Trop J Nat Prod Res, January 2021; 5(1):62-65 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Levels of Antinuclear and Anti-double Stranded Antibodies in Sera of Breast Cancer Patients

Sadeq K. Hachim[1]*, Aesha S. Ali[2], Mohammed R. Ali[3]

[1]Department of Nursing Technology, Middle Technical University, Institute of Medical Technology,Baghdad, Iraq
[2]Department of Microbiology, College of Medicine, Ninevah University, Ninevah, Iraq
[3]Department of Microbiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq

ARTICLE INFO

Article history:
Received 22 September 2020
Revised 21 December 2020
Accepted 23 January 2021
Published online 03 February 2021

ABSTRACT

Antinuclear antibodies (ANAs) and anti-double stranded deoxyribonucleic acid antibodies (ADSADs) are autoantibodies that have been linked to biomarkers of systemic autoimmune diseases. Although their involvement in relation to breast cancer is yet to be examined. Therefore, this study evaluated the serum concentration of ANAs and ADSDAs in breast cancer patients, with a view to finding diagnostic biomarkers. One hundred female patients with breast cancer which were recently diagnosed depending on histopathology and hormonal receptor criteria by an oncologist, and another twenty-five females as healthy control group were recruited for the study. Direct interview of the female patients and healthy (control) group was conducted in addition to the information from patients' registration records in the hospital. An aliquot of 5 mL of intravenous blood was collected from each subject to recover serum samples after centrifugation. An enzyme-linked immunosorbent assay (ELISA) was used to measure the concentrations of ANAs and ADSADs in the sera. The mean age of breast cancer patients was 55.60 years, while that of the healthy control group was 56.76 years. There was a highly significant ($P < 0.05$) association between ANA of breast cancer patients and grade, as well as stage (MCP < 0.01). On the contrary, the concentration of ADSADs was showing no significant association with the grade (MCP > 0.05) and stage (MCP > 0.05) of breast cancer patients. Conclusively, our findings suggest the use of ANA as a diagnostic biomarker for breast cancer patients.

***Keyword**:* Autoantibodies, ADSAD, ANA, Biomarker, Breast cancer.

Introduction

Autoantibodies are self-reactive antibodies, which are produced by the immune system that target one or more of an individual's own proteins. They are found in cell organelles such as mitochondria, chromatin, centromeres and are responsible for many autoimmune diseases. Autoimmune disorders are either widespread or highly specific for a particular cell type in a unique organ of the body (e.g. thyroglobulin in cells of the thyroid gland) and these autoimmune diseases are tissue specific. Conversely, auto antigen can be found in proteins, nucleic acids, carbohydrates and lipids.[1] Antinuclear antibodies (ANAs) are autoantibodies that form the immune biomarkers of systemic autoimmune diseases.[2] These biomarker autoantibodies may be present in certain situations in a way that the diagnosis of such disorder is ambiguous and this may indicate diagnostic task as soon as the test is persistently positive.[2] For example, autoimmune disorders such as systemic lupus erythematosus (SLE) or rheumatoid arthritis (RA) may possibly indicate positivity for autoantibodies when diagnosed some years earlier than their actual occurence.[3] In this situation, a positive ANAs will reflect pre-autoimmunity condition. Though not well thought of to be autoimmune, it is more likely be an indication of such specific disease. They are capable of providing adequate antigenic activation for its emergence. Positive ANA may be the cause of neoplastic diseases. A number of authors have reported that ANA is regularly identified in the sera of patients with lung, breast, head and neck cancers such as in SLE and RA.[4] As a reaction to the presence of DNA fragment in the blood, anti-double stranded deoxyribonucleic acid antibodies (ADSADs) are produced. As ANA is associated with the detection of SLE, so also ADSDA is used in the diagnosis of autoimmune diseases.[5] ADSDA plays a dual role, in that it hydrolyzes extracellular DNA, in addition to having a cytotoxic activity on tumor cells.[6] Also, it has been reported to be present in serum samples of patients with SLE, rheumatologic illness, infections and malignancy.[7]

Breast cancer is often considered as one of the most common tumors at this time, and as a universal health problem.[8] The risk of occurrence of this tumor rises with individual's life span. Regardless of its prevalence, death rate has decreased significantly over the past 10 years.[9] This is as a result of a combination of early detection and enhanced treatment options. Therefore, the aim of the current research was to determine the levels of autoantibodies (ANAs and ADSDAs) in sera of breast cancer patients, with a view to finding potential diagnostic biomarkers. The presence of ANA in breast cancer patients without any evidence of autoimmune disease may attract a consideration of the likelihood that this disease may be diagnosed early.[10] Although, their involvement in relation to breast cancer is yet to be examined, it was hypothesized that the ANAs and ADSDAs in the sera of newly diagnosed Iraqi breast cancer patients were significantly elevated.

Materials and Methods

Study population and design

From 5th December, 2018 to 30th November, 2019, one hundred (100) newly diagnosed breast cancer patients attending the National Center

*Corresponding author. E mail: sadeqkadhim08@gmail.com, Sadeq.kadhim2016@yahoo.com
Tel: 00964 771 108 3673

Citation: Hachim SK, Ali AS, Ali MR. Levels of Antinuclear and Anti-double Stranded Antibodies in Sera of Breast Cancer Patients. Trop J Nat Prod Res. 2021; 5(1):62-65. doi.org/10.26538/tjnpr/v5i1.6

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

for Cancer, Baghdad City, Iraq were recruited for the study. The female patients with breast cancer were classified according to the World Health Organization 2012 classification which depends on histopathology and hormonal receptor. Twenty-five women were included as healthy control groups. The inclusion criteria included the females patients with recently diagnosed breast cancer (not more than three months), not suffering from other types of cancer, and must be free from any sign of metastasis. The study design was descriptive, case–control and hospital-based according to questionnaire chart admistered by direct interview.

Ethical clearance and consent to participate

The research was carried out after the Iraqi health consultants had received an ethical authorization. An informed consent was obtained from each of the participating subjects of the study.

Sample collection

Five milliliter (5 mL) of whole blood sample was collected in gel tube from each subject included in the study. The blood samples were separated by centrifugation and the serum samples were decanted into another plain tube and then stored at -20^{o}C.

Enzyme-linked immunosorbent assay

The enzyme-linked immunosorbent assay (ELISA) kit (DRG Co, USA) was used to estimate the concentration of ANAs and ADSDAs in the serum samples by indirect ELISA method following the manufacturer's instructions.

Statistical analysis

The statistical analysis of this case control study was performed with the statistical package for social sciences (SPSS) 21.0 and Microsoft excel 2013. Categorial data was formulated as percentage count.

Results and Discussion

In this study, all participants were females with recently diagnosed breast cancer. The mean age of breast cancer patients was 55.62 years and the control group was 56.76 years (Table 1). Age range of breast cancer patients was from 33 to 72 years, while the healthy control group ranged from 38 to 72 years. Breast cancer patients were classified into Grades I, II and III comprising 39, 32 and 29 individuals, respectively (Table 2). For the stages of breast cancer patients, 26, 22, 30 and 22 individuals were grouped into Stages I, II, III and IV, respectively as shown in Table 3.

The results obtained for the association between ANA of breast cancer patient group and grades are shown in Table 4. There was a large grade correlation with the ANA of breast cancer patients, as there was high proportion of positive cases, relative to the negative cases. The overall number of positive cases was 76%, while the negative cases were 24% of the one hundred breast cancer patients recruited for the study. Therefore, a correlation of ANA with grades of breast cancer showed a high significant association (MCP < 0.01).

Similarly, the results of the association between ANA of the breast cancer group and stages (Table 5), indicated that there was high percentage of positive cases (76%), when compared with negative cases (24%), out of the one hundred breast cancer patients examined.

Association between ADSDA of breast cancer group and grades was investigated and the results obtained are presented in Table 6. The correlation between ADSDA concentration and grades of breast cancer patients was identified to be a non-significant association ($P \geq 0.05$). When compared to negative cases (61%), the overall positive cases (7%) of ADSDA were very low in breast cancer patients. Also, the correlation of ADSDA concentration with stages of breast cancer group indicated a non-significant association ($P \geq 0.05$) as showed in (table 7). Positive cases of ADSDA was very low (7%), compared to the negative cases (61%), while the determinant was 32%, as observed in this study.

The results obtained in this research showed significant increase in the concentration of ANAs in serum samples of breast cancer patients. There was a high positive percentage in the ANA of breast cancer patients, compared to the negative cases. Conversely, the amount of ADSDAs in serum samples showed no substantial association with breast cancer, as low positive percentage was obtained. It is possible to relate the high percentage of ANAs in patients' sera to cancerous cell death, which can be due to exposure to nuclear material from immune system cells. This observation is in accordance with the finding of Mohammed and Najim where they found out that there were 9 patients with positive ANA in 20 breast cancer patients examined (i.e. 45%).[11]

Table 1: Descriptive statistics of age of studied groups

Parameters	Age of study groups (Year)	
	Breast cancer	Control
N	100	25
Mean	55.62	56.76
Std. Deviation	8.65	8.22
Minimum	33.00	38.00
Maximum	72.00	70.00

Table 2: Distribution of breast cancer group according to grade

Grade of breast cancer	No.	%
I	39	39
II	32	32
III	29	29
Total	100	100

Table 3: Distribution of breast cancer group according to stage

Stage of breast cancer	No.	%
I	26	26
II	22	22
III	30	30
IV	22	22
Total	100	100

Table 4: Association between antinuclear antibodies of breast cancer group and grade

Grade		ANA of breast cancer group		Total
		Negative	Positive	
I	No.	12	27	39
	%	12	27	39
II	No.	6	26	32
	%	6	26	32
III	No.	6	23	29
	%	6	23	29
Total	No.	24	76	100
	%	24	76	100

ANA: Antinuclear antibody; X2 = 1.638; $P \leq 0.05$ (S)

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 5: Association between antinuclear antibodies of breast cancer group and stage

Stage		ANA of breast cancer group		Total
		Negative	Positive	
I	No.	21	5	26
	%	21	5	26
II	No.	0	22	22
	%	0	22	22
III	No.	1	29	30
	%	1	29	30
IV	No.	2	20	22
	%	2	20	22
Total	No.	24	76	100
	%	24	76	100

ANA: Antinuclear antibody; MCP < 0.01 (HS)

Table 6: Association between anti dsDNA antibodies of breast cancer group and grade

Grade		ADSDA of breast cancer group			Total
		Negative	Indeterminate	Positive	
I	No.	25	12	2	39
	%	25	12	2	39
II	No.	25	5	2	32
	%	25	5	2	32
III	No.	11	15	3	29
	%	11	15	3	29
Total	No.	61	32	7	100
	%	61	32	7	100

ADSDA: Anti dsDNA antibody; MCP > 0.05 (NS)

Table 7: Association between anti dsDNA of breast cancer group and stage

Stage		Anti dsDNA of breast cancer group			Total
		Negative	Indeterminate	Positive	
I	No.	16	9	1	26
	%	16	9	1	26
II	No.	15	6	1	22
	%	15	6	1	22
III	No.	20	9	1	30
	%	20	9	1	30
IV	No.	10	8	4	22
	%	10	8	4	22
Tota l	No.	61	32	7	100
	%	61	32	7	100

ADSDA: Anti dsDNA antibody; MCP > 0.05 (NS)

In a similar research, Madrid and colleagues also showed an association of autoantibodies with breast cancer in their study, it was suggested that a significant promise existed for developing panels of breast cancer-specific and premalignant-phase autoantibodies.[12] More so, autoantibody establishment in the pre-malignant process and prospective studies using approved autoantibody panels could allow identification of the risk of breast cancer in asymptomatic women.[12]
In our study, a high percentage of positive ANA (76%) was recorded for patients with breast cancer and this observation is comparable to the study of Wasserman *et al*, who found out that ANA had 35% positivity in patients with breast cancer.[13] Our finding suggests the potential use of ANA as a biomarker for precancer status detection or a follow-up procedure for pathogenesis of breast cancer with respect to stages and grades. Besides, our results indicated that there was no substantial association between ADSDA concentration and stages, as well as grades of breast cancer. There were low total positive cases of ADSDA for both stages and grades (each with 7%) of breast cancer in relation to total negative cases for stages and grades (each with 61%). This finding is consistent with the study of Berg *et al*., where they discovered that there was a low concentration of ADSDAs in sera of breast cancer cases.[14] Breast cancer patients with high level of ADSDA has a good chance of recovery due to the anti-tumor activity of autoantibodies, which when present in high titer level results in limited growth of cancer cells.[15]

Conclusion

The findings from this study indicated that both ANA and ADSDA can serve as potential biomarkers for breast cancer, in relation to stage and grade classifications. However, ANA showed more association with grades and stages of breast cancer, hence it may be a preferred indicator for breast cancer progression.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby state that the work offered in this article is original and that any legal responsibility for claims relating to the content of this article will be borne by them.

Acknowledgements

The research was supported by the Ministry of Health and Middle Technical University, Baghdad, Iraq.

References

1. Haugbro K, Nossent JC, Winkler T, Figenschau Y, Rekvig OP. Anti‐dsDNA antibodies and disease classification in antinuclear antibody positive patients: The role of analytical diversity. Ann Rheum Dis. 2004; 63:386-394.
2. Pisetsky DS. Antinuclear antibody testing–misunderstood or misbegotten? Nat Rev Rheumatol. 2017; 13:495-502.
3. Robertson JM and James JA. Preclinical systemic lupus erythematosus. Rheum Dis Clin North Am. 2014; 40:621-635.
4. Madrid FF, VandeVord PJ, Yang X, Robert LK, Pippa MS, Michael JK, Jose LG, John ET. Antinuclear antibodies as potential markers of lung cancer. Clin Cancer Res. 1999; 5(1):393-400.
5. Madrid FF, Karvonen RL, Ensley J, Michael J, Michael K, Jose LG, Huda A, Naimei T, John ET. Spectra of antinuclear antibodies in patients with squamous cell carcinoma of the lung and of the head and neck. Cancer

Detect Prev. 2005; 29:59-65.
6. Imran A, Neelam F, Tariq M. Incidence of circulating antinuclear antibodies in cancer patients. Indian J Med Sci. 2003; 57:113-116.
7. Pavlovic M, Kats A, Cavallo M, Chen R, Hartmann JX, Shoenfeld Y. Pathogenic and epiphenomenal anti‐DNA antibodies in SLE. Autoimmune Dis. 2010; 2011:462841.
8. Attar SM and Koshak EA. Medical Conditions associated with a positive anti-double-stranded deoxyribonucleic acid. Saudi Ned J. 2010; 31:781-787.
9. Akram M, Iqbal M, Daniyal M, Khan AU. Awareness and current knowledge of breast cancer. Biol Res. 2017; 50:33.
10. Griffiths C and Brock A. Twentieth century mortality trends in England and Wales. Office for National Statistics. Health Stat Q. 2003; 18:5-17.
11. Mohammad DO and Najim RH. Relation of anti‐nuclear antibodies with 11 different types of cancer. Tikrit J Pharma Sci. 2011; 7:51-54.
12. Madrid FF, Maroun M, Olivero OA, Long M, Stark A, Grossman L, Bander W, Dong J, Burke M, Nanthanson SD, Zarbo R, Chitale D, Chavez RZ, Peebles C. Autoantibodies in breast cancer sera are not epiphenomena and may participate in carcinogenesis. BMC Cancer. 2015; 15:407.
13. Wasserman J, Glas U, Blomgren H. Autoantibodies in patients with carcinoma of the breast. Correlation with prognosis. Clin Exp Immunol. 1975; 19:417-422.
14. Berg WA, Campassi C, Langenberg P, Sexton MJ. Breast imaging reporting and data system. inter-and intra-observer variability in feature analysis and final assessment. Am J Roentgenol. 2000; 174:1769-1777.
15. Qinghua C, Wei X, Zhenke W, Lin X, Kang L, Yiwei C, Sidong Xiong. Anti-double stranded DNA monoclonal antibody induced by tumor cell-derived DNA inhibits growth of tumor *in vitro* and *in vivo* via triggering apoptosis. DNA Cell Biol. 2008; 27:91-100.