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The relationship between homocysteine , folate, B12 serum level and breast cancer in sample of women in Baghdad city –Iraq.

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ABSTRACT

The study included eighty cases of women with breast cancer (as patients group) with age range from 30 to 8 years, The control group included twenty healthy women. The level of Homocysteine, vitamin B12 and folate were estimated in the serum of patients group and compared with the control and it has been compared to some other aspects such as aBody mass index (BMI), type of feeding and Family history. The statistical analysis revealed a significant difference ($p \leq 0.05$) between the mean of Homocysteine level (13.18 ± 0.61) µmol/l for patients group compared with mean (9.78 ± 0.60) µmol/l of control group . It was also found a significant difference ($p \leq 0.05$) between the mean of folate level for patients group and control group , (8.64 ± 0.40) ng/ml and (13.19 ± 1.19) ng/ml for both groups, respectively,.It was noticed that there was interaction between the mean of homocysteine, B12 and folate level in serum with age, marital status ,type of feeding and family history.

Introduction:

Cancer is a major public health problem diffuse in world [1]. Breast cancer is a disease where cells is grow and divided in the tissue without natural control [2].In Iraq, it position first among cancers diagnosed in women, but studies on carcinoma among women in Iraq's western regions are limited [3]. In 2014,one studies was noticed that Iraqi women have higher percentage of infiltrated ductal carcinoma (86%), and (4%) for the invasive lobular carcinoma[4]. In Hilla, Iraq, almost (71.3%) from tumors in breast tissue were diagnosed at age under than fifty years [5]. Risk factors for developing breast cancer include: gander, obesity, lack of physical exercise, The influence of alcohol, hormonal drugs through menopause, radiation, age , number of children and family history [6,7]. For the last several years, a relationship has been established between breast cancer and elevated level of homocysteine (Hcy)[8] .

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The increased level of Hcy in serum of cancer patients is probably due to deficiency in folate, B12, and B2 which are considered as important factors in Hcy metabolism.[9] Low folate levels prevent the methionine homocysteine cycle and lead to decreased methylation[10]. However, disruption in themethionine-homocysteine biosynthesis indicated by elevated Hcy is hypothesized to play a role. A disruption in this cycle is postulated to lead to two potential cancer-causing processes: abnormal DNA methylation, and the production of reactive oxygen species that can lead to oxidative stress.[11]. The synthesis of methionine from Hcy is dependent on folate (5-methyltetrahydrofolate), but also of vitamin B-12, which acts as a cofactor to the enzyme methionine synthase. Since the transference of 5,10-methylenetetrahydrofolate to 5-methyl tetrahydro folate is practically an irreversible response [12].

Materials and Methods

A sample of 100cases was studied including 80 patient and 20 control .woman aged from 30 to80 years with radiotherapy, chemotherapy and hormone therapy. All sample of patients were Collected from Al– Amal National Hospital for cancer Management– Baghdad, for the period from September 2015 to November 2015 . Random sample of twenty apparently healthy person were used as control group

with age ranged from 25 to 70 years. In all cases and control full history and complete physical examinations including age , marital status, number of children and body mass index (BMI) were done by using a short structured questionnaire. the biochemical tests were conducted in Al nadaer Clinical laboratory and Al- Bailasan laboratory.

Blood samples were collected from patients and control groups . The quantity (2.5ml) from blood were collected in tubes without any anti-coagulant, these tubes were immediately placed on crushed ice, protected from light, then the blood samples were allowed to clot for 30 min and serum was collected. The serum was again centrifuged at 4000 rpm at 4°C in cooling centrifuge and then the purified serum samples were used for evaluation of homocysteine, B12 and folate concentrations.

Measurement of Homocysteine (Hcy) concentration.

Quantitative determination of Hcy in human serum was performed using an sandwich enzyme immunoassay technique kit (ST AlA-PACK Homocysteineassay) using a Tosoh AlA System Analyzer.(Tosoh , Japan)

Measurement of folate concentrations

Determination of folate was performed by using an Electro Chemicaluminescence technology kit(Cobas e411 analyzer(REF: 04476433 190) data sheet at (Roche,Germeny).

Measurement of B12 concentration

Determination of vitamin B12 was performed by using an Microplate Enzyme Immunoassay, Colorimetric. By using Cobas e 411 kit (FolateIII) kite (Roche ,Germany).

Statistical Analysis

All data were analyzed using the Statistical Analysis System- SAS (2012) program, version 9.1 for windows [13]. A one – way analysis of variance (ANOVA),Chi-square(Preasoning),T-test(in depending) and Least significant difference (LSD) were used to test the significance difference between the group means of patients and control group.

Result and Discussion

1-Homocysteinemeasurement

Result of serum homocysteine measurement revealed a significant increase($p \leq 0.05$)in patients group(13.18 ± 0.61)µmol/l compared with mean of control group(9.78 ± 0.60)µmol/l (Tab 1).

Table. 1 .The mean ± SD of homocysteine level for patients and control group

Group	No.	Mean ± SD µmol/L
Patients	**80**	**13.18 ± 0.61**
Control	**20**	**9.78 ± 0.60**
T-value	**---**	**2.495 ****
***P*-value**	**---**	**0.0064**
**** (*P≤0.01*).**		

The Hcy values are within normal value, but there is an increase in the pateint group comparison with control group ,The increased level of total Hcy in serum of patients was probably due to deficiency in folate and B_{12} or perhaps even to a genetic predisposition causative to both phenomenonon the other hand, methylene tetrahydro folate (MTHFR) polymorphisms, which are a well known factor for hyperhomocysteinemia[14]. The MTHFR catalyzes the formation of 5-methyltetra hydrofolate from 5,10-methylenetetrahydrofolate; the folate form necessary for the remethylation of homocysteine to methionine. The 677 $C \rightarrow T$ mutation results in a reduced specific MTHFR activity in isolated, which leads to higher total of Hcy concentrations [15].

2-Folate measurement

Measuring of folate in serum revealed significant association ($P \leq 0.01$) with breast cancer, but tend to decrease among patients (8.64 ± 0.40) ng/ml compared to control group(13.19 ± 1.19)ng/ml that shown in (Tab 2).

Table. 2 . The mean ± SD of folate level in serum for patients and control group.

Group	No.	Mean ± SD ng/ml
Patients	**80**	**8.64 ± 0.40**
Control	**20**	**13.19 ± 1.19**
T-value	**---**	**1.982 ****
***P*-value**	**---**	**0.0001**
**** (*P≤0.01*).**		

Folate is play a role in the pathway of methylation of DNA and cell division and thus the safety of genome, the research community agrees that the function of folate in tumor increase depends strongly on the timing of an person's onset for folate consumption and the presence of cancer cell formation[16].

It was reported that there was invert relationship between depressed folate concentration and increase the happening of breast cancer [17], the same results were observed in other study included 13 samples of the control group and 9 samples of infected women[18].

Ericson(2010) was reported in a case study that no significant difference was shown between folate

and breast cancer[19].While[20,16]confirmed the significant association.

3-Age group

It has been noticed that the age of breast cancer patients have been distributed as follow, 9(11.25%) patients with age group ranged from 30 to 39 years , 28 (35.00%) patients ranged from 40 to 49 years, 30 (37.50%) patients ranged from 50 to 59 and13 (16.25%) patients ranged for 80 years, this distribution may explain that breast cancer may increase with age group (50-59) and (40 -49)Tab 3.

Statistical analysis revealed the patients with age group ranged from 30 to 39 years have higher mean of folate level (11.03 ±1.35)ng/ml with significant *(P≤0.05)* ,while the mean of B_{12} level was(442.69 ± 44.12) pmol/l in age group 50 to 59 and increases in patients with age group 80 years (601.05 ±145.9) pmol/l, While homocysteine showed convergent values in all ages.

Table.3 . Effect of age group on the (mean ± SD) of Homocysteine ,B_{12} and folate level for patients group.

Age group (year)	No. (%)	Mean ± SD		
		Hcy µmol/l	B_{12} pmol/l	Folat ng/ml
30-39	9 (11.25%)	13.46 ± 2.01	592.26 ±239.4	11.03 ±1.35
40-49	28 (35.00%)	12.90 ± 0.86	599.85 ± 81.9	8.59 ± 0.66
50-59	30 (37.50%)	13.58 ± 1.19	442.69 ± 44.12	8.22 ± 0.64
80years	13 (16.25%)	13.29 ± 1.24	601.05 ±145.9	8.07 ± 9.75
LSD value	---	2.316 NS	289.54 NS	2.043 *
P-value	---	0.397	0.371	0.0411
NS: Non-significant.				

Usually affects breast cancer women between the ages of 40 and 50 years, but it may affect the girls from the age group younger than thirty, and up to the age of 17, is associated with the cause hormonal changes that occur in a woman's body, because these blocks increased symptoms increased the proportion of estrogen, The ease with increasing the proportion of progesterone [21].

In some countries, the happening of breast cancer, the average increases with age, and 85% of breast cancer are showed in women with age more than 50 years, pointing out that one in 12 women with tumor in breast tissue before the age of 75, [21].It was also noticed a total plasma homocysteine increases with increasing age because of younger people are more active than older people [22].

It was appear the relationships between the level of vitamin B_{12} and breast cancer in women who were postmenopausal. The risk of breast cancer was more than multiplied doubled in women with vitamin B_{12} levels in the lowest levels of B_{12} compared to women with higher levels, this was confirmed by [23 ,24] .

4-Marital Status

The statistical analysis indicates that, B_{12} and Hcy values tend to increase slightly among in unmarried women compared to women married, There is a non-significant differences of homocysteine, B_{12} and folate (0.3194µmol/l),(0.2837pmol/l) and (0.2761ng/ml), respectively (Tab. 4).

Table. 4 .Effect of Marital Status in the mean± SD of Homocysteine , B12 and folate level for patients group .

Marital Status	No. (%)	Mean ± SD		
		Hcy µmol/L	B_{12} pmol/l	Folat ng/ml
Married	69 (86.25%)	13.07 ± 0.65	523.60 ± 49.69	8.86 ± 0.45
Single	11 (13.75%)	14.60 ± 1.69	644.74 ± 164.0	7.29 ± 0.65
LSD-value	---	2.094 NS	164.37 NS	1.988 NS

***P*-value**	---	**0.3194**	**0.2837**	**0.2761**
NS: Non-significant.				

The increase risk of breast cancer happening by reproductive factors, which contain absence of pregnancy, delaying the first pregnancy and changes in the menstrual cycle. All of these factors lead to increased exposition to breast estrogen and increase the incidence of breast cancer. Unmarried women are more likely to be diagnosed with breast cancer at later stages than married women [25] This was confirmed by [26]. While Palestinian women in North West Bank which also showed that there is non- significant difference between cases and controls in regard to marital status [27].

5-Type of suckling:

Married woman with breast cancer were divided in three categories depending on the type of suckling: natural suckling , artificial and both types. It was noticed that the ratio for these type were 24 (37.50%) , 4 (6.25%) and 36 (56.25%) Respectively. Mean± of Hcy , B_{12} and folate level with breast cancer were signification (*P≤0.01*) that explained in (Tab. 5)**.**

Table.5. Effect the type of suckling in the mean level SD of Homocysteine ,B_{12} and folate for patients**.**

Type of suckling	**No. (%)**	**Mean ± SD**		
		Hcy µmol/L	**B_{12} pmol/l**	**Folateng/ml**
Natural	**24 (37.50%)**	**13.93 ± 1.36**	**423.51 ± 39.94**	**9.06 ± 0.82**
Artificial	**4 (6.25%)**	**12.02 ± 3.50**	**328.28 ± 97.60**	**6.56 ± 1.39**
N + A	**36 (56.25%)**	**12.96 ± 0.73**	**548.88 ± 76.41**	**8.77 ± 0.62**
LSD-value	**---**	**2.178 NS**	**207.46 ***	**2.155 ***

***P*-value**	---	**0.3729**	**0.0496**	**0.0461**
NS: Non-significant.				

Feeding of the most important agents that play a large role in saving against breast cancer, specially.

In the early ages women who give birth and breastfeed their children breastfed before the age of twenty may have greater protection in contrast, for example, give birth to their first child at the age of thirty doubles the risk of breast cancer compared to couples to their first child at the age of 25 not to give birth to a child doubles the risk of injury three times. One study indicated the role of breast feeding to slightly lower breast cancer risk, especially if it is continued for 1 to 2 years. [28],This was confirmed by [29] . But there is no proof that Hcy , B_{12} and folate that effect with breastfed in this study or any studies.

6- Family history:

Result of serum homocysteine measurement revealed significant increase ($p\leq$ 0.05) with breast cancer patients that positive family history ,where the mean of homocysteine level(14.66 ± 1.16)µmol/l compared with mean of patients that negative family history(12.41 ± 0.64) µmol/l .Furthermore B_{12} was showed non- significant increase ($p\geq$0.05) with positive family history for patients with mean (583.84 ± 78.24) pmol/l compared with mean (512.68 ± 61.49) pmol/l in negative family history**,** Measuring of folate level in serum of patients group(9.08 ± 0.65) ng/ml with family history risk , but decreases slightly in group with negative family history (8.37 ± 0.51)ng/ml as shown in (Tab. 6).

Table.6. Effect of family history in the mean ±SD of Homocysteine ,B_{12} and folate level for patients.

Family history	**No. (%)**	**Mean ± SD**		
		Hcy µmol/L	**B_{12} pmol/l**	**Folatng/ml**
Yes	**31 (38.75%)**	**14.66 ± 1.16**	**583.84 ± 78.24**	**9.08 ± 0.65**
No	**49 (61.25%)**	**12.41 ± 0.64**	**512.68 ± 61.49**	**8.37 ± 0.51**

LSD-value	---	**1.0755 ***	**106.48 NS**	**1.784 NS**
***P*-value**	---	**0.0477**	**0.3690**	**0.2966**
NS: Non-significant.				

Family history of breast cancer is one of the most fully-determined breast cancer danger factors. Studying family history of tumor can highlight the hereditary tendency to develop the disease , several breast cancer susceptibility genes have been fixed. Change in gene with high pen trance breast cancer genes, such as *BRCA1* and *BRCA2*, confer a greater than tenfold relative danger of breast cancer and are responsible for disease in a substantial portion of very high risk families [30].It has been noticed the relationship between breast cancer and family history by [31].Women whose first-degree relatives have had breast cancer have been showed to be at increased danger of being absorbed with tumor in breast tissue. Moreover, the risk has been noticed to increase with increasing number of influenced first-degree relatives [32].

Reference

1-Ahmedin ,J.; Rebecca ,S.; Elizabeth ,W.; Taylor ,M.; Jiaquan ,X and Michael, J. T.2007. Cancer Statistics. *CA Cancer Journal Clinical*, 57: 43-66.

2- Girish, C.; Vijayalakshmi, P.;Ramesh, M.; Chandu, B. and Sreekanth, N.(2014).A review on breast cancer . *IJPBS*,4: *47-54.*

3- Al-Isawi,A.(2016) Breast Cancer in Western Iraq: Clinico pathological Single Institution Study. *Advances in Breast Cancer Research*. 5: 83-89.

4-AL-Bedairy, I. H.; AL.Azzawie,H.F.; AL-Kazaz, A.K and Ghanim, M.(2014). Immunohistochemical evaluation of human epidermal growth factor receptor 2 and estrogen and progesterone receptors in Iraqi breast carcinoma women. *International Journal of Advanced Research*, 2: 168-177.

5- Al-Alwan, N. and Al-Rufaee, F.L .(2010) .Comparative demographic and clinicopathological study on the behavior of mammary carcinoma in three Iraqi governorates (Baghdad, Hilla and Karbala). *Journal Medicine Baghdad* , 52:419-423.

6- Ceber, E .; Mermer, G. and Okcin F. (2013). Breast cancer risk and early diagnosis applications in Turkish women aged 50 and over. *Asian Pacific Journal of Cancer Prevention* , 14: 5877-82.

7- Fitzpatrick,L. D.; Hodgson, N.; Ciliska, D .; Peirson, L.; Gauld, M. and Liu, Y . (2011).Canada Breast Cancer Screening. (Online) Hamilton, ON: McMaster University. Review .

8- Wu LL, W. 2002 .Hyperhomocysteinemia is a risk factor for cancer and a new potential tumor marker. *Clinical Chimistry*. 322:21-28.

9-Brosnan, J.2001**.** Homocysteine in the kidney. In: Ralph C, Jacobsen DW, editors. Homocysteine in health and Disease. Cambridge: Cambridge University Press.

10- Amy, S. 2007.Investigation in to the relationship between physical activity and total plasma homocysteine . Department of Community Health. Queen's University Kingston, Ontario, Canada.

11-Wu, L.; Wu, J.2002.Hyperhomocysteinemia is a risk factor for cancer and a new potential tumor marker. *Clinical Chimistry*. 322 :21-28.

12-Smulders, Y.M; Smith ,D.E; Kok, R.M; Teerlink, T.; Swinkels, D.W; Stehouwer, C.D; Jakobs, C.2006 . Cellular folatevitamer distribution during and aftercorrection of vitamin B_{12} deficiency: a case for the methylfolate trap. *British Journal of Haematology*, 132:623-629.

13- Statistical Analysis System (SAS) . 2012., User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.

14-Jazet, I. M.;de Groot ,G.H;Tuijnebreyer,W.E. ;Fogteloo, A.J. 2007. Cardiovascular risk factors after bariatric surgery: Do patients gain more than expected from their substantial mass loss? *European Journal of Internal Medicine* . 18:39-43 .

15-Liselotte, M.;Sabroe , 2003. Hyperhomocysteinemia due to folate deficiency. Impact on haemostasis and vascular biology. Dan Med Bull 51:129 -130 .

16- Smith, A.D; Kim, Y.I;Refsum, H. 2008. Is folic acid good for everyone? *The American journal of clinical nutrition*, 87:517-533.

17-Xinran, X. and Jia, C. 2009. One-carbon metabolism and breast cancer: An epidemiological perspective.*J Genet Genomics* , 36: 203–214.

18-Lewis, S.J.; Harbord, R.M.; Harris, R.; Smith, G.D. 2006 . Meta-analyses of observational and genetic association studies of folate intakes or levels and breast cancer risk. *J. Natl. Cancer Inst*,98:1607–1622.

19–Ericson, U. 2010. Folate Associations with breast cancer depending on intake, metabolism, genetic variation and estrogen receptor status. Department of Clinical Sciences in Malmo, Lund University.

20- Stolzenberg, S. R. ; Chang, S.C; Leitzmann, M.F;. Johnson, K.A.; Johnson, C.; Buys, S.S.; Hoover, R.N.; Ziegler, R.G.2006. Folate intake, alcohol use and postmenopausal breast cancer risk in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. *The American journal of clinical nutrition*, 83:895-904.

21- Cancer Registry of Norway . 2012. Cancer incidence, mortality, survival and prevalence in Norway. Report .

22- Sallis, J. 2000. Age-related decline in physical activity: a synthesis of human and animal studies. *Medicine and Science in Sports and Exercise.* 32:1598-1600.

23- Ulrika, E. 2010. Associations with breast cancer depending on intake,metabolism, genetic variation and estrogen receptor status. Nutrition Epidemiology Research Group Department of Clinical Sciences in Malmo - Lund University.

24- Mahabir, S.; Susan, E.; Laura, J.; David, J.; Beverly, A.; Terry,J. H. and Philip, R. T. 2008. Measures of adiposity and body fat distribution in relation to serum folate levels in postmenopausal women in a feeding study. *European Journal of Clinical Nutrition* , 62: 644–650.

25-Kuo, T.M.; Mobley, L.R.;Anselin, L. 2011. Geographic disparities in late-stage breast cancer diagnosis in California. *Health and Place*, *17*: 327-334.

26-Kravdal, H. and Syse, A. 2011 . Changes over time in the effect of marital status on cancer survival. *BMC Public Health*, *11*: 1-3.

27- Alaa ,D .2009.Risk factors of breast cancer among Palestinian women in North West bank ,thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Public Health, Faculty of Graduate Studies, An-Najah National University, Nablus, Palestine.

28- Gartner, L.M; Morton, J.; Lawrence, R.A. 2005. "Breastfeeding and the use of human milk". *Pediatrics* 115 : 496–506.

29-Chung, M.; Raman, G.; Chew, P.; Magula, N.; DeVine, D.; Trikalinos, T.; Lau, J .2007. "Breastfeeding and maternal and infant health outcomes in developed countries, Evidence report technology assessment *(Full Rep)*, 153: 1–186.

30- Turnbull, C.; Rahman, N. 2008.Genetic predisposition to breast cancer: past, present, and future .Annual Review of Genomics and Human Genetics,9:321-45.

31- Schwartz, G.F.; Hughes, K.S.; Lynch, H.T.; Fabian, C.J.; Fentiman, I.S.; Robson, M.E.; Domchek, S.M.; Hartmann, L.C.; Holland, R.; Winchester, D.J. 2008. Proceedings of the international consensus conference on breast cancer risk, genetics, and risk management. *Cancer*,113: 2627-2637.

32-Saxena, S. ; Rekhi, B.; Bansal, A.; Bagga, A.; Murthy, N.S. 2005. Clinicomorphological patterns of breast cancer including family history in a New Delhi hospital, India-A cross-sectional study. *World Journal of Surgical Oncology* , 3: 67- 74.

علاقة مستوى الهوموسستين، الفوليت،B12 المصل وسرطان الثدي في عينة من النساء في مدينة بغداد – العراق

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شملت الدراسة على عينة تكونت من 80 حاله من النساء المصابات بسرطان الثدي بأعمارتراوحت من (30−80) سنه بالإضافة الى عينة شملت على عشرين من النساء الصحيحات أستعملت كمجموعة سيطره. قدر مستوى الهوموسستين, فيتامين B12 والفوليت في مصل الدم لكل من مجموعة المرضى والسيطره كذلك تم دراسة مؤشر كتلة الجسم ,نوع الرضاعه وتاريخ العائله الوراثي. اظهرت نتائج التحليل الاحصائي عند مستوى الأحتماليه ($p\leq0.05$) فروقات معنويه بين متوسطي مستوى الهوموسستين للنساء المصابات ومجموعة السيطره, حيث بلغت قيمة المتوسطين ± 13.18 (0.61)µmol/l و(l9.78 ± 0.60) µmol/l لكل منهما وعلى التوالي. كذلك لوحظ هنالك فرق معنوي بين مستويات الفوليت للنساء المصابات وبين مجموعة السيطره بمتوسط قدره ng/ml (8.64 ± 0.40) و ng/ml (13.19 ± 1.19) لكلا المجموعتين وعلى التوالي . ايضا تم ملاحظة وجود فرق معنوي عند مستوى الأحتماليه ($p\leq0.05$) لكل من قيم الهوموسستين, B12 والفوليت مع تأثير عامل العمر , الحاله الزوجيه, نوع الرضاعه وتاريخ العائله بالأصابه بالمرض.