

Evaluation of oxidative stress status and antioxidant capacity in patients with renal cell carcinoma

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Introduction We evaluated and compared the serum oxidative stress and antioxidant enzymes in patients with renal cell carcinoma (RCC) and the control group.

Material and methods The prospective study consisted of 97 patients with RCC (Group 1) and 80 age and sex matched healthy volunteers (Group 2). Group 1 and 2 were compared concerning serum mean total oxidant status (TOS), total antioxidant capacity (TAC), paraoxonase-1 (PON-1), arylesterase, total thiol, catalase (CAT), myeloperoxidase (MPO) and ceruloplasmin.

Results Patients' mean age was 58.5 ± 12.3 and 56.9 ± 15.8 years, respectively, in Group 1 and 2. No statistically significant differences were detected between the groups in terms of oxidative stress parameters and antioxidant capacity measured in the serum of patients including, TOS, TAC, PON1, arylesterase, total thiol, CAT, MPO, and ceruloplasmin levels ($p > 0.05$ for all parameters). The PON-1 value was significantly higher in patients with pT1 stage than pT3 stage ($p = 0.007$). The arylesterase value was significantly higher in patients with Fuhrman's nuclear grade 3 than grade 2 ($p = 0.035$). There was no correlation between these parameters level and Fuhrman's nuclear grade, stage, or histopathological tumor type.

Conclusions Our results demonstrated that evaluation of these parameters in the serum of patients with localized RCC may not be used as a marker to discriminate between patients with RCC and healthy people.

Key Words: renal cell carcinoma ↔ oxidative stress ↔ antioxidant capacity

INTRODUCTION

Renal cell carcinoma (RCC) is the most common solid lesion in the kidney and constitutes approximately 90% of all kidney malignancies. It has various histopathological types and specific genetic characteristics [1]. The exact etiology of RCC is still unclear; however, tobacco exposure, obesity, and hypertension seem to be the most accepted risk factors. Moreover, numerous other potential etiologic factors such as viruses, lead compounds, and various chemicals have been identified in animal models.

Oxidative stress is defined as an imbalance between reactive oxygen species (ROS) and antioxidant capacity.

Oxidative stress has a crucial role in many pathological conditions including oncogenesis. ROS can induce the carcinogenesis process and sustain tumor progression by damaging DNA [2]. It has been demonstrated that important changes occurred in the balance between oxidative stress and antioxidant status at a cellular level during tumor growth process [3].

In literature there are several studies showing both elevated ROS levels and elevated antioxidant enzyme levels in patients with RCC [4–10]. The oxidative stress issue in RCC is still controversial. In this study, we aimed to evaluate the serum total oxidant status (TOS), total antioxidant capacity (TAC), and paraoxonase-1 (PON1) levels in pa-

tients with RCC and compare these enzyme levels with control groups. We also compared arylesterase, thiols, catalase (CAT), myeloperoxidase (MPO) and ceruloplasmin levels between these two groups. To the best of our knowledge, our study would be the first that investigates serum TOS, TAC, PON-1 and arylesterase levels in patients with RCC.

MATERIAL AND METHODS

We designed a multi-center prospective study. Blood samples were obtained from two institutions. A total of 97 patients with RCC (Group 1) were included in this study between May 2010 and August 2013. Eighty healthy volunteers' age and sex were matched and selected as the control group (Group 2). The local Institutional Review Board approved the study protocol. Written informed consent was obtained from all participants.

All patients were evaluated clinically and underwent relevant hematological, biochemical and radiological investigations. Smoking and antioxidant or vitamin supplements were stopped at least 4 weeks before the study. Kidney lesions were removed either with radical nephrectomy or a nephron sparing surgery. Tumors were graded according to the Fuhrman's nuclear grading system [11]. Renal cancer was staged according to the American Joint Committee on Cancer TNM system [12].

Blood samples

Blood samples were obtained following an overnight fasting state. Samples were withdrawn from a cubital vein into blood tubes and immediately stored in ice at 4 °C. The serums were then separated from the cells by centrifugation at 3000 rpm for 10 min and were directly frozen and stored at -80°C until analysis.

Measurement of total oxidant status (TOS)

Serum TOS levels were determined using a novel automated measurement method, developed by Erel [13]. In this method, oxidants present in the sample oxidise the ferrous ion–o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion makes a coloured complex with xylenol orange in an acidic medium. The colour intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide, and the results are expressed in terms of micromolar hydrogen peroxide equivalent per litre ($\mu\text{mol H}_2\text{O}_2$ equiv. l^{-1}).

Measurement of total antioxidant capacity (TAC)

Serum TAC levels were determined using a novel automated measurement method, developed by Erel [14]. In this method, hydroxyl radical, which is the most potent radical, is produced via Fenton reaction. In the classical Fenton reaction, the hydroxyl radical is produced by mixing of ferrous ion solution and hydrogen peroxide solution. In the most recently developed assay by Erel, the same reaction is used [15]. In the assay, ferrous ion solution, which is present in Reagent 1, is mixed with hydrogen peroxide, which is present in Reagent 2. The sequential-produced radicals, such as brown-coloured dianisidiny radical cation produced by the hydroxyl radical, are also potent radicals. In this assay, the antioxidative effect of the sample against the potent free radical reactions, which is initiated by the produced hydroxyl radical, is measured. The assay got excellent precision values, which are lower than 3%. The results are expressed in mmol Trolox equiv. l^{-1} .

Measurement of paraoxonase-1 (PON-1) activities

PON1 activity was determined using paraoxon as a substrate and measured by increases in the absorbance at 412 nm due to the formation of 4-nitrophenol, as already described [15]. The activity was measured at 25°C by adding 50 μl of serum to 1 ml Tris-HCl buffer (100 mM at PH 8.0) containing 2 mM CaCl_2 and 5.5 mM of paraoxon. The rate of generation of 4-nitrophenol was determined at 412 nm. Enzymatic activity was calculated by using the molar extinction coefficient 17100 $\text{M}^{-1} \text{cm}^{-1}$.

Measurement of arylesterase activities

Arylesterase activity was measured using phenylacetate as a substrate. Serum was diluted 400 times in 100 mM Tris-HCl buffer, pH = 8.0. The reaction mixture contained 2.0 mM phenylacetate (Sigma Chemical Co) and 2.0 mM CaCl_2 in 100 mM Tris-HCl buffer, pH = 8.0. Initial rates of hydrolysis were determined by following the increase of phenol concentration at 270 nm at 37°C on a CE 7250 spectrophotometer (Cecil Instruments Limited, UK) [16]. Enzyme activities were expressed in international units (U) or kilo units (kU) per 1 litre of sera.

Measurement of total thiol activities

Serum total thiol concentration or sulfhydryl groups (SH) were measured by the methods originally described by Elman and modified by Hu [17, 18]. Here, thiols interact with 5, 5'-dithiobis-(2-nitrobenzoic

acid) (DTNB), forming a highly colored anion with maximum peak at 412 nm ($\epsilon_{412} = 13,600 \text{ M}^{-1} \text{ cm}^{-1}$). Here, this method was adapted to an automated biochemistry analyzer (Cobas C 501; Roche, Mannheim, Germany).

Measurement of catalase (CAT) activity

Catalase activity was measured using hydrogen peroxide (H_2O_2) as substrate. The disappearance of H_2O_2 was followed at 240 nm, and enzyme activity was expressed in units per liter of serum (U/L) at 25°C [19].

Measurement of myeloperoxidase activity (MPO)

Serum MPO activity was determined by the method of Klebanoff and Clark and was based on kinetic measurement of the formation rate of the yellowish-orange product of the oxidation of *o*-dianisidine with myeloperoxidase in the presence of hydrogen peroxide (H_2O_2) at 460 nm [20]. One unit of myeloperoxidase was defined as that degrading 1 μmol of H_2O_2 per minute at 25°C. A molar extinction coefficient of $1,3 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ of oxidized *o*-dianisidine was used for the calculation. Myeloperoxidase activity was expressed in units per liter of serum (U/L).

Measurement of ceruloplasmin activity

Serum ceruloplasmin level was measured using an automated measurement method defined by Schoinsky et al. [21].

Lipid profile analysis

The levels of triglyceride, total cholesterol (TC), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were determined using a colorimetric method (Cobas C 501) with an automatic analyzer (Roche, Mannheim, Germany).

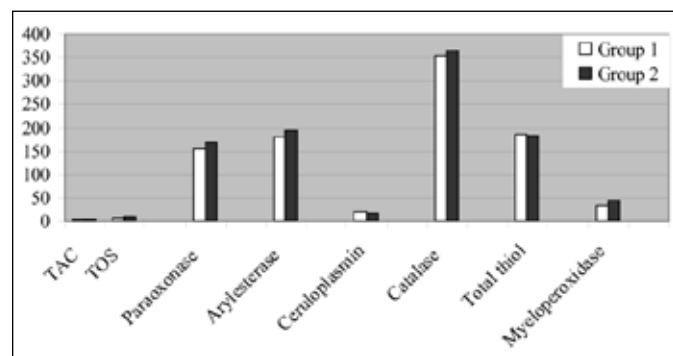


Figure 1. Oxidant and antioxidant parameters were similar in Group 1 and 2.

Table 1. Summary of pathological results in patients with renal cell carcinoma

	N (%)
Pathological type	
Clear cell	71 (73.2)
Multicystic type	4 (4.1)
Papillary type 1	8 (8.2)
Papillary type 2	5 (5.2)
Chromophobe	7 (7.2)
Clear + Sarcomatoid	1 (1.0)
Clear + papillary	1 (1.0)
Pathological stage	
pT1a	28 (28.9)
pT1b	27 (27.8)
pT2a	16 (16.5)
pT2b	13 (13.4)
pT3a	13 (13.4)
Fuhrman's nuclear grade	
Grade 1	8 (8.2)
Grade 2	54 (55.7)
Grade 3	27 (27.8)
Grade 4	8 (8.2)

Statistical analysis

SPSS version 11.5 software (Chicago, Illinois, USA) was used for data analysis. Parameters were expressed as mean \pm standard deviation. The Shapiro-Wilk test was used for a normal distribution before analysis. For independent samples, a *t* test was used to compare Group 1 and Group 2. The Pearson correlation test was used to evaluate the association between all parameters. *P*-values less than 0.05 were considered significant.

RESULTS

A total of 97 patients were enrolled in the study. There were 71 (73.2%) males and 26 (26.8%) females in the RCC group and the mean age was 58.5 ± 12.3 years (range 32 to 83). The healthy control group had 62 (77.5%) males and 18 (22.5%) females with a mean age of 56.9 ± 15.8 years (range 17 to 86) ($p > 0.05$). 45 patients (46.4%) were active smokers. Tumors were located on the right side in 54 patients (55.7%). Tumors were removed via radical nephrectomy and nephron sparing surgeries in 73 patients (75.3%) and 24 patients (24.7%), respectively. Table 1 shows pathological characteristics of the patients. Pathological examination revealed that 71 patients (73.2%) had clear cell carcinoma and significant amount of patients had early staged tumors. 55 patients (56.7%) were pT1 and 29 patients (29.9%) were pT2. Surgical margins were negative in all specimens.

Total oxidant status, total antioxidant capacity, and antioxidant enzymes are demonstrated in Table 2.

Table 2. Comparison of oxidative and antioxidative status parameters of Group 1 and Group 2

Parameters	Group 1 Mean \pm standart deviation (n = 97)	Group 2 Mean \pm standart deviation (n = 80)	p-value*
TAC (mmol Trolox equivalent l ⁻¹)	2.11 \pm 0.24	2.09 \pm 0.25	0.691
TOS (μ m)	6.21 \pm 7.83	6.97 \pm 5.21	0.461
PON-1 (U l ⁻¹)	153.85 \pm 110.93	167.10 \pm 118.84	0.445
Arylesterase (U l ⁻¹)	178.42 \pm 71.19	192.37 \pm 79.78	0.221
Ceruloplasmin (mg dl ⁻¹)	18.93 \pm 6.23	17.40 \pm 5.19	0.082
CAT (IU mg ⁻¹)	353.34 \pm 165.51	365.50 \pm 225.04	0.680
Total thiol (mm)	184.33 \pm 53.45	182.52 \pm 45.20	0.810
MPO (pmol l ⁻¹)	34.05 \pm 54.25	44.63 \pm 53.62	0.196
ESR (mm hr ⁻¹)	37.05 \pm 35.11	26.43 \pm 25.55	0.025

TAC – total antioxidant capacity; TOS – total oxidant status; PON-1, paraoxonase-1; CAT – catalase; MPO – myeloperoxidase; ESR – erythrocyte sedimentation rate; *P < 0.05

There were no statistically significant differences between the RCC group and control group in terms of TOS, TAC, PON-1, arylesterase, total thiol, CAT, MPO, and ceruloplasmin levels (Figure 1). When antioxidants levels were matched to the pathological stage, the only statistically significant differences were found in PON-1 levels in pT1 and pT3 tumors. PON-1 level was 163.7 ± 117.9 U l⁻¹ in pT1 and 101.7 ± 54.3 U l⁻¹ in pT3 ($p = 0.007$). Fuhrman's nuclear grade 2 and 3 were the most common types in our patients (Table 1). Arylesterase levels were higher in Fuhrman's nuclear grade 3 patients (Grade 2: 162.2 ± 60.2 U l⁻¹, Grade 3: 203.1 ± 87.1 U l⁻¹, $p = 0.035$). No correlations were found between total oxidant status, total antioxidant capacity, or antioxidant enzymes level and Fuhrman's nuclear grade, tumor stage, or tumors' histopathological type. A positive significant correlation was found between Fuhrman's nuclear grade and smoking ($r = 0.029$, $p = 0.003$). Also, there was a negative significant correlation between ceruloplasmin levels and smoking ($r = -0.275$, $p = 0.006$). Both groups were similar in terms of routine biochemical parameters and lipid profile.

DISCUSSION

The etiology of renal cell carcinoma is not clear; however, oxidative stress is considered to be a factor in the development of RCC, as well as other cancers [3, 4]. Oxidative stress can induce growth factors and hypoxia inducible factor (HIF), and thereby accelerate tumor growth.

Oxidative status and antioxidants can be measured either in the blood samples or tissue samples [5]. Markers studied in tissue samples represent the alterations in the tumor itself and adjacent tissues, but markers studied in blood samples reflect the systemic processes associated with the cancer. The advantages of studying markers in the blood samples include giving information about the status of oxidative stress before surgery, independency of the additional oxidative stress of the surgery itself, simplicity of retrieving blood samples – venous blood samples are usually more than enough-, and reproducibility. In the vast majority of previously published studies, tissue samples retrieved from kidney tumors were preferred over blood samples [4–11]. We preferred to study the blood samples due to their simplicity to attain, preservation, and their potential in evaluating the status of the systemic oxidative stress before surgery.

Ganesamoni et al. reported an increase in oxidative stress parameters in patients with RCC compared to normal controls [4]. They reported a significantly higher superoxide dismutase (SOD) level and lower catalase level in tumor tissue when compared to normal kidney tissue. They reported no association with SOD level and tumor grade, stage and tumor histopathology [4]. However, lipid peroxidation was significantly higher in RCC tissue than in normal renal tissue. Moreover, higher nuclear grade of RCC was associated with increased lipid peroxidation in tumor tissue [4]. They reported a lower serum glutathione in association with higher grade RCC and metastatic disease; furthermore, systemic ROS and nitric oxide levels did not decrease significantly following surgery in patients with metastatic disease [4].

We haven't found any difference in the levels of systemic oxidative stress indices and antioxidant indices between the patients with RCC and the control group. However, the level of PON-1 was significantly lower in pathologic stage T3 patients than pathologic stage T1 ($p = 0.007$). The arylesterase enzyme was significantly higher in patients with higher nuclear grade ($p = 0.035$). Other than the above mentioned differences between the study and control group, there were no associations with oxidant and antioxidant indices, and nuclear grade, stage, or tumor histopathology. We studied the levels of oxidative and anti-oxidative indices in venous blood samples; however, 84 out of 97 (86.6%) patients with pT1-2 disease were without any evidence of local lymph node dissemination or systemic metastasis. In our opinion, in patients with localized low stage disease, blood levels of oxidative and anti-oxidative stress indices do not dramatically rise due to low tumor bulk and less neoplastic metabolic events. Levels of indices

in tissue samples of RCC might be more relevant than blood samples. Accordingly, we decided to study tissue samples as well in future participations of the study. Glutathione peroxidase and catalase activities have been reported significantly decreased in RCC [10], although we did not find a significant association with RCC and anti-oxidant levels in our series. Likewise, advanced stages are not associated with a significant change in antioxidant enzyme activities as of our series [10]. Another study comparing the levels of CAT, SOD, glucose 6-phosphate dehydrogenase (G6PD), and glutathione peroxidase were not different in RCC tissues and normal renal cortex tissues [9]. We similarly have found no difference of antioxidant levels in blood samples between patients with RCC and the control group.

Total level of thiol was similar in RCC and control groups. In a previously published study, it has been demonstrated that normal kidney tubular cells can be stained, but with a variable intensity for anti-oxidant enzymes such as peroxiredoxins and thiol-containing proteins [7]. The presence of peroxiredoxin-2 was associated with better prognosis and nitrotyrosine was associated with higher nuclear grade [7]. Similarly, Soini et al. reported increased nitrotyrosine staining in higher grades of RCC, but no association with apoptosis and antioxidant enzymes in RCC [8]. Oberley et al. reported increased products of lipid peroxidation in clear cell variant of RCC, although there was not much of an increase in other types of kidney cancers, such as papillary renal cell carcinoma, Wilms' tumor, and transitional cell carcinoma of the renal pelvis [22]. Okamoto et al. compared the DNA oxidation levels, measuring the 8-hydroxy-2'-deoxyguanosine utilizing biochemical techniques in RCC and normal kidney tissues showing higher levels of DNA oxidation than normal kidney tissues [23].

Antioxidant enzymes are found in a variety of localizations and diverse types of cells [24]. Manganese superoxide dismutase (MnSOD) is located in the mitochondria, whereas CuZnSOD is located in the cytoplasm, within the lysosomes, and also in the nucleus.

Catalase is primarily located in peroxisomes, whereas glutathione peroxidase can be found within all sub-cellular compartments [24]. Frederiks et al. reported that five out of 8 human RCC specimens had high glucose-6-phosphate dehydrogenase (G6PD) activity, even though G6PD activity was neither associated with tumor histopathology nor nuclear grade, size of the tumor, or metastases [6]. They also demonstrated the peroxisomes in the normal epithelial layer of the proximal tubules by electron microscopy. In contrast, peroxisomes could not be detected in cancer cells irrespective of tumor histopathology or G6PDH activity [6].

Despite that we have not found any difference in oxidant and antioxidant levels between RCC and normal control group, this subject is controversial according to literature. A majority of the publications referring this subject are predominantly done using the tumor tissue retrieved from the nephrectomy specimens. New studies need to focus on cellular biology of oxidation, anti-oxidant defense mechanisms against oxidative stress, and genetic details of this complex phenomenon. The future perspective of this subject will lead to new developments on diagnosis, treatment, and follow-up of RCC.

CONCLUSIONS

Serum oxidative stress indices and anti-oxidant levels/activity are not associated with RCC, however a vast majority of the patients were with localized low stage disease, which might be a confounding effect and considered to be a reason of type 2 errors with sampling error. Levels of paraoxonase-1 were significantly lower in advanced stage disease than lower stages. There were no correlations between oxidant and anti-oxidant level, and nuclear grade, stage. or tumor histopathology, but there were correlations between high arylesterase levels and higher nuclear grade.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

References

1. Kovacs G, Akhtar M, Beckwith BJ, et al. The Heidelberg classification of renal cell tumors. *J Pathol.* 1997; 183: 131-133.
2. Klaunig JE, Kamendulis LM. The role of oxidative stress in carcinogenesis. *Annu Rev Pharmacol Toxicol.* 2004; 44: 239-267.
3. Pelicano H, Carney D, Huang P. ROS stress in cancer cells and therapeutic implications. *Drug Resist Update.* 2004; 7: 97-110.
4. Ganesamoni R, Bhattacharyya S, Kumar S, et al. Status of oxidative stress in patients with renal cell carcinoma. *J Urol.* 2012; 187: 1172-1176.
5. Gago-Dominguez M, Castelao JE. Lipid peroxidation and renal cell carcinoma: Further supportive evidence and new mechanistic insights. *Free Radic Biol Med.* 2006; 40: 721-733.
6. Frederiks WM, Bosch KS, Hoebein KA, van Marle J, Langbein S. Renal cell carcinoma and oxidative stress: The lack

- of peroxisomes. *Acta Histochem.* 2010; 112: 364-371.
7. Soini Y, Kallio JP, Hirvikoski P, et al. Oxidative/nitrosative stress and peroxiredoxin 2 are associated with grade and prognosis of human renal carcinoma. *APMIS.* 2006; 114: 329-337.
8. Soini Y, Kallio JP, Hirvikoski P, et al. Antioxidant enzymes in renal cell carcinoma. *Histol Histopathol.* 2006; 21: 157-165.
9. Lusini L, Tripodi SA, Rossi R, et al. Altered glutathione anti-oxidant metabolism during tumor progression in human renal-cell carcinoma. *Int J Cancer.* 2001; 91: 55-59.
10. Pljesa-Ercegovic M, Mimic-Oka J, Dragicevic D, et al. Altered antioxidant capacity in human renal cell carcinoma: role of glutathione associated enzymes. *Urol Oncol.* 2008; 26: 175-181.
11. Fuhrman SA, Lasky LC, Limas C. Prognostic significance of morphologic parameters in renal cell carcinoma. *Am J Surg Pathol.* 1982; 6: 655-663.
12. Edge SB, Byrd DR, Compton CC, Fritz AG, Green FL, Trotti A (eds). *AJCC cancer staging handbook.* 7 th edn, New York: Springer, 2010; pp. 479-489.
13. Erel O. A new automated colorimetric method for measuring total oxidant status. *Clin Biochem.* 2005; 38: 1103-1111.
14. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clin Biochem.* 2004; 37: 277-285.
15. Eckerson HW, Wyte CM, La Du BN. The human serum paraoxonase/arylesterase polymorphism. *Am J Hum Genet.* 1983; 35: 1126-1138.
16. Haagen L, Brock A. A new automated method for phenotyping arylesterase (EC 3.1.1.2) based upon inhibition of enzymatic hydrolysis of 4-nitrophenyl acetate by phenyl acetate. *Eur J Clin Chem Clin Biochem.* 1992; 30: 391-395.
17. Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys.* 1959; 82: 70-77.
18. Hu ML. Measurement of protein thiol groups and glutathione in plasma. *Methods Enzymol.* 1994; 233: 380-385.
19. Catalase Aebi H. In Bergmeyer HU ed. *Methods of Enzymatic Analysis.* New York and London: Academic Pres Inc., 1974; pp. 673-677.
20. Clark RA, Klebanoff SJ. Role of the myeloperoxidase-H₂O₂-halide system in concanavalin A-induced tumor cell killing by human neutrophils. *J Immunol.* 1979; 122: 2605-2610.
21. Schosinsky KH, Lehmann HP, Beeler MF. Measurement of ceruloplasmin from its oxidase activity in serum by use of o-dianisidine dihydrochloride. *Clin Chem.* 1974; 20: 1556-1563.
22. Oberley TD, Toyokuni S, Szweda LI. Localization of hydroxynonenal protein adducts in normal human kidney and selected human kidney cancers. *Free Radic Biol Med.* 1999; 27: 695-703.
23. Okamoto K, Toyokuni S, Uchida K, et al. Formation of 8-hydroxy-2'-deoxyguanosine and 4-hydroxy-2-nonenal-modified proteins in human renal-cell carcinoma. *Int J Cancer.* 1994; 58: 825-829.
24. Muse KE, Oberley TD, Sempf JM, Oberley LW. Immunolocalization of antioxidant enzymes in adult hamster kidney. *Histochem J.* 1994; 26: 734-753. ■