

RESEARCH ARTICLE

Assessment of Interleukin -8 , Interferon - Γ and Vitamin – D in Group of Patients with different types of Tumor in Babylon Province of Iraq

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ABSTRACT

Chemokines such as interleukin – 8 and interferon - γ in association with Vitamin D play a critical role in the early innate immune response to invading pathogens. Significant progress has been made over the past years in the understanding of cytokine function as well as Vit. D. Cytokines especially IL-8 and interferon - γ agonists are currently under investigation in anticancer therapies for their ability to activate immune cells and promote inflammation. Here we assess the state of Cytokines (IL – 8 and IFN- γ) and Vitamin D level in patients with different type of tumors and their relation to patient's demographic and clinical features. Case control study among all newly diagnosed patients with different types of tumor registered in the malignant registry center of Babylon, clinically diagnosed and confirmed by consultant physician including 210 patients were conducted from January until August 2016. Among all patients who are eligible for study, we chose 100 patients and the others were excluded because either they have started medication for tumor or have more severe cases. Some of them refused to participate in the study. Consent was taken from all patients in addition to the center permission for this study. Serum level of IL-8, INF- γ was assessed by ELISA in the laboratory department of Merjan medical city/ immunology unit using specific available kits. Mean age of patients was 49.9 years, mostly at 40s age group. No significant statistical difference was there according to the gender of patient and control groups. More patient and control groups were from urban area. Majority of patients had breast malignancy (52%) and the other sites represented different sites of uterine, colonic, liver, ovarian, thyroid, stomach, and gall bladder and prostatic malignancies. Ten percent of patients have family history of malignancy and chronic disease associated with malignancy is present in 44% of the patients. Significant low concentration of IL-8, INF- γ and Vitamin D was found in different types of tumors in initial diagnosis with obvious significant correlation of high IL-8 concentration with demographic and clinical features of the patients. A significant positive correlation of patient ages with tumor types, IL-8 and Vitamin D as well as same correlation of tumor type with IL-8 and Vit.D is observed. According to cytokines and vitamin-D there is a direct correlation between IL-8 and Vit –D. Assessment of such types of receptors may play a role in anti-tumor therapy and research of related aspect. As cytokines are critical mediators of the immune system, further studies are needed to shed more light on the relationship between vitamin D and cytokine levels, as well as on the possible immune modulatory action of vitamin D on the immune system.

Keywords: Interleukin -8, INF- γ , Vit.D, ELISA, Cytokines.

1. INTRODUCTION

Cytokines are chemicals made by some immunological cells. They are crucial in controlling the growth and activity of other

immune system and blood cells. Interleukins are a group of cytokines that act as chemical signals between white blood cells. Interferons are chemicals that help the body resist virus

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infections and cancers. It boosts the ability of certain immune cells to attack cancer cells. It may also slow the growth of cancer cells directly, as well as the blood vessels that tumors need to grow [1].

Tumor cells can acquire the expression of various chemokine(s) and/or receptor(s) to exploit these signaling pathways for their own growth and survival. Co-opting the IL-8/IL-8R axis is now known to be an established occurrence in human cancer, and has been shown to promote tumor progression by multiple means. Functional studies have revealed that tumor-derived IL-8 can function in a paracrine manner to alter the composition of immune cells within the Tumor Micro Environment (TME), and also in an autocrine fashion to facilitate oncogenic signaling, angiogenesis, and pro-metastatic qualities like invasion and resistance [2]. Recent advances now suggest that tumor-derived IL-8 can bias the TME into an immunosuppressive state by increasing the infiltration of neutrophils and myeloid-derived suppressor cells, TME induction via multiple means has been shown to promote IL-8 in numerous experimental systems. For example, treatment of colon cancer cells with TNF- and TGF- upregulated IL-8 expression [3]. Conversely, treatment of tumor cells with IL-8 is known to promote EMT; IL-8 induces EMT in colon cancer cells, nasopharyngeal carcinoma cells, and breast cancer cells [4]. IL-8-driven cancer stemness appears to be a common mechanism that is conserved across multiple tumor types, and, importantly, that this mechanism is amenable to therapeutic intervention [5]. Cancer treatment is in the midst of a revolution brought about by the successful development and implementation of immunotherapies. The collective work of many laboratories indicates that the IL-8/IL-8R axis is a common pathway to resistance for tumor cells. IL-8 is able to activate stemness and anti-apoptotic processes that circumvent killing by cytotoxic immune cells [6]. Th1-type cytokine, Interferon-gamma (IFN-gamma) is of particular relevance, stimulates several anti-proliferative, tumoricidal biochemical pathways in macrophages and other cells and also tumor cell lines. Although the anti-proliferative strategy of the immune system aims to inhibit the growth of malignant cells, it can also affect T-cell response and thus contribute to the development of immunodeficiency in cancer

patients and may develop as a long-term side-effect of the anti-proliferative and pro-apoptotic mechanisms elicited within the Th1-type immune response, and enhanced production of pro-inflammatory cytokine IFN-gamma seems to be critically involved [7].

The Interferons (IFNs) are a family of cytokines that protect against disease by direct effects on target cells and by activating immune responses. The production and actions of IFNs are finely tuned to achieve maximal protection and avoid the potential toxicity associated with excessive responses. IFNs are back in the spotlight owing to mounting evidence that is reshaping how we can exploit this pathway therapeutically [8].

IFN γ is a cytokine that plays a pivotal role in antitumor host immunity, elicits potent antitumor immunity by inducing Th1 polarization, CTL activation, and dendritic cell tumoricidal activity. However, there are significant discrepancies in our understanding of the role of IFN γ as an antitumor cytokine. Several mechanisms, including IFN γ insensitivity and the down regulation of the MHC complex, have been regarded as the reasons for this discrepancy, but they do not fully explain it, although IFN γ is thought to be a representative antitumor cytokine, it actually has dual roles: one as a hallmark of antitumor immunity and the other as an inducer of the immune escape phenomenon through various mechanisms. The optimization of immunotherapy according to the local immune environment is important [9].

Vitamin - D is the name given to a group of fat-soluble prohormones (substances that usually have little hormonal activity by themselves but that the body can turn into hormones). Vitamin - D helps the body use calcium and phosphorus to make strong bones and teeth. Skin exposed to sunshine can make vitamin D, and vitamin D can also be obtained from certain foods. In studies about cancer cells, vitamin D has been found to have several activities that might slow or prevent the development of cancer, including promoting cellular differentiation, decreasing cancer cell growth, stimulating cell death (apoptosis), and reducing tumor blood vessel formation (angiogenesis). A number of epidemiologic studies have investigated whether people with higher vitamin D intakes or higher blood levels of vitamin D have lower risks of specific cancers. Researchers are also

beginning to study vitamin D analogs--chemicals with structures similar to that of vitamin D--which may have the anticancer activity of vitamin D but not its ability to increase calcium levels [10].

The potential role of vitamin D in functioning of the immune system is supported by identifying vitamin D receptors in most immune system cells including macrophages, neutrophils, dendritic cells, and T-lymphocytes. Accordingly, vitamin D status is thought to be one of the key parameters contributing to healthy functioning of the immune system, including regulation of the cytokine environment. Clinically, vitamin D deficiency was found to be associated with several autoimmune inflammatory diseases such as multiple sclerosis, type I diabetes, and rheumatoid arthritis [11].

2. PATIENTS, MATERIALS AND METHODS

- **Patient data base:** We collected all data available about the patients registered in the malignant registry center of Babylon with different types of tumors; including 100 adult patients are clinically diagnosed and confirmed by consultant physician in the specialist center.
- **Study design and setting:** This is a case control study among group of patients with different types of tumors registered in malignant registry center of Babylon living in the community of middle Euphrates of Iraq and of different areas from Babylon, Najaf, Karbalaa, Diwania, Simawa and Kut cities. This study was carried out from January until August 2016 to evaluate the status of IL-8, INF- γ and Vit.D in association with medical laboratory department of that center.

2.1. Cases definition and ascertainment

- **Inclusion criteria:** A total of 210 newly diagnosed patients with different type of tumors were registered in the malignant registry center of Babylon, and 100 of the patients are eligible for study. We aimed at those who are newly diagnosed without medication and agree to participate in the study.
- **Exclusion criteria:** We excluded 110 patients from the study either because they started medication for tumor or they had more severe, complicated and critical

cases and some patients refused participation in the study.

- **Permission from patients:** The study protocol was done under permission from the center. In addition to verbal consent, the laboratory studies were obtained from each individual participating in this study.
- **Assessment of exposure:** After assessment, the criteria required to the patients to be eligible for the study was found to be the status of IL-8, INF- γ and Vit.D.
- **Potential confounders:** Chronic disease like hypertension, DM, allergy are considered as confounder factors that may relatedly affect the concentration of IL-8 , INF- γ and Vit.D .
- **Validation:** The validation of the result to IL-8, INF- γ and Vit.D level was not done due to our limitation in the cost, time and technical facility.
- **Power calculation:** Assuming that the patients have such status of IL-8, INF- γ and Vit.D in review of similar articles, a sample size of 100 patients and control group of 100 individuals had a power of 80% at a type 1 error rate of 5%. Statistical analyses were done by using Chi -square, ANOVA, Odd ratio and T-Test of SPSS program.
- **Control group:** 100 individuals related to patients with age and sex matching are consider as control group and are selected randomly with role of one every three sequence. The individuals' flowchart included in this report is shown in figure B1.

2.2. Materials and methods

Venous blood was taken from all cases and control groups in sterile venipuncture. Four mls were collected in plain tube for serum separation. Small aliquot was achieved for deep freezing until all samples was analyzed using ELISA technique of special kits provided by Elab- Seince Company. This analysis was done in the laboratory department of Merjan medical city/ immunology unit using semi-automated ELISA according to corresponding associated manual principle of Bioelisa Company.

3. RESULTS

Demographic features of patients and control groups are demonstrated in table A1, that show mean age of patients to be 49.9 years and control 50.1 years with no significant statistical difference. Most of the patients rank in the age group of 40s while the control group at 50s decade. No significant statistical difference according to the gender of patient and control groups were observed but with prominent female to male occurrence of malignancy. More patient and control group are from urban area (68%) than rural one (32%) with significant statistical difference (P value 0.031).

Majority of patients have breast malignancy (52%) and the other sites represented 8% for each uterine and colonic malignancies, 6 % for liver, ovarian, thyroid and stomach malignancies, and 4% for gall bladder and prostatic malignancies. Ten percent of patients have family history of malignancy. Presence of chronic disease associated with malignancy is present in 44% of patients.

Table A2 shows the highly statistical significant low concentration of IL-8 in patients (90.38 ± 29.41) than control groups (107.47 ± 12.44) while the INF- γ concentration is present in no significant low concentration (50.11 ± 16.89) than control group (58.70 ± 6.62). The Vitamin D result revealed a high significant low concentration (32.19 ± 10.05) than control (61.64 ± 15.06), although the patient Vit.D level were within lower normal limit.

Table A3 reveal that IL-8 concentration abnormality have no significant association with patient demographic features like gender, address, type of malignancy, family history of malignancy and even presence or absence of chronic disease, while concentration abnormality of INF- γ show significant association with patient features of gender, address, type of malignancy, family history of malignancy as showing in table A4. The Vitamin D concentration showing result is mentioned in table A5.

The result of present study show significant positive correlation of patient ages with tumor types, IL-8 and Vitamin D as well as same tumor type with IL-8 and Vit.D. According to cytokines and vitamin-D there is a direct correlation between IL-8 and Vit -D, while there is no significant negative correlation between INF- γ with both IL- 8 and Vit-D as show in table A6.

4. DISCUSSION

4.1. Demographic features

Mean age of our patients in this study come close to the age of control group with no significant statistical difference due to our selection of control group matched in age and gender to the patients in an attempt to reduce bias of some confounders like age. Most of the patients rank in the age group of 40s decade mostly to predominant evidence of breast malignancy in the patient group and certainly the breast cancer is one of the most common malignancy in our society. No significant statistical difference according to the gender of patient and control groups due to the same explanation of control selection criteria and their matching to patients group. More patient and control group are from urban area with significant statistical difference and this may be explained by hypeine level and life style. Women who use oral birth control pills for most of their life have a slightly higher chance of developing breast cancer. Also those who live a sedentary lifestyle are at an increased risk of breast cancer, [12]. Majority of the patients have breast malignancy (52%) and this percentage is consistent with the percentage of Babylon center of malignancy. Also this result is consistent with the study done by [13] which stated that, breast cancer is the second most common cancer among women in the world. Breast cancer occurs almost entirely in women, but men are able to get it, too. [14] mentioned that the geographical variation in breast cancer incidence or mortality rates is compared statistically with solar UVB amounts. Such studies in Australia, China, France, Nordic countries, Spain and the United States have found lower breast cancer rates in regions of higher solar UVB.

Cancer statistics are hard to come by, since only 50 % of the healthcare in Iraq is public, the other half of our healthcare is provided by the private sector, and that sector is deficient in their reporting of statistics. Hence, all of our statistics in Iraq must be multiplied by two. Any official numbers are likely to be only half of the real number.

In Babylon Province, cancer rates have been escalating at alarming rates, due to using of depleted uranium weapons by US forces during and following the 2003 invasion. Breast was by far the most common site of

cancer accounted for 16% of all Iraqi patients. Lungs and the bronchi was the second most common site of cancer. Leukemia was the third most common cancer in Iraq accounting for 7% of all cancers. The four most commonly diagnosed types of cancer among males were cancers of the lung and bronchus, bladder, leukemia and Non- Hodgkin lymphoma (NHL), accounting for about 37.7% of estimated cancer cases in males. The four most commonly diagnosed types of cancer among females were cancers of the breast, leukemia, uterus including cervix and corpus) and cancers of brain and CNS, accounting for about 47.5% of estimated cancer cases in females. Breast cancer alone is accounted for 31% of all new cancer cases among females [15].

The top 10 cancer incidence in Iraq was breast cancer followed by lung cancer, leukemia, bladder cancer, brain and CNS, non-Hodgkin's lymphoma, colo-rectal cancer, stomach cancer, skin cancer excluding melanoma, larynx cancer. Cancer incidence rate significantly increases after 2000 in comparison with the period before 2000. Cancer incidence in Iraq is relatively high and trends are up going in terms of quantity and variables related like age, sex, etc. [16].

Ten percent of patients have family history of malignancy and can't compare with control group of those selected apparently healthy without personal or family history of malignancies. Presence of chronic disease like hypertension, DM, allergy associated with malignancy are present in 44% of patients and are considered as confounder factors that may alter the result.

IL-8 expression has been detected in numerous cancer types, including solid tumors (brain, breast, cervical, colon, gastric, lung, melanoma, mesothelioma, ovarian, prostate, renal, and thyroid) and hematological malignancies (AML, CLL, Hodgkin's lymphoma). Furthermore, a direct link between high serum IL-8 expression and disease progression has been reported in clinical studies of breast, colon, ovarian, and prostate cancers, as well as in melanoma [17].

Breast cancer is not the only malignancy in which the IL-8/IL-8R axis is associated with CSC properties. IL-8 inhibition reduced stemness marker expression, colonosphere formation, and tumor growth in colon cancer cell lines [5].

Vitamin D deficiency has been associated with breast and colon cancer, asthma, cardiovascular diseases, preeclampsia, multiple sclerosis, inflammatory bowel diseases, systemic lupus erythematosus, rheumatoid arthritis, and others. Maintenance of an adequate vitamin D level is essential in the prevention of a wide variety of health disorders as it modulates multiple genes, immune, and inflammatory responses [18]. Some reports showed a positive relationship with IFN- γ and IL-10. Others showed a significant inverse relationship with serum TNF- α but no relationship with CRP, IL-6, or IL-10, or even no significant changes in serum levels of IL-4, IL-5, IL-10, IL-13, IL-2, IL-6, IFN- γ , or TNF- α . On the other hand, there is accumulating evidence to support an immunoregulatory role of vitamin D, and other studies are showing an association between vitamin D deficiency and a wide range of diseases [19].

Chronic vitamin D deficiency may create a tumor microenvironment with increased inflammation. This type of tumor microenvironment could be more common or could cause tumors to become more aggressive. However, the vitamin D inflammation pathways have not been a well-explored mechanism in tumor pathogenesis and progression [20, 21].

As cytokines are critical mediators of the immune system, further studies are needed to shed more light on the relationship between vitamin D and cytokine levels, as well as on the possible immunomodulatory action of vitamin D on the immune system. Such studies should include both healthy and diseased populations.

5. CONCLUSION

Significant low concentration of IL-8, INF- γ and Vitamin D was found in different types of tumors. Initially their diagnosis showed obvious and significant correlation of high IL-8 concentration with demographic and clinical features of the patients while not to INF- γ and Vit.D level. A significant positive correlation of patient ages with tumor types, IL-8 and Vitamin D as well as same correlation of tumor type with IL-8 and Vit.D is observed. According to cytokines and vitamin-D there is a direct correlation between IL-8 and Vit -D. Assessment of such types of receptors may play a role in anti-tumor therapy

and research of related aspects. As cytokines are critical mediators of the immune system, further studies are needed to shed more light on the relationship between vitamin D and cytokine levels, as well as on the possible immunomodulatory action of vitamin D on the immune system.

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APPENDIX A

Table A1. Demographic features of patients and control groups

Characteristic	Patient No. 100	Control No. 100	P. value
Age Mean (Range) yrs	49.9 (27 -74)	50.1 (28 -74)	0.183
Groups			
21 -30 Years	6.0	5.0	
31 -40 Years	14.0	11.0	
41 - 50 Years	30.0	28.0	
51 -60 Years	26.0	29.0	
> 60 Years	24.0	27.0	
Gender			
Male	22.0	26.0	0.097
Female	78.0	74.0	
Address			
Urban	68.0	62.0	0.031
Rural	32.0	38.0	
Type of malignancy: No. (%)			
Breast malignancy	52.0 (52%)		
Liver malignancy	6.0 (6%)		
Uterine malignancy	8.0 (8%)		
Ovarian malignancy	6.0 (6%)		
Gall Bladder malignancy	4.0 (4%)		
Thyroid malignancy	6.0 (6%)		
Stomach malignancy	6.0 (6%)		
Colon malignancy	8.0 (8%)		
Prostate malignancy	4.0 (4%)		
Family history of malignancy			
Presence	10.0		
Absence	90.0		
Chronic disease (Hypertension, DM, allergy)			
Presence	44.0		
Absence	56.0		

Table A2. Status of IL-8, INF- γ and Vit. D concentration in patients and control groups

	N	Patient (M \pm SD)	Control (M \pm SD)	Significance
Interlukin – 8 Concentration pg/ml	100	90.38 \pm 29.41	107.47 \pm 12.44	0.001 (H.S)
Interferon – γ concentration pg/ml	100	50.11 \pm 16.89	58.70 \pm 6.62	0.116 (N.S)
Vitamin –D concentration pg/ml	100	32.19 \pm 10.05	61.64 \pm 15.06	0.000 (H.S.)

Table A3. Association of IL-8 state with patient parameters

Characteristic		No. Patient with normal concentration of IL-8	No. Patient with abnormal concentration of IL-8	P. value
Gender	Male	13.0%	9 .0%	0.12
	Female	44.0%	34.0%	
Address	Urban	32.0%	30.0%	0.36
	Rural	27.0%	11.0%	
Type of malignancy	Breast Ca.	31.0%	27.0%	0.086
	Liver Ca.	4.0%	2.0%	
	Uterine Ca.	3.0%	5.0%	
	Ovarian Ca.	4.0%	2.0%	
	Gall Bladder	2.0%	0.0%	
	Thyroid Ca.	2.0%	4.0%	
	Stomach Ca.	4.0%	2.0%	
	Colon Ca.	3.0%	3.0%	
Prostate Ca.	0.0%	2.0%		
Family history of malignancy				1.25
	Presence	6.0%	4.0%	
	Absence	48.0%	42%	
Chronic disease (Hypertension, DM, allergy)				0.93
	Presence	16 .0%	28.0%	
	Absence	24.0%	32.0%	

Table A4. Association of INF - γ state with patient parameters

Characteristic		No. Patient with normal concentration of INF- γ	No. Patient with abnormal concentration of INF- γ	P. value
Gender	Male	4.0%	18.0 %	0.012
	Female	4.0%	74.0 %	
Address	Urban	56.0 %	6.0 %	0.023
	Rural	36.0 %	2.0 %	
Type of malignancy	Breast Ca.	49.0%	9.0%	0.020
	Liver Ca.	5.0 %	1.0%	
	Uterine Ca.	6.0%	2.0%	
	Ovarian Ca.	4.0%	2.0%	
	Gall Bladder	2.0%	0.0%	
	Thyroid Ca.	6.0%	0.0%	
	Stomach Ca.	3.0%	3.0%	
	Colon Ca.	4.0%	2.0%	
Prostate Ca.	0.0%	2.0%		
Family history of malignancy				
	Presence	17.0 %	5.0%	0.041
	Absence	67.0 %	11.0%	
Chronic disease (Hypertension, DM, allergy)				
	Presence	26.0%	5.0%	0.58
	Absence	58.0%	11.0%	

Table A5. Association of Vitamin –D state with patient parameters

Characteristic		No. Patient with normal concentration of Vit. D	No. Patient with abnormal concentration of Vit.D	P. value
Gender	Male	6.0%	16.0 %	0.012
	Female	7.0%	71.0 %	
Address	Urban	11.0 %	51.0 %	0.023
	Rural	6.0 %	32.0 %	
Type of malignancy	Breast Ca.	9.0%	49.0%	0.020
	Liver Ca.	2.0%	4.0 %	
	Uterine Ca.	1.0%	7.0%	
	Ovarian Ca.	2.0%	4.0%	
	Gall Bladder	0.0%	2.0%	
	Thyroid Ca.	0.0%	6.0%	
	Stomach Ca.	2.0%	4.0%	
	Colon Ca.	3.0%	3.0%	
Prostate Ca.	2.0%	0.0%		
Family history of malignancy				
	Presence	6.0%	16.0 %	0.041
	Absence	10.0%	68.0 %	
Chronic disease (Hypertension, DM, allergy)				
	Presence	9.0%	22.0%	0.58
	Absence	10.0%	59.0%	

Table A6. Pearson correlation state of studied parameters

		Age	Tumor Types	Interleukin -8	Interferon -Y	Vitamin -D
Age	Pearson Correlation	1				
	Sig. (2-tailed)					
Tumor Types	Pearson Correlation	.678**	1			
	Sig. (2-tailed)	.000				
Interleukin -8	Pearson Correlation	.302**	.269*	1		
	Sig. (2-tailed)	.008	.020			
Interferon -Y	Pearson Correlation	-.029-	.053	-.177-	1	
	Sig. (2-tailed)	.803	.651	.128		
Vitamin -D	Pearson Correlation	.402**	.465**	.321**	-.008-	1
	Sig. (2-tailed)	.000	.000	.005	.946	

APPENDIX B

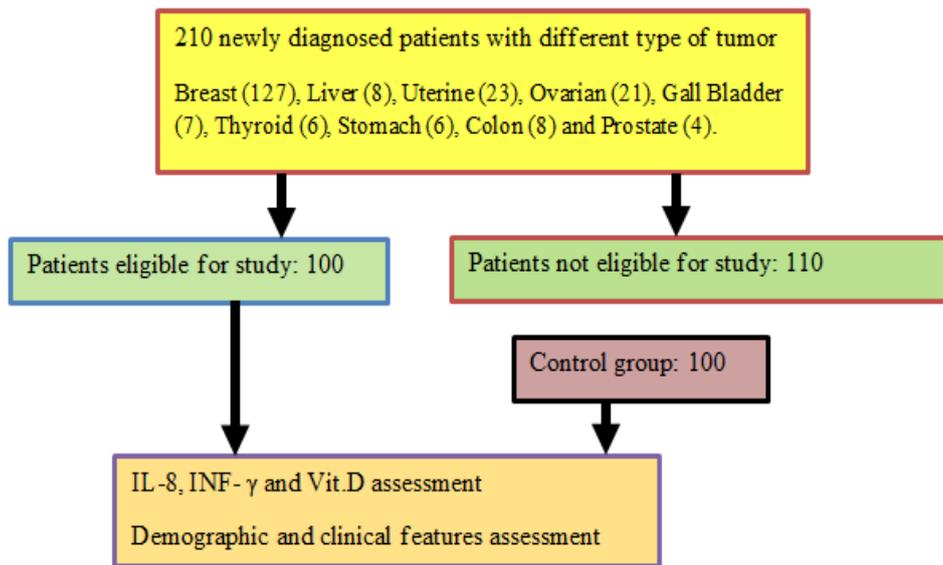


Figure B1. Flowchart of individuals included in this study