International Journal of Medicine and Pharmaceutical Science (IJMPS)
ISSN(P): 2250-0049; ISSN(E): 2321-0095
Vol. 6, Issue 1, Feb 2016, 31- 36
© TJPRC Pvt. Ltd.

TRANS STELLAR
•Journal Publications •Research Consultancy

Original Article

HOW USEFUL IS URINE PROTEIN IN THE DIAGNOSIS AND PROGNOSIS OF BREAST CANCER IN WOMEN?

NADHAM K. MAHDI[1], HIBA QASSEM ALI[2] & MOHAMMED H. AL-JAWHER[3]

[1, 2]*Department of Microbiology, College of Medicine, University of Basrah, Basrah, Iraq*

[3]*Department of Surgery, College of Medicine, University of Basrah, Basrah, Iraq*

ABSTRACT

Objective: Is to assess the possible use of urine protein as a source of antigen in a serological examination in breast cancer diagnosis.

Method: This study enrolled 30 females, aged 27-70 years from Basrah Hospitals. Those women were diagnosed to have breast cancer by fine needle aspiration biopsy. Also 20 apparently healthy women from out-patients department were involved in this study as a control group. Urine samples were collected from patients and control groups and stored at -20^{o}C. Blood samples were also collected from patients as well as control group, centrifuged and then serum stored at -20^{0}C.

Isolation and purification of antigen from breast cancer patients urine were done. Gel filtration chromatography was carried out after dialysis. Paper electrophoresis was performed by using cellulose acetate paper. Enzyme immunoassay for detection of antigen by serum antibody.

Results: Urine protein was isolated by ammonium sulfate precipitation and purified by gel cghromatography, then used as a coating antigen for detection of specific antibody in the serum of breast cancer patients by ELISA test. The readings of the optical density for breast cancer patients (0.82$\pm$0.24 nm) was significantly higher than the control group (0.19$\pm$0.09 nm), thus it gaves a positive results for breast cancer patients and negative for the control group.

Conclusion: ELISA test by using urine protein coating antigen provide a hope for the development of non-invasive breast cancer screening and or diagnostic laboratory test.

KEYWORDS: Breast Cancer, ELISA, Protein, Women, Urine

Received: Nov 05, 2015; **Accepted:** Dec 23, 2015; **Published:** Jan 20, 2016; **Paper Id.:** IJMPSFEB20164

INTRODUCTION

Breast cancer is the third most frequent cancer in the world [(1).] It is the first cancer leading death among females in Basrah [(2).] Early detection of breast cancer by screening or early diagnosis of the disease has been linked to decrease in morbidity and mortality of the illness [(3).] The available screening, diagnostic and prognostic procedures are either invasive (tissue biopsy) [(4),] had a low efficacy (clinical breast examination) [(5)] or expensive (mammography) [(6)].

Many serum protein have been used as tumour markers in diagnosis and prognosis of breast cancer [(7,8).] The existing serum markers are of limited value in either screening or diagnosing of early breast cancer, but might be useful in prognosis and monitoring therapy of breast cancer [(7,8).] Therefore, the aim of this study is to assess the possible use of urine protein as a source of antigen in serological test in breast cancer diagnosis.

PATIENTS AND METHOD

This study enrolled 30 females, aged 27-70 years from Basrah Hospitals. Those women were diagnosed to have breast cancer by fine needle aspiration biopsy. Also 20 apparently healthy women from out-patients department were involved in this study as a control group. Urine samples were collected from patients and control groups and stored at -20^{o}C. Blood samples were also collected from patients as well as control group, centrifuged and then serum stored at -20^{0}C. The work has been approved by the ethical committee of the College of Medicine, University of Basrah, Basrah, Iraq.

Isolation and purification of antigen from breast cancer patients urine were done [9]. Gel filtration chromatography [9] was carried out after dialysis [10]. Paper electrophoresis [11] was performed by using cellulose acetate paper (Fischer Science, USA). Enzyme immunoassay for detection of antigen by serum antibody [12, 13]

RESULTS

Gel chromatography: The results of spectrophotometer readings of the purified protein from the urine of the patients group and the healthy control group were shown in Figure 1 that the crude protein consist of a number of protein components for both patients and control group samAples. At the same time, most of the fractions of patients samples showed high optical density with a peak of 0.7, while such high readings not found in samples of the healthy control group (Figure 1).

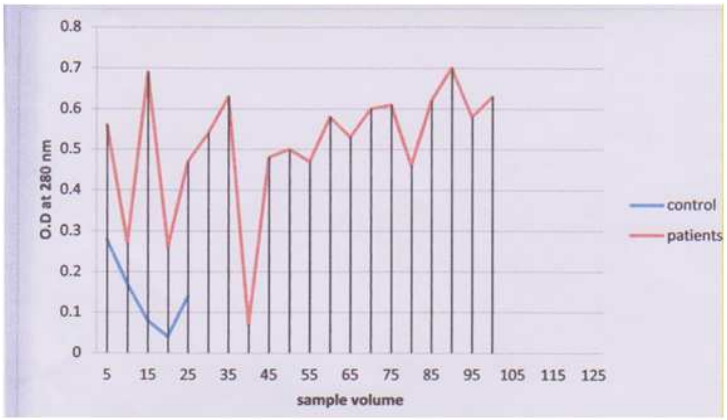

Figure 1: Spectrophotometer Readings of the Purified Protein from Patients and Healthy Control Urine

Paper electrophoresis: It showed a band at the same level of the band from the control protein papain (mw 23500 D), and another band appear at the level of control protein bovine serum albumin (mw 67000 D). No band appear at the level of the control protein ova albumin. Control samples express only one band at the level of control protein bovine serum albumin (Table 1).

Table 1: Results of Paper Electrophoresis of Purified Protein from Patients and Control

Control Bands	Patients Test Urine	Women Control Urine
Papain (mw 23500 D)	+	-
Ova albumin (mw 35000 D)	-	-
Bovine serum albumin (mw 67000 D)	+	+

Purified protein ELISA test: The results of ELISA test on the purified urine protein coated ELISA plate showed high level of readings from patient's samples with maximum reading of 1.57 nm, while the readings for the control group

samples was generally low with maximum reading 0.37 nm. The mean of reading for the patients (0.8283±0.2442) was significantly higher than that for the controls (0.19±0.09) (Table 2).

Table 2: Results of ELISA Test Readings for Patients in Comparison to Control Group

Optical Density	Patients N=30	Control N=20	P Value
Mean	0.8283	0.19	< 0.05
S.D	0.2442	0.09	

The cut off value of the test determined by the application of the formula: (X+2xS.D) on the readings of the samples of the healthy control group.

Cut off value = (X+2xS.D) = 0.378. So the resultant readings from breast cancer patients samples in this test would be all above the cut off value (0.378) (Figure 2).

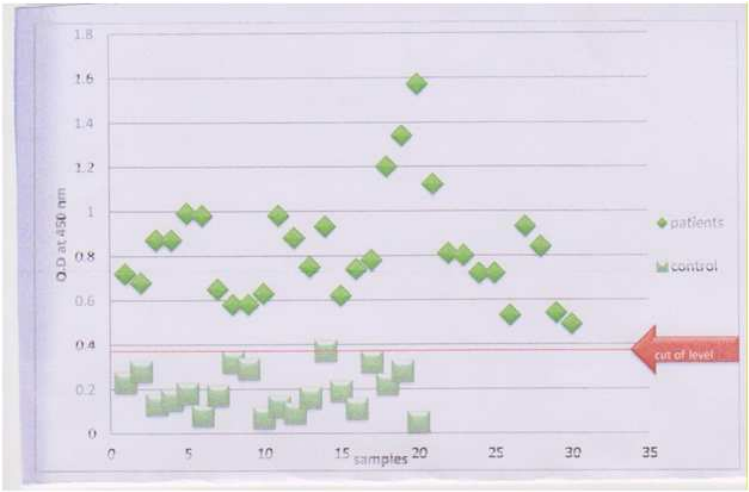

Figure 2: The Distribution of the Optical Density of ELISA Test for the Serum Antibody Detected for Patients and Control Group

DISCUSSIONS

Many studies focused on detection of protein in patients serum [(14),] plasma [(15),] breast fluid [(16)] and urine [(17)] as breast cancer markers. But they lack sensitivity specially for early stage disease and a lack of specificity. Therefore, the available markers are of no value in either screening or diagnosing early breast cancer [(7).] As a result, this insist for continuous searching to reach a satisfactory noninvasive laboratory test for breast cancer screening and/or diagnosis.

Many proteins had been found to be over expressed and up regulated in breast cancer patients urine such as Matrix Metaloproteinase-9 [(18),] Survivin protein [(19)] and Epidermal Growth Factor-related protein [(20)] that act as a diagnostic and/or prognostic tumour markers. The present study demonstrate the beneficial use of urine proteins as an antigen for serological diagnosis of breast cancer to the best of our knowledge for the first time in Meddle East including Iraq. The results of urine protein gel filtration showed greater protein concentration in breast cancer patients urine than healthy control urine.

Paper electrophoresis reveal a protein band appeared almost at the level of the control protein papain (mw 23500 D) which is not found in healthy control protein. However, McDevitt *et al* [(21)] found protein with molecular weight

24000 dalton in urine of cachectic cancer (lung, colon, breast, prostate, cervix and pancreas) patients but they claim absence of the protein in patient without cachexia.

CONCLUSIONS

The present study demonstrate that ELISA test of purified urine protein for detection of specific breast cancer antibody in patient's serum was all positive for breast cancer patients, while the test was negative for the control group, with highly significant difference between patients and control group. McDevitt *et al* [21] detected a specific antibody for the purified antigen (cachectic patients urine protein) in tumour bearing mice serum. Whereas, the present study tested serum from breast cancer patients with a coating antigen from breast cancer patients only and at time of diagnosis with different disease stages. So, it was specific test for breast cancer and the readings that obtained from ELISA test display its ability to detect different disease stages. Thus, it has a value in diagnosis of breast cancer and it promises for the development of reliable non-invasive breast cancer laboratory test that may confer women (at risk) a simple and safe screening test.

REFERENCES

1. *Parkin DM, Pisani P, Ferlay J. Global Cancer Statistics 1999. Cancer Journal for Clinicians 1999; 49: 33-64.*
2. *Iraqi Cancer Board, Iraqi Cancer Registry, Ministry of Health 2005.*
3. *Guidelines for early detection of breast cancer, Cairo, World Health Organization, Regional Office for the Eastern Mediterranean (EMRO Technical Publication Series 31) 2006.*
4. *Tiwari M. Role of fine needle aspiration cytology in diagnosis of breast lumps. Kathmandu University Medical Journal 2007; 5(2): 215-217.*
5. *Elmore JG, Barton MB, Moceri VM et al. Ten-Year risk of false positive screening mammograms and clinical breast examination. New England Journal of Medicine 1998; 338 (16): 1089-1096.*
6. *Lindfors KK, Rosenquist CJ. The cost-effectiveness of mammographic screening strategies. Journal of American Medical Association 1995; 274(11): 881-884.*
7. *Duffy MJ. Serum tumour markers in breast cancer: Are they of clinical value?. Clinical Chemistry 2006; 52(3): 345-351.*
8. *Berberoglu U, Ceyhan B, Ercakmak N, et al. The value of new tumour marker CA 15-3 in diagnosis and monitoring of patients with breast cancer Journal of Islamic Academy of Sciences 1989; 2(2): 113-117.*
9. *Protein Purification. A Manual for Biochemistry Protocols. http://www.worldscibooks.com/lifesci/6269.html.*
10. *Andrew SM, Titus JA, Zumstein L. Dialysis and concentration of protein solutions. Current Protocols in Immunology 1997; 3: 1-5.*
11. *Wright CA, Ross GC. Electrophoretic studies in some planorbid egg proteins. Bulletin World Health Organization 1965; 32: 709-712.*
12. *Fernandez-Botran R, Vetvicka V. Advanced Methods in Cellular Immunology. Boca Raton London, New York, Washington, D. C. CRC Press 2000.*
13. *Direct ELISA Protocol. www.abcam.com/technical.*
14. *Lv Y-G, Yu F, Yao Q, et al. The role of surviving in diagnosis, prognosis and treatment of breast cancer. Journal of Thoracic Disease 2010; 2: 100-110.*

15. *Pusztai L, Mendoza TR, Reuben JM et al. Changes in plasma levels of inflammatory cytokines in response to paclitaxel chemotherapy. Cytokine 2004; 25(3): 94-102.*
16. *Petrakis NL, Doherty M, Lee R, et al. Immunoglobulin levels in breast fluids of women with breast cancer. Clinical Immunology and Immunopathology 1977; 7(3): 386-393.*
17. *Roy R, Wewer UM, Zurakowski D, et al. ADAM 12 cleaves extracellular matrix proteins and correlates with cancer status and stage. The Journal of Biological Chemistry 2004; 279 (49): 51323-51330.*
18. *Pories SE, Zurakowski D, Roy R, et al. Urinary metalloproteinases non-invasive biomarkers for breast cancer risk assessment. Cancer Epidemiology, Biomarkers and Prevention 2008; 17(5): 1034-1042.*
19. *Guney N, Soydine HO, Derin D, et al. Serum and urine surviving levels in breast cancer. Medical Oncology 2006; 23(3): 335-339.*
20. *Eckert K, Granetzny A, Fischer J, et al. A Mr 43000 Epidermal Growth Factor-related protein purified from the urine of breast cancer patients. Cancer Research 1990; 50: 642.*
21. *McDevitt TM, Todorov PT, Beck SA, et al. Purification and characterization of a lipid-mobilizing factor associated with cachexia-inducing tumors in mice and humans. Cancer Research 1995; 55: 1458-1463.*