The importance of alpha-1 antitrypsin (α1-AT) and neopterin serum levels in the evaluation of non-small cell lung and prostate cancer patients

Zeyad J. El-Akawi [1], Aymen M. Abu-awad [2], Abdelmonem M. Sharara [3], Yousef S. Khader [4]

1 Department of Biochemistry and Molecular Biology, Faculty of Medicine, Jordan University of Science and Technology, Irbid, Jordan.
2 Princess Iman Center of Research and Laboratory Sciences, King Hussein Medical Center, Royal Medical Services, Amman-Jordan.
3 Department of Chest Diseases, King Hussein Medical Center, Royal Medical Services, Amman-Jordan.
4 Department of community Medicine, Public Health and Family Medicine, Faculty of Medicine, Jordan University of Science and Technology, Irbid, Jordan.

Correspondence to: Zeyad El-akawi, MD, MA, PhD.
Associate Professor, Biochemistry and Molecular Biology,
Vice Dean, Faculty of Medicine
Jordan University of Science and Technology, Irbid-Jordan.
TEL: +962-2-720-1000 EXT. 23837, 23783; FAX: +962-2-7201064;
E-MAIL: zakawi@just.edu.jo

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Abstract

OBJECTIVE: Increased serum levels of alpha-1 antitrypsin (α1-AT) and neopterin were observed in many diseases including different types of cancer. The aim of this work is to determine α1-AT and neopterin serum levels in newly diagnosed untreated non-small cell lung and prostate cancer patients and to test their relation to cancer staging.

METHODS: Radial Immunodiffusion and ELISA methods were used to determine α1-AT and neopterin serum levels, consequently.

RESULTS: α1-AT and neopterin mean serum levels were found to be elevated in non-small-cell lung and prostate cancer patients. In non-small cell lung cancer patients α1-AT was 454.5±129.2 mg/dL (p<0.0005) and neopterin was 7.9±4.2 ng/mL (p<0.0005). In prostate cancer patients α1-AT was 462.7±116.9 mg/dL (p<0.0005) and neopterin was 8.1±3.1 ng/mL (p<0.0005). These elevated levels were significantly correlated with the stage of cancer. The mean serum level of α1-AT in stages I, II, III, and IV among non-small cell lung cancer patients were 305.1, 453.6, 490.3 and 616.0 mg/dL respectively, and the mean serum levels for neopterin were 4.0, 7.0, 8.1 and 14.9 ng/mL, correspondingly. The mean serum level of α1-AT in stages A, B, C, and D among prostate cancer patients were 342.9, 418.5, 467.8 and 593.5 mg/dL respectively and the mean serum levels for neopterin were 4.9, 6.6, 8.7 and 11.6 ng/mL, correspondingly.

CONCLUSIONS: Based on the above mentioned findings α1-AT and neopterin serum levels should be considered in the follow up as well as in the prognosis of cancer patients.

ORIGINAL ARTICLE

INTRODUCTION

Human alpha-1 antitrypsin (α1-AT) is the major component of α1-globulin electrophoresis bands of plasma proteins. It is known as alpha-1 protease inhibitor (α1-Pi), it inhibits serine proteases and acts as an acute phase glycoprotein (Carrell *et al.* 1982). Several studies performed to test for the correlation between α1-AT serum levels and neoplastic diseases demonstrated that these levels get elevated in different types of malignancies such as hepatocellular carcinoma, multiple myeloma, pancreatic carcinoma, prostatic carcinoma, primary carcinoma of the lung, cervical carcinoma, gastric cancer, laryngeal carcinoma, nasopharyngeal carcinoma, breast cancer and colorectal carcinoma (Robinaovitz *et al.* 1992; Chio and Oon 1979; Pelllinieme *et al.* 1995; Tountas *et al.* 1985; Ward *et al.* 1977; Bata *et al*, 1977; Patenoster *et al.* 1980; deLorimier *et al.* 1993; Krecicki and Leluk 1992; Mattison *et al.* 1981; Amiguet *et al.* 1998). Elevated α1-AT levels in cancer patients attracted the attention of many scientists in term of using this parameter in the diagnosis as well as in the follow up of cancer patients. Schena *et al.*, 1985 showed that α1-AT represents a sensitive but little specific diagnostic index of neoplastic disease (Schena *et al.* 1985). In addition, It has been demonstrated that α1-AT is an alarming factor but not sufficiently specific to become a diagnostic tool for malignancy (Debrowska *et al.* 1997; Demidove *et al.* 1990; Pirisi *et al.* 1996). Neopterin is a pteridine that is released from stimulated human monocytes and macrophages. It is considered as a nonspecific biochemical marker of activated cellular immune response (Fuchs *et al.* 1992). Increased serum levels of neopterin were observed in lung, prostate, gastrointestinal tract, pancreatic, hepatic and breast cancers (Murr *et al.* 1999a;b). Yildirim *et al.* (2008) demonstrated that serum neopterin levels were significantly elevated in patients with metastatic breast cancer and these levels seem to be indicator of metastatic cancer rather than a marker for local cancer (Yildirim *et al.* 2008). In this work we aimed to determine α1-AT and neopterin levels in the serum of newly diagnosed untreated non-small cell lung and prostate cancer patients and their relation to cancer staging.

MATERIAL AND METHODS

Blood samples were obtained from one hundred newly diagnosed untreated male cancer patients at King Hussein Medical Center (K.H.M.C.) and Albashir Hospital in Amman. Fifty patients, mean age was 54 (35–70) years, with non-small cell lung cancer distributed based on the stage of the disease as follows: 13 patients with stage I, 14 patients with stage II, 14 patients with stage III, and 9 patients with stage IV. The other fifty patients, mean age was 61 (50–70) years, with prostate cancer distributed based on the cancer staging as follows: 11 patients with stage A, 13 patients with stage B, 12 patients with stage C and 14 patients with stage D. Cancer staging were performed by specialist physicians. One hundred healthy, age matched, non-smokers individuals were included in this study as a control group. Blood samples for the control group were obtained from blood bank in K.H.M.C., Prince Iman Research and Lab Sciences Center in Amman. To perform serum analysis for both, α1-AT and neopterin, venous blood was drawn into plain tubes then these tubes were centrifuged for 10 min to separate the serum. Serum was divided into two tubes, the first tube was directly used for the determination of α1-AT levels by Radial Immunodiffusion (RID) technique and the second tube was stored at 2–8 °C protected from light to be used for neopterin analysis using Enzyme Linked Immunosorbent Assay (ELISA) technique.

Statistical analysis

Statistical analysis was performed using t-test and Duncan's Post Hoc Test of multiple comparisons. Differences between mean levels were considered significant if obtained *p*-value was less than 0.05.

RESULTS

As shown in Table 1, the mean serum levels of α1-AT and neopterin in non-small cell lung cancer patients are 454.5 ± 129.2 mg/dL and 7.9 ± 4.2 ng/mL, respectively. Those for prostate cancer are 462.7 ± 116.9 for α1-AT and 8.1 ± 3.1 for neopterin. α1-AT and neopterin levels in the control group are 146.2 ± 24.3 mg/dL and 1.7 ± 0.4 ng/mL, correspondingly. As data indicated, there is a significant increase in α1-AT and neopterin serum levels in patients with non-small cell lung cancer and prostate cancer compared with those of the control groups. The *p*-values are less than 0.05 in both cases. Table 2 shows α1-AT and neopterin serum levels in non-small cell lung cancer patients at different cancer stages. In this table we demonstrated the increase in both α1-AT and neopterin levels with the advancement of cancer, from stage I to stage IV, and this increment is very significant as *p*-values indicated. In Table 3 we demonstrated α1-AT and neopterin levels in prostate cancer patients at stages A, B, C and D were we showed the significant increase of these levels with cancer progression from stage A to stage D.

DISCUSSION

Proteolytic enzymes play an important role in cancer physiology, but the role of the body's natural inhibitors of these enzymes in this process is not very well studied. α1-AT is the major serine protease inhibitor in the human plasma. Researchers have been trying to find a correlation between α1-AT and the process of neoplasia that may help in the diagnosis and the follow up of cancer patients (Carrell *et al.* 1982; Schena *et al.* 1985; Debrowska *et al.* 1997; Demidove *et al.* 1990; Pirisi *et al.*

1996). Neopterin is another biological compound that produced by human monocytes upon their stimulation by IFN-γ. It has been reported that the increase in neopterin in the serum of cancer patients depends on tumor type and stage (Fuchs *et al.* 1992; Murr *et al.* 1999a;b). In this work, one hundred newly diagnosed (untreated) male patients were involved. Fifty patients with non-small cell lung cancer and other fifty with prostate cancer. The reason behind our choice of untreated and newly diagnosed cancer patients was to avoid the interference of the treatment factor with α1-AT and neopterin serum levels in these patients.

We demonstrated that all patients (100%) with non-small cell lung and prostate cancer have elevated serum α1-AT concentrations. Non-small cell lung and prostate cancer patients have shown significantly higher mean serum levels of α1-AT 454.5 ± 129.2 (p<0.0005) and 462.7 ± 116.9 mg/dL (p<0.00005), respectively, than those in male controls 146.2 ± 24.3 mg/dL. These findings are in agreement with what was reported in earlier studies (Bata *et al.* 1977; Micksche and Kokron 1977; Daddi *et al.* 1976; Harris *et al.* 1976). Daddi *et al.* (1976) demonstrated that in more than 90% of lung cancer patients the level of α1-AT was significantly higher than those in the sera of controls. Ward *et al.* (1977) and Zietek *et al.* (1996) found that α1-AT serum levels and activity were increased with the development of prostate cancer. Patients with non-small cell lung and prostate cancer had also shown a significant rise in the serum concentration of neopterin. Neopterin mean serum levels were 7.9 ± 4.2 (p<0.0005) and 8.1 ± 3.1 ng/mL (p<0.00005), correspondingly, while the mean level in controls was 1.7 ± 0.4 ng/mL. This finding is in accordance with what was observed by Fuchs *et al.* (1992) and Lewenhaupt *et al.* (1986). They found that neopterin serum level and activity were increased with advancement of lung and prostate cancer. In addition, we demonstrated that serum levels of α1-AT and neopterin were significantly higher in non-small cell lung cancer patients with stage IV than those with stage I–III prior to treatment and the correlation between the serum levels of α1-AT and neopterin and the cancer staging is a direct one as shown in Table 2. Our observation came to support Daddi *et al.* (1976) findings who demonstrated that the level of α1-AT in lung cancer patients might vary during the course of the disease. In this study, we found that the mean serum levels of α1-AT in stage A, B, C, and D among prostate cancer patients were 342.9, 418.5, 467.8 and 593.5 mg/dL, respectively and the mean serum levels for neopterin were 4.9, 6.6, 8.7 and 11.6 ng/mL, correspondingly (p<0.0005). Similar to our findings were demonstrated by Ward *et al.* (1977) where they showed that there were differences in α1-AT serum levels between prostate cancer patients with different stages of the disease (Ward *et al.* 1977). Therefore, we can use α1-AT test as one of the acute-phase protein in cancer patient that might help in evaluating the advancement and the activity of the neoplastic process as well as in prognosis. In non-small cell lung and prostate cancer patients, serum levels of neopterin were significantly higher in cancer patients with stage IV than those with stage I–III prior to treatment, there was a striking correlation of neopterin with malignancy stage for both types of cancer. This finding is in accordance with that observed by Murr *et al.* 1999. They demonstrated that neopterin serum levels might have a predictive value to the stage of the tumor.

There is a higher neopterin mean value in patients with adenocarcinoma and non-small cell lung cancer stage IV than with stages I to III. Our findings were supported by Mohamed *et al.* (2001) who showed that the neopterin levels were elevated in patients with lung

Tab. 1. α1-AT and neopterin mean serum levels in patients with non-small cell lung cancer, prostate cancer, and control group.

Group	Number of individuals	α_1-AT mg/dL Mean ± SD	Neopterin ng/mL Mean ± SD
Lung cancer	50	454.5 ± 129.2*	7.9 ± 4.2#
Prostate cancer	50	462.7 ± 116.9**	8.1 ± 3.1##
Control	100	146.2 ± 24.3	1.7 ± 0.4

SD - standard deviation
* p<0.0005; ** p<0.00005;
# p<0.0005; ## p<0.00005

Tab. 2. α1-AT and neopterin mean serum levels at different stages in non-small cell lung cancer patients.

Stage	Number of individuals	α_1-AT mg/dL Mean ± SD	p-value*	Neopterin ng/mL Mean ± SD	p-value*
Control	100	146.2 ± 24.3		1.7 ± 0.4	
I	13	305.1 ± 31.6	<0.0005	4.0 ± 0.6	<0.0005
II	14	453.6 ± 90.8	<0.0005	7.0 ± 2.8	<0.0005
III	14	490.3 ± 88.7	<0.0005	8.1 ± 2.3	<0.0005
IV	9	616.0 ± 81.4	<0.0005	14.9 ± 2.3	<0.0005

* One-tailed P-value for the difference between control group and each stage using Duncan's Post Hoc Test of multiple comparisons.

Tab. 3. α1-AT and neopterin mean serum levels in patients with different stages of prostate cancer.

Stage	Number of individuals	α_1-AT mg/dL Mean ± SD	p-value*	Neopterin ng/mL Mean ± SD	p-value*
Control	100	146.2 ± 24.3		1.7 ± 0.4	
A	11	342.9 ± 63.8	<0.0005	4.9 ± 1.0	<0.0005
B	13	418.5 ± 86.8	<0.0005	6.6 ± 1.6	<0.0005
C	12	467.8 ± 69.7	<0.0005	8.7 ± 1.4	<0.0005
D	14	593.5 ± 70.3	<0.0005	11.6 ± 2.5	<0.0005

* One-tailed P-value for the difference between control group and each stage using Duncan's Post Hoc Test of multiple comparisons.

cancer, especially non-small cell lung carcinoma. In conclusion, α1-AT and neopterin serum levels were elevated in all non-small cell lung and prostate, newly diagnosed, cancer patients. A striking correlation was demonstrated between α1-AT and neopterin serum levels and cancer stages in both types of cancer. Therefore, α1-AT and neopterin serum levels should be considered as additional tumor markers for the diagnosis as well as for the evaluation of the advancement and the activity of the neoplastic process in non-small cell lung and prostate cancer patients.

REFERENCES

1 Amiguet JA, Jimenez J, Monreal JI, Hernandez MJ, Lopez–Vivanco G, Vidan JR, *et al.* (1998). Serum proteolytic activities and antiproteases in human colorectal carcinoma. J. Physiol. Biochem. **54**(1): 9–13.

2 Bata J, Colobert L, Biron A, Brune J. (1977): Study of various serum proteins in lung cancer. Immunoglobulins A, G, M, haptoglobin, alpha-1 antitrypsin, alpha-2 macroglobin. Ann. Biol. Clin. (Paris) **35**(4): 297–303.

3 Carrell RW, Jeppson JO, Laurell CB, Brennan SO, Owen MC, Vaughan L, *et al.* (1982). Structure and variation of human alpha-1 antitrypsin. Nature. **298**: 329–333.

4 Chio LF, Oon CJ. (1979). Changes in serum alpha-1 antitrypsin, alpha-1 acid glycoprotein and beta-2 glycoprotein-1 in patients with malignant hepatocellular carcinoma. Cancer. **43**(2): 596–604.

5 Daddi G, Mancini PA, Parola D, Contini A. (1976). Alpha-antitrypsin increase in lung cancer. Boll lst Sieroter Milan **55** (6): 510–512.

6 Debrowska M, Mantur M, Panasiuk A, Prokopowicz J. (1997). Does the concentration of alpha-1 proteinase inhibitor reflect the transformation of liver cirrhosis to liver carcinoma? Neoplasma. **44**(5): 305–307.

7 deLorimier A, Park F, Aranha GV, Reyes C. (1993). Hepatoid carcinoma of the stomach. Cancer. **71**(2): 293–296.

8 Demidove VP, Iakubovskaia RI, Karmakova TA, Avdeev GI, Komissarov AB, Sorokin EE, *et al.* (1990). Alpha-1 proteinase inhibitor in breast cancer. Vopr. Onkol. **36**(1): 23–29.

9 Fuchs D, Weiss G, Reibnegger G, Wachter H. (1992). The role of neopterin as a monitor of Cellular Immune Activation in Transplantation, Inflammatory, Infectious, and Malignnat Diseases. Crit. Rev. Clin. Lab. Sci. **29** (3,4): 307–341.

10 Harris CC, Cohen MH, Connor R, Primack A, Saccomanno G, Talamo RC. (1976). Serum alpha-1 antitrypsin in patients with lung cancer or abnormal sputum cytology. Cancer. **38** (4): 1655–1657.

11 Krecicki T, Leluk M. (1992). Acute phase reactant proteins: an aid to monitoring surgical treatment of laryngeal carcinoma. J. Laryngol. Otol. **106**. (7): 613–615.

12 Lewenhaupt A, Ekman B, Eneroth P, Eriksson A, Nilssson B, Nordstrom L. (1986). "Serum levels of neopterin as related to the prognosis of human prostatic carcinoma". Eur. Urol. **12**(6): 422–425.

13 Ljungberg B, Grankvist K, Rasmuson T. (1995). Serum acute phase reactant and prognosis in renal cell carcinoma. Cancer. **76**(8): 1435–1439.

14 Mattison P, Cove DH, Walsh L, Howell A, McConkey B, Morrison JM. (1981). Serum proteins as tumor markers for breast cancer. Br. J. Cancer. **43**(4): 542–545.

15 Merlini G, Perfetti V, Gobbi PG, Quaglini S, Franciotta DM, Marinone G, Ascari E. (1993). Acute phase proteins and prognosis in multiple myeloma. Br. J. Haematol. **84**(4): 595–601.

16 Micksche M, Kokron O. (1977). Serum levels of alpha-1 antitrypsin and alpha-2 macroglobulin in lung cancer. Osterr Z Onkol. **3** (5–6): 116–119.

17 Mohamed KH, Mobasher AA, Yousef AR, Salah A, EL-Naggar IZ, Ghoneim AH, *et al.* (2001). BAL neopterin: a novel marker for cell-mediated immunity in patients with pulmonary tuberculosis and lung cancer. Chest. **119**(3): 776–780.

18 Murr C, Bergant A, Widschwendter M, Heim K, Schrocksnadel H, Fuchs D. (1999). Neopterin is an independent prognsotic variable in female with breast cancer. Clin. Chem. **45**(11): 1998–2004.

19 Murr C, Fuith LC, Widner B, Wirleitner B, Baier-bitterlich G, Fuchs D(1999). Increased neopterin concentrations in patients with cancer: indicator of oxidative stress. Anticancer Res. **19**(3A): 1721–1728.

20 Patenoster D, Cartei G, Campagnutta E, Maggino T, Valente S, Ongaro G. (1980). Acute reactive stage proteins in gynaecologic cancers. Eur. J. Gynaecol. Oncol. **1**(2): 94–98.

21 Pelllinieme TT, Irjala K, Mattila K, Pulkki K, Rajamaki A, Tienhaara A, *et al.* (1995). Immunoreactive interleukin-6 and acute phase proteins as prognostic factors in multiple myeloma. Blood. **85**(3): 765–771.

22 Pirisi M, Fabris C, Soardo G, Toniutto P, Vitulli D, Bartoli E. (1996). Prognostic value of serum alpha-1 antitrypsin in hepatocellular carcinoma. Eur. J. Cancer. **32A**(2): 221–225.

23 Robinaovitz M, Gavaler JS, Kelly RH, Prieto M, Van Thiel DH. (1992). Lack of increase in heterozygous alpha-1 antityrpsin deficiency phenotypes among patients with hepatocellular and bile duct carcinoma. Hepatology. **15**(3): 407–410.

24 Schena M, Quadri A, Ragazzoni G, Corti P. (1985). Alpha-1 antitrypsin as a tumor marker. Quad. Sclavo. Diagn. **21**(1): 87–96.

25 Tountas Y, Sparos L, Theodoropoulos C, Trichopoulos D. (1985). Alpah-1 antitrypsin and cancer of the pancreas. Digestion. **31**(1): 37–40.

26 Ward AM, Cooper EH, Houghton AL. (1977). Acute-phase reactant proteins in prostatic cancer. Br. J. Urol. **49**(5): 411–418.

27 Yildirim Y, Gunel N, Coskun U, Pasaoglu H, Aslan S, Cetin A. (2008). Serum neopterin levels in patients with breast cancer. Med.Oncol. **25**(4): 403–407.

28 Zietek Z, Iwan-Zietek I, Kotschy M, Tyloch F. (1996). Activity of alpha-1 antitrypsin in blood of patients with prostatic tumor. Pol. Tyg. Lek. **51**(6–9): 89–90.