World Cancer Research Journal WCRJ 2022; 9: e2363

GENOMIC AND SERUM TUMOR MARKERS IN EGYPTIAN FEMALES WITH AND WITHOUT FAMILY CANCER HISTORY

H. H. SALEM[1], A. M. ELMAGHRABY[1], M. A. SHALABY[2], A. M. ELFRAMAWY[1], S. A ABDAL-AZIZ[1], N. M. HASSANIN[3], A. S. HAGGAG[1], S. M. HAMMAD[4]

[1]Nucleic Acid Research Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), City of Scientific Research and Technological Applications, Sarta City, Alexandria, Egypt
[2]Medical Biotechnology Research Department, Gebri, Sarta City, Alexandria, Egypt
[3]Central Lab, Sarta City Alexandria, Egypt
[4]Plant Protection and Biomolecular Diagnosis Department, Arid Lands Cultivation Research Institute, Sarta City Alexandria, Egypt

Abstract – *Objective:* *Multiple variables affect the probability of development of cancer. The present study aimed to screen Egyptian females for early prognostic cancer markers such as carcinoembryonic antigen (CEA), the soluble form of transmembrane mucin protein (CA15-3), MUC1 and important sex hormones (Progesteron, Oestrogen, and Prolactin) and three germline BRCA1/2 founder mutations.*

Patients and Methods: *Forty-five DNA samples were screened for 185delAG and 5382insC in the BRCA1 and 6174delT in the BRCA2 genes using polymerase chain reaction (PCR)-directed mutagenesis. Each sample of the 185delAG and the 6174delT mutations was confirmed using Restriction Fragment Length Polymorphism (RFLP) analysis. Nine suspected PCR products of 185delAG and the forty-five amplicons of 6174delT mutations were further confirmed using Sanger sequencing. Sex hormones (Progesteron, Oestrogen, and Prolactin) and cancer antigens (CA 15-3 and CEA) concentrations were quantitatively determined in serum samples using ELISA.*

Results: *We found significant associations only for oestrogen (p-value=0.036), while non-significant (p-value= 0.123) hyperprolactinemia with cancer history. But none of the individuals carried the BRCA1/2 studied mutations while new variants were detected; (delA) in position 93865, deletion (delA) or substitution of A by G (A/G) in position 93858 and (insA) in position 93844 with frequency of 50%, 50%, 25% and 25%, respectively, in subjects with cancer history.*

Conclusions: *The serum level of oestrogen could be a useful non-invasive cancer marker while significant association of hyperprolactinemia and the new BRCA1/2 variants with cancer needs extra study.*

KEYWORDS: *Breast cancer, Sex hormones, Tumor markers, Genes.*

INTRODUCTION

There are currently blood-born biomarkers that have been suggested for cancer predicting and follow-up. CEA markers for colon, pancreatic, lung and colorectal cancer and CA15-3 for breast cancer have proven to be reliable indicators of early disease as they present in the primary levels in normal individuals and will be elevated in individuals with disease in different stages [1,2]. These markers were used in different clinical practices [3,4]. In addition, sex hormones and its specific

DOI: 10.32113/wcrj_20227_2363

Corresponding Author: N. M. Hassanin, PhD; e-mail: nagahhassanin69@gmail.com

receptors may contribute to the growth and development of several tumors, including breast, endometrium, ovarian, head, neck and prostate carcinomas[5-8]. The tumour promoting effect of prolactin and its association with breast cancer was reported[9-11]. BRCA1/BRCA2 pathogenic variants in BRCA1 and BRCA2 can be involved in a higher risk of developing breast and ovarian cancer, prostate cancer and pancreatic cancer[12-14]. Furthermore, BRCA1/BRCA2 gene mutation biomarkers have been applied to the clinical management of breast/ovarian cancer patients [15]. In addition, Foulkes[16] included BRCA1/BRCA2 in the final list of established breast and ovarian cancer susceptibility genes. The current study aimed to evaluate CEA, CA15-3, oestrogen, progesterone, prolactin and BRCA1/BRCA2 gene mutations as cancer biomarkers in Egyptian females with and without cancer history. We started an extensive mutation screening study with these investigations and further studies will be carried out.

PATIENTS AND METHODS

Subjects

Forty-five female volunteers aged 25-52, with and without family history of cancer were included in this study. One of them was subsequently diagnosed with breast cancer and another one with a uterine benign tumor case (underwent tumor completely removed with surgery). These volunteers agreed to donate blood samples, personal and family cancer histories information for research purposes. This study was performed in compliance with relevant laws, institutional guidelines and the Declaration of Helsinki. It also was approved by the Faculty of Medicine Ethical Committee, Alexandria University (RB NO: 00007555-FWA NO: 00015712).

Sample collection

Venous blood (5 mL) was collected from each participant. Aliquots received in freezing tubes containing EDTA (anticoagulant) were used for DNA extraction and the remaining aliquots (without anticoagulant) were used for serum preparation. Serum was prepared according to a standard protocol[17] and stored at -20ºC.

CA 15-3 and CEA assays

Cancer antigen CA15-3 and Carcinoembryonic antigen (CEA) concentrations were quantitatively determined in serum samples using sandwich enzyme-linked immunosorbent (ELISA) kit (Calbiotech CA 15-3 ELISA kit, Spring Valley, CA USA) and sandwich enzyme immunoassay test kit (CEA enzyme immunoassay test kit, Chemux BioScience, Inc., San Francisco, CA USA), respectively, according to the manufacturers' instructions.

Hormones testes

In this study, serum concentrations of progesterone, oestrogen and prolactin were quantitatively measured using ELISA assays. The manufacturers' instructions of competitive EIA kit (enzyme immunoassay test kit, Chemux BioScience, Inc., San Francisco, CA USA), DRG Instruments (GmbH, Frauenbergstr, Germany) DRG Estradiol ELISA kit (DRG International, Inc., Springfield, NJ, USA) and Enzyme immunoassay test kit (Chemux BioScience, San Francisco, CA, USA), used in Progesteron, oestrogen and prolactin tests, respectively.

Genomic Marker Assays DNA extraction

Genomic DNA was extracted from whole blood by using Thermo Scientific Gene JET genomic DNA purification kit (2014 Thermo Fisher Scientifi, Waltham, MA, USA) according to the manufacturers' instructions.

BRCA1 and BRCA2 mutations detection PCR

Detection of Jewish founder mutations; 185delAG and 5382insC mutations in the BRCA1 Gene and 6174delT mutation in the BRCA2 gene was carried out using a Polymerase Chain Reaction (PCR), as previously described[18]. 8% denaturing polyacrylamide gel used to detect the mutations because each of the mutation alleles had either a deletion or an insertion (the size of PCR products of normal and mutant alleles of each mutation differs by one or two nucleotides). For confirmation of the 185delAG and the 6174delT mutations, RFLP and sequencing assays were carried out.

RFLP

The PCR product for each sample of the 185delAG and the 6174delT mutations was digested with (20 U/µl) of HinfI (SibEnzyme) and (12 U/µl) of BbrPI (ViVantiS), respectively in a 20 µl final volume according to the manufacturer's instructions. The digested fragments were analyzed electrophoretically on 15% non-denaturing polyacrylamide gel[18].

Sanger sequencing

Nine suspected PCR products were confirmed for 185delAG mutation using sequencing with the forward primer (forward direction) and the forty-five amplicons of 6174delT mutation were confirmed using sequencing with reverse primer (reverse direction). The sequencing primers were selected where their sequences did not harbour the interested polymorphisms. The band was excised from the agarose gel and purified according to the instruction manual of EZ-10 spin column DNA gel extraction kit (bio basic). Purified PCR products were sequenced using Genetic Analyzer (Applied Biosystems, Hitachi, Japan) at (Gatc lab, Germany).

Statistical analysis

Significant association between studied markers and each of age, weight, presence of children and cancer history of studied individuals were tested using the chi-square (χ^2) goodness of fit Statistical Package for the Social Sciences (SPSS) version 22 (IBM Corporation, Armonk, NY, USA). *p*-values of ≤0.05 were considered significant.

RESULTS

The demographic characteristics of the participants are shown in Table 1. It was noticed that the mean age was 38.5. Among the studied individuals, 26 (57.8%) were > 40 and 19 (42.2%) were ≤40, 71.1% married, 28.9% not married, and 37.8% of these participants had cancer family history.

TABLE 1. Volunteers demographics.

Variable	*Frequency*
Age	
> 40	26 (57.8%)
≤ 40	19 (42.2%)
Marital Status	
Married	32 (71.1%)
Not Married	13 (28.9%)
Breast-feeding	
Ever	30 (66.7%)
Never	15 (33.3%)
Having children	
Children	31 (68.9%)
No children	14 (31.1%)
Cancer history	
With cancer history	17 (37.8%)
Without cancer history	28 (62.2%)

BRCAI/BRCA2 mutations analysis

Forty-five PCR products for each of 185delAG and 5382insC mutations in the BRCA1 gene and 6174delT mutation in the BRCA2 genes have been typed by PAGE single strand conformation polymorphism (SSCP). All samples showed the same profile for each mutation. These results indicated that mutant allele of each mutation was absent or the efficiency of PAGE typing for detection of mutant allele was low. In addition, RFLP patterns showed that all PCR products for each sample of the 185delAG and the 6174delT mutations were wild-type therefore, each PCR product of the 185delAG (170-bp) digested with *Hin*fI restriction enzyme (which recognizes 5'G↓ANTC3'-3'CTNA↓G5'; N=A or C or G or T) and produced two bands 150-bp and 20-bp. While each PCR of the 6174delT mutation (148-bp) gave cut (127 and 21-bp) with BbrPI which recognize5'CAC↓GTG3'-3'GTG↓CAC5'. These results confirmed that mutagenic primers introduced a restriction site into all the studied PCR products. Furthermore, sequence results did not detect either the185delAG BRCA1 nor 6174delT BRCA2 mutant allele in the current PCR products. In addition, the sequence of nine 185delAG BRCA1 samples were aligned with the reference sequence (NG_005905.2) (Figure 1). Novel alterations in BRCA1, which were not reported from the main reference databases, were noticed; there was a deletion (delA.) in all samples except sample no. 31 and 17 in position 93865, in samples no. 10, 12, 31, and 36 there was a deletion (delA) and substitution of A by G in samples no. 1, 15 in position 93858 and there was (insA) in position 93844 in sample no.17 only. The association of frequency of the new BRCA1 unknown variants with cancer history was shown in Table 2. The accession numbers of these samples are GenBank MW432525, GenBank MW462238-MW462241, and GenBank MW531176-MW531179.

Also, alignment of forty-five 6174delT BRCA2 sample sequences and reference sequences (NG_012772.3) showed that forty-one sample sequences had 99% sequence identity. Figures 2(a and b) displayed graphics of alignment between any of these samples sequences and reference sequence to determine the location of target mutation in both gene and chromosome. The deposited accession numbers of forty-one sample sequences are: GenBank MZ027671-MZ027672, GenBank MW928852-MW928887, GenBank MZ054388-MZ054389, and GenBank MW600293. It also noticed that samples no. 29 and 44 had 83% similarity with reference sequence and samples no. 1 and 7 had different nucleotide sequences.

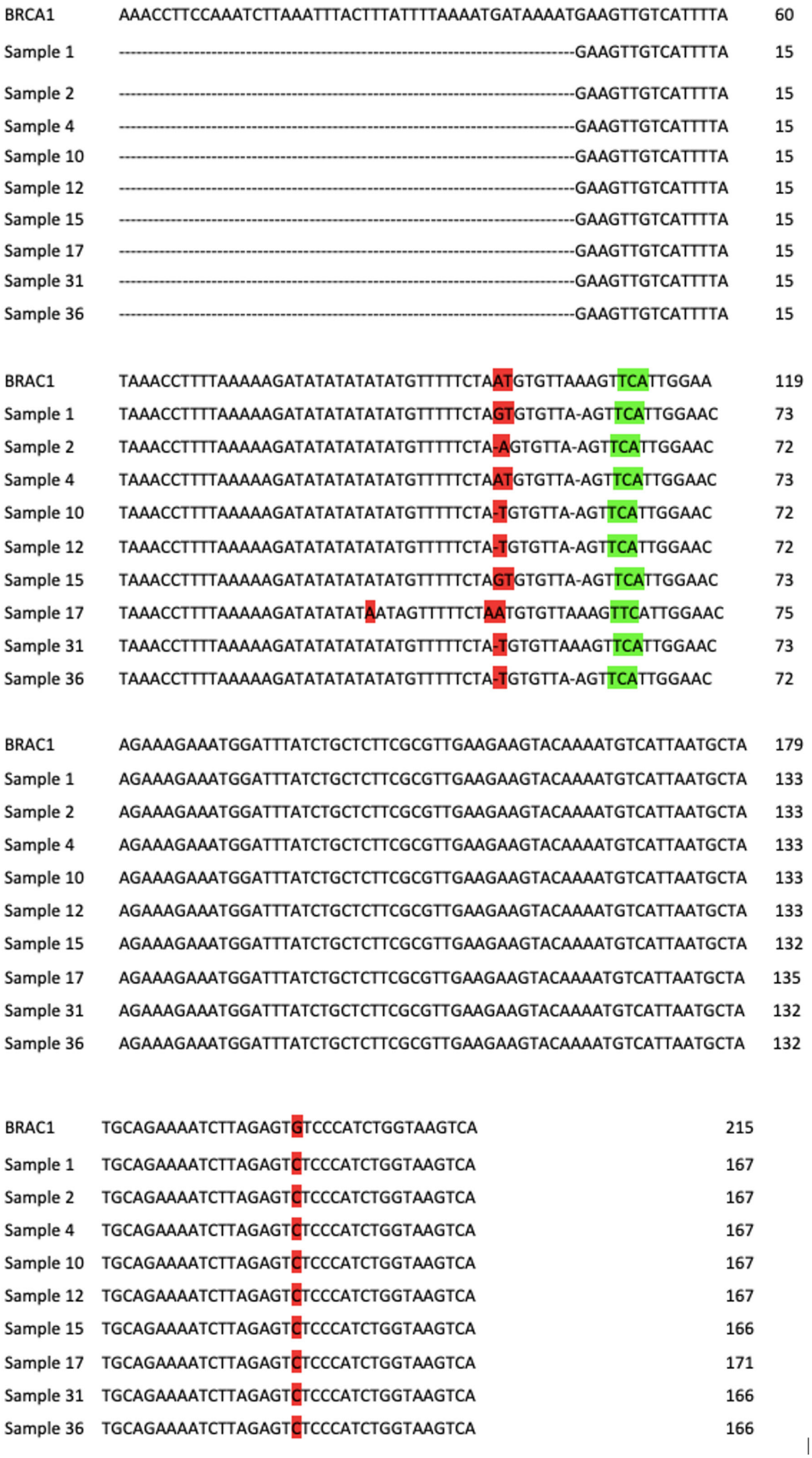

Fig. 1. The sequence alignment of BRCA1 reference sequence (NG_005905.2) with the nine selected samples. The green border line indicates the start codon in Exon 2 of BRCA1 gene. The red border line indicates the 185delAG and new variant position in the sequences.

TABLE 2. Frequency of the new BRCA1 unknown variants in selected samples with and without cancer history.

Variants	*Location in BRCA1 gene*	*Frequency in individuals NO. (%)*	
		With cancer history (total number=4)	*Without cancer history (total number=5)*
Deletion of nucleotide a (delA)	93865	2 (50%)	5 (100%)
Deletion of nucleotide a (delA)	93858	2 (50%)	3 (60%)
Substitution of nucleotide a by g(A/G)	93858	1 (25%)	1 (20%)
Insertion of nucleotide a(insA)	93844	1 (25%)	0 (0%)

(a)

(b)

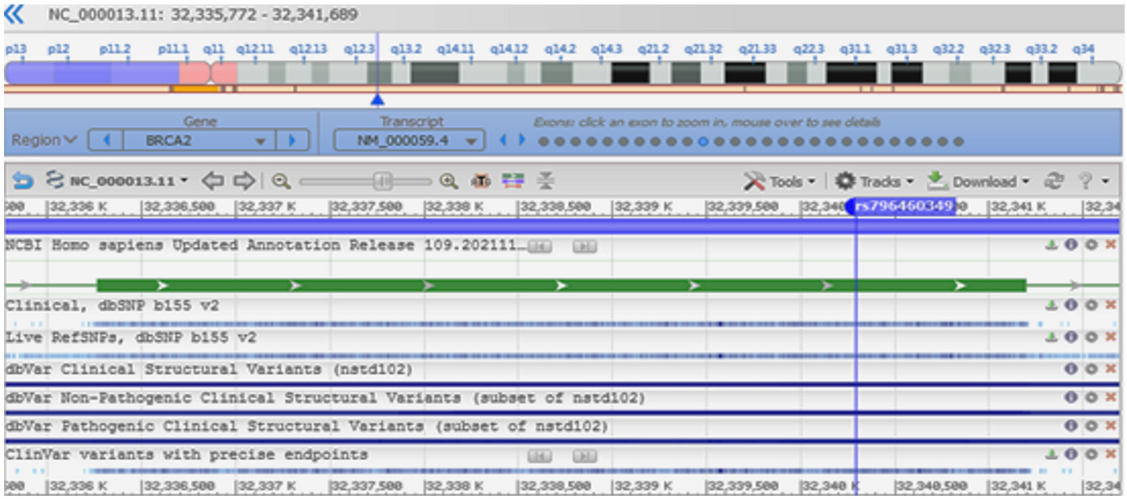

Fig. 2. (a) Graphic of alignment between samples and reference sequences (NG_012772.3), red C determines the target 6174delT (rs796460349) variant position in BRCA2 gene (exon 11). (b) Graphic of alignment between sample sequences and reference sequence (NG_012772.3), blue box determines the rs796460349 BRCA2 variant position on chromosome 13. Figure 2a and 2b are part of our current study and deposited accession number (https://www.ncbi.nlm.nih.gov/nuccore/MZ054388.1/).

TABLE 3. Hormonal and cancer biomarker level in serum.

Hormone	Hormone and tumor markers level percentage		
	High level%	Low level %	Normal level %
Prolactine	75.6	0	24.44
Progesterone	0	17.78	82.22
Oestrogen	2.2	26.7	71.1
CEA	2.2	0	97.8
CA15-3	8.9	0	91.1

High level: above normal range, Low level: below normal range, Normal level: within normal range.

Serum Biomarkers

Table 3 and Figure 3 demonstrate the percentage of people with normal, elevated and reduced Progesterone, prolactin, estradiol, CEA and CA15-3. Data revealed that serum level of CEA of all samples was in normal range (< 5 ng/ml) except for sample no. 36 which had elevated CEA level (49.8 ng/ml) although it was included in no cancer history group. Similarly, CA15-3 serum level of all samples was at normal level (<35 U/ml) while samples no. 10, 13, 25 and 26 had raised CA15-3 levels. 40, 37.5, 38 and 36 U/ml, respectively, had no history of cancer. Of 45 women screened with prolactin test, 34 women (of different age, different menopausal status and 15 of them with cancer history) had high prolactin levels (16-100 ng/ml) compared to cut-off value (15 ng/ml).

Serum progesterone of 37 women (82.22%) was in normal ranges which vary from 0.06-25 ng/ml according to the phase of menstrual cycle and age. Decreased levels were detected in 8 women (17.78%) with age; 32, 38, 39, 40, 44, 45, 46 and 49. Interestingly, estradiol of 32 women was in normal ranges (18.4-549 pg/ml). Reduction in serum estradiol level was detected in samples of 12 women, 7 of them with history of cancer and 8 of them with age >40. Moreover, estradiol level was elevated in a sample of women who were diagnosed with breast cancer after taking its sample. Furthermore, the association of studied serum biomarkers with each of age, body weight, presence of children and cancer history of the individuals was analyzed using the Pearson's chi-square test. It was noticed that the association was significant only for oestrogen with cancer history as shown in Table 4. Also, data analysis showed non-significant increase in all biomarkers abnormality for women who were 40 years or older.

DISCUSSION

Current study used some known cancer biomarkers for the early detection of cancer in Egyptian women. Data of PCR, RFLP and sequencing showed that neither the studied 185delAG, 5382insC BRCA1 nor 6174delT BRCA2 mutant allele detected in the current PCR products. These data are in accordance with Hussien et al[19] who reported that 185delAG founder mutation was expressed at low frequency (3%) whereas the 5382insC in BRCA1

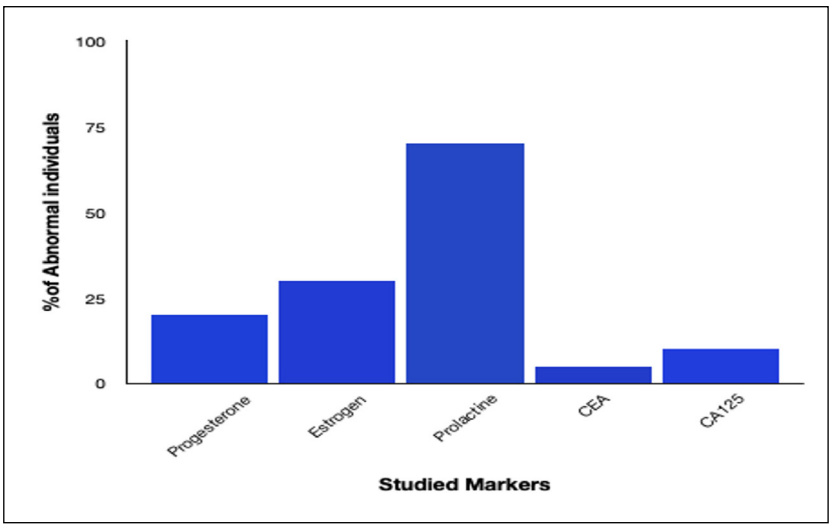

Fig. 3. Percentage of participants with abnormal level of serum tumor markers.

TABLE 4. Results of chi-square serum biomarkers data analysis.

Variable	*Progesterone*			*Oestrogen*			*Prolactin*			*CA15-3*			*CEA*		
	*%N***	*%Å*	*Pv.¹*	*%N*	*%A*	*P v.*	*%N*	*%A*	*P v.*	*%N*	*%A*	*P v.*	*%N*	*%A*	*P v.*
Age≥40	73.7	26.3	.200	57.9	42.1	.094	21.1	78.9	.651	89.5	10.5	.741	94.7	5.3	.237
Age<40	88.5	11.5		80.8	19.2		26.9	73.1		92.3	7.7		100.0	0.0	
With cancer history	88.2	11.8	.411	52.9	47.1	.036	11.8	88.2	.123	100.0	0.0	.103	100.0	0.0	.431
Without cancer history	78.6	21.4		82.1	17.9		32.1	67.9		85.7	14.3		96.4%	3.6	
Body weight≥70k	80.0	20.0	.466	74.3	25.7	.379	25.7	74.3	.711	91.4	8.6	.889	97.1	2.9	.589
Body weight<70	90.0	10.0		60.0	40.0		20.0	80.0		90.0	10.0		100.0	0.0	
Have children	77.4	22.6	.210	67.7	32.3	.458	32.3	67.7	.070	87.1	12.9	.159	96.8	3.2	.497
Have no children	92.9	7.1		78.6	21.4		7.1	92.9		100.0	0.0		97.8	2.2	

: normal: within normal range,: abnormal: above or below normal range and¹: *p*-value.

and 6174delT in BRCA2 were not detected within the Eastern Egyptian population. Also, Zoure et al[20] did not detect 185delAG (c.68_69delAG (exon2)) in the population of Burkina Faso. On the other hand, Debaky et al[21] found that the5382insC and 185 delAG mutations showed a frequency (56%) and (22%), respectively, in females of Qalubia governorate (Egypt). They also found that incidence of BRCA1 gene mutations in the breast cancer group and in the groups with family history were higher than its incidence in the control group and in the groups without family history. It was reported that the prevalence of BRCA1/2 germline variants is variable among different ethnic groups [22]. BRCA1/2 variants showed a frequency between17.6% to 29.8% in white European and Australian people while 9.4% to 21.7% in Asian countries [23] and 8% to 37% within Italian families with BC and/or OC cases[24,25]. New mutations in current study which were detected need further study to its identification. It was found that neither CEA nor CA15-3 could not detect cancer in women who were diagnosed as breast cancer via mammography while they revealed positive results with healthy women with no cancer history. These results indicated that CEA and CA15-3 lack sensitivity for early disease detection and may be useful as pre-diagnostic markers of a more aggressive tumour phenotype and metastatic disease during active therapy, in agreement with several authors such as Harris et al[26], Mari´c et al[27], Atoum et al[28], Shao et al[29], and Kazarian "et al[30]. The current study showed that about 75% of women had increased prolactin levels including 2.9% with breast cancer. In addition, non-significant hyperprolactinemia in women with cancer history was noticed. This finding indicated that the high prolactin level may be correlated to disease state. Cohen et al[31] found non-significant increase in prolactin level in patients with active disease while Sethi et al[32] and Ali et al[11] reported that both breast and prostate cancer were associated significantly with increased prolactin level. With respect to serum progesterone and oestrogen, the current study demonstrated decreased progesterone levels in (17.78%) of women with age: 32, 38, 39, 40, 44, 45, 46 and 49. Interestingly, reduction in serum estradiol level was also detected in 26.7% of samples, 66.7% of them with age >40 and 58.3% of them with history of cancer. Also, data analysis showed non-significant increase in all biomarkers abnormality for women who were 40 years or older but significant association only for oestrogen with cancer history. These results indicated that the reduction in progesterone and oestrogen related non-significantly with age in accordance with Perheentupa and Huhtaniemi[33] and Neal-Perry et al[34] who reported an overall decline in concentrations of estrogens and progesterone in females with age. Additionally, it was reported that estrogen levels decrease significantly in women with menopause [35]. Moreover, the current study showed high estradiol and prolactin levels while low progesterone levels in a sample of women who were diagnosed with breast cancer after taking its sample. These results indicated that high estradiol and prolactin levels while low progesterone may serve as indicators of tumour or cancer as previously reported that either insufficient progesterone action or an excess in oestrogen can result in endometrial pathologies such as endometrial hyperplasia or endometrial adenocarcinoma[36].

CONCLUSIONS

The serum level of oestrogen could be a useful non-invasive cancer marker while significant association of hyperprolactinemia and the new BRCA1/2 variants with cancer needs extra study.

Acknowledgments:

Workgroup is indebted to sample donors for their participation in the study.

The authors would like to thank Dr. James E. Freund and Dr.Ehab M. Hassouna for their proofreading of this manuscript.

Ethical approval:

The study's Ethics committee approval was taken from faculty of Medicine Alexandria University, Ethics committee, released decision number 50 on January 3, 2013

Availability of data and materials:

The sequence dataset is published in the ncbi database. The other dataset generated and/or analysed during the current study are not publically available but are available from the authors upon reasonable request.

Conflict of interest:

The authors declare that they have no competing interests and no conflict of interest.

Funding:

This work was supported by the research fund of the Genetic Engineering & Biotechnology Research Institute (GEBRI), City of Scientific Research & Technological Applications, Alexandria, Egypt. It is not part of an approved student thesis.

Author contributions:

All authors distributed their inputs for; samples obtaining & processing, conducting the experiments, data analysis and manuscript preparation.

Consent for publication:

A written informed consent was obtained from each participant for publication.

REFERENCES

1. Ballesta AM, Molina R, Filella X, Jo J, Gimenez N. Carcinoembryonic antigen in staging and follow-up of patients with solid tumours. Tumour Biol 1995; 16: 32-41.
2. Chatterjee SK, Zetter BR. Cancer biomarkers: knowing the present and predicting the future. Future Oncol 2005; 1: 37-50.
3. Stieber P, Molina R, Chan DW, Fritsche HA, Beyrau R, Bonfrer JM, Filella X, Gornet TG, Hoff T, Jager W, van Kamp GJ, Nagel D, Peisker K, Sokoll LJ, Troalen F, Untch M, Domke I. Clinical evaluation of the Elecsys CA 15-3 test in breast cancer patients. Clin Lab 2003; 49: 15-24.
4. Duffy MJ. Serum tumour markers in breast cancer: Are they of clinical value? Clin Chem 2006; 52: 345-351.
5. Fan CY, Malhelm MF. Expression of androgen receptors, epidermal growth factor receptor & transforming growth factor alpha in salivary duct carcinoma. Arch Otolaringol Head Neck Surg 2001; 127: 1095-1097.
6. Glas AS, Hollema H, Nap RE, Plukker JT. Expression of oestrogen receptor, progesterone receptor, and insulin-like growth factor receptor-1 and of MIB-1 in patients with recurrent pleomorphic adenoma of the parotid gland. Cancer 2002; 94: 2211-2216.
7. Mannarini L, Kratochvil V. Human Papillomavirus in head and neck region: A review of literature. Acta Otorhinolaryngol Ital 2009; 29: 119-126.
8. Badawy A, Elnashar A. Treatment options for polycystic ovary syndrome. Int J Womens Health 2011; 3: 25-35.
9. Ingram DM, Nottage EM, Roberts AN. Prolactin and breast cancer risk. Med J Aust 1990; 153: 469-473.
10. Bernichtein S, Touraine P, Goffin V. New concepts in prolactin biology. J Endocrinol 2010; 206: 1-11.
11. Ali Kh J, Hassan HS, Oleiwi AS. Relationship of Prolactin Serum Levels and Breast Cancer with Haematological Factors Among Cases in Karbala Province, Iraq. Int J Med Sci Public Health 2018; 7: 82-87.
12. Kuchenbaecker KB, Hopper JL, Barnes DR, Phillips KA, Mooij TM, Roos-Blom MJ, Jervis S, Van Leeuwen FE, Milne RL, Andrieu N. Risks of breast, ovarian, and contralateral breast cancer for BRCA1 and BRCA2 mutation carriers. J Am Med Assoc 2017; 317: 2402-2416.
13. Edwards SM, Evans DG, Hope Q, Norman AR, Barbachano Y, Bullock S, Kote-Jarai Z, Meitz J, Falconer A, Osin P Fisher C, Guy M, Jhavar SG, Hall AL, O'Brien LT, Gehr-Swain BN, Wilkinson RA, Forrest MS, Dearnaley DP, Ardern-Jones AT, Page EC, Easton EC, Elmi RA. Prostate cancer in BRCA2 germline mutation carriers is associated with poorer prognosis. Br J Cancer 2010; 103: 918-924.
14. Iqbal J, Ragone A, Lubinski J, Lynch HT, Moller P, Ghadirian P, Foulkes WD, Armel S, Eisen A, Senter L, Singer CF, Ainsworth P, Kim-Sing C, Tung N, Friedman E, Llacuachaqui M, Ping S, Narod SA. The incidence of pancreatic cancer in BRCA1 and BRCA2 mutation carriers. Br J Cancer 2012; 107: 2005-2009.
15. Torres D, Bermejo JL, Rashid MU, Briceno I, Gil F, Beltran A, Ariza V, Hamann U. Prevalence and penetrance of BRCA1 and BRCA2 germline mutations in Colombian breast cancer patients. Sci Rep 2017; 7: 4713.
16. Foulkes WD. The ten genes for breast (and ovarian) cancer susceptibility. Nat Rev Clin Oncol 2021; 18: 259-260.
17. Sambrook J, Fritsch EF, Maniatis T. Molecular Cloning: A Laboratory Manual (2nd ed.). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press 1989.
18. Abeliovich D, Kaduri L, Lerer I, Weinberg N, Amir G, Sagi M, Zlotogora J, Heching N, Peretz T. The founder mutations 185delAG and 5382insC in BRCA1 and 6174delT in BRCA2 appear in 60% of ovarian cancer and 30% of early-onset breast cancer patients among Ashkenazi women. Am J Hum Genet 1997; 60: 505-514.
19. Hussien YM, Gharib AF, Ibrahim HM, Abdel-Ghany ME, Elsawy WH. Analysis of BRCA1 and BRCA2 Mutations in Eastern Egyptian Breast Cancer Patients. Bull ESPS 2011; 31: 107-116.
20. Zoure AA, Slaoui M, Bambara AH, Sawadogo AY, Compaoré TR, Ouédraogo NLM, El Mzibri M, Attaleb M, Traoré SS, Simpore J, Bakri Y. BRCA1 c.68_69delAG (exon2), c.181T>G (exon5), c.798_799delTT and 943ins10 (exon11) mutations in Burkina Faso. J Public Health Afr 2018; 9: 663.
21. El-Debakey FE, Azab NI, Alhusseini NF, Eliwa SK, Musalam HR. Breast Cancer Gene 1 (BRCA 1) Mutation in Female Patients with or without Family History in Qalubia Governorate. Am J Sci 2011; 7: 82-93.
22. Santonocito C, Rizza R, Paris I, Marchis LD, Paolillo C, Tiberi G, Scambia G, Capoluongo E. Spectrum of germline BRCA1 and BRCA2 variants identified in 2351 ovarian and breast cancer patients referring to a reference cancer hospital of Rome. Cancers 2020; 12: 1286.
23. Rashid MU, Muhammad N, Naeemi H, Khan FA, Hassan M, Faisal S, Gull S, Amin A, Loya A, Hamann U. Spectrum and prevalence of BRCA1/2 germline mutations in Pakistani breast cancer patients: Results from a large comprehensive study. Hered Cancer Clin Pract 2019; 17: 27.
24. Capalbo C, Ricevuto E, Vestri A, Ristori E, Sidoni T, Buone O, Adamo B, Cortesi E, Marchetti P, Scambia G. BRCA1 and BRCA2 genetic testing in Italian breast and/or ovarian cancer families: Mutation spectrum and prevalence and analysis of mutation prediction models. Ann Oncol 2006; 17: 34-40.
25. Santonocito C, Scappaticci M, Guarino D, Bartolini A, Minucci A, Concolino P, Scambia G, Paris I, Capoluongo E. Identification of twenty-nine novel germline unclassified variants of BRCA1 and BRCA2 genes in 1400 Italian individuals. Breast 2017; 36: 74-78.
26. Harris L, Fritsche H, Mennel R, Norton L, Ravdin P, Taube S, Somerfield MR, Hayes DF, Bast Jr RC. American Society of Clinical Oncology update of recommendations for the use of tumour markers in breast cancer. J Clin Oncol 2007; 25: 5287-5312.
27. Mari´c P, Ozreti´c P, Levanat S, Ore˘skovi´c S, Antunac K, Beketi´c-Ore˘skovi´c L. Tumor markers in breast cancer: evaluation of their clinical usefulness. Coll Antropol 2011; 35: 241-247.
28. Atoum M, Nimer N, Abdeldayem S, Nasr H. Relationships among serum CA15-3 tumour markers, TNM staging, and oestrogen and progesterone receptor expression in benign and malignant breast lesions. Asian Pac J Cancer Prev 2012; 13: 857-860.
29. Shao Y, Sun X, He Y, Liu C, Liu H. Elevated levels of serum tumour markers CEA and CA15-3 are prognostic parameters for different molecular subtypes of breast cancer. PLoS One 2015; 10: e0133830.
30. Kazarian A, Blyuss O, Metodieva G, Gentry-Maharaj A, Ryan A, Kiseleva EM, Prytomanova OM, Jacobs IJ, Widschwendter M, Menon U, Timms JF. Testing breast cancer serum biomarkers for early detection and prognosis in pre-diagnosis samples. Br J Cancer 2017; 116: 501-508.
31. Cohen AD, Cohen Y, Maislos M, Buskila D. Prolactin Serum Level in Breast Cancer Patients. IMAJ 2000; 2: 287-289.

32. Sethi BK, Chanukya GV, Nagesh VS. Prolactin and cancer: Has the orphan finally found a home. Indian J Endocr Metab 2012; 16: 195-198.
33. Perheentupa A, Huhtaniemi I. Ageing of the human ovary and testis. Mol Cell Endocrinol 2009; 299: 2-13.
34. Neal-Perry G, Nejat E, Dicken C. The neuroendocrine physiology of female reproductive ageing: an update. Maturitas 2010; 67: 34-38.
35. Ozdemir BC, Dotto G-P. Sex Hormones and Anticancer Immunity. Clin Cancer Res 2019; 25: 4603-4610.
36. Kim JJ, Chapman-Davis E. Role of Progesterone in Endometrial Cancer. Semin Reprod Med 2010; 28: 81-90.