Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.47750/jptcp.2023.30.13.031

Evaluation some immune aspects in Iraqi women patients with breast cancer

Israa Allawi Luaibi Al-Hraishawi[1*], Ziad M. Alkhozai[2], Hadeel Jabar Neama Almuoswi[3]
[1,2,3]Department of Medical Biotechnology, College of Biotechnology, University of Al-Qadisiyah, Iraq.
***Corresponding Author:** Israa Allawi Luaibi Al-Hraishawi, Department of Medical Biotechnology, College of Biotechnology, University of Al-Qadisiyah, Iraq,
Email: asraaallawei@gmail.com

Submitted: 24 March 2023; Accepted: 10 April 2023; Published: 13 May 2023

ABSTRACT

The current experiment have been conducted to to determinate the activity of natural killer cells of CD56 and dendritic cells of CD1a by estimating the expression for both markers depending on flow cytometery technique . Blood samples are taken from 60 patients women with breast cancer to represent patients group and 40 women's without breast cancer to represent control group, the ages of both groups ranged from 30 to more than 50 years in the Al.Diwaniyah General Teaching Hospital in AL-Qadisyaha province .

Also ROC curve analyzed is used to demonstrate the accuracy and validity of previous parameters in detecting women with breast cancer and to estimate the sensitivity and specificity.

The results of this study show that the patients group have recorded highly significant ($p<0.01$) superiority in natural killer cells of CD56 (23.780) comparison with control group which is (10.708) , in addition to the patients group have appeared significant increase in dendritic cells of CD1a on control group by 5.741..

Age groups have showed significant ($p<0.05$)effect on natural killer cells of CD56 , there is decline in natural killer cells of CD56 with age progress, while the decline in dendritic cells of CD1a with age progress do not reach to significant level.

Analysis of ROC curve for CD56 and CD1a has been showed that these characters are excellent indicators to detecting actually patients with breast cancer,the sensevity and specificity for CD56 and CD1a is 90.90,20 and 25% respectively. The cut-off for CD56 and CD1a is more 11.85 and more than 2.65 respectively.

Keywords: *breast cancer, Naturall killer cells, dendritic cells,CD56,CD1a.*

INTRODUCTION

Breast cancer is one of the most prevalent cancers affecting women. with a mortality rate of more than one million per year throughout the world. In 2020, there were 2.3 million women diagnosed with breast cancer and 685, 000 deaths globally. The most common cancer in the world as of the end of 2020 was breast cancer, which had been diagnosed in 7.8 million women in the previous five years (WHO,2020). In women, breast cancer accounted for almost 24.5% of cases, placing it first for incidence and mortality in the vast majority of countries worldwide(Sung *et al*.,2020). and 4.4 million cases are predicted in 2070(Soerjomataram and Bray,2021).

Despite being frequently thought of as a disease that only affects women, breast cancer (BC) can also affect men. Male Breast Cancer (MaBC) is uncommon (Makdissi et al .,2022).The development of this condition is influenced by both genetic and environmental factors (Benvidi *et al*., 2015).This condition ranks as the second leading cause of cancer-related death in women between the ages of 45 and 55 years (Jemal *et al*.,2009), and is the second most common reason for cancer-related death. Breast cancer affects almost one in eight women, and the majority of the time it requires complete tissue removal, chemotherapy, radiotherapy, and hormone therapy (Heravi -Karimovi *et al*.,2006).Age, menarche, menopause, parity, breastfeeding, use of exogenous hormones or oral contraceptives, obesity, lack of exercise, diet, smoking, alcohol consumption, and family history of breast cancer or other cancers are just a few of the breast cancer-associated risk factors that have been suggested to play a role in the development of breast cancer. Breast cancer is regarded as clinically heterogeneous and Various pathological conditions and clinical behaviors are present in complex disease. Therefore, it is generally accepted that the accumulation of genetic abnormalities contributes to the development of breast cancer that is becoming more and more invasive. This heterogeneity is strictly linked to individuals and tumors of genetic variability(Cavallaro *et al*., 2012).Natural Killer cells are innate immune system lymphocytes that are programmed to identify and eliminate "modified-self" cells, such as cancerous and virus-infected cells, without the need for pre-sensitization (Taouk *et al* .,2019),CD56 (also known as neural cell adhesion molecule, NCAM-1) was discovered to be a polySia chain carrier protein on NK and CD3+ T cells (Sato and Kitajima ,2021). The development of adaptive immune responses depends on dendritic cells (DC), the most powerful antigen-presenting cells (Liu and Nussenzweig ,2010),CD1a expression as a marker for dendritic cells , It appears that epithelial cells express CD1a molecules. during the antigen capture and processing stage, associated DC, decreasing in density as the DC phenotype reaches its maximum potential to present an antigen (Cernadas *et al*.,2009).

This work was aimed to monitor the role of Natural killer and Dendritic cell in patient of breast cancer in Al-Qadisiyah province/Iraq.

MATERIALS AND METHODS

Study group

This study included 60 female patients with the breast cancer of AL-Qadisyah provience who attended to oncology unit of AL-Diwaniyah teaching hospital . Their age range from(30–40), (40–50),50 years and over .The study included apparently healthy control 40 individual.

Blood samples

Two ml of blood from 60 patients 40 healthy women were collected ,kept in a plastic tube containing an anticoagulant (EDTA) , and transported to the laboratory within 24h for immunological tests by flowcytometry .

Inclusion Criteria

The included criteria were all women patients with breast cancer.

Exclusion Criteria

Excluded criteria all men infected with breast cancer,women who was suffering from other chronic disease and cancers.

Ethical approval

This work was done meeting all the formal regulations of AL- Qadisyah university and the ministry of health of iraq also all patients were informed and formal acceptance were obtained.

Principle of flow cytometry :

The basic principle of flow cytometry was the passage of cells in single file in front of a laser so they could been detected, counted and sorted. Cell components were fluorescently labeled and then excited by the laser to emit light at varying wavelengths (Bendall et al., 2012).

Procedure

The counts of CD56 and Cd1a T-cell were determined by using , PerCp Anti-Human CD3Antibody (Miltenyi Biotic), PE Anti-Human CD56 antibody [RPA-T4] and FITC Anti-Human Cd1a antibody [OKT-8] kits (Elabscience Biotechnology/ USA) according to the manufacturer's instructions:. one hundred µl of anticoagulated (edta, acd) whole blood was added to the bottom of a 12x75 mm polystyrene tube, with percp anti-human cd3antibody and pe anti-human cd56 antibody [rpa-t4] or with fitc anti-human cd1a antibody [okt-8], vortex and incubated in the darkness at room temperature for 45 minutes.one ml of reagent A and Reagent B respectively of (Lysing and Fixation Kit

Elabscience Biotechnology/USA) was added to each sample and vortex. Incubated for 20 minute in the dark.Five minutes of centrifuging at 5500 rpm. Resolve in (1 ml) of pbs after removing the supernatant. After that, centrifuge at (5500 rpm) for 5minutes. Poured off supernatant and resuspend in (600 µl) of pbs. Mixed by micropipette and transfer to could tube , vortex.Samples were investigated by Bricyte E6-Flow cytometer (Bricyte E6 clinical Software, Mindray/Bio-Medical Electronics/China).The results were compared to the normal values according to normal value .

Statistics analysis

The results of this study were analyzed by using factorial analysis treatment groups with two levels (patients group and control group)and the second factor was age groups with three levels (30-40,40-50 and 50year and over),general linear method was used according to spss (2013).Receiver operating characteristic (ROC) curve was used to estimate true positive cases and true negative cases and to calculate the sensitivity and specificity also to know the accuracy of used test ,indetection the patients with breast cancer (zweignd and campbell,1993).

RESULTS AND DISCUSSION

All data of female patients who attended to the hospital of ages were the number of the category was from 30-40 years (9) and the percentage was (15%) ,and the number of the category 40-50 years was (15) and the percentage 25% ,The number of the category 50 years and over was (36) and the percentage 60%. as showen in figure(1).

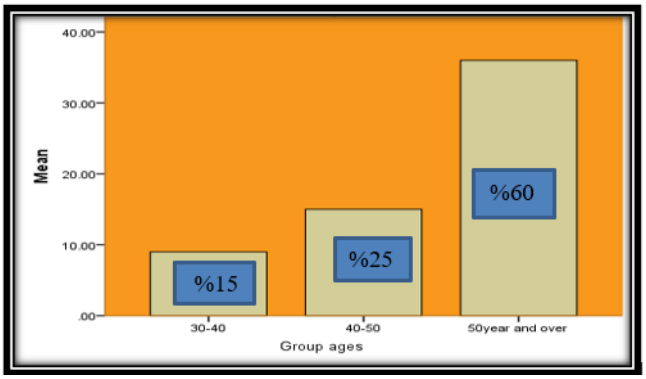

FIGURE 1: Distribution of patients with breast cancer according to age

Profiling of immune marker (CD56) on natural killer cells

Table (1)shows the overall mean of CD56 expression on NKs is 18.551.

The results show that the mean value of the patients and control groups are 23.780 and 10.7080 ,respectively , the patients group was significantly ($p<0.01$) superior to the control group by 13.072.

TABLE 1: The means ± standard deviations (SD) of CD56 expression on NKs

Study groups		Age groups	Mean	±SD	Number
Patients group		30-40	35.040	13.249	9
		40-50	22.242	8.748	15
		50 year and over	11.167	3.379	36
		Total	23.780 A	12.105	60
Control group		30-40	14.367	2.048	22
		40-50	12.117	1.844	12
		50 year and over	8.941	2.653	6
		Total	10.708 B	3.118	40
Total		30-40	24.852 A	15.19	31
		40-50	21.117 A	8.57981	27
		50 year and over	9.587 B	3.005	42
		The Overall mean	18.551	11.515	100

The CD56 marker is one of the adhesion molecules involved in the interaction between NK cells and target cells (Robertson *et al* .,1990).The increment in the expression of CD56 marker on Natural Killer cells is due to activation of NK cells upon the non-specific immune response(Poli *et al* .,2009).

Lanier(2005) and Roberti, *et al*.,(2012) have been found the increased expression of CD56 marker on NKs due to the activation of natural killer cells, NK cells directly recognize their target through a complex array of regulatory (activating and inhibitory) receptors that monitor cell surfaces of autologous cells for an aberrant expression of self-ligands and cell stress markers, which frequently occurs in tumors.

Naturall killer cells activation results in the polarization of cytotoxic granules (containing cytolytic efector molecules such as granzymes and perforins) toward the immunological synapse and in their directed exocytosis.

The, following membrane perturbation by perforin at the immune synapse, granzymes enter the target cells where they can cleave numerous substrates in the cytoplasm and nucleus leading to the induction of apoptosis (Boivin *et al*.,2009). ,Although the perforin/granzyme pathway is the main mechanism of NK-mediated cytotoxicity, NK cells can also induce apoptosis in target cells through the tumor necrosis factor (TNF) receptor pathway (Zamai *et al*.,1998).

Active NK cells can secrete a variety of cytokines and chemokines that might exert direct anti-tumor activity or promote innate and adaptive responses(Klingemann,2013).

Hematopoietic expression of CD56 seems to be confined to activated immune cells exhibiting some level of cytotoxic properties(Van Acker *et al*.,2017).

The highly expresssion of CD56 marker on Natural Killer cells might be involved in the formation and stabilizing of the immunological synapse allowing enhanced NK activation and stimulation of target cell death (Taouk *et al*,2019). (Figure 2) shows the expression of CD56 marker on NKs according to patients groups that use the flow cytometry technique.In this findings ,this may added to concept that cancer cells may activate the non-specific immunity via natural killer cells.

.

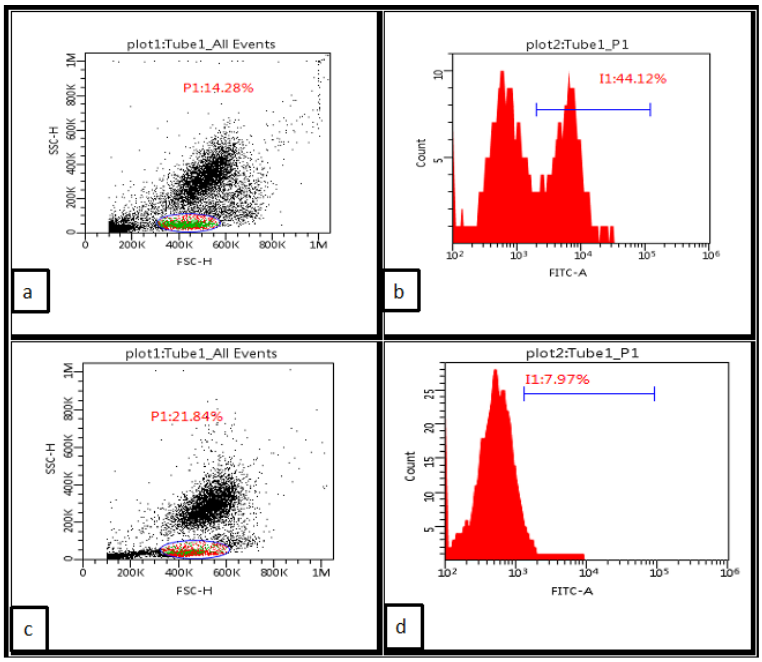

FIGURE 2. Diagrams showing a forward scatter height (FSC-H) and side scatter height (SSC-H) the CD56 markers according to study groups that use the flowcytometry technique. (a,b) : CD56 markers percentage in Breast Cancer Women. (c,d) : CD56 markers percentage in control groups

Age groups appear significantly ($p<0.01$) effect on CD56 marker .there are decline in CD56 marker with progress ages (Table 2) .

The decline may be due to the decrease in immunity function with progressing age is termed immunity aging . decline in CD56 marker which may have considerable implications for NK cell function in elderly cohort (chidrawar *et al*.,2006).

Similar results have been stated by castle(2000).Also ,the statistical analysis shows there is no significant effect of interaction among treatment and age groups

The Recevier Operating Chraracteristic curve of the CD56 marker

(Figure 3) shows the area under the curve (AUC) of CD56 marker for both patients and control groups where is 0.907,and this value is an excellent indicater of the reliability of CD56 marker indicating cancer patients.it is found that the value of cut-off point is more than 11.85,this value means that any women more than 11.85 is patient with cancer breast .

The percentage of sensitivity at this point is 90%, this percentage means that 90%(54) of the patients are actually cancerous and 10%(6) of total patients are false negative also ,it is found that specifity percentage is 20%,meaning that 80% (32) of healthy control are actually healthy and 20% (8) of them are false positive (Table 2).

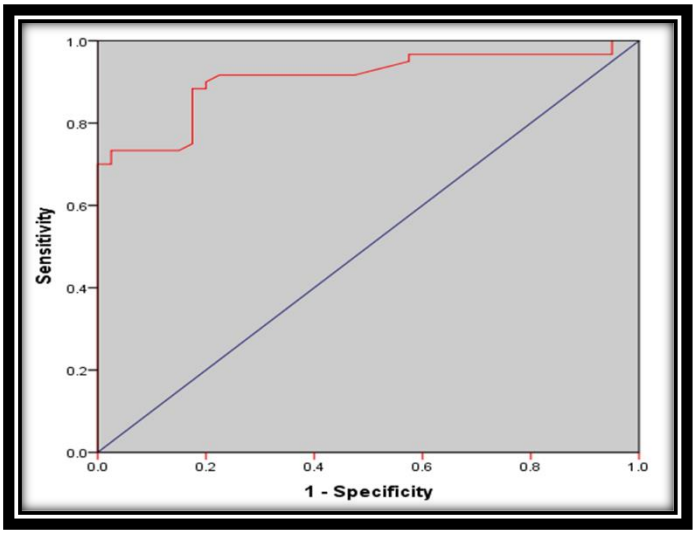

Figure (3) :- The recevier operating chraracteristic curve of the CD56

TABLE 2: The parameters of the Receiver operating characteretic curve of the CD56Marker.

parameters	Patients group		Control group	
Number	60		40	
AUC	0.907			
Standard error	0.03			
95% CI *	0.849-0.966			
Sensivity	90 %			
Specificity	20%			
Cut-off point	>11.85			
Criterion	True positive	54	True negative	32
	False negative	6	False negative	8
	Total positive cases	62	Total positive cases	38

Estimation of immuno-marker (CD1a) marker expression on dendritic cells

Table (3) explains the expression of study groups and age groups for both groups (patient and control groups) the expression of CD1a on dendritic cells , The overall mean of CD1a on dendritic cells is 5.746 ,it was significantly ($p<0.01$) affected by study group , patients group have inquired higher expression CD1a of dendritic cells compared to control group and the differences between these groups are 5.741.

TABLE 3: The means ± standard deviations of CD1a expression on DCs

Study groups	Age group	Mean	±SD	Number
	30-40	8.446	4.332	9

Patients group	40-50	8.427	3.703	15
	50 year and over	5.786	4.870	36
	Total	8.042 A	4.303	60
Control group	30-40	2.958	1.275	22
	40-50	2.850	0.973	12
	50 year and over	1.793	0.6491	6
	Total	2.301 B	1.064	40
Total	30-40	7.647 a	4.480	31
	40-50	5.996 a	3.967	27
	50 year and over	2.952b	3.165	42
	Overall mean	5.746	4.413	100

The increase in CD1a expression of dendritic cell may due to induction to the tumour environment which has a capability to initiate an immune response to the malignancy in the host organism, which results lead to a better prognosis (Szpor *et al*.,2021).

The reason for increased CD1a expression on DCs may led to activation crucial adaptive immune response regulators and essential for T cell-mediated cancer immunity. To produce co-stimulatory signals that activate T lymphocytes, dendritic cells must mature before they can carry tumor antigens to the draining lymph nodes and cross-present tumor antigens.

Dendritic cells undergo an upregulation in the cell surface expression of the CD83 protein throughout the maturation process, which is crucial for controlling the adaptive immunological responses that are mediated by dendritic cells,these data are concordant with the data obtained by Fujimoto and Tedder,(2006),while the results of this study contradict the finding by researchers Coventry *et al*.,(1996) ; hillenbrand *et al*.,(1999);Coventry *et al*.,(2002) found The low CD1a expression on dendritic cell due to the anti-tumour immune response is ineffective;possible mechanisms include loe DC cell number ,poor antigen capture or presentation ,failure of DC maturation ,lack of IL-2 cytokine production ,down-regulation of cytokine receptors.

Reichert *et al*.,(2001) demonstrated the increase in DCs is often associated with better survival and lower recurrence rates in cancer patients.

The high level of DC in breast cancer appears to be associated with a poor clinical outcome(Treilleux *et al*.,2004; Sisirak *et al*.,2012).

(Figure 4) shows The expression of CD1a marker of dendritic cells according to patients groups and control groups.

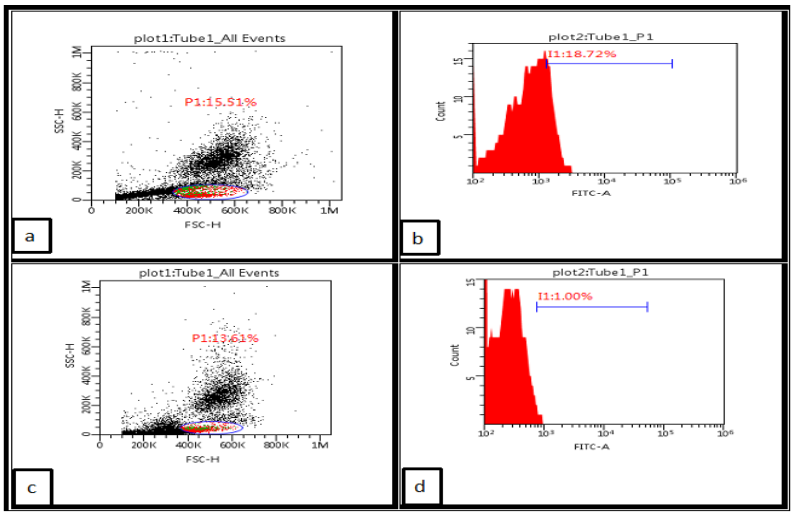

FIGURE 4: Diagrams showing a forward scatter height (FSC-H) and side scatter height (SSC-H) the CD1a marker on dendritic cells according to study groups that use the flowcytometry technique. (a,b) : CD1a markers percentage in Breast Cancer Women. (c,d) : CD1a markers percentage control groups.

The age group have significantly ($p<0.05$) effect on CD1a,there are significantly (0.05) decline in CD1a with age progress,the decrese of this trait may be belong to the decrease in immunity function with progressing age is termed immunity senescence. Some times due to other chronic diseases or some immunosuppressive drugs ,life style also may play a role.

Agrawal and Gupta ,(2011) demonstrated The response of DCs to infections is compromised either due to a reduction in their frequency or due to reduced expression of PRRs on CDC and PDC subsets. The effect seems to be primarily on the cytokine secretion by DCs. The antigen capture and migratory capacity of CDCs is also severely affected with age, suggesting that the functions related to motility of DCs may be affected. The capacity of DCs to prime T cells is also impaired.

The Recevier Operating Chraracteristic curve of the CD1a marker

The result of the ROC for CD1a (figure 5),refers that the CD1a has a high sensetivity (90%) and a low specificty (25%).CD1a The two criteria indicate that the CD1a is efficient for detection of patients cases with breast cancer .

The value of cut-off is more than 2.65 which means that any women with value more than 2.65 is patient with breast cancer(Table 4).

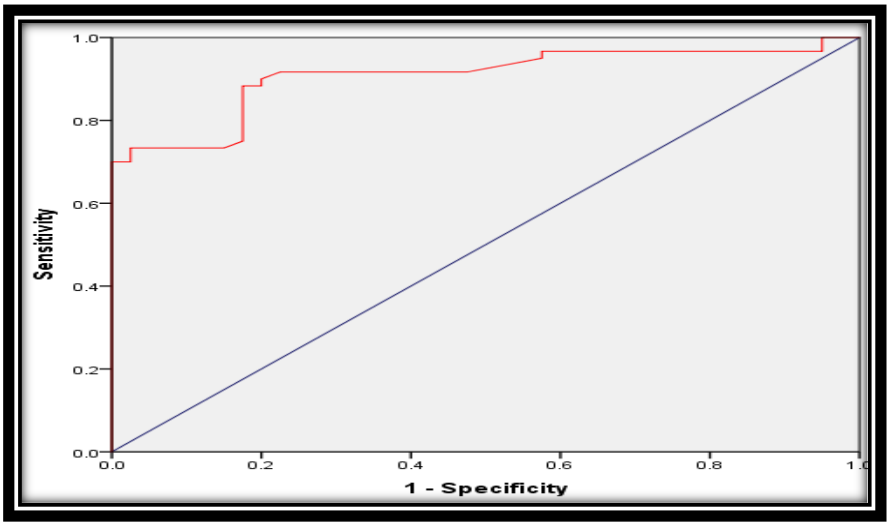

FIGURE 5: The recevier operating chraracteristic curve of the CD1a

TABLE 4: The parameters of the Receiver operating characteretic curve of the CD1a Marker.

parameters	Patients group	Control group
Number	60	40
AUC	0.916	
Standard error	0.028	
95% CI*	0.862-0.970	
Sensivity	90 %	
Specificity	25%	
Cut-off point	>2.65	
Criterion		
	True positive 54 False negative 6 Total positive cases 64	True negative 30 False negative 10 Total positive cases 36

REFRENCES

1. Agrawal, A., & Gupta, S. (2011). Impact of aging on dendritic cell functions in humans. Ageing research reviews, 10(3), 336-345.
2. Bendall, SC.; Nolan, GP.; Roederer, M. and Chattopadhyay, PK. (2012). A deep profiler‟s guide to cytometry. Trends Immunol. , 33: 323 332.
3. Benvidi, A., Dehghani Firouzabadi, A., Dehghan Tezerjani, M., Moshtaghiun, S. M., Mazloum-Ardakani, M., & Ansarin, A. (2015). A highly sensitive and selective electrochemical DNA

biosensor to diagnose breast cancer. Journal of Electroanalytical Chemistry, 750, 57–64.
4. Boivin, W. A., Cooper, D. M., Hiebert, P. R., & Granville, D. J. (2009). Intracellular versus extracellular granzyme B in immunity and disease: challenging the dogma. *Laboratory investigation*, *89*(11), 1195-1220.Breen N, Gentleman JF, Schiller JS. (2011). Update on mammography trends: comparisons of rates in 2000, 2005, and 2008. Cancer; 117: 2209- 2218.
5. Castle, S. C. (2000). Clinical relevance of age-related immune dysfunction. Clinical Infectious Diseases, 31(2), 578-585.
6. Cavallaro, S., Paratore, S., de Snoo, F., Salomone, E., Villari, L., Buscarino, C., Ferraù, F., Banna, G., Furci, M., Strazzanti, A., Cunsolo, R., Pezzino, S., Gangi, S.and Basile, F. (2012). Genomic analysis: toward a new approach in breast cancer management. Crit. Rev. Oncol. Hematol., 81: 207-223
7. Cernadas, M., Lu, J., Watts, G., & Brenner, M. B. (2009). CD1a expression defines an interleukin-12 producing population of human dendritic cells. Clinical & Experimental Immunology, 155(3), 523-533.
8. Chidrawar, S. M., Khan, N., Chan, Y. L., Nayak, L., & Moss, P. A. (2006). Ageing is associated with a decline in peripheral blood CD56bright NK cells. Immunity & Ageing, 3(1), 1-8.
9. Coventry BJ, Lee PL, Gibbs D, Hart DN (2002) Dendritic cell density and activation status in human breast cancer –CD1a, CMRF-44, CMRF-56 and CD-83 expression. Br J Cancer 86: 546 – 551
10. Coventry, B. J., Weeks, S. C., Heckford, S. E., Sykes, P. J., Bradley, J., & Skinner, J. M. (1996). Lack of IL-2 cytokine expression despite Il-2 messenger RNA transcription in tumor-infiltrating lymphocytes in primary human breast carcinoma: selective expression of early activation markers. *The Journal of Immunology*, *156*(9), 3486-3492.
11. Fujimoto, Y., & Tedder, T. F. (2006). CD83: a regulatory molecule of the immune system with great potential for therapeutic application. *Journal of medical and dental sciences*, *53*(2), 85-91.
12. Heravi Karimovi, M., Pourdehqan, M., Jadid Milani, M., Foroutan, S. K., & Aieen, F. (2006). Study of the effects of group counseling on quality of sexual life of patients with breast cancer under chemotherapy at Imam Khomeini Hospital. *Journal of mazandaran university of medical sciences*, *16*(54), 43-51.
13. Hillenbrand, E. E., Neville, A. M., & Coventry, B. J. (1999). Immunohistochemical localization of CD1a-positive putative dendritic cells in human breast tumours. *British journal of cancer*, *79*(5), 940-944.
14. Jemal, A., Siegel, R., Ward, E., Hao, Y., Xu, J., & Thun, M. J. (2009). Cancer statistics, 2009. *CA: a cancer journal for clinicians*, *59*(4), 225-249.
15. Klingemann, H. G. (2013). Cellular therapy of cancer with natural killer cells—where do we stand?. *Cytotherapy*, *15*(10), 1185-1194.
16. Lanier, L. L. (2005). NK cell recognition. *Annu. Rev. Immunol.*, *23*, 225-274.
17. Liu, K., & Nussenzweig, M. C. (2010). Origin and development of dendritic cells. Immunological reviews, 234(1), 45-54.
18. Makdissi, F. B. A., Santos, S. S., Bitencourt, A., & Campos, F. A. B. (2022). An introduction to male breast cancer for urologists: epidemiology, diagnosis, principles of treatment, and special situations. *International braz j urol*, *48*, 760-770.
19. Poli, A., Michel, T., Thérésine, M., Andrès, E., Hentges, F., & Zimmer, J. (2009). CD56bright natural killer (NK) cells: an important NK cell subset. *Immunology*, *126*(4), 458-465.
20. Reichert, T. E., Scheuer, C., Day, R., Wagner, W., & Whiteside, T. L. (2001). The number of intratumoral dendritic cells and ζ-chain expression in T cells as prognostic and survival biomarkers in patients with oral carcinoma. Cancer: Interdisciplinary International Journal of the American Cancer Society, 91(11), 2136-2147.
21. Roberti, M. P., Mordoh, J., & Levy, E. M. (2012). Biological role of NK cells and immunotherapeutic approaches in breast cancer. *Frontiers in Immunology*, *3*, 375.
22. Robertson, M. J., Caligiuri, M. A., Manley, T. J., Levine, H. E. R. B. E. R. T., & Ritz, J. E. R. O. M. E. (1990). Human natural killer cell adhesion molecules. Differential expression after activation and participation in cytolysis. *Journal of immunology (Baltimore, Md.: 1950)*, *145*(10), 3194-3201.Romagne F, Vivier E. Natural killer cell-based therapies. F1000 Med Rep 2011; 3: 9.
23. Sato, C., & Kitajima, K. (2021). Polysialylation and disease. Molecular Aspects of Medicine, 79, 100892.
24. Sisirak, V., Faget, J., Gobert, M., Goutagny, N., Vey, N., Treilleux, I., ... & Bendriss-Vermare, N. (2012). Impaired IFN-α Production by Plasmacytoid Dendritic Cells Favors Regulatory T-cell Expansion That May Contribute to Breast Cancer ProgressionpDC in Breast Cancer Pathophysiology. Cancer research, 72(20), 5188-5197.
25. Soerjomataram, I., & Bray, F. (2021). Planning for tomorrow: Global cancer incidence and the role of prevention 2020–2070. *Nature reviews Clinical oncology*, *18*(10), 663-672.
26. Spss,(2013).statistical pachage of social science.version22,aplication Guide:copy Right by spss Inc.USA.

27. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021.
28. Szpor, J., Streb, J., Glajcar, A., Frączek, P., Winiarska, A., Tyrak, K. E., ... & Hodorowicz-Zaniewska, D. (2021). Dendritic cells are associated with prognosis and survival in breast cancer. *Diagnostics*, *11*(4), 702.
29. Taouk, G., Hussein, O., Zekak, M., Abouelghar, A., Al-Sarraj, Y., Abdelalim, E. M., & Karam, M. (2019). CD56 expression in breast cancer induces sensitivity to natural killer-mediated cytotoxicity by enhancing the formation of cytotoxic immunological synapse. Scientific reports, 9(1), 8756.
30. Treilleux, I., Blay, J. Y., Bendriss-Vermare, N., Ray-Coquard, I., Bachelot, T., Guastalla, J. P., ... & Lebecque, S. (2004). Dendritic cell infiltration and prognosis of early stage breast cancer. Clinical Cancer Research, 10(22), 7466-7474.
31. Van Acker, H. H., Capsomidis, A., Smits, E. L., & Van Tendeloo, V. F. (2017). CD56 in the immune system: more than a marker for cytotoxicity?. Frontiers in immunology, 8, 892.
32. World Health Organization ,2020.Estimated of the new cases in 2020,worldwide .New Global Cancer Data.(WHO,2020).
33. Zamai, L., Ahmad, M., Bennett, I. M., Azzoni, L., Alnemri, E. S., & Perussia, B. (1998). Natural killer (NK) cell–mediated cytotoxicity: differential use of TRAIL and Fas ligand by immature and mature primary human NK cells. *The Journal of experimental medicine*, *188*(12), 2375-2380.
34. Zweignd,M.H.,campbell.G.(1993).Receiver-operating characteristic (ROC) plots : a fundamental evaluation tooin clinical medicine .clinical chemistry.,39:561-577.