate biopsy samples in which chronic inflammatory infiltrates predominate (8, 15).

Among these inflammatory components, the presence of high levels of IL-6 has been demonstrated to be a poor prognosis factor that affects directly the growth of tumors such as non-small-cell lung carcinomas and renal cell carcinomas (16, 17). However, in other types of cancer, such as ovarian cancer, there is no correlation between the concentrations of pro-inflammatory cytokines and severity index of cancer (18), which is similar to what we found in this study.

In this study, serum levels of the pro-inflammatory cytokines IL-6 and TNF-α were found increased in both the group at risk of developing prostate cancer and the group already diagnosed with prostate cancer, compared to the levels detected in the control group. Despite this tendency, we fail to detect any significant differences due to the high dispersion of the data.

Previous studies have demonstrated a correlation between the amount of MCP-1 and the severity of prostate cancer as assessed by Gleason score (19, 20). However, we fail to reproduce these findings. This could be attributed to differences in methodology, as immunohistochemistry was also used in previous studies to evaluate the expression of MCP-1 in prostate tissue.

Although the evidence indicates that pro-inflammatory cytokines increase in cancerous processes, they may also increase secondary to cancer-unrelated processes. Therefore, these cytokines would show a high sensitivity but low specificity with respect to prostate tumor.

The correlation analysis of the serum levels of pro-inflammatory cytokines in the risk and cancer groups revealed that the increase in IL-6 is associated with an increase in TNF-α. This finding is in accordance with what was found in a study conducted by Alcaide et al., in which the high expression of IL-6 was associated with a high expression of TNF-α (21). In addition to this positive correlation,

the incorporation of plasma concentrations of IL-8, a cytokine involved in local inflammatory events, may serve as an index that correlates with the severity and prognosis of prostate cancer (22).

The fact that PSA did not show significant differences despite tumor appearance is likely due to the effect that obesity has on the levels of this marker. Thus, due to hemo-dilution present in overweight or obese patients, there is a decrease in the differences between plasma levels of the various groups studied (23).

Conclusion

The results obtained provide general information about the behavior of the pro-inflammatory cytokines in prostate cancer. However, they do not demonstrate a direct relationship between serum levels and neoplastic progression. Yet, these findings do not rule out a potential link between prostate cancer and serum levels of other pro-inflammatory cytokines.

In this context, and based on the evidence presented, it becomes necessary to have further detailed studies that evaluate the relation between inflammation and prostate cancer.

In summary, in patients with prostate cancer or at risk of developing this neoplasia, serum levels of IL-6 are positively correlated with serum levels of TNF-α, and the levels of pro-inflammatory cytokines were did not correlate with histopathologic grading.

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	Frequency	Percentage	PSA	IL-6	TNF- α	MCP-1
Normal	2	33,8	1,6	254,0	529,8	38,4
Overweight	15	28,8	2,6 + 2	193,5 + 159,52	415,5 + 178,1	204,6 +238
Slight obesity	23	44,2	5,7 + 10	165,7 + 94,7	352,0 179,3	47,2 + 71,6
Moderate obesity	10	19,2	4,8 + 6	135,0 + 105,4	343,7 31,43	24,4 + 22,4
Morbid obesity	2	3,8	0,8 + 0,1	95,79 + 47,3	421,26 199,9	155,1 + 195
Total	52	100,0				

Table 1. Group classification based on BMI. PSA: Prostate specific antigen. Data are expressed as median values.

Gleason Score	Frequency	Percentage	BMI	IL-6	TNF- α	MCP-1
4	1	5,9	27,5	368,31	-	-
6	5	29,4	30,1	105,4 + 5,6	413,58 + 70,45	112,6 + 140,7
7	10	58,8	30,9	210,1 + 169, 3	379,6 + 137,8	52,6 + 47,6
8	1	5,9	29,5	67,6	349,80	1,29
Total	**17**	**100,0**				

Table 2. Group classification based on Gleason scores. BMI: Body mass index. Data are expressed as median values.

	Control group (n=15)		At risk group (n=20)		Cancer group (n=17)		*P*
AGE	57,1	+ 6,5	59,7	+ 6,7	65,1	+ 5,5	0,02*
BMI	34,5	+ 5,3	31,6	+ 4,8	30,6	+ 2,5	0,04*
PSA	1,1	+ 0,6	4,4	+ 3,7	5,4	+ 9,8	0,12
COL	218,6	+ 51,5	230,4	+ 47,4	223,8	+ 50,2	0,78
TGL	241,1	+ 160	213,6	+ 127,4	183,9	+ 85,1	0.45
HDL	46,2	+ 5,5	47,2	+ 5,1	47,6	+ 5,6	0.75
LDL	143,8	+ 46,6	162,6	+ 41,2	138,3	+ 38,1	0,18
UA	7,6	+ 3,0	7,3	+ 3,2	6,4	+ 2,1	0,46
LEP	16,6	+30,4	6,7	+ 6,2	6,7	+ 7,1	0,19
MCP-1	125,8	+ 256,9	114,7	+ 126,7	60,9	+ 77,8	0,60
IL-6	134,8	+ 89,1	233,3	+ 148,4	226,0	+ 179,7	0,11
TNF-α	302,5	+ 49,4	443,8	+ 234,7	371,5	+ 104,8	0,05

Table 3. Summary of the parameters under study. BMI: body mass index; PSA: prostate specific; COL: total cholesterol; TGL: triglycerides; HDL: HDL cholesterol; LDL: LDL cholesterol; UA: uric acid; LEP: leptin. *: p value p <0,05