Prognostic value of auto-antibodies in the serum of Omani patients with gastric cancer

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ABSTRACT

Objectives: To investigate the prevalence of a group of different autoantibodies, in Omani patients with gastric cancer, and to examine whether their presence correlates with clinical course of the disease.

Methods: Ninety-three Omani patients with gastric cancer, and 100 gender-matched blood donors were investigated for the presence of 15 different auto-antibodies against nuclear antigens (ANA), extractible nuclear antigens (ENA), Scleroderma antigen (Scl-70), Sjogren syndrome antigen A/B (SSA/B), Smith antigen (Sm), ribonucleoprotein (RNP), Jo-1 antigen, double stranded DNA (ds-DNA), parietal cell antibodies (APCA), reticulin antibodies (ARA), smooth muscle antibodies (ASMA), proteinase 3 (PR3), myeloperoxidase (MPO), and mitochondria antibody (AMA). Antinuclear antigen were detected using human epithelial cells-2 (Hep-2 cells). Anti-dsDNA antibodies were measured using Crithidia lucilia slides; APCA, ARA, and ASMA were examined using mouse liver, kidney, and stomach sections. Other autoantibodies were detected using commercially available ELISA kits. Seventy-three out of the 93 patients with gastric cancer were divided into 4 groups (stages I to IV) according to disease severity. This study was conducted in the period of 2001-2005 in the Department of Microbiology and Immunology Laboratories of the College of Medicine and Health Sciences, Sultan Qaboos University, Muscat, Oman.

Results: Approximately 40% of the autoantibodies investigated were found to be significantly higher in patients with gastric cancer than in normal controls. These autoantibodies are ANA (57.3 versus 14%, $p<0.0001$), anti-ENA (38.7 versus 13.9%, $p<0.01$), anti-Scl-70 (29 versus 5%, $p<0.001$), ARA (19.8 versus 3.1%, $p<0.0001$), ASMA (72.9 versus 31.6%, $p<0.01$), and anti-PR3 (21.5 versus 5.3% $p<0.01$). Generally, the presence of auto-antibodies was more frequent in stage III and IV compared to stage I and II. However, some autoantibodies (ENA, SSA, Scl-70, and ASMA) were more common in stage II than stage IV.

Conclusion: Auto-antibodies are more prevalent in the serum of patients with gastric cancer compared to healthy controls. Some of these auto-antibodies may prove to be important markers of prognostic values in patients with gastric cancer.

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Association between certain cancers and the presence of auto-antibodies has been previously reported especially during the last 2 decades.[1] Various abnormalities of the humoral immune response are common in patients with malignant diseases.[2] This may be associated to the disarray of the immune system caused by the malignant diseases or may be an attempt by the immune system to react against the tumor cells.[3] In addition, cell-mediated immunity is impaired by the dysfunction of the immune response, resulting in the uncontrolled growth of the malignant cells.[3] In one hand, immunofluorescence technique

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revealed elevated titers of antinuclear auto-antibodies (ANA) among cancer patients.[4] On the other hand, some studies reported that no increased incidence of ANA in malignant conditions.[5] Similarly, contradictory results were reported with anti-parietal cells antibodies (APCA).[6] Moreover, high levels of antibodies to single stranded DNA (ssDNA), ribonucleoproteins (RNP), and Smith (Sm) antigens were reported in sera of patients with lymphoma.[2] In breast cancer, both anti-centromere protein (anti-CENP) (33% versus 8%) and anti SSB (44% versus 24%) auto-antibodies had higher positivity compared to controls.[7] Furthermore, elevated levels of auto-antibodies against ssDNA, ds-DNA, cardiolipin, actin, myosin, tropomyosin and ganglioside antibodies were detected in serum of patients with gastric cancer, but found not to be statistically significant.[8] Several studies have shown that patients with autoimmune conditions such as Sjogren syndrome and rheumatoid arthritis develop neoplastic diseases especially lymphoma, more frequently than the general population.[1] This may indicate an important role of such auto-antibodies in the development and progression of cancer.[1] The aim of the present study was to investigate the prevalence of a panel of 15 different auto-antibodies in the sera of patients with gastric cancer and to examine whether their presence correlates with clinical course of the disease.

Methods. Serum samples were obtained from 93 consented patients with gastric cancer (49 males and 44 females), attending the Oncology Clinic, Sultan Qaboos University Hospital (SQUH), Muscat, Oman, during the period of 2001-2005. The patients were aged between 22-85 years, average of 55 years. Seventy-three patients were grouped into 4 different stages according to the severity of the disease (during the data analysis the clinical staging was available for 73 patients; therefore correlation was made with these patients). A total of 100 healthy blood donors (50 males and 50 females), aged between 20-54 years, average of 34 years, served as the study control group. None of the patients or blood donors had a previous history of autoimmune disease, or any disease that could be associated with presence of auto-antibodies. This has been verified by a questionnaire and during their clinical investigations. All participants submitted their written informed consent before being enrolled in the study. Clotted blood samples were obtained from participants and the separated sera were stored at -20°C and tested for auto-antibodies within 6-12 months. Antinuclear antigen were detected using human epithelial cells-2 (Hep-2 cells). Crithidia lucilia slides were used to identify anti-ds-DNA. For the detection of antibodies to parietal cells (APCA), reticulin antibodies (ARA), smooth muscle antibodies (ASMA), we used mouse liver-kidney-stomach sections as the substrate. All slides were purchased from Binding Site, UK. Tests were performed according to manufacture instructions. Briefly, sera were screened at 1/40 dilution except for anti-dsDNA, at a screening dilution of 1/10. A volume of 30 μl of diluted sera was added onto appropriate slide and incubated for 30 minutes at room temperature. Slides were washed twice in phosphate buffer saline (PBS), and fluorescence isothiocyanate (FITC)-conjugated polyvalent goat anti-human IgG was added and incubated for further 30 minutes at room temperature. After that, slides were washed again in PBS, and examined under fluorescence microscope (OLYMPUS, USA). Commercially available ELISA kits for the detection of antibodies to extractible nuclear antigens (ENA), SS-A, SS-B, Sm, Scleroderma antigen (Scl-70), RNP, Jo-1, and mitochondria antigen (M2), were purchased from INOVA diagnostic, USA. Enzyme-linked immunosorbent assay kits for the measurement of antibodies to proteinase-3 (PR3), and myeloperoxidase (MPO), were obtained from Binding Site, UK. Antibodies were tested following the manufacturer instructions. Briefly, diluted sera (1:100) were added to ELISA plate coated with the specific antigen, and incubated for 30 minutes at room temperature. Plates were washed 3 times, and purified peroxidase labeled antibody to human IgG or IgM was added, and incubated for further 30 minutes at room temperature. All plates were washed again and the substrate (TMB) was added. After 30 minutes, the reaction was stopped with 0.3M sulfuric acid or 3M phosphoric acid. The optical density (OD) was read at 450 nm on a microplate reader (Labsystem, Multiskan MS, Finland). The data were analyzed using EPI Info Statistical Program, and the percentage of the high titer sera samples was compared to the normal controls by the Chi-Square Test. Fisher exact results were used for values less than 5. The probability values <0.05 were considered significant.

Results. Gastric cancer patients showed significantly higher percentage of some positive auto-antibodies than the controls. Antinuclear antigens were present in 55 of the 93 gastric cancer patients (59.1%) compared to 14 of the 100 normal controls (14%), $p<0.0001$ (**Table 1**). Moreover, in gastric cancer patients, the ANA titer range was between 1:40 to ≥1:640, whereas in normal controls the titer does not exceed 1:80. The most common Hep-2 pattern was the speckled, which was dominant in both gastric cancer and in normal controls (approximately 65% of total positive ANA). Several other patterns

were observed in gastric cancer patients and not in normal controls including: cytoplasmic reaction such as ribosomal pattern (2 patients), other cytoplasmic reaction (4 patients), centriole (2 patients), multiple nuclear dots (one patient), and a strong unusual pattern with multiple large cytoplasmic dots (one patient). Similarly, anti-ENA was elevated, in patients with gastric cancer (38.7%) compared to normal controls (13.9%), $p<0.001$. Scleroderma antigen-70 was significantly ($p<0.001$) dominant in gastric cancer patients (29%) compared to normal controls (5%), **Table 1**. Significant statistical differences were observed between gastric cancer patients and normal controls with ARA (20.4% versus 2%, $p<0.0001$), ASMA (75.3% versus 24%, $p<0.01$), and anti-PR3 (21.5% versus 5.3%, $p<0.01$) (**Table 1**). Furthermore, in gastric cancer patients the titer range for ARA was between 1:40 to 1:280, and for normal controls was between 1:40 to 1:160. Similar pattern was observed with ASMA; gastric cancer patients had higher titer range (1:40 to 1:1280) of ASMA than normal controls (1:40 to 1:160). In addition, some auto-antibodies (SS-A, Sm, RNP, APCA, and MPO), were slightly more frequent in gastric cancer patients compared to normal controls, but statistically insignificant. Anti-ds-DNA and anti-mitochondrial antibodies were not detected in gastric cancer patients or in normal controls (**Table 1**).

Table 2 shows the frequency of auto-antibodies in different stages of the gastric cancer. Two patients were in stage I, 14 in stage II, 25 in stage III, and 32 in stage IV. The total number of positive auto-antibodies in stage III (49%) and IV (34%) was significantly ($P<0.05$) higher than stage I (3.2%) and stage II (13.5%). However, the percentages of some auto-antibodies (ENA, Sjogren syndrome antigen [SSA], Scl-70, and ASMA) were slightly higher in stage II than stage IV; the variation in the number of patients in each group and number of positive cases, such elevation is not statistically significant.

Discussion. The presence of auto-antibodies in the serum of normal people and in patients with cancer has been the field of intensive study for the last decade. Auto-antibodies against ssDNA, and extractible nuclear antigens[2] cardiolipin,[2,5] anti-parietal cell antibodies,[6] and thyroid antibodies,[9] have been reported to increase in many types of cancer. In this present study, we have statistically shown higher occurrence of 6 auto-antibodies (ANA, ENA, Scl-70, ARA, ASMA, and PR3) out of 15 auto-antibodies studied in patients with gastric cancer compared to the healthy controls. These auto-antibodies when present may indicate a worse prognosis, and may be used as prognostic markers for patients with gastric cancer. A high prevalence of ANA has been reported in patients with malignant disease,[4] in spite of some conflicting results,[5] the clinical and biological significance of these auto-antibodies has not been well characterized. It is still not clear to what extent the contribution of

Table 1 - Frequency of different auto-antibodies in Omani patients with gastric cancer.

Auto-antibody	Number of positive/total number (%)				*P*-value
	Normal controls		Patients with gastric cancer		
Antinuclear auto-antibodies	14/100	(14.0)	55/93	(59.1)	<0.0001
Anti-reticulin antibodies (R1)	2/100	(2.0)	19/93	(20.4)	<0.0001
Extractible nuclear antigens	13/93	(13.9)	36/93	(38.7)	<0.001
Scleroderma antigen-70	4/80	(5.0)	27/93	(29.0)	<0.001
Sjogren syndrome antigen-A	8/93	(8.6)	12/93	(12.9)	NS
Sjogren syndrome antigen-B	1/93	(1.1)	1/93	(1.1)	NS
Smith antigen	1/93	(1.1)	2/93	(2.15)	NS
Ribonucleoprotein	2/93	(2.2)	3/93	(3.2)	NS
Jo-1 antigen	1/93	(1.1)	1/93	(1.1)	NS
Anti-smooth muscle antibodies	24/100	(24.0)	70/93	(75.3)	<0.01
Proteinase 3	5/93	(5.3)	20/93	(21.5)	<0.01
Anti-parietal cell antibodies	1/100	(1.0)	3/93	(3.2)	NS
Myeloperoxidase	0/93	(0.0)	1/93	(1.2)	NS
Double standard-DNA	0/93	(0.0)	0/93	(0.0)	-
Anti-mitochondria antibody	0/93	(0.0)	0/93	(0.0)	-

NS - Not significant, negative sign - Not detected

these auto-antibodies to cellular transformation might be.[4,10] We have demonstrated a significant prevalence of ANA among gastric cancer patients compared to healthy controls. Low ANA titers (<1:80) were most often detected in both, healthy individuals and gastric cancer patients, but titers from 1:160 to ≥1:640 were detected in 16 patients with gastric cancer (29%) but not in the healthy control group. Our results are in agreement with previous studies showing positive ANA occurring in up to 28% of patients with various types of malignancies.[4,10,11] Extractible nuclear antigens are soluble nuclear and cytoplasmic components that are targets for auto-antibodies. To date, over 100 different antigens have been described of which only 6 (SSA, SSB, Sm RNP, Scl-70, and Jo-1) are sufficiently important to be tested on routine clinical basis. Auto-antibodies to these antigens are usually associated with autoimmune diseases.[12-14] Elevated levels of auto-antibodies against ENA were reported in untreated lymphoma patients.[15] Our observations were in a partial agreement with the study of Gergely et al.[15] Among the 6 different ENA tested, only Scl-70 (Topoisomerase-1) was significantly (p<0.001) elevated in patients with gastric cancer (29%) compared to normal controls (5%). Topoisomerase-1 is a DNA binding protein that regulates DNA topology. This enzyme is the target of anti-neoplastic agents such as camptothecins.[16] Antibodies to Scl-70 are specific markers for scleroderma patients,[17] and elevation of these auto-antibodies in gastric cancer has not been reported before. To our knowledge this is the first report demonstrating high level of anti-Scl-70 antibodies in gastric patients' sera. We do not know the significance of this observation at present, but it deserves more future research. Anti-reticulin antibodies and ASMA were not well characterized in gastric cancer patients and we have shown in this study a high occurrence of these auto-antibodies in such patients. Type-1 ARA is strongly associated with celiac disease,[18] and also can be found in approximately 5% of healthy people.[19] An increased incidence of ARA was observed in carcinomas of the urinary bladder, prostate and kidney, when compared to healthy controls.[20] No previous study to our knowledge has demonstrated an association of ARA or ASMA with gastric cancer. The significance of the correlation of ARA and ASMA with gastric cancer requires further investigation.

Moreover, we have demonstrated that the occurrence of the auto-antibodies studied in gastric cancer patients at late stages (III and IV) were at higher levels compared to patients in early stages (I and II). This could be related to the disruption of the immune system. Our observation was in contrast with previous studies,[4,21,22,23] which showed that occurrence of auto-antibodies (ssDNA, actin, ganglioside, and APCA) in patients with early stages of their diseases (I and II) was higher compared to patients at late stages (III and IV). Moreover, Reitzig et al[23] demonstrated that APCA were more often found in patients with early than with advanced gastric cancers and attributed this to loss of antigens or binding of antibodies in immune complexes. In our study, low prevalence of APCA was found in both gastric cancer patients and normal controls. Furthermore, a slight increase in some auto-antibodies, such as ASMA and SSA, was observed in early stages (stage II) than late stages (stage IV), but statistically insignificant. Discrepancy between our observations and previous findings may be related to the difference in the types of the auto-antibodies examined in those studies. Further studies are required to characterize the role

Table 2 - Prevalence of auto-antibodies in patients at different stages of gastric cancer.

Stage of the gastric cancer	No. of patients (%)	Number of positive auto-antibodies (%)							Total of positive auto-antibody
		ANA	ENA	Scl-70	SSA	ARA	ASMA*	PR3	
Stage I	2 (1.1)	1 (50)	0	0	0	1 (50)	1 (50)	1 (50)	4 (3.2)
Stage II	14 (8.0)	6 (42.8)	4 (28.5)	3 (21.4)	2 (14.2)	0	2 (14)	0	17 (13.5)
Stage III	25 (14.4)	20 (80)	11 (44)	9 (36)	5 (20)	6 (24)	3 (12)	8 (32)	62 (49)
Stage IV	32 (18.4)	16 (50)	7 (21.8)	6 (18.7)	1 (3.1)	6 (18.7)	1 (3)	6 (18)	43 (34)

*ASMA has a titer of ≥1:160, ANA - Antinuclear auto-antibodies, ENA - Extractible nuclear antigens
Scl-70 - Scleroderma antigen-70, SSA - Sjogren syndrome antigen-A, ARA - Anti-reticulin antibodies, ASMA - Anti-smooth muscle antibodies
PR3 - Proteinase 3

of auto-antibodies in malignancy and to assess their diagnostic and/or prognostic values.

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