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Determination of Interferon Gamma Protein in Serum of Breast Cancer Patients Using the ELISA

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Abstract

Interferon gamma (IFN-γ) is a cytokine involved in the induction and modulation of various immune responses. INF-γ has recently been associated with breast cancer and the stage of the disease. Also, it can be used as a cancer marker in determining disease activity. The ELISA test is a technique for identifying antigens in biological materials. The ELISA test is used for the detection of antibodies against target antigen through highly antibody-antigen interactions. In this study, the blood samples were taken from 88 Iraqis, 30 healthy controls, and 58 breast cancer patients (ranging from 35 to 52 years of age). During a visit to the AL Amal National Hospital for Cancer Treatment in Baghdad, Iraq, these patient samples were taken October 2020 - January 2021. Depending on the color changes to yellow, the results of this study were determined under wavelength of 450 nm to reveal the optical density (OD) via spectrophotometrically. The OD value is proportional to the amount of human IFN-γ in the sample of the healthy control and the cancer patients. The blood concentration of IFN-γ was substantially greater in women with breast cancer than in the control group (43.15 ± 4.68 pg/ml vs. 18.8 ± 3.89 pg/ml), respectively. Our results showed an elevation of Interferon-gamma (IFN-γ) in most of the serum samples from women with breast cancer. The conclusions of this study are the main findings, INF-y protein was employed as a marker and assistance in diagnosing breast cancer based on the results of the calculation of protein concentration from the ELISA test.

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1. Introduction

The only member of the type II interferon family is interferon gamma (IFN) [1]. The JAK-STAT1 system and a specific heterotetrameric receptor complex are required for canonical IFN signaling. The IFN receptor is a tetramer complex consisting of two IFNGR1 and two IFNGR2 subunits [2]. JAK1 and JAK2 are constitutively

associated with IFNGR1 and IFNGR2 in this complex. IFN is recognized by IFNGRs, which undergo a conformational change and activate and transphosphorylates JAK proteins [3].

STAT1 is phosphorylated by JAK1 and 2, resulting in the formation of a homodimer known as gamma-activated factor. It is enriched in the nucleus known as gamma-activated sites, or GAS, on the regulatory regions of target genes, modulating IFN target gene transcription in a cell type-dependent manner [4]. Interferon regulatory factor 1 (IRF1), a transcription factor that induces other IFN target genes, is one of the early IFN induced genes [4]. In addition to canonical IFN signaling, IFN upregulates non-canonical pathways for IFNGRs as well as some genes in a STAT1-independent manner [5].

In addition, IFN signaling can activate NF-B, which regulates gene expression [6, 7]. Breast cancer (BC) is a major public health issue around the world. More than 70% of breast cancer cases are estrogen receptor-positive (ER+), whereas “triple-negative BC” is highly metastatic and lacks ER, progesterone receptor (PR), and epidermal growth receptor (EGR) (HER2) [8, 9]. Numerous IFN-target genes, on the other hand, have been linked to chemotherapy resistance. IFN, in particular, has the ability to cause apoptosis and cell cycle arrest [10, 11]. IFN autocrine signaling has also been discovered in BC cells. Short stimulation intervals (0.5 – 2 h) activate some IFN-target genes in specific cell types [12, 13].

The ELISA test however, is a technique for identifying antigens in biological materials [14, 15]. Like other forms of immunoassays, an ELISA uses antibodies to detect a target antigen through highly precise antibody-antigen interactions [16, 17]. Cancer is one of the most pressing health issues of our day and the main cause of mortality among the population. Cancer poses a greater risk to me than it does to those with corona, which is dangerous in asthma cases [18]. Therefore, nanomaterials should not be exposed to cancer patients since they are harmful to their health [19, 20].

This study aims to use the ELISA technique to assess the concentration of INF-protein in the blood serum of breast cancer patients and healthy controls. The ELISA test is a method of detecting antigens in biological samples.

2. Experimental Procedure

1.1. Sample Collection: Blood samples were obtained from 88 Iraqi, 30 healthy control and 58 patients with breast cancer withage ranges (35-52) years. These patient samples were taken at the AL Amal National Hospital for Cancer Treatment in Baghdad, Iraq (October 2020 - January 2021). The blood sample was collected in a gel tube free of anticoagulant to separate the serum from measuring the concentration of IFN-γ protein using the ELISA technique. All serum samples were kept frozen at 4℃.

1.2. IFN-y Protein Concentration Assay: Is the gold standard immunoassays used for detecting and measuring the concentration of Human IFN-γ in human serum. The components of this kit are: Micro ELISA Plate, Reference Standard, Concentrated Biotinylated Detection Ab, Concentrated Horseradish Peroxidase Conjugate (HRP Conjugate), Reference Standard & Sample Diluent, Biotinylated Detection Ab Diluent, HRP Conjugate Diluent, Concentrated Wash Buffer, Substrate Reagent, Stop Solution and Plate Sealer.

1.3. Calculated the Concentration of the IFN-γ: the color changes to yellow was determined under the wavelength of 450 nm for revealing the optical density (OD) via spectrophotometer. The OD value is proportional to the amount of human IFN-γ in the sample of healthy and patients. Finally, comparing the OD of all samples to the standard curve (Figure 1).

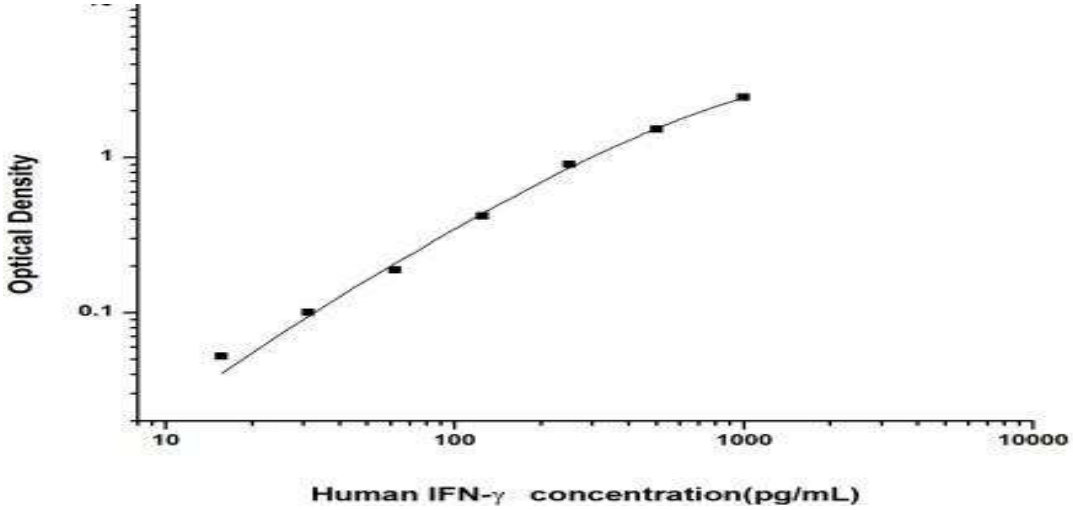

Figure 1: Standard curve for the concentration INF-y protein in serum.

1.4. Procedure Technique: All components were brought to room temperature. Then, the washing buffer was prepared for washing the plate after each step. Furthermore, the reference standard and diluent for samples were set before gently flipping and mixing it with a pipette. Then serial dilutions were made as 1000, 500, 250, 125, 62.5, 31.25, 15.63, 0 picogram /ml (Figure 2).

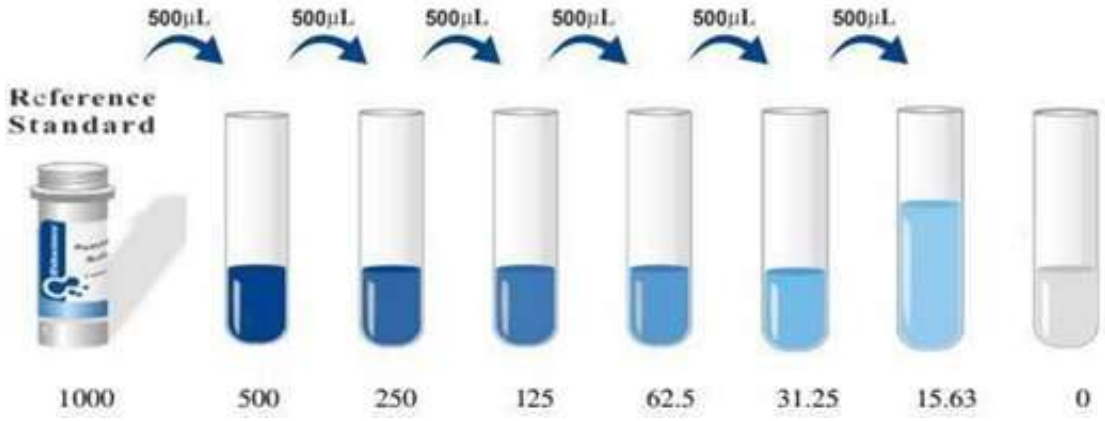

Figure 2: Dilutions of standard solutions.

Subsequently, the stock tube centrifuged and diluted the 100 concentrated biotinylated detection Ab with biotinylated recognition Ab diluent to 1 working solution. Afterward, concentrated Horseradish Peroxidase (HRP) conjugate diluent is used to dilute the 100 concentrated Horseradish Peroxidase (HRP) conjugate to 1work solution. Later, each solution concentration was put in duplicate, side by side, to one well each and the plate was sealed with an incubator at 37℃ for 90 min. Consequently, solutions should be introduced to the bottom of the micro ELISA plate well, avoiding contacting the inner wall and creating bubbling. Then, each well was drained from liquid without cleaning it, then adding 100 of biotinylated detection Antibody solution that performs to each well right away. Also, the sealer for plates was used to seal the plate which gently combined all of the ingredients incubate at 37℃ for 1 hour. Furthermore, the solution was removed from each well by aspirating or decanting it., and then adding 350 l of wash buffer to every well. Aspirating or decanting the solutions from each well after 12 minutes of soak and wiping them off with clean absorbent paper. This washing

procedure should be done three to four times. Additionally, 100 l of HRP Conjugate working solution was poured into each hole. The Plates Sealer was used to seal the plates, incubating them for thirty minutes at 37°C. Aspirating or decanting the solution out of each well. The processes continued 3–5 times for a total of five washes. Each was completed with 90 micro liters of Substrate Reagent. Then, a fresh coat of plate sealer was applied. It was incubated at 37°C for about 15 minutes. The amount of light in the room should have been sufficient to be kept away from the plate. Depending on the actual change in color, the time to react can be decreased or prolonged, but not further than 30 min. Each well was filled with 50 microliters of Stop Solution. The stop solution wasmixed in with the wash buffer in the same sequence. In conclusion, Eachwell's optical density (OD value) was estimated simultaneously utilizing a microplate reader set to 450 nm. ELISA plate composed from 96 wells as shown in (Figure 3)

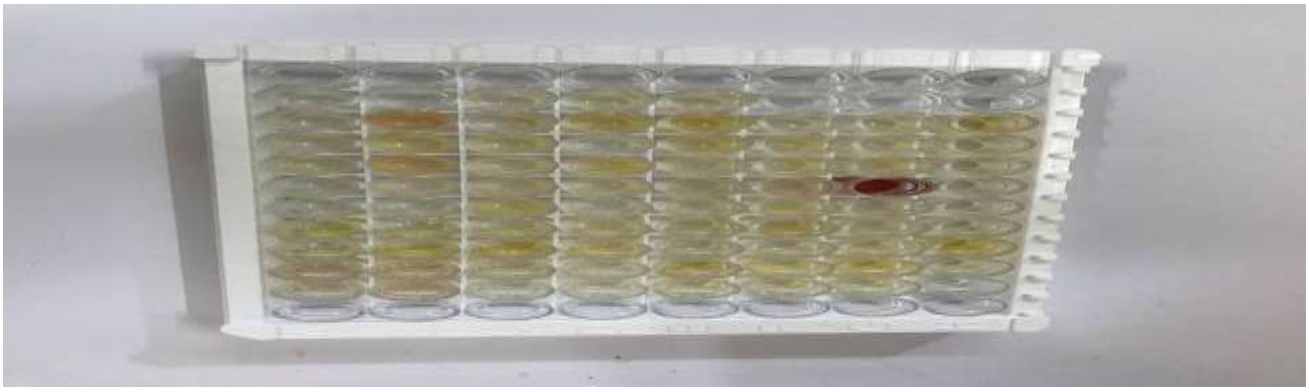

Figure 3: ELISA plate.

3. Results and Discussion

Breast cancer cells morphologically changed after 24 hours of IFN stimulation. As a result, it washypothesized that: 1) IFN may influence gene expression patterns in a longer-term manner than previously thought (brief exposure: 1–2 h). 2) In both cell types, most of these IFN-regulated genes might describe some of the reported morphological alterations [12]. This study identified that the serum IFN-γ concentration was measured for all breast cancer patients group and healthy control of the present study. The results demonstrated an elevation of Interferon-gamma (IFNγ) in the majority of the breast cancer patient's serum samples. The serum concentration of IFN-γ among women who have breast cancer was significantly higher than that in the control group (43.15 ± 4.68 pg/ml vs. 18.8 ± 3.89 pg/ml, respectively) (Figure 4 and Table 1, 2, and 3), which was in agreement with another study [13].

In addition, Rohani Borj *et al.* showed that the concentration of IFN-γ in blood serums and adjacent tissues of the tumor in patients with malignant breast tumors was significantly higher than that in the benign breast tumor samples [14]. Furthermore, many studies showed the key role of interferon-γ (IFN-γ) in cellular immunity activation and, consequently, the antitumor immune response [15]. Based on its anti-proliferative functions, pro-apoptotic, and cytostatic, IFN-γ is considered potentially useful for adjuvant immunotherapy for different forms of cancer [13, 15].

Table 1: Concentration of Interferon gamma (IFN-γ) in serum of healthy subjects and women with breast cancer

IFNγ Concentration pg/ml	Group	No. of Samples	Mean ± Std. Error	Std Mean	P Value
	Breast cancer	**58**	**46.15 ± 4.68**	**1.35**	**0.046**
	Healthy (control)	**30**	**17.8 ± 3.89**	**1.7**	

Table 2: The results of the Concentration of Interferon gamma (IFNγ) in serum of healthy subjects.

NO	Group	Description	Concentration pg/ml
1	A	Healthy (control)	12
2	A	Healthy (control)	16
3	A	Healthy (control)	19
4	A	Healthy (control)	17
5	A	Healthy (control)	12
6	A	Healthy (control)	18
7	A	Healthy (control)	19
8	A	Healthy (control)	15
9	A	Healthy (control)	15.4
10	A	Healthy (control)	17
11	A	Healthy (control)	18.2
12	A	Healthy (control)	19.8
13	A	Healthy (control)	14.6
14	A	Healthy (control)	17.8
15	A	Healthy (control)	14.6
16	A	Healthy (control)	19.8
17	A	Healthy (control)	12.6
18	A	Healthy (control)	11.9
19	A	Healthy (control)	15.8
20	A	Healthy (control)	13.1
21	A	Healthy (control)	15.8

22	A	Healthy (control)	11.4
23	A	Healthy (control)	12.6
24	A	Healthy (control)	17.4
25	A	Healthy (control)	16.6
26	A	Healthy (control)	11.8
27	A	Healthy (control)	18.9
28	A	Healthy (control)	16.2
29	A	Healthy (control)	17
30	A	Healthy (control)	16.8

Table3: The results of the Concentration of Interferon gamma (IFNγ) in serum of cancer patients.

31	**B**	**Breast cancer**	**53.4**
32	B	Breast cancer	60.2
33	B	Breast cancer	53.4
34	B	Breast cancer	67.8
35	B	Breast cancer	57.9
36	B	Breast cancer	53.1
37	B	Breast cancer	44.6
38	B	Breast cancer	41.9
39	B	Breast cancer	51.8
40	B	Breast cancer	57.2
41	B	Breast cancer	50.2

42	B	Breast cancer	57.8
43	B	Breast cancer	45.8
44	B	Breast cancer	43.4
45	B	Breast cancer	63.4
46	B	Breast cancer	42.2
47	B	Breast cancer	53
48	B	Breast cancer	61.4
49	B	Breast cancer	53.4
50	B	Breast cancer	55.8
51	B	Breast cancer	51.4
52	B	Breast cancer	59.8
53	B	Breast cancer	62.2
54	B	Breast cancer	67.4
55	B	Breast cancer	50.2
56	B	Breast cancer	52.6
57	B	Breast cancer	59.4
58	B	Breast cancer	63.8
59	B	Breast cancer	45.7
60	B	Breast cancer	49.8
61	B	Breast cancer	55.8

62	B	Breast cancer	43.8
63	B	Breast cancer	44.2
64	B	Breast cancer	60.2
65	B	Breast cancer	54.6
66	B	Breast cancer	57
67	B	Breast cancer	49.6
68	B	Breast cancer	46.1
69	B	Breast cancer	50.6
70	B	Breast cancer	57.4
71	B	Breast cancer	60.6
72	B	Breast cancer	45.7
73	B	Breast cancer	61.8
74	B	Breast cancer	63.4
75	B	Breast cancer	56.6
76	B	Breast cancer	58.2
77	B	Breast cancer	49
78	B	Breast cancer	61.4
79	B	Breast cancer	52.6
80	B	Breast cancer	55.4
81	B	Breast cancer	48.6

82	B	Breast cancer	49.4
83	B	Breast cancer	45.8
84	B	Breast cancer	54.6
85	B	Breast cancer	51.8
86	B	Breast cancer	63.4
87	B	Breast cancer	56.6
88	B	Breast cancer	48.6

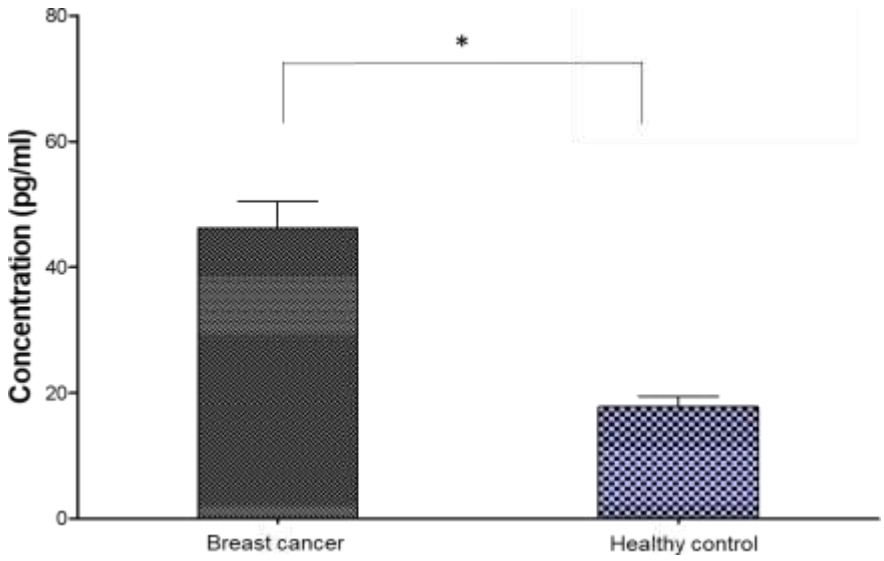

Figure 4: Level of Interferon gamma (IFN-γ) in serum of healthy subjects and women with cancer**.**

Nonetheless, several investigations found that IFNGR1 expression was lower in mammary cancers. In addition, secreted substances from the same cancer cells, stroma, and immune cells enriched the microenvironment of these tumors [16]. Because T lymphocytes and natural killer cells secrete IFN, persistent interferon signaling may have a deleterious impact on IFNGR1 expression in BC cells [17].

In addition, the expression of certain IFN-downregulated genes found in our investigation was higher in breast tumor samples than in normal mammary tissue. According to the findings, the IFN/ IFNGR1 signaling pathways are disrupted in BC patients' breast tumors, resulting in the de-repression of certain target genes such as HTR7, KIF20B, PSMD8, AURKA, BIRC3, and RALA. AURKA, a kinase-encoding gene, is linked to BC progression in terms of incidence, and its inhibitors are being studied as potential therapeutics for the disease [16]. The downregulation of AURKA by IFN shows that the interferon's anti-tumor actions may be mediated through transcriptional suppression of BC-related genes. IFNGR1 expression was reduced in stage 4 advanced mammary tumors. As a result, IFN signaling may decline, altering the regulation of its target genes and jeopardizing its anti-tumor efficacy [17].

Nonetheless, the mechanisms underpinning IFN-mediated repression regulation remain to be explained further. Furthermore, it focused on common IFN-regulated genes in BC cells, and it is critical to identify the BC cell type. Finally, it is also worth noting that IFN's overall gene regulation may play a role in the ultimate cellular behavior in BC. Some researchers have discovered a unique immune control route for HER2-expressing malignancies. We show that the ubiquitin-proteasomal pathway is important for maintaining HER2 expression in HER2pos and luminal BCs and that reduced expression is linked to poor survival. While HER2-targeted medications have improved survival in HER2positive BC patients, those with locally progressed or metastatic disease frequently become refractory or resistant to these treatments found that a Th1 immune response, mediated predominantly by IFN-g, can improve the effectiveness of all HER2-targeted drugs evaluated in both susceptible and resistant BC cells [21]. In addition, the study established a critical relationship between Th1 immunity and HER2-mediated responses, presenting a fresh potential to add to the arsenal of HER2pos cancer treatments [21]. Enhanced infiltrating lymphocytes have previously been linked to increased PCR chances and long-term survival in HER2positive BC [22].

This research identifies a method through which IFN-y increases the activity of the ubiquitin-proteasomal pathway, causing HER2 to be broken down from the cell surface, resulting in apoptosis and tumor senescence [23]. IFN-g, when used in conjunction with HER2-targeted treatments, can help enhance tumor growth inhibition and proliferation arrest. However, chemotherapy used in combination with HER2-directed medicines has many side effects. In addition, when used in conjunction with HER2-directed therapy, low doses of IFN-g or vaccines stimulating the anti-HER2 CD4 Th1 response have been shown to significantly affect HER2 tumor growth [24, 25]. When paired with IFN-g, even those resistant to antibody conjugates like T-DM1 improve, implying that combining Th1 immune therapies can effectively reduce the concentration and improve the effectiveness of all HER2-directed therapies.

Compared to cells that were susceptible to HER2-targeted drugs, cells resistant to HER2-targeted therapies were more sensitive to the growth arrest and senescence induction mediated by IFN-g [21, 24]. In patients who grow resistant to HER2-directed therapeutic regimens, boosting anti-HER2 CD4 Th1 responses may be very effective [26]. As a result, Th1 immunity via IFN-g may offer an alternate immunological approach to treating HER2 resistance, with IFN-g and HER2-directed therapy possibly benefiting even sensitive tumors [22, 24]. HER2 cancers have not been particularly responsive to checkpoint therapy, but because IFN-g promotes antigen presentation and PD1 expression on T cells, it is possible that HER2 BC will be as well. There is no apparent mechanism for how trastuzumab and pertuzumab increase PCR and survival in HER2 BC [27]. Pertuzumab is thought to interfere with HER2-HER3 dimerization, resulting in reduced proliferation [20,22, 28].

4. Conclusions

The primary outcomes of this study are as follows: firstly, based on the obtained results of the protein concentration estimation from the ELISA test, INF-y protein was used as a marker and assistant in diagnosing breast cancer.

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Conflict of Interest

The authors declare that they have no conflict of interest.

References

[1] M. Ivanchenko, G. Thorlacius, M. Hedlund, V. Ottosson, L. Meneghel, S. Björkander, A. Ossoinak, J. Tingström, K. Bremme, E. Sverremark, and K. Gemzell, “Natural killer cells and type II interferon in Ro/SSA and La/SSB autoantibody-exposed newborns at risk of congenital heart block,” *Annals of the Rheumatic Diseases*, vol. 80, no. 2, pp. 194-202, 2021.

[2] Q. Xu, D. Deng, H. Guo, H. Yuan, W. Zhang, B. Wang, Y. Lu, D. Chen, and S. Zhang, “Comprehensive comparison of thirteen kinds of cytokine receptors from the endangered fish Chinese sturgeon (Acipenser sinensis),” *Developmental & Comparative Immunology*, vol. 123, pp. 104-132, 2021.

[3] E. Huarte, M. Peel, A. Juvekar, P. Dubé, S. Sarah, L. Stephens, B. Stewart, B. Long, P. Czerniak, J. Oliver, and P. Smith, "Ruxolitinib, a JAK1/JAK2 selective inhibitor, ameliorates acute and chronic steroid-refractory GvHD mouse models," *Immunotherapy*, vol. 13, no. 12, pp. 977-987, 2021.

[4] C. Tecalco, M. Macías, J. Ramírez, and B. Méndez, "Identification of genes modulated by interferon gamma in breast cancer cells," *Biochemistry and Biophysics Reports*, vol. 27, pp.101-153, 2021.

[5] F. Fei, P. Siegal, and S. Wei, "Characterization of estrogen receptor-low-positive breast cancer," *Breast Cancer Research and Treatment*188(1):225-235, 2021.

[6] K. Yamagata, E. Uzu, Y. Yoshigai, C. Kato, and M. Tagami, "Oleic acid and oleoylethanolamide decrease interferon-γ-induced expression of PD-L1 and induce apoptosis in human lung carcinoma cells" *European Journal of Pharmacology*, vol. 903, pp. 174116, 2021.

[7] Ali M. Sameen, Majid S. Jabir, and Mohammed Q. Al-Ani,"Therapeutic combination of gold nanoparticles and LPS as cytotoxic and apoptosis inducer in breast cancer cells," AIP Conference Proceedings 2213, 020215 (2020) https://doi.org/10.1063/5.0000161.

[8] Majid S. Jabir, Aya A. Hussien, Ghassan M. Sulaiman, Nahi Y. Yaseen, Yaser H. Dewir, Mona S. Alwahibi, Dina A. Soliman & Humaira Rizwana (2021) Green synthesis of silver nanoparticles from Eriobotrya japonica extract: a promising approach against cancer cells proliferation, inflammation, allergic disorders and phagocytosis induction, *Artificial Cells, Nanomedicine, and Biotechnology*, vol. 49, 2021, DOI: 10.1080/21691401.2020.1867152.

[9] Al-Ziaydi AG, Al-Shammari AM, Hamzah MI, Kadhim HS, Jabir MS. Newcastle disease virus suppress glycolysis pathway and induce breast cancer cells death. Virusdisease. 2020 Sep;31(3):341-348. doi: 10.1007/s13337-020-00612-z. Epub 2020 Jun 27. PMID: 32904847; PMCID: PMC7458979.31(3):341-348

[10] L. Vučković, A. Klisic, J. Raonić, and J. Vučinić, "Comparative study of immunohistochemical determination of breast cancer molecular subtypes on core biopsy and surgical specimens," *European Review for Medical and Pharmacological Sciences*, vol. 25, pp. 3990-6, 2021.

[11] R. Mardis, "A decade's perspective on DNA sequencing technology," *Nature*, vol. 470 (7333), pp. 198-203, 2011.

[12] R. Mardis, "DNA sequencing technologies: 2006–2016," *Nature protocols*, vol. 12(2), pp. 213-8, 2017.

[13] A. Bagale, "Influence of Gamma-Secretase Inhibitor On Cytokine-Induced Apoptosis in Breast Cancer Cell Lines" (Doctoral dissertation, Kent State University), 2020..

[14] M. Rohani Borj, AR. Andalib, A. Mohammadi, SM. Hoseiniharouni, H. Pourghadamyari, H. Azizi, T. Golmahammadi, K. Moghaddas Ghahfarokhy, "Evaluation of IL- 4, IL-17, and IFN-γ Levels in Patients with Breast Cancer", *Int. J. Basic Sci. Med.*, vol. 2, no. 1, pp. 20-24, 2017.

[15] Q. Duan, H. Zhang, J. Zheng, and L. Zhang, "Turning cold into hot: firing up the tumor microenvironment," *Trends in Cancer*, vol. 6, no. 7, pp. 605-618, 2020.

[16] J. Klarquist, R. Cantrell, A. Lehn, K. Lampe, M. Hennies, k. Hoebe, and M. Janssen, "Type I IFN Drives Experimental Systemic Lupus Erythematosus by Distinct Mechanisms in CD4 T Cells and B Cells," *ImmunoHorizons*, vol. 4, no. 3, pp. 140-52, 2020.

[17] M. García, and M. Redondo, "Protein kinase targets in breast cancer," *International journal of molecular sciences*, vol. 18, no. 12, pp. 25-43, 2017.

[18] Basima Q. H. Primer and Probe Designing to Detect SNP rs 4073 in Interleukin-8 Gene in Iraqi Patients with Bronchial Asthma. Journal of Applied Sciences and Nanotechnology, 2021; 1(3): 51-57.

[19] N. Hussein, and M. Khadum, "Evaluation of the Biosynthesized Silver Nanoparticles Effects on Biofilm Formation," *Journal of Applied Sciences and Nanotechnology*, vol. 1, no. 1, pp. 23-31, 2021.

[20] A. Taha, "Perspectives of Photonics Technology to Diagnosis COVID–19 Viruses: A Short Review," *J. Appl. Sci. Nanotechnol.*, vol. 1, pp.1-6, 2021.

[21] T. Triulzi, L. Forte, V. Regondi, M. Di, C. Ghirelli, L. Carcangiu, L. Sfondrini, A. Balsari, and E. Tagliabue, "HER2 signaling regulates the tumor immune microenvironment and trastuzumab efficacy," *Oncoimmunology*, vol. 8, 1, pp. 1512942, 2019.

[22] J. Eustace, F. Madden, J. Fay, M. Collins, W. Kay, M. Sheehan, S. Furney, B. Moran, A. Fagan, G. Morris, and A. Teiserskiene, "The role of infiltrating lymphocytes in the neo-adjuvant treatment of women with HER2-positive breast cancer," *Breast Cancer Research and Treatment*, vol. 187, no. 3, pp. 635-45, 2021.

[23] L. Castejón, T. Montoya, C. Alarcón, and M. Sánchez, "Potential protective role exerted by secoiridoids from Olea europaea L. in cancer, cardiovascular, neurodegenerative, aging-related, and immunoinflammatory diseases," *Antioxidants*, vol. 9, no. 2, pp. 149, 2020.

[24] I. García,, M. Ricote, A. Ruiz, B. Fraile, R. Paniagua, and M. Royuela, "Influence of IFN-gamma and its receptors in human breast cancer," *BMC cancer*, vol. 7, no. 1, pp. 1-1, 2007.

[25] H. Fluhr, T. Seitz, and M. Zygmunt, "Heparins modulate the IFN-γ-induced production of chemokines in human breast cancer cells," *Breast cancer research and treatment*, vol. 137, no. 1, pp. 109-118, 2013.

[26] H. Yuan, Q. Yang, L. Zhu, P. Liu, C. Wang, and B. Wang, "Interleukin-7 enhances the in vivo anti-tumor activity of tumor-reactive CD8+ T cells with induction of IFN-gamma in a murine breast cancer model," *Asian Pacific journal of cancer prevention*, vol. 15, no. 1, pp. 265-271, 2014.

[27] P. Toomey, K. Kodumudi, A. Weber, L. Kuhn, E. Moore, A. Sarnaik, and S. Pilon, "Intralesional injection of rose bengal induces a systemic tumor-specific immune response in murine models of melanoma and breast cancer," *PloS one*, vol. 8, no. 7, pp. e68561, 2013.

[28] B. Alomashi, and J. Al-Kilabi, "Association of IL-17 and IFN-g in Patients of Breast Cancer Infected and non-Infected with Toxoplasmosis," *Annals of the Romanian Society for Cell Biology*, vol. 26, pp.11172-11181, 2021.