Serum levels of interleukin-18 and nitrite+nitrate in renal cell carcinoma patients with different tumor stage and grade

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Interleukin (IL-18) and nitric oxide (NO) are immunoregulatory cytokines that have been suggested to participitate in the multistep process of carcinogenesis. In this study, serum IL-18 and nitrite+nitrate levels (as an index of NO generation) were measured in 26 patients with histologically confirmed renal cell carcinoma (RCC) before surgical procedure and in 8 healthy controls. RCC patients showed significantly higher serum IL-18 levels when compared to the control subjects ($p<0.001$). Serum IL-18 levels were found to be similar in patients with T1 and T2, T3, T4 tumors ($p>0.05$). Patients with grade 3 and 4 tumors showed significantly higher serum IL-18 levels when compared to the patients with grade 1 and 2 tumors ($p<0.05$). No significant difference was found in serum nitrite+nitrate levels between RCC patients and control subjects ($p>0.05$). Serum nitrite+nitrate levels were lower in patients with grade 3 and 4 tumors than those with grade 1 and 2 tumors, but this was not statistically significant ($p=0.063$). In conclusion, elevated serum level of IL-18 in RCC patients than in controls and extra-increase of this level in those with high grade tumors may reflect the degree of human defence mechanisms against tumor cells in RCC.

Key words: interleukin-18, nitric oxide, renal cell carcinoma, nitrate, nitrite

Interleukin-18 (IL-18) is a novel cytokine that was previously known as an interferon-γ (IFN-γ) inducing factor [22]. It is synthesized by activated macrophages, kupffer cells, intestinal epithelial cells, keratinocytes and osteoblasts as a biologically inactive form that requires cleavage with interleukin-1β converting enzyme (caspase-1) to become active [22]. IL-18 has been shown to have potent antitumor activities that are mediated by induction of apoptosis [21] and inhibition of angiogenesis [25]. It has been reported that serum level of IL-18 may be a useful marker to monitor the clinical course of patients with non-Hodgkin's lymphoma [28], gastric [14] and colon carcinoma [23]. Recently, increased serum IL-18 levels in patients with metastatic breast carcinoma compared to non-metastatic patients and controls were reported by our study group [10]. HARA et al [11] reported that administration of recombinant IL-18 inhibited the tumor growth of renal carcinoma cells.

Nitric oxide (NO) has been reported to be an important bioactive molecule that mediates a variety of actions such as vasodilatation, host defence and carcinogenesis [4, 20, 30]. NO appears to have both promoting and inhibitory effect on carcinogenesis. For example, NO causes DNA damage and promotes angiogenesis [20]. On the other hand, it can induce apoptosis and suppress tumor growth [5]. The balance between these different effects of NO may depend on its concentration in cancer tissue [17, 18]. Moreover, some specific actions of NO in tumor biology are thought to be related with its interactions with other molecules including IFN-γ and IL-18 [2, 3]. It has been demonstrated that changes in serum IFN-γ levels after IL-18 induction were positively correlated with NO activity [3].

In this study, we aimed to investigate the importance of serum IL-18 and NO activity in renal cell carcinoma (RCC) patients.

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Material and methods

Twenty-six patients with histologically confirmed RCC were included in this study. Serum IL-18 and nitrite+nitrate levels were measured in the patients before surgical procedure and in 8 healthy controls. After surgery, the macroscopic and histologic features, including tumor stage (TNM) and grade as defined by FUHRMAN et al [7] were evaluated. The tumor staging was defined according to the International Union Against Cancer TNM classification [27]. T1 tumor (T1: tumor ≤7 cm in greatest dimension limited to the kidney) was found in 13 patients and T2, T3, T4 tumor (T2: tumor >7 cm in greatest dimension limited to the kidney, T3: tumor extends into major veins or invades the adrenal gland or perinephric tissues, T4: tumor invades beyond Gerota's fascia) in 13 patients. Pathological examination of tumors revealed that 19 patients had grade 1 and 2 tumor and 7 patients had grade 3 and 4 tumor. To eliminate the influence of other diseases, we excluded patients with infectious diseases, diabetes mellitus, lung disease and synchoronous secondary malignancies. Control group included 8 healthy subjects. Serum samples were obtained from patients and control subjects after an overnight fasting and stored at –70 °C until analysis.

Statistical analysis. The results were presented as in mean ± SD. Student't test, Mann Whitney U test and Pearson's correlation analysis were used in statistical analysis. P values less than 0.05 were accepted as significant.

IL-18 measurement. ELISA for determination of IL-18 in serum was performed according to previously described method [29]. Immediately after blood sampling, serum was obtained by centrifugation at x800 g at 4 °C for 15 minutes and stored at –70 °C until use. The IL-18 level in each sample was determined by using commercially available ELISA kits (BioSource International human IL-18 Colorimetric solid phase Sandwich ELISA, California, USA). Sensitivity was determined by assaying serially diluted hIL-18 Calibrator. The mean absorbance plus 2 standard deviations for Calibrator diluted to 6.25 pg/ml was lower than the mean absorbance minus 2 standard deviations for Calibrator diluted to 12.5 pg/ml. The minimal detectable dose is therefore 12.5 pg/ml.

Nitrite and nitrate measurement. Nitrite was measured by using the Griess reaction and the results are given as micromoles per liter [9]. Nitrate was measured by using the enzymatic one-step assay with nitrate reductase [1]. The method was based on the reduction of nitrate to nitrite by nitrate reductase in the presence of β-NADPH. We equilibrated tubes at 25 °C containing 250 μL of 100 mmol/L potassium phosphate buffer (pH 7.5) and 50 μL of 12mmol/l β-NADPH with 100 μL of sample. To start the enzymatic reaction, we added 40 μL of 500 U/L nitrate reductase. We incubated the tubes in the dark for 45 minutes. The concomitant oxidation of β-NADPH was monitored by the decrease in absorbance at 340 nm. The method of standard addition was used to minimize the effect of interfering substances via serum. The results were expressed as micromoles per liter. We also used samples with internal standard, serum blanks and reagent blank.

Results

There was no significant difference among RCC patients with different tumor stage, grade and control subjects in terms of age and gender (p>0.05) (Tab. 1). RCC patients showed significantly higher serum IL-18 levels when compared to the control subjects (638.2 ± 296.7 vs 167.6 ± 39.5 pg/ml, p<0.001) (Tab. 1). Serum IL-18 levels were found to be similar in patients with T1 (595.1 ± 335.0 pg/ml) and T2, T3, T4 (680.6 ± 259.4 pg/ml; p>0.05) tumors (Tab. 1, Fig. 1). Patients with grade 3, 4 tumors (908.8 ± 315.6 pg/ml) showed significantly higher serum IL-18 levels when compared to the patients with grade 1, 2 tumors (538.5 ± 223.9 pg/ml; p<0.05) (Tab. 1, Fig. 2).

No significant difference was found in serum nitrite+nitrate levels between RCC patients (28.6 ± 17.4 μmol/l) and

Table 1. Characteristics of patients and control subjects

	n	Gender F/M)	Age (median) (years)	IL-18 (mean) (pg/ml)	Nitrite+nitrate (mean) (μmol/l)
All patients	26	7/19	58.5(35–78)	638.2 ± 296.7*	28.6 ± 17.4
Control subjects	8	2/6	61.0(55–65)	167.6 ± 39.5	29.9 ± 22.6
Tumor stage					
T1	13	3/10	59.0(35–74)	595.1 ± 335.0	28.8 ± 8.9
T2,T3,T4	13	3/10	58.0(39–78)	680.6 ± 259.4	28.3 ± 22.9
Grade					
1+2	19	4/15	60.0(35–75)	538.5 ± 223.9	31.7 ± 18.5
3+4	7	3/4	54.5(35–75)	908.8 ± 315.6**	20.0 ± 8.4

*p<0.001, **p<0.05, F/M – female/male.

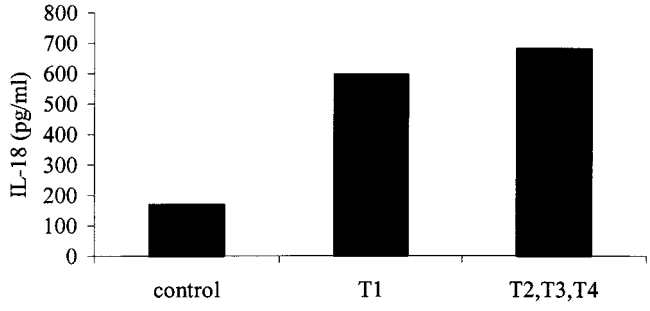

Figure 1. Serum IL-18 levels in patients according to tumor stage.

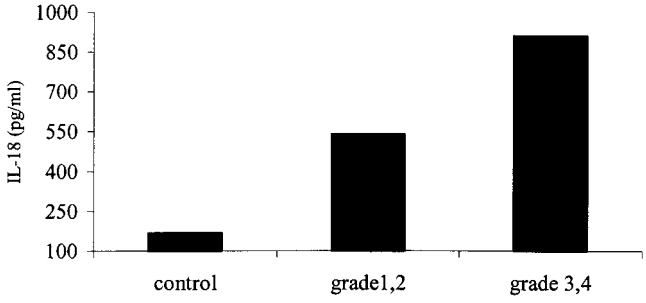

Figure 2. Serum IL-18 levels in patients according to tumor grade.

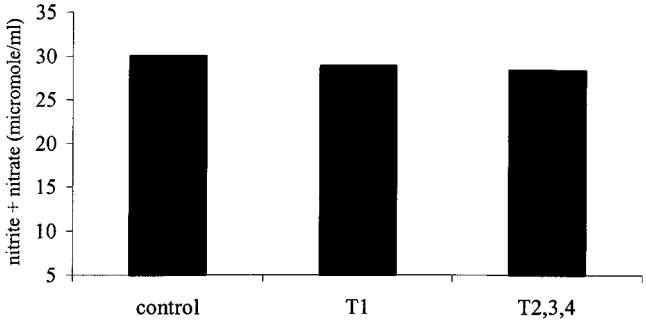

Figure 3. Serum nitrite+nitrate levels in patients according to tumor stage.

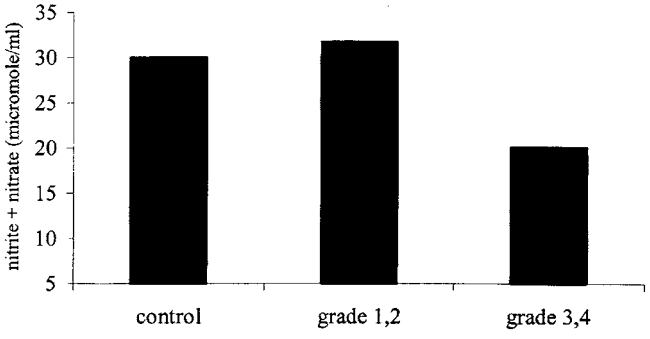

Figure 4. Serum nitrite+nitrate levels in patients according to tumor grade.

control subjects (29.9 ± 22.6 μmol/l, $p>0.05$). Serum nitrite+nitrate levels were not found to be different in patients with T1 (28.8 ± 8.9 μmol/l) and T2, T3, T4 (28.3 ± 22.9 μmol/l) tumors ($p>0/05$) (Tab. 1, Fig. 3). Serum nitrite+nitrate levels were found to be lower in patients with grade 3, 4 tumors (20.0 ± 8.4 μmol/l) than those with grade 1, 2 tumors (31.7 ± 18.5 μmol/l), but this was not statistically significant ($p=0.063$) (Tab. 1, Fig. 4). No correlation was found between serum IL-18 and nitrite+nitrate levels in RCC patients ($p>0.05$).

Discussion

IL-18 is a multifunctional cytokine of a 18,3 Kd [22]. IL-18 has been reported to increase immune defence against tumor growth through induction of IFN-γ production 8. IL-18 has also been reported to have potent antitumor activity that are mediated by inhibition of tumor angiogenesis [25], reduction of tumorigenesis [8] and induction of apoptosis [21]. Recently, its antitumor effect has been demonstrated in RCC [11, 12]. In our previous study, we demonstrated an association between serum IL-18 levels and metastatic activity of breast carcinoma [10]. KAWABATA et al [14] reported that the survival rate of patients with gastric cancer who had high serum IL-18 levels was significantly lower than of patients who had low serum IL-18 levels. It has been reported that patients with non-Hodgkin's lymphoma who had serum level over 2000 pg/ml were the high risk group in non-Hodgkin's lymphoma [28].

In our study, all patients showed higher serum IL-18 activity when compared to the control subjects. Increase in serum levels of IL-18 may be associated with host defence mechanism to suppress tumor growth and metastasis.

RCC represents a wide variation in biologic behavior and clinical course [26]. Tumor stage and histologic grade are the most useful markers to predict the prognosis of patients with RCC after nephrectomy [6, 32]. In some studies, proliferative, apoptotic index and angiogenic activity were found to be elevated in RCC patients with high grade and stage tumors [24, 31, 33]. In our study, patients with high grade tumors had increased serum IL-18 levels when compared to the patients with low grade tumors. This increase of serum IL-18 levels in high grade tumors may be an indirect reflection of elevated apoptotic, angiogenic and antiproliferative activity of tumor, because as mentioned above, antitumor effects of IL-18 stimulated by host defence system are mediated through these three mechanisms [8, 21, 25]. We found no significant difference in serum IL-18 levels of RCC patients in terms of tumor stage.

It has been reported that antitumor effects of IL-18 are not limited to the activity of IFN-γ. The production of other cytokines stimulated by IL-18 including IL-1β and NO might be involved in this process [13, 25].

NO partipicates in the multistep process of carcinogenesis, but its precise function in tumor biology is not clearly understood [19]. NO may prevent tumor growth by stimulation of apoptosis [5]. On the contrary, it can also promote tumor growth and metastasis by inducing angiogenesis [20]. It is believed that increased NO production may exert antiproliferative effects and decreased production may facilitate tumor growth [17, 18]. In this study, we measured serum nitrite+nitrate levels as an index of NO generation [16]. Serum nitrite+nitrate levels were not different in RCC patients and healthy controls. Serum nitrite+nitrate levels were found to be similar in patients who had T1,T2 and T3, T4 tumors. Interestingly, in contrast to serum IL-18 activity, serum nitrite+nitrate levels were found to be lower in patients with high grade tumors than in those with low grade

tumors and this difference was close to be statistically significant (p=0.063).

CHIKANO et al [3] demonstrated that IL-18 caused marked increases in serum NO levels and thus inhibited IFN-γ production from T and natural killer cells by damaging these cells. It has been reported that endogenous NO inhibits IL-18 and IFN-γ production by causing the S-nitrosation of caspase-1 [15]. As we found higher IL-18 and lower NO activity in serum of patients who had high grade tumors, it can be speculated that there may be an interaction between IL-18 and NO activity and, excessive synthesis of one of them may be regulated by a feed back mechanism between IL-18 and NO.

In conclusion, RCC patients showed higher serum IL-18 activity when compared to the healthy controls. Serum IL-18 levels were found to be significantly higher in patients who had high grade tumors than those in with low grade tumors. Patients with high grade tumors had a trend to represent lower NO activity. Elevated serum IL-18 levels in patients with RCC may reflect the degree of defence mechanism against the growth of tumor cells. In our small group of patients, we can only suggest that serum IL-18 activity may be a candidate to be a prognostic marker. Further studies with larger populations are required to determine the importance of serum IL-18 levels as a prognostic marker in RCC patients.

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