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Study Relationship Between IL-17 , IL-17R and Other Biochemical Parameters in Iraqi Patients with Colorectal Cancer

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Dedication

To …

The two greatest in my life

My mother & My father

To …

My wife …

My brother …

My sister …

My children …

To everyone who supported me …

Mezher

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I praise God almighty very much for giving me the confidence , strength and patience to conduct this study . I sincerely like to express my deep gratitude to:

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List of Abbreviations:

5-FU	5-fluorouracil
ACP	Acid Phosphatase
BMI	Body mass index
CEA	Carcinoembryonic antigen
CRC	Colorectal cancer
DNA	Deoxyribose nucleic acid
ERK	Extracellular signal-regulated kinase
Hb	Hemoglobin
IL-17	Interleukin-17
IL-17RA	Interleukin-17A receptor
NK	Natural killer cells
NMR	Nuclear magnetic resonance
P	Phosphorus
Th17	T-helper17
TNM	Tumor, nodes , metastases
UVB	Ultraviolet B
VAT	Visceral fatty tissue
VDR	Vitamin D receptor
VEGF	Vascular endothelial growth factor
γδT	Gamma delta T cells

Summary

Colorectal cancer CRC is the third most commonly diagnosed cancer in males and the second in female , and it is one of a heterogeneous disease.

Venous blood samples were taken from 48 males and 40 female who are suffering from CRC disease as group one (G1) without taking a chemotherapy dose . The same patients group two (G2) after taking the first chemotherapy dose , while group three (G3) whose patients in (G1) and (G2) after second taking a chemotherapy dose . Group four (G4) consist of (30 males and 30 females) healthy Iraqi control . All the blood sample were analyzed for (IL-17), (IL-17RA), vitamin D, CEA, total acid phosphatase, magnesium, phosphorus and Body mass index.

Results in the present study show :

- A significant increase in IL-17 , IL-17RA , CEA , acid phosphatase , magnesium and phosphorus in G1 of male patients.
- A significant increase of BMI and a high significant increase in IL-17 , IL-17RA, in G1 of female patients than other groups, while there was high significant increase in G2 in female patients.
- Non significant decrease was observed in BMI and high significant decrease in vitamin D in G1 of male patients.
- High significant decrease of vitamin D in G1 of female patients.
- There was a high significant increase in BMI, and magnesium and significant increase in IL-17 , acid phosphatase in G1 female (B1F) as a compared with the same group of male (B1M) . However, there was non significant decrease in IL-17RA, vitamin D, in Iraqi female patients (B1F) and non significant increase in phosphorus in the same patients as a compared with male patients (B1M).
- The results in G2 as a comparative study (male and female) show a high significant increase in IL-17 and non significant different in BMI, IL-17RA, CEA and phosphorus in Iraqi female patients A1F . A high significant decreasing also shown in Magnesium in A1F group than A1M .
- The data in this study also was studied in G3 and show a high significant increase in IL-17RA and phosphorus and significant increase in CEA , while there was a high significant decrease in

vitamin D in Iraqi female patients A2F as a compared with Iraqi male patients A2M .

- There was a high significant negative (-ve) correlation between (IL-17RA and CEA) and between (IL-17 and phosphorus) . A significant positive (+ve) and negative (-ve) correlation was shown between (IL-17 and CEA), (IL-17 and magnesium) respectively in the patients male G1 before taking chemotherapy dose . However, in the same groups of female patients there was a high significant positive (+ve) correlation between (IL-17 & CEA), (IL-17RA & acid phosphatase), (IL-17RA & magnesium) . A high significant negative (-ve) correlation was found between (IL-17RA & CEA).
- In male patients as G1 find a high significant negative (-ve) correlation between (IL-17RA & CEA), (IL-17 & Acid phosphatase) and (IL-17 & phosphorus) . There was a high significant positive (+ve) correlation also between (IL-17 & IL-17RA). However, in female patients G2 we find in the recent study there was a high significant negative (-ve) correlation between IL-17 and(BMI, IL-17RA) and between IL-17RA and (CEA, vitamin D).
- The correlation result in Iraqi male G3 show a high significant negative (-ve) correlation between (IL-17RA & CEA) and (IL-17 & phosphorus) and high significant positive (+ve) correlation between (IL-17 & IL-17RA) . In female patient in the same group a high significant negative (-ve) correlation also find between (IL-17RA & CEA).

A conclusions could be drown that is the first observe the correlation of IL-17 and IL-17RA in serum of Iraqi male and female colorectal cancer before and after taking chemotherapy dose . Therefore , it is indicated that IL-17 and IL-17RA may be a good tumor marker for diagnosis a colorectal cancer.

Contents Index

List of Figures

List of Tables

Chapter One

Introduction

1.1 Colorectal cancer :

Colorectal cancer (CRC) is a single disease that refers to colon and rectal cancer [1]. It is one of the most common cancers in the world, each year, there are between 1 million and 2 million new cases. For this reason it is the third most common type of cancer and fourth most prevalent in terms of cancer-related death [2].

The abnormal growth of epithelial cells that form the lining of the colon or rectum is a cause of CRC , some small growths (known as polyps) have the potential to develop to cancerous, although most of them are benign. It is estimated that the risk of CRC is associated with two thirds of the polyps in the colon and rectum which are pre-malignant [3].

The early detection and disposal of benign tumors before they become cancerous through screening and awareness. It can be considered a root to reduce the mortality of CRC and the rate of treatment is higher when cancer is detected at an early stage [4].

Colorectal cancer is one of a heterogeneous disease. This is indicated by clinical experience supported by various pathohistological and molecular biology data [5].

1.2 Causes and risk factors :

Behavioral factors influencing the development of colorectal cancer are including : age, lifestyle, environmental and genetic factors [6]. The risk factors of this cancer can be divided into two categories, one of which can be changed and the other can not be changed [7].

***Nonchangeable risk factors*:** These factors comprise sex, age, heredity (chronic family history of the disease), chronic colitis, inflammatory bowel disease or Crohn's disease, as well as the history of benign tumors , so the individual can not control these factors [8].

***Changeable risk factors*:** The risk of colorectal cancer can be reduced by these behavioral factors that can be changed or managed, dangerous health behaviors are reported to be associated with more than half of all cancers. The changing factors includes smoking, moderate to heavy levels of alcohol consumption, obesity, an unbalanced diet, physical inactivity, and excessive consumption of red meat and processed meat products [6,9] .

Risk factors that increase the chance of colorectal cancer can be explained as follows:

1- Age:

Age is the main risk factor for colorectal cancer , this risk increased after the fifth decade of life, while colorectal cancer rarely appears below the age of fifty (except for inherited cancer) [10].

The importance of age as a risk factor in men and women Its be equally and more than 50% of colorectal cancers are personated after age seventy, while only 10% are personated before age fifty five. nevertheless, the risk of men promoting advanced adenoma or cancer is round double that regarding women [11].

2- Lifestyle: Modest changes can be made to decrease other risk factors associated with lifestyle from where dietary and physical activity. For example, the risk of colorectal cancer is believed to increase through a stable lifestyle, although this relationship is not fully determined between inactivity and colorectal cancer. On the other hand, it has been shown that metabolic rates and bowel movements can be increased through physical activity, which in turn reduces blood pressure and increases metabolism efficiency . Obesity is also associated with sedentary lifestyle, which is another important risk factor for colorectal cancer. noteworthy. Food intake and elevated levels of visceral fatty tissue (VAT) are associated with this increased risk . The development of colorectal cancer is promoted by the (VAT), which is an effective hormonal component of total body fat, which the inflammation occurs in the colon and rectum through inflammatory cytokines that secrete them. In the same context , the risk of colorectal cancer is strongly associated with the type diet, so that the chances of colorectal cancer increase by up to 70% due to those unhealthy dietary habits . Moreover, the risk of colorectal cancer is increased by smoking and alcohol use. In alcohol consumption, the carcinogen that increases the risk of colorectal cancer among humans is acetaldehyde (the main metabolite of ethanol). Whereas polymorphisms of alcohol metabolism enzymes have a role in it [10].

3- Family history: If a direct relative has contracted the disease before, the risk of the disease will double in the person. If there are more than one relative has had colorectal cancer the risk will be greater [12]. Genetic factors may cause a third of the risk of colorectal cancer and the risk of colorectal cancer is increased in individuals with a history of colorectal cancer or colorectal adenoma. The level of risk relies on the grade of kinship , the number of relatives affected, and stage of life at which the index person was diagnosed with colorectal cancer [13].

4- Genetics: familial adenomatous polyposis (FAP) is a common disease in individuals with genetic disorders that increase the risk of colorectal

cancer because the individual is exposed to the formation of benign tumors [12]. Colorectal cancer can be minimized through regular colonoscopy with the removal of benign tumors [14]. The adenomas occurs in 95% of individuals aged 35 years and infected with FAP. Colorectal cancer can not be prevented when the colon is not eradicated. When the adenomas develops into more than 20-30 tumors, it is recommended to use a colectomy. On the other hand, colectomy may be necessary in individuals with FAP attenuated in order to avoid cancer later in life. While colonoscopy and polypectomy may be adequate in individuals with a limited number of adenomas [11].

5- Colorectal polyps or inflammatory bowel diseases: Inflammatory bowel disease is one of the most common diseases in developed countries , which has long been afflicted by patients, also has an increased risk of colorectal cancer [15,16]. As the severity and duration of the disease increase the risk of cancer. Ulcerative colitis and Crohn's disease are the most common forms of inflammatory bowel disease [17, 18] . Monitor the screening to detect cancer lesions and use of medicines that control the inflammation led to the decline of the disease over time [19].

1.3 Symptoms of colorectal cancer:

The colorectal cancer site and its ability to metastasize are considered signs and symptoms for CRC which are largely dependable. These symptoms include fever, weight loss, blood in the stool, loss of appetite, constipation . While the common symptom in people over the age of 50 is anemia, hemorrhage, nausea and vomiting . Weight loss and rectal bleeding are among the most obvious symptoms that should be noted with interest . In the absence of these symptoms, the rest of the other symptoms are a good sign of many of different gastrointestinal diseases . The right side of the colon is the ascending colon and cecum, where colorectal cancer occurs. Fecal occlusion and anemia are the most obvious causes. This is because of the site of the intestinal wall these tumors tend to outward. However, constipation occurs in the tumors of the descending colon, ie tumors on the left side. These tumors are often circumferential [20]. Patients referred to the hospital, who account for about 85%, suffer from one or more of the following symptoms very serious: [21]

- Rectal hemorrhage
- A conglomerate in the abdomen or rectum
- Change in bowel conduct
- Abscesses or lesions that represent symptoms around the anus

1.4 Diagnosis of colorectal cancer:

Survival rates can be improved by early diagnosis of colorectal cancer. However, early symptoms may mix with symptoms of other diseases, such as abdominal pain [22]. This indicates that when the disease is diagnosed they will have advanced disease [23] .

During colonoscopy or sigmoidoscopy, a biopsy is taken from the tumor as a first step towards diagnosis, and after confirming the presence of cancer, the extent of the disease is determined by imaging tests of the patient's abdomen, chest and pelvis. These tests involve computed tomography (CT) scan, positron emission tomography (PET) scan, nuclear magnetic resonance (NMR), through which the doctor can clearly identify the stages of cancer, using the TNM classification system [24] .

The basic tests for the screening are four that include adaptable sigmoidoscopy, a fecal occult blood test and a stool DNA test and a colonoscopy. In order to screen right half of the colon only sigmoidoscopy can be used where 42% of malignant tumors are found [25]. Virtual colonoscopy thru a CT study indicates over on a portion with standard colonoscopy for figuring out malignancies and extensive adenomas but is high-priced, linked with radiation presentation, and can not void any featured unusual trends like standard colonoscopy scan [26].

The traditional example of a screening technique is Fecal occult blood testing . To determine which one is most likely to develop colorectal cancer are used , the finding of occult blood in the stool . In this way those who should be subject are chosen endure colonoscopy, as a conclusive diagnostic test. [27]

1.5 The stages of colorectal cancer:

The TNM staging system is the most widely used to classify the system. It is regarded the most descriptive and accurate . So T means the profundity of the tumor, and to which stage penetrated the colon wall, while N indicate to the sharing of lymph nodes, and M indicates the grade of metastases that have occurred or whether the tumor has prevalence or not. [28]

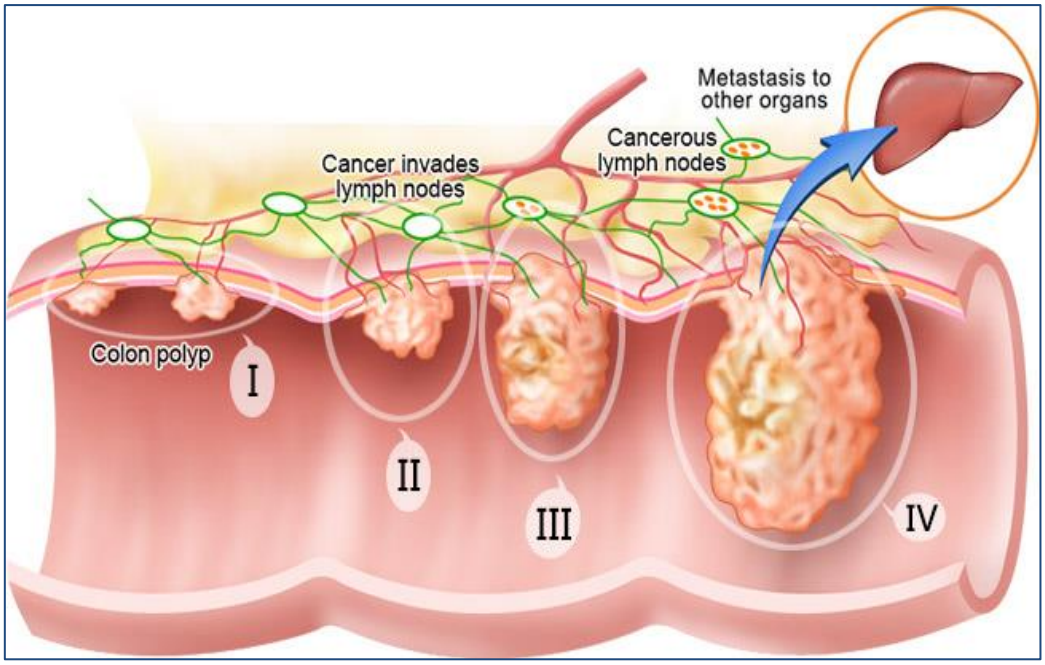

Figure (1-1): The stages of colorectal cancer [29]

The most common staging for colorectal cancer is described via the tumor, node, metastasis (TNM) staging system . The level of invasion or spread of the tumor to other organs (malignancy) is determined for the patient into stages I-IV [30].

During the same stage, the perfect forecast system supply homogeneity , proper discrimination amidst different stages, and monotonous of gradients that foretells to stay alive that are harmonious with the riskiness of cancer staging [31] .

Table (1-1): The stages of colorectal cancer [30, 32]

Stage	Classification
Stage I	The tumor is localized to the lining of the colon . T1-T2, N0, M0
Stage II	The tumor grows into the outer lining of the colon or surrounding tissue. T3-T4, N0, M0
Stage III	The cancer has metastasized to the lymph nodes. Any T,N1-N2,M0
Stage IV	The cancer has metastasized to distant organs in the body. Any T, Any N,M1

1.6 Chemotherapy :

In some cases, chemotherapy is used before surgery. This will help shrink the tumor before eradicating it . In other condition where cancer spreads and enters the lymph nodes, the chemotherapy is necessary to prolong the patient's expected life. Drugs may comprise oxaliplatin, leucovorin, irinotecan, uracil-tegafur (UFT), and capecitabine, or 5-fluorouracil [33]. Combining chemotherapy and radiation might be helpful . But, in most cases, this is not used as curative technique, since the bowels are highly sensitive to radiation [34]. The tumor is extracted by surgery that is subject to colorectal cancer patients. It is either done by laparotomy or laparoscopy, which is a minimally invasive procedure, it is removed surgically if other tumors are metastasized to the lungs or liver [24].

Chemotherapy may assistance those with locally advanced or metastatic disease and may even change initially unresectable metastatic disease to resectable disease [35].

1.7 Cytokines:

Cytokines are tiny, non-structural proteins with low molecular weights that contribute to the complex regulation of inflammation and immunity. The development of the immune and inflammatory response has long been considered to include hematopoietic cells, lymphocytes, and many pro-inflammatory and anti-inflammatory cells, and cytokines mediate in the complex interactions of these cells [36]. Cytokines are pleiotropic proteins or tiny glycoproteins with a molecular weight less than 30 kDa (<200 amino acids). Cytokines are generated by a number of cell kinds, such as leukocytes which organize immunity, inflammation and hematopoiesis [37].

Cytokines are classified according to their secretion to [38] :

- T cells and regulate the immune response)
- Proinflammatory cytokines (cytokines that amplify and perpetuate the inflammatory process)
- Growth factors (cytokines that promote cell survival and result in structural changes in the airways)
- Chemokines (cytokines that are chemotactic for inflammatory cells)
- Anti-inflammatory cytokines (cytokines that negatively modulate the inflammatory response) .

Cytokines are the intercellular messengers in the immune system where they combine function of several cell types in different body compartments into a cohesive immune response. They have evolved over the years and now include the interferons, the interleukins, the

chemokine family, mesenchymal growth factors, the tumor necrosis factor family and adipokines. Cytokines are produced by induce a response from every cell, except red blood cells. In response to diverse stimulus cytokines are secreted from various cells inclusiveness white blood cells [36].

The origin of the word cytokine is derived from a blending of two Greek words - "cyto" indicate to cell and "kinos" indicate to the cell motion. Cytokines are essentially the cell signaling molecules that assistance cell to cell communication in immune responses and catalyze the motion of cells towards sites of inflammation, infection and contusions . Cytokines arose from the previous forms as intracellular molecules before the manifestation of receptors and signaling molecules . The cytokines are an extensive family of molecules that are categorized in varied different ways due to lack of a unified classification system. They are found in peptide, protein and glycoprotein forms . Cytokines are the key modulators of a wide range of bodily functions. Several cytokines exhibit some redundancy in function and share overlapping properties along with their cell surface receptors. Being mediators of inflammation and immune response, these molecules have been targeted as therapeutics in various diseases. So, a better understanding of the history and biological aspect of these cytokines can facilitate the development of agents to improve the modulation of inflammatory response for the treatment of autoimmune, infectious and neoplastic diseases [39].

The cytokine pattern that is released from the cell depends primarily on the nature of the antigenic stimulus and the type of cell being stimulated. Cytokines compromise leukocytes to respond to a microbial stimulus [40].

The history of cytokine development exhibit them as soluble factors created by one cell and acting on other cell. The activity of cytokines was established and recognized in (1940- 1960). It was soon apparent that the production of these factors could be regulated by activation with an antigen or a nonspecific mitogen . Uniform nomenclature have been developed to designate cytokines as interleukins in relation to their role among leukocytes. [41].

Cytokines can act on their target cells in an autocrine, paracrine, and/or endocrine style to promote systemic and/or localized immune responses. On the other hand, cytokines have multiple activity, on various target cells. It also affects the function of other cytokines in an additive, support, or antagonistic style [42]. Immune cells have the ability to secrete cytokines. On the other hand, they can also be produced by a wide range of cells in response to infection or can be created or released from cells in response to cellular damage when cell safety is at risk. Therefore,

cytokines are Important mediators of communication for the immune system and are necessary for host defense against pathogens . Acting through a chain of preserved signaling pathways that program transcriptional pathways over controlling many biological processes, such as cell growth, cell differentiation, apoptosis, development, and survival . They can also reprogram cells in the local tissue environment to improvement certain types of immune responses [43].

1.8 Cytokine Receptors:

The nature of the target cell associated with specific cytokines is due to existence the specific membrane receptors. Cytokines and their receptors show very high attraction for each other and have dissociation constants ranging from 10^{-10} to 10^{-12}M . Because of this high convergence, biological effects are produced by cytokines in picomolar concentrations. They show autocrine, paracrine, and endocrine actions and mediate cellular intercommunication [44].

Most cytokine receptors are composed of a multisubunit complex: a unique and specific ligand-binding subunit and a signal-transducing subunit, which may be structurally identical to other members of the cytokine receptor superfamily [45]. In spite of hematopoietic cytokine receptors are grouped together due to the existence of common elements within their extracellular domains. They can be divided into more families based on the number of subunits and/or the existence of one or more shared subunits [46]

The physiological responses of cytokines on binding to receptors involve increase of cellular and humoral immune response, regulation of hematopoiesis, induction of inflammatory response, control of cellular proliferation and induction of wound healing and differentiation . On contrary, the cytokines frequently induce the synthesis of another cytokines leading to a series of activity in which the last cytokines impact the activity of the previous cytokines which secreted it. Where they work for a very restricted period of time because of their short half-life within the blood stream and extracellular fluids [41].

1.9 Interleukin-17 (IL-17A) :

The human IL-17A gene product is a protein consisting of 150 amino acids and has a molecular weight of 15 kDa. It is secreted as a disulfide linked homodimer of 30–35 kDa of glycoprotein [47]. The common feature to many cancers is the poor regulation of growth factor pathways. Although there are no published reports showing genetic linkage of either IL-17 cytokines or receptors straight to cancers, there is directory that IL-17s are active in cancers [48].

These reports show a role of IL-17 cytokines in increasing tumor. though, other evidence indicate that IL-17A may protect against tumors by encouraging rejection of the immune tumor [49].

After tumor forming in colon, immune system responds against neoplastic cells. Immune responses involve immune cells increasing, phenotype alteration, production and release of cytokine such as interleukin- 17 (IL-17) [50].

Interleukin-17 is a proinflammatory cytokine, which is related with cancer development [51]. The major source of IL-17 is a subpopulation from CD4+ T cells recognized as T-helper17 (Th17) cells [52]. Interleukin-17 bind to a heterodimer of IL-17(receptor A) it's a proinflammatory cytokine has been shown to play a critical roles in colon carcinogenic [53] , and its sometimes contradictory roles in human malignancies . [54,55] It is linked to rapid progression of colorectal cancer , therapy resistance and exerts its protumorigenic activity through its type A receptor (IL-17RA) , but it promotes colonic tumorigenesis is unknown. [56, 54]

Many kinds of cancers have been found tumor infiltrating Th17 cells [57,58]. Although, the name of Th17 cells comes from their ability to produce IL-17, they are also releasing other cytokines [59].

Interleukin-17 family comprise of six cytokines with IL-17A as the prototype with five additional members, IL-17B, IL-17C, IL-17D, IL-17E (also called IL-25) and IL-17F. IL-17A and IL-17F have the elevated homology and IL-17E has the lower [60].

The published data showed that IL-17 level was increased in serum and tumor tissues for CRC patients [50,61]. Furthermore, many studies have shown that IL-17 has an important role in malignancy and diagnosis of CRC [62,63] .

A large body of evidence indicates that IL-17 is a major proinflammatory cytokine due to stimulating a mass of cytokines secretion by distinct cell types, which recruit monocytes and neutrophils into the place of infections [64]. Furthermore, IL-17 promotes of antimicrobial peptides from epithelial cells and facilitates host defense against inflammation [65].

IL-17 is an inflammatory cytokine is produced by a large variety of leukocytes, as explained in Figure (1-2), including T cells, neutrophils, lymphoid tissue inducer-like cells (LTi-like cells), and natural killer cells (NK cells) [66]. IL-17 secretion is regulated by the cooperation of the inflammatory cells, cytokines, and antigens which coexist in the specific inflammatory microenvironment [67].

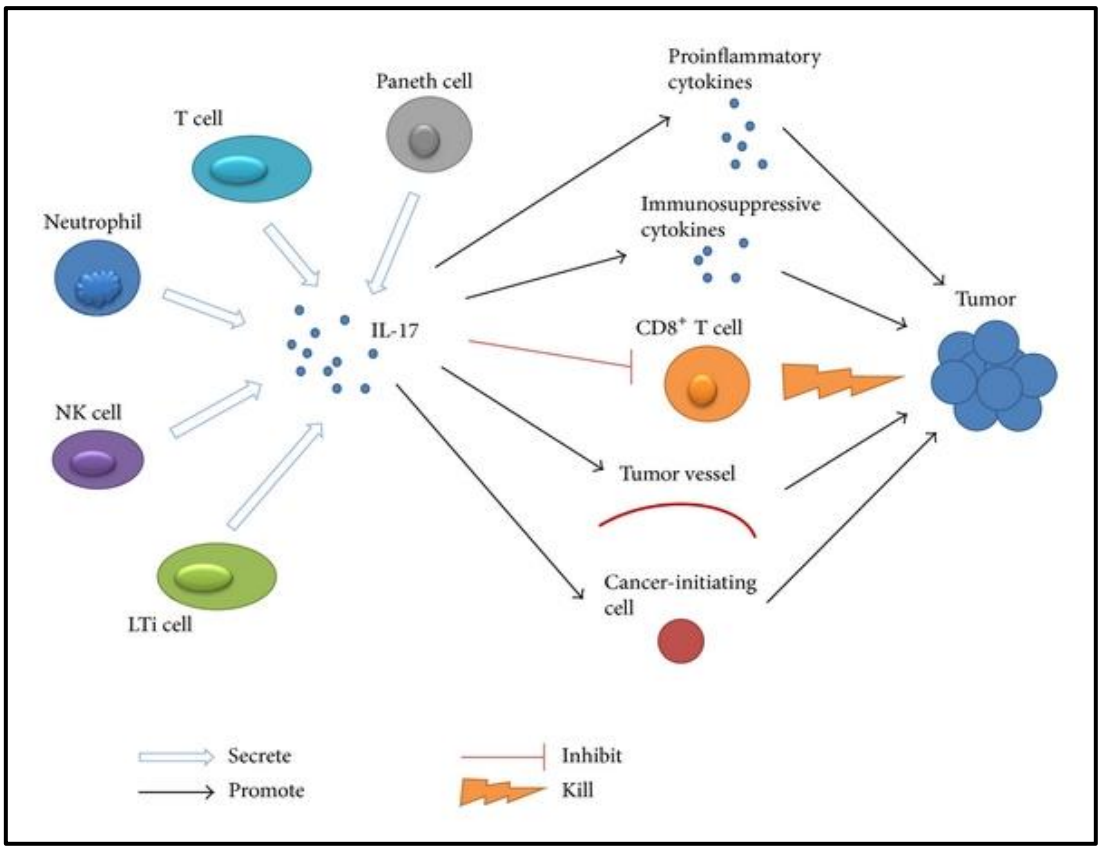

Figure (1-2): The protumor activity of IL-17 in CRC microenvironment. Blue colored arrow Indicates cells producing IL-17. Black arrow Indicates the latter stimulated by the former. T-shaped arrow Indicates process inhibited by IL-17 and lightning arrow Indicates attack on tumor cells. [67]

1.10 The Role of IL-17 in Cancer Promotion

Highly expression of IL-17 in many human tumors, such as ovarian cancer, cervical cancer, breast cancer, liver cancer, esophageal cancer, stomach cancer and CRC [68].

This suggests that IL-17 is an important inflammator cytokine which binds innate and adaptive immunity. Moreover, some studies have proven that IL-17 has an active role in allergies, autoimmune diseases, allograft transplantation, and cancer [69]. Newly, several studies have shown that IL-17 plays either a protumor or antitumor role in different cancer types [70].

Some researchers suggest that IL-17 promotes tumor starting and progression by suppressing the immune response to anti-tumors. As well, some studies indicate that IL-17 can inhibit tumor growth. This evidence suggests that IL-17 may have partial antitumor effect by enhancing immune response in the tumor starting stage. [67].

1.11 IL-17 as a Promoter in colorectal cancer advancement:

According to studies conducted in other cancers, mounting evidence has shown that IL-17 can also promote tumor advancement in CRC [71]. Table 1-2 illustrates these studies related to activities to promote colorectal cancer for IL-17. Most researchers appreciate IL-17 as a promoter in CRC progression . Besides, Table 1-2 illustrates studies with IL-17 which indicating its possible antitumor role in CRC. Based on these results, It was found that the protomer activity of IL-17 in CRC microenvironmentmay exert in several aspects [67]:

1. Promoting tumorelicited inflammation which facilitates the proliferation and survival of malignant cells,
2. Forming an immunosuppressive tumor microenvironment by chemoattracting immunosuppressive cells and cytokines,
3. Suppressing cytotoxic cells-mediated immunosurveillance against tumor,
4. Fostering tumor angiogenesis to promote tumor growth and metastasis, and
5. Inducing cancer-initiating cells, which facilitates tumor malignant progression and escaping from host immune surveillance as shown in Figure (1-2).

Progress is a process involving interactions between tumor and host cellular immunity in the microbial environment of the tumor. Tumor cells secrete pro-inflammatory mediators and immune cells produce cytokines that all lead to tumor growth. [72]. Studies indicated that the number of Th-17 cells are significantly higher in CRC tissues [62,73].

IL-17 is generated by Th17 cells and it is an significant cytokine In many immune responses such as type2 immune response. In pro-inflammatory responses, IL-17 plays an important function in activation and induction of neutrophils. Neutrophils are the essential sources of cytokines attached to Th2-type immune response that promote negative feedback, suppress neutrophilia, decrease tissue devastation, and also induce IL-17 production [74].

Published data showed that the level of IL-17 was significantly higher in CRC tissues [75]. These data showed that the higher regulation of

IL-17 starts from the stage of the benign tumor and is higher in the cancer stage but is not associated with the TNM criteria for the tumor [50].

Table (1-2): Role of IL-17 in CRC [67].

	Species	Mediators	Findings
Tumor promoting role	Human	VEGF	IL-17 induces both CRC cell lines and primary cancer cells to produce VEGF.
	Human	HIF-1α and c-myc	IL-17 and TNFα cooperatively stimulate glycolysis in CRC cells via induction of HIF-1α and c-myc expression.
	Mouse	Stat 3	IL-23/IL-17 signaling activated by microbial products promotes STAT3 phosphorylation in CRC epithelial cells.
	Mouse	VEGF, KC, and PGE2	IL-17 promotes angiogenesis via induction of a variety of proangiogenic factors secretion from fibroblasts and tumors.
	Mouse	IL-6, IL-23, and IL-1β; KC and Cox-2; CD4 T cells	IL-6, IL-23, IL-1β, KC, and Cox-2 are decreased and function of CD4 T cells alters in ApcMin/+ mice, resulting in abrogating spontaneous intestinal tumorigenesis.
	Mouse	IL-6, STAT3, and TNF-α; cyclin D1, cyclin-dependent kinase 2, and cyclin E	IL-17A knockout decreases IL-6, STAT3, TNF-α, cyclin-D1, cyclin-dependent kinase 2, and cyclin E and inhibits CAC tumorigenesis.
	Mouse	G-CSF, VEGF, and Bv8 NF-κB and ERK signaling	IL-17 induces the expression of G-CSF through NF-κB and ERK signaling, enhancing proangiogenic function via VEGF and Bv98 and promoting tumor growth.
Tumor inhibiting activity	Mouse	IFN-γ NK cells and T cells	IL-17-deficient decreases IFN-γ+ NK and tumor-specific IFN-γ+ T cells and promotes tumor growth and lung metastasis.
	Human	Claudin ERK MAPK pathway	IL-17 enhances the development of the tight junctional barrier mediated by claudin of T84-cell monolayers via ERK MAPK pathway in intestine.

Different family members of IL-17 have Numerous roles in tumorigenesis. while IL-17A has a pro-tumorigenic effect on CRC [76]. Furthermore, raised level of IL-17 has been detected in the sera of CRC patients and thus it can be a favorable diagnostic marker in patients with CRC [77].

Published data indicated that IL-17, which is a pro-inflammatory cytokine, can directly impact the angiogenesis of CRC by attaching to the IL-17 receptors (IL-17R) on the endothelial cells and activating these cells to produce the vascular endothelial growth factor (VEGF) . VEGF can indirectly stimulate cancer cells to generate angiogenic factors , and molecules that can decrease IL-17 production or can prevent the

downstream of IL-17 are among the choices for treatment of CRC [62 ,78]. None of these drugs can induce an anti-tumor effect on their own, and these drugs should be used with chemotherapy drugs such as oxalplatin, 5-fluorouracil and anti-angiogenic agents [79].

1.12 The Interleukin-17A receptor :

The IL-17R family involves IL-17RA, IL-17RB, IL-17RC, IL-17RD, and IL-17RE. [80] . IL-17RA is the cognate receptor for IL-17. IL-17RA links both IL-17A and IL-17F. Although it is associated with IL-17A a higher degree of affinity [60]. IL-17RA is expressed largely on hematopoietic cells, and is expressed at low levels on osteoblasts, fibroblasts, endothelial cells, and epithelial cells. In humans IL-17RA can form a heterodimer with IL-17RC that binds human IL-17A and IL-17F [80]. The best functional data exist for IL-17RA, and many experimental samples deal with models of infectious diseases in which IL-17R signaling is protective by initiating granulopoiesis and orchestrating neutrophil trafficking [81].

All of which are type I transmembrane proteins. IL-17RA (or IL-17R) was the first described IL-17 receptor [82]. Unlike other cytokine receptors, the IL-17RA subunits are preassembled on the plasma membrane before ligand binding, enabling it to respond rapidly and specifically to its ligand . Although the accurate receptor complex of IL-17A has not been obviously elucidated, the IL-17A receptor combines at least two IL-17RA subunits and one IL-17RC subunit [80] . IL-17A and IL-17F activity are canceled when IL-17RA is depleted completely [81]. This may explain why IL-17RA functions as a common subunit to all other IL-17Rs in the family [83].

IL-17 Receptor (IL-17R) activates extracellular signal-regulated protein kinase (ERK), c-jun N-terminal kinase (JNK) and p38 MAP kinase pathways [84] . Subsequently, ACT1 (also known as CIKS), an activator of NF-kB that previously linked to B cellactivating factor and CD40L signaling, was found to contain a SEFIR domain (similar expression of fibroblast growth factor gene and IL-17Rs) domain [85]. It is recruited within minutes after IL-17A stimulation and binds IL-17RA through SEFIR-dependent interactions [86].

Moreover, ACT1 contains a TRAF6-binding motif and thus has the ability to bind TRAF6 and TGF-b-activated kinase 1 to deliver downstream signals, resulting in activation of the canonical NF-kB pathway. Deficiency in ACT1 (NF-κB activator 1) renders cells unresponsive to IL-17A , strongly suggesting its essential role in downstream signaling of IL-17RA. Consequently, the ACT1/TRAF6/NF-

kB pathway has now been elucidated and may be the most important signal pathway of IL-17A ,as explained in Figure (1-3). [83 , 86]

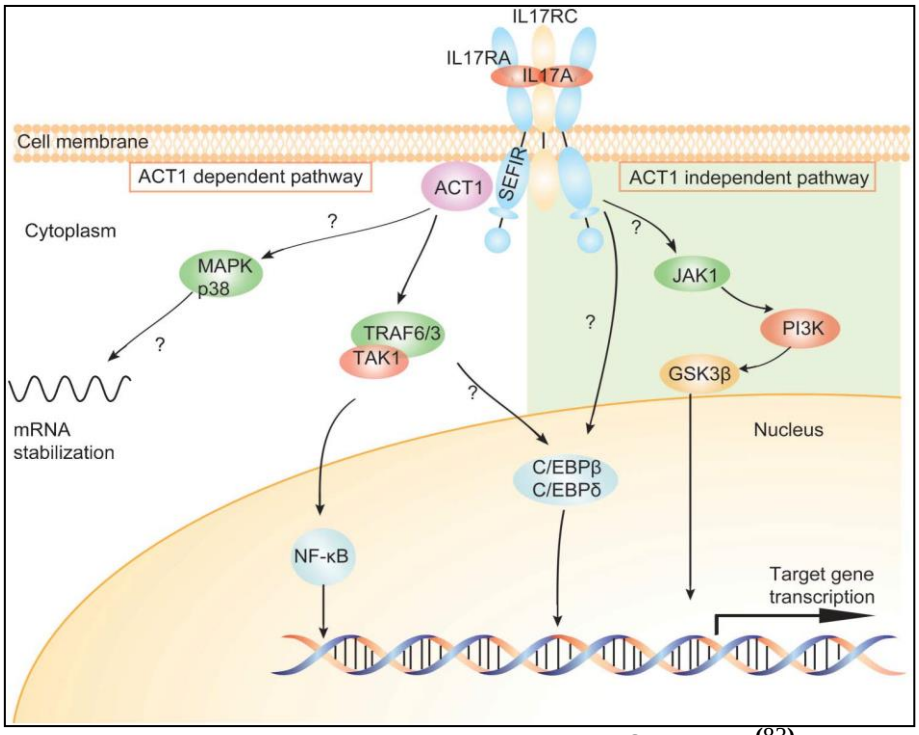

Figure (1-3): Signal pathways of IL-17. [83]

Interleukin-17RA was first specified as the receptor for IL-17A; moreover, subsequent studies have showed interaction with other family members. Although ubiquitously expressed, the major focus of IL-17RA biology has concentrated on stromal cells, which are the crucial targets for IL-17A and IL-17F. The regulation of IL-17RA expression has not been well studied but elevated IL-17RA expression has been detected in human inflammatory diseases such as arthritic joints from patients with Rheumatoid Arthritis (RA) , suggesting a role in autoimmunity [87]. In line with these reports, individual risk patterns within the IL-17RA gene that increase susceptibility to Crohn's disease were identified through genetic studies. [88].

1.13 Vitamin D:

The vitamin D system includes a group of fat-soluble pro-hormones and their respective metabolites .There are two main forms of vitamin D in nature : vitamin D2 (ergocalciferol) photochemically synthesized in plants , and vitamin D3 (cholecalciferol) synthesized in the skin of animals and humans in response to sunlight too, inparticular to ultraviolet B radiations of appropriate wavelength (270–300 nm) . In most countries in Europe and in the US the requirement of vitamin D is

given by 90% of the 7-dehydrocholesterol cholesterol synthesis in the skin from solar irradiation and only about 10% are taken up by the diet [89].

The classical synthetic pathway involves 25- and 1- alpha-hydroxylation of vitamin D2 and D3 , in the liver and kidney, respectively . First hydroxylation occurs within the liver and lead to the formation of 25 (OH) D or calcidiol ; second hydroxylation occurs within the kidneys and constitutes the most biologically active hormonal form of vitamin D (1,25 (OH)2D) , or calcitriol , as explained in Figure 1-4 [90] .

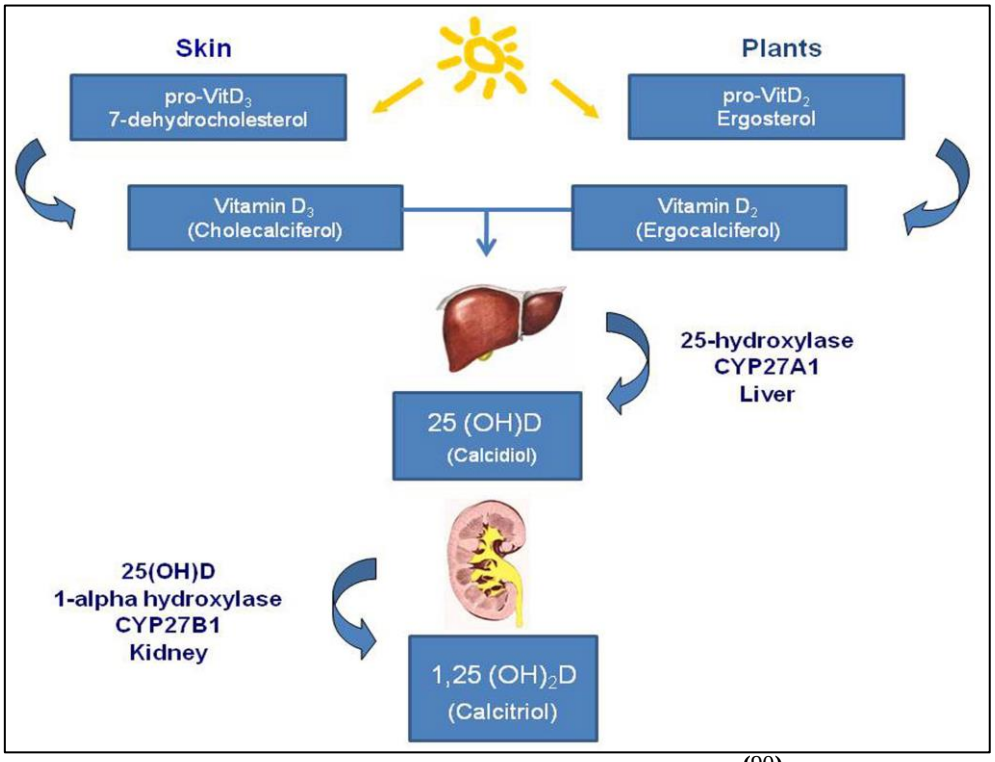

Figure (1-4) Vitamin D synthesis [90]

1.14 Role of vitamin D in the development of cancer :

Many studies have suggested that low vitamin D levels are a risk factor for human cancers, and vitamin D deficiency is associated with the incidence and mortality of many types of cancers, including breast, colon and prostate cancer [91].

There are several levels of evidence supporting the relationship between vitamin D and cancer :

- Low circulating levels of vitamin D are linked with increased risk of progression of cancer .
- A high intake of vitamin D is associated with a decreasing risk of cancer.

- Cancer aggressiveness is lower in the summer when vitamin D production is higher.
- Polymorphisms of genes encoding proteins involved in the signal pathway of vitamin D influence the risk of developing cancer .

This relationship is supported by in vitro studies and epidemiologic studies . A lot of in laboratory studies have demonstrated that exposure of tumor cells to high concentrations of vitamin D compounds prevent their proliferation and induce differentiation . Several epidemiological studies have indicated a correlation between the expected factors to reduce vitamin D levels (e.g., geography and latitude , history of sun exposure, lifestyle) and increased cancer incidence, highlighting the protective effects of sunlight and high levels of vitamin D on different types of tumors [92].

1.15 Vitamin D and Colorectal cancer:

Several epidemiological studies have been developed after the protective role of vitamin D in colorectal cancer has been proposed. The important point was to note that mortality from colorectal cancer were increasing with geographical latitude . The gradual decrease in vitamin D levels was taken into account as a result of the low UV-B radiation seen at higher latitudes to explain the geographical pattern of cancer mortality. [93].

It is now well established that vitamin D and its metabolites act as inhibitors of colorectal cancer progression via several underlying mechanisms, some of which have been clarified during past few years [94]. Moreover, a recent and extensive European observational study also confirmed a strong inverse relationship between 25(OH)D concentrations and risk of developing colorectal cancer [95].

1.16 Carcinoembryonic antigen (CEA) :

Carcinoembryonic antigen (CEA), an autoantigen that is expressed at low levels in normal intestinal epithelia. It is remarkably regular in most colorectal cancer (CRC) [96]. It is a tumor marker that is used in the diagnosis of colorectal cancer. It is also used before surgery staging and postoperative follow-up of patients, in particular patients who using chemotherapy [97]. CEA affects tumorigenesis by promoting tumor cell survival and by inducing tumor angiogenesis [98] .

CEA is used as a preoperative test in patients with colorectal cancer if it would help in staging and surgical treatment planning.

Although raised preoperative CEA may correlate with poorer prognosis, CEA is the appropriate marker for monitoring metastatic colorectal cancer during systemic therapy [99]. Until now, serum CEA level is still considerably used as a marker to monitor the recurrence after surgery, but is rarely a sign of disease prediction. [100].

Currently, the American Society of Clinical Oncology and the European Group on Tumor Markers have recommended that the serum CEA test as a vital indicator for predicting recurrent CRC after therapeutic resection. Even so, the effectiveness of CEA as a preoperative and postoperative marker of CRC still has to be evaluated. In particular, it remains unclear how accurate a negative CEA value is for excluding primary and recurrent CRC, and under what conditions CEA values are inaccurate . Reference values of serum CEA for non-smokers are less or equal 3.0 ng/mL, some smokers may have elevated CEA, usually up to 5.0 ng/mL. Serum markers are not specific for malignancy, and values may vary by method [99]. Elevated CEA levels are also more common in smokers and in patients with inflammatory conditions but rarely exceed 10 ng/mL. The test can be also elevated in a variety of other carcinomas, including lung, breast, gastrointestinal, and gynecologic cancers [101].

CEA is not specific to any cancer type, but its concentrations in blood may predict the therapeutic effect, progression, and prognosis of the disease. CEA is most widely used in detecting gastrointestinal cancers, especially colorectal cancer. Several organizations recommend the measurement of both preoperative and postoperative levels of CEA in patients with colorectal cancer [102,99].

In some studies, high CEA concentrations in patients with CRC stage II and III were found to be potentially indicative of more aggressive types of cancer [103]. Earlier, the colorectal working group of American joint committee on cancer proposed to include CEA baseline concentration to the traditional TNM classification as the so-called C-stage. C-stage was proposed to be divided into Cx, C0 (CEA < 5 ng/mL) and C1 (CEA > 5 ng/mL) substages [104].

Constant increase in CEA levels is typically associated with a progression of the disease, even though radiological tests may prove otherwise [99 ,102]. However, chemotherapy can also result in temporary increase in CEA concentration, which must be also taken into account. Therefore, it is not recommended to test CEA levels within 2 week of chemotherapy , whereas in patients on oxaliplatin, tests can be carried out after 4 to 6 week [105].

According to previous studies, the serum concentration of soluble tumor markers in obese populations is lower compared with that in non-obese subjects [106].

Serum carcinoembryonic (CEA) is a set of glycoproteins involved in cell adhesion . An early study on CEA showed an association between tumor stage and elevated CEA. On the other hand, there is an inverse relationship between CEA levels and tumor grade: well-differentiated tumors tend to produce more CEA than poorly differentiated tumours . CEA has no value in screening for bowel cancer as the positive predictive value is too low . However, measuring CEA during postoperative follow-up is common because CEA levels may rise with cancer recurrence, which is important for early detection, treatment at a curable stage, and therefor to improve survival [107].

1.17 Acid Phosphatase (ACP) :

The measurement of changes in enzymatic activity is usually employed as an important clinical assessment tool for detecting, diagnosing, screening and monitoring diseases and pathological processes. Some of the enzymes used in diagnosis include acid phosphatase (in malignant diseases) [108] .

The acid phosphatase enzyme is used to release the attached phosphoric group and is called phosphomonoesterase. Studies suggest that phosphatase enzymes are importance in regulating metabolic processes such as kinase enzymes [109]. The importance of acid phosphate phosphatase antagonists is prominent in the diagnosis of some diseases such as Prostatic acid phosphatase (PAP) in the blood of prostate cancer patients because of the increased number of cells that make the enzyme [110].

1.18 Magnesium:

Magnesium is the most common divalent cation, found in the ionized form Mg^{2+} and forms Mg-ATP complexes with adenosine triphosphate (ATP) [111]. Magnesium plays a complex function in our body but accounts for only 0.05% of body weight. This element is regarded as the macro element in the human body and important for human health [112]. Magnesium is involved in the maintenance of normal muscle and nerve function, heart rhythm, bone strength, and immune system. Consequently, daily proper intake of this element is required. The recommended dosage of Mg for adults is 300-420 mg/day . Magnesium

can be obtained from all kinds of foods and water. Magnesium rich foods include grains, nuts, vegetables, and fruits. Actual intake of Mg is determined by various factors [113].

Magnesium deficiency increases genomic instability and it has been reported to be inversely associated with a risk of colorectal cancer (CRC). That organo-Mg inhibits inflammation related mouse colon carcinogenesis by modulating the proliferative activities and chromosomal instability of CRC and suppressing colonic inflammation may suggest potential use of organo-Mg for clinical chemoprevention trials of CRC in the inflamed colon . A complex relationship links magnesium and cancer. Impaired magnesium homeostasis is reported in cancer patients, and frequently complicates therapy with some anticancer drugs. More studies should be undertaken in order to disclose whether a simple and inexpensive intervention to optimize magnesium intake might be helpful in the prevention and treatment of cancer [114].

1.19 Phosphorus intake and cancer:

Phosphorus is found in the mineral structure of bones and teeth and in soft tissues where it participates mainly in phosphorylation processes and acid-base equilibrium. Its deficiency is not common because it is present in most foods and its absorption is relatively high [115].

Although studies on the interaction between variations in dietary P supply and the immune system are rare, the overall picture from current studies indicates that dietary P has a positive impact on the adaptive immune response due to modulations of lymphocyte proliferation and antibody response [116].

Several types of cancer have been reported to be associated with high-P intake, including lung, colon, breast, ovary, and endometrial cancer, among others [117]. Phosphorus is a vital element for the cells and organisms [118]. It has been already reported, few decades ago, that phosphorus increases in the blood in various conditions and especially in cancer due to increase the requirements of the rapidly growing cells. It seems that might face the axiom that “there is no growth of cancer in the body without an increase of phosphorus in the blood” [119]. The main explanation might be the fact that phosphorus is the nutrient for the cancer cells [120].

1.20 Body mass index (BMI) :

Body mass index (BMI), which is defined as the relation of one's weight in kilograms divided by the square of one's height in meters, is a useful tool in clinical practice for assessing adult weight and nutritional

status. Higher BMIs are related to morbidity and prognoses [121,122]. The BMI categories are shown in Table 1-3 .

Table (1-3): Categories of BMI [123]

underweight	15-19.5
Normal weight	20-24.9
Overweight	25- 29.9
Class I obesity	30-34.9
Class II obesity	35.9
Class III obesity	≥ 40

One of the primary risk factors for colorectal cancer is obesity, a condition typically assessed using a scale known as the body mass index (BMI) [124]. Understanding the association between BMI and the prognosis of colorectal cancer is highly important to provide body weight guidelines for colorectal cancer patients. Studies have clearly identified that being underweight is associated with increased risk of death, probably due to cancer progression-associated weight loss [125,126]. A reverse association between weight change and cancer risk has been observed [127]. Inconsistent findings have also been observed among studies which examined the association between being obese and the prognosis of colorectal cancer; some reported increased mortality [128] , while others reported reduced the mortality among obese colorectal cancer patients [125] .

1.21 Aim of the study:

The objective of this study is to:

1- Valuation of Interleukin-17 (IL-17), Interleukin-17RA (IL-17RA) in male and female patients suffering from colorectal cancer before and after taking chemotherapy.

2- Found correlation relation for Interleukin-17 and Interleukin-17RA with BMI, Vitamin D, CEA, ACP , Mg ,and P in these patients, in order to be marker in early diagnosis of these patients.

3- Study the difference between male and female patients with CRC by that parameters.

Chapter Two
Materials
&
Methods

2.1 Materials:

Table (2-1) Chemicals and kits that used and It's suppliers.		
No.	**Chemicals**	**Suppliers**
1.	Acid Phosphatase - kit	Human Germany
2.	CEA - ELISA Kit	Elabscience China
3.	Interleukin-17 - ELISA kit	Elabscience China
4.	Interleukin-17 receptor A - ELISA kit	Elabscience China
5.	Magnesium liquicolor - Kit	Human Germany
6.	Phosphorus liquirapid - Kit	Human Germany
7.	VIT. D – Kit	BioMérieux U.S.A

2.2 Instruments and Suppliers :

Table(2-2) : Instruments and their Suppliers		
No.	**Instruments**	**Suppliers**
1.	80-1 Electronic Centrifuge	Hettich Germany
2.	HumaReader HS	Human Germany
3.	Oven	Memmert , Germany
4.	Spectrophotometer	EMclab Germany
5.	Spectrophotometer medical PD-303	Apel Japan
6.	VIDAS® 30	BioMérieux USA

2.3.1 Study groups :

The sample of blood were collected from (148) males and females whose average age (50-56) years were enrolled in this study in Oncology teaching hospital in Baghdad from December 2018 to June 2019 . They were divided into four groups as follows:

1- The colorectal cancer patients a (48 males) and (40 females) before taking a dose of chemotherapy (G1).

2- The same patients in G1 after three weeks taking a first dose of chemotherapy (G2).

3- The same patients in G1 and G2 , after three weeks taking the second dose of chemotherapy (G3) .

4- Healthy control group as group four (G4) , consist of 30 males and 30 females .

All patients in G2 and G3 were taking 5-fluorouracil (5FU) , oxaliplatin and leucovorin chemotherapy . The patients with diabetic mellitus and renal failure were excluded .

2.3. 2 Collection of blood samples:

Disposable plastic syringes were used to withdraw 5 ml of venous blood from each patients and control group. The blood sample was trans fenced inside plain test tube to get the serum to determine IL-17, IL-17RA, vitamin D, CEA, acid phosphatase , phosphors and magnesium .

2.4 Methods:

2.4.1 Determination of interleukin-17 (by ELISA kit) :

Principle:

This ELISA kit uses the Sandwich – ELISA principle, and the micro Elisa plate provided in this kit has been pre-coated with an antibody specific to human IL-17 . The standards and samples were added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for human IL-17 and avidin-horseradish peroxidase (HRP) conjugate were added successively to each micro plate well and after that incubated, and free components were washed away. The substrate solution was added to each well. Only those wells that contain human IL-17 , biotinylated detection antibody and Avid in-HRP conjugate were appeared blue in color. A reaction stop solution was added to terminate the substrate enzyme reaction to turn yellow. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value was proportional to the concentration of human IL-17. The concentration of human IL-17 can calculate in the samples by comparing the OD of the samples to the standard curve.

Procedure:

1- Standard working solution: the standard was put in centrifuge at 10,000 × g for 1min . 1.0 ml of Reference standard & sample Diluent was added , then it was stood for 10 min and inverted it gently several times, after it dissolved fully and mixed it thoroughly with a pipette.

This reconstitution produced a working solution of 2000 pg/ml , then serial dilutions were made as needed . The recommended dilution gradient was as follows: 2000, 1000, 500, 250, 125, 26.5, 31.25, 0 pg/ml.

Seven EP tubes were taken , then 500µL of Reference Standard & Sample Diluent were added to each tube, then 500µL of the 2000 pg/mL working solution was pipetted to the first tube and mixed up to produce a 1000 pg/mL working solution . Then, 500µL of the solution from the former tube was pipetted into the latter one according to these steps, it was (dilution method).

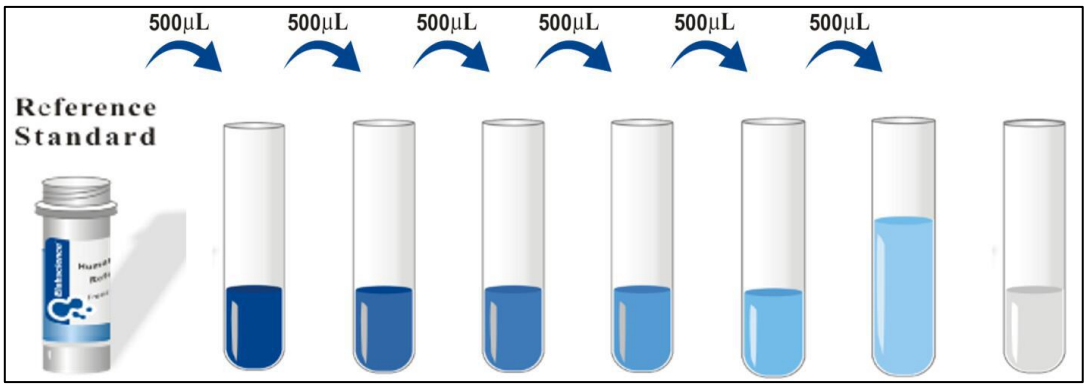

Figure (2-1): Standard solution of IL-17

2- The standard working solution was added to the first two columns : Each concentration of the solution was added in duplicate , to one well each , side by side (100 µL for each well). Then the plate was covered with the sealer provided in the kit and incubated for 90 min at 37˚C .

3- The liquid was removed out of each well and without wish . Then, 100µL of Biotinylated Detection Ab working solution was added to each

well, after that was covered with the plate sealer , gently mixed up and incubated for 1 hour at 37˚C .

4- Thirty mL of concentrated wash buffer was diluted with 720 mL of deionized or distilled water , It was used to prepare 750 mL of wash buffer .

5- Three hundred fifty µL of wash buffer was added to each well to aspirate or decant the solution from each well . The solution was soaked for (1-2) minutes and aspirated or decanted from each well and pat it dry against clean absorbent paper. This wash step was Repeated 3 times in total.

6- A hundred µL of HRP Conjugate working solution was added to each well. Then , it was covered with the plate sealer and incubated for 30 minutes at 37˚C .

7- The solution was aspirated or decanted from each well and the wash process was repeated for five times as conducted in step3.

8- Ninety µL of Substrate reagent was added to each well, and it was covered with a new plate sealer then incubated for about 15 minutes at 37˚C to protect the plate from light.

9- Fifty µL of stop solution was added to each well.

10- A micro-plate reader set was used to 450 nm to determine the optical density (OD value) of each well at once .

2.4.2 Determination of interleukin-17 receptor A (by ELISA kit) :

Principle:

This ELISA kit uses the Sandwich – ELISA principle. The micro Elisa plate provided in this kit has been pre-coated with an antibody specific to human IL-17RA . Standards and samples were added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for human IL-17RA and Avidin-Horseradish Peroxidase (HRP) conjugate were added successively to each micro plate well and after that incubated, and free components

were washed away. The substrate solution was added to each well. Only those wells that contain human IL-17RA, biotinylated detection antibody and Avid in-HRP conjugate were appeared blue in color. A reaction stop solution was added to terminate the substrate enzyme reaction to turn yellow. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value was proportional to the concentration of human IL-17RA. The concentration of human IL-17RA can calculate in the samples by comparing the OD of the samples to the standard curve.

Procedure:

1- Standard working solution: the standard was put in centrifuge at 10,000 × g for 1min . 1.0 ml of Reference standard & sample Diluent was added , then it was stood for 10 min and inverted it gently several times, after it dissolved fully and mixed it thoroughly with a pipette.

This reconstitution produced a working solution of 4000 pg/ml , then serial dilutions were made as needed . The recommended dilution gradient was as follows: 4000, 2000, 1000, 500, 250, 125, 26.5, 0 pg/ml.

Seven EP tubes were taken , then 500µL of Reference Standard & Sample Diluent were added to each tube, then 500µL of the 2000 pg/mL working solution was pipetted to the first tube and mixed up to produce a 1000 pg/mL working solution . Then, 500µL of the solution from the former tube was pipetted into the latter one according to these steps, it was (dilution method).

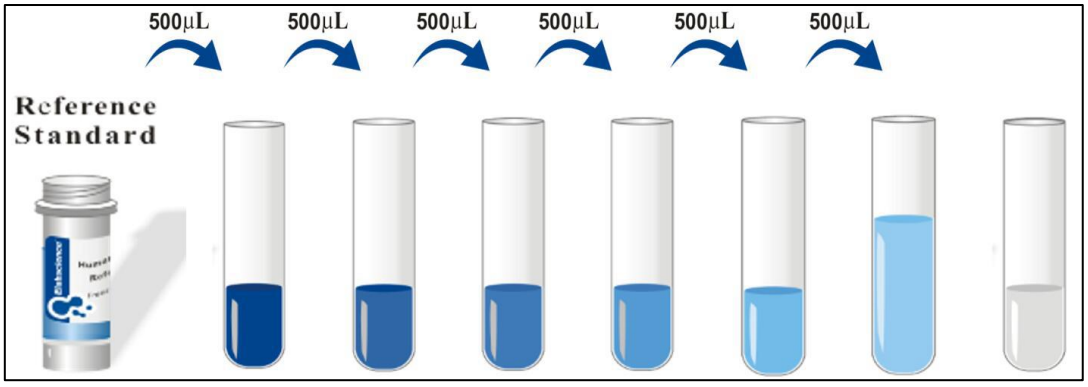

Figure (2-2): Standard solution of IL-17RA

2- The standard working solution was added to the first two columns : Each concentration of the solution was added in duplicate , to one well each , side by side (100 µL for each well). Then the plate was covered with the sealer provided in the kit and incubated for 90 min at 37˚C .

3- The liquid was removed out of each well and without wish . Then, 100µL of Biotinylated Detection Ab working solution was added to each well, after that was covered with the plate sealer , gently mixed up and incubated for 1 hour at 37˚C .

4- Thirty mL of concentrated wash buffer was diluted with 720 mL of deionized or distilled water , It was used to prepare 750 mL of wash buffer .

5- Three hundred fifty µL of wash buffer was added to each well to aspirate or decant the solution from each well . The solution was soaked for (1-2) minutes and aspirated or decanted from each well and pat it dry against clean absorbent paper. This wash step was Repeated 3 times in total.

6- A hundred µL of HRP Conjugate working solution was added to each well. Then , it was covered with the plate sealer and incubated for 30 minutes at 37˚C .

7- The solution was aspirated or decanted from each well and the wash process was repeated for five times as conducted in step3.

8- Ninety µL of Substrate reagent was added to each well, and it was covered with a new plate sealer then incubated for about 15 minutes at 37˚C to protect the plate from light.

9- Fifty µL of stop solution was added to each well.

10- A micro-plate reader set was used to 450 nm to determine the optical density (OD value) of each well at once .

2.4.3 Evaluation of 25OH Vitamin D using VIDAS® 25 OH Vitamin D Total : [129]

The VIDAS® 25 OH vitamin D total is an automated quantitative test for the determination of 25 hydroxyvitamin D Total in human serum

or plasma using the ELFA (Enzyme Linked Fluorescent Assay) technique. It reflects vitamin D produced cutaneously and that obtained from food and supplements for a reliable indication of vitamin D status. This method is very well correlated to the liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) reference method. So it offers the same high degree of precision and is easy to perform in any lab for rapid results.

Principle:

The principle assay combines an enzyme immunoassay competition method with a final fluorescent detection (ELFA). The solid phase receptacle (SPR®) was served as the solid phase as well as the pipetting device for the assay. And reagents for the assay are ready-to-use and predispensed in the sealed reagent strips. All steps of the assay were performed automatically by the instrument. The reaction medium was cycled in and out of the SPR in the several times. The sample is mixed with pre-treatment reagent to separate vitamin D from its binding protein. The pre-treated sample then collected and transferred into the well whose contains an alkaline phosphatase (ALP)- labeled anti-vitamin D antibody (conjugate). And vitamin D antigen present in the sample and the vitamin D antigen coating the interior of the SPR compete for binding sites on the anti-vitamin D antibody-ALP conjugate. During the final detection step, the substrate (4-Methylumbelliferyl phosphate) is cycled in and out of the SPR. And conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methylumbelliferone), the fluorescence measured at 450 nm. The intensity of the fluorescence was inversely proportional to the concentration of vitamin D antigen present in the sample. The results were calculated by the instrument in relation to the calibration curve stored in memory, and then printed out.

Procedure:

1. The required was only removed reagents from the refrigerator.

2. One "VITD" strip and one "VITD" SPR® were used from the kit for each sample, control or calibrator to be tested. It must be sure the storage pouch has been carefully resealed after the required SPRs have been removed.

3. The test was identified by the "VITD" code on the instrument.

4. The samples were Clarified by centrifugation if it necessary.

5. The calibrator was mixed, control and samples by using a vortex type mixer (for serum or plasma separated from the pellet).

6. Ensure that samples, calibrators, controls and diluent were freed of bubbles, before pipetting .

7. The calibrator, control, and sample test portion was 100 µL in this test.

8. The "VITD" SPRs and "VITD" strips were Inserted into the instrument. The color labels with the assay code on the SPRs and the Reagent Strips must be match.

9. The assay was initiated as directed in the User's Manual. All the assay steps were performed automatically by the instrument.

10. After pipetting , the vials was reclosed and return them to 2–8°C.

11. The assay was completed within approximately 40 minutes. Once the assay was completed, then results were analyzed automatically by the computer. After the assay was completed, the SPRs and strips were removed from the instrument.

12. The used SPRs and strips were disposed into an appropriate recipient.

2.4.4 Determination of CEA (by ELISA kit) :

Principle:

This ELISA kit uses the sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to human CEA. Standards , samples were added to the micro ELISA plate wells and combined with the specific antibody. A biotinylated detection antibody specific was added successively for human CEA and avidin-horseradish peroxidase (HRP) conjugate are to each micro plate well and incubated. Free components were washed away. The substrate solution was added to each well. Only those wells that contain human CEA, biotinylated detection antibody and Avidin-HRP conjugate will appear the color blue. The enzyme-substrate reaction

was terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured by spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of human CEA. We can calculate the concentration of human CEA in the samples by comparing the OD of the samples to the standard curve.

Procedure:

1. A hundred µL standard or sample was added to each well and incubated for 90 min at 37℃ , after that removed the liquid.

2. A hundred µL biotinylated detection Ab was added. Incubated for 1 hour at 37℃ , then aspirated and washed 3 times.

3. A hundred µL HRP conjugate was added , and incubated for 30 min at 37℃, then aspirated and washed 5 times.

4. Ninety µL of substrate reagent was added also incubated for 15 min at 37℃.

5. fifty µL stop solution was added , and read at 450 nm immediately, then results were calculated.

2.4.5 Determination of total Acid phosphatase:[130]

Principle:

p-Nitro phenyl phosphate + H_2O $\xrightarrow{Acid\ phosphatase}$ p.nitrophenol + phosphate

Reagents:

- Reagent -1- Buffer

Citrate buffer pH 4.8	55 mmol/L

- Reagent -2- Substrate

p-Nitro phenyl phosphate	5.5 mmol/L

- Reagent -4-

Sodium Hydroxide	200 mmol/L

Preparation of Solution :

1- Buffer : reagent -1-

The content was ready for using.

2- Substrate reagent -2-

The contents of 10 mL reagent (2) was reconstituted with 10 ml of buffer reagent (1). It was stabled for 5 days at +2 to +8 ˚C .

3- Sodium hydroxide reagent (4)

For 10 assays 10 ml of reagent (4)was diluted with 90 ml of D.W.

Procedure:

	Reagent blank	serum
Solution -2-	1 ml	1ml

Solution -2- Was incubated for exactly 5 min at 37 ˚C , then was added to:

Serum	/	0.2ml

And incubated for exactly 30 minutes at 37 ˚C, then was added to:

Diluted NaOH	10 ml	10 ml

The content were mixed and the absorbance of the serum against the reagent blank was read at 405 nm.

Calculation :

Total Acid phosphatase U/L = 101 × A_{sample}

A_{sample} = Absorbance of serum at 540 nm.

2.4.6 Determination of serum magnesium:[131]

Principle:

Magnesium ion in an alkaline medium from a coloured complex with xylidyl blue. The absorbance increase is proportional to the magnesium concentration in the sample. Glycoletherdiamine-N, N, N', N'-tetraacetic acid (GEDTA) was used as masking agent for calcium ions.

Reagents:

Reagent: 2 × 100 ml colour reagent:

CAPS	50 mmol/L
GEDTA	13 mmol/L
Xylidyl blue	0.09 mmol/L
Sodium azide	0.095 %
Activators	

Standard : 1 × 3 ml standard

Magnesium(II)	10 mg/dl or 1.03 mmol/L
Sodium azide	0.095 %

Procedure:

Pipette into cuvettes	Regent blank	Sample or standard
Sample / standard	---	10 µl
Distilled water	10 µl	---
Reagent	1000 µl	1000 µl

Mixture was incubated for 10 minutes at 20 … 25 ˚C. The absorbance of the sample was measured and the standard against the reagent blank within 60 minutes (ΔA).

Calculation of the magnesium concentration:

$$C = 2.5 \times \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \quad (mg/dl)$$

or

$$C = 1.03 \times \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \quad (mmol/L)$$

2.4.7 Determination of phosphorus :[132]

Principle:

Phosphate reacts with molybdate in strong acidic medium to from a complex. The absorbance of this complex in the near UV is directly proportional to the phosphate concentration.

$$7H_3PO_4 + 12(Mn_7O_{24})^{6-} + 51\ H^+ \longrightarrow 7[P(Mn_{12}O_{40})]^{3-} + 36H_2O$$

Reagents:

Reagent: 2 × 100 ml reagent :

Ammonium heptamolybdate	0.3 mmol/L
Sulphuric acid (pH < 1.0)	160 mmol/L
Detergent	1%
Activators and stabilisers	

Standard : 1 × 5 ml standard

Phosphorus	10 mg/dl or 3.2 mmol/L

Procedure:

Pipette into cuvettes	Regent blank	Sample or standard
Sample / standard	---	10 µl
Reagent	1000 µl	1000 µl

Mixture was incubated at least 1 minute at room temperature. The absorbance of the sample was measured and the standard against the reagent blank within 60 minutes (ΔA).

Calculation of the phosphorus concentration:

$$C = 10 \times \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \quad (mg/dl)$$

or

$$C = 2.3 \times \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \quad (mmol/L)$$

2.5 : Statistical analysis :

The statistical analysis of this prospective study performed with the Graph Pad Prism® 7 and Microsoft Excel 2013. Numerical data with normal distribution were described as a mean and standard deviation. Independent sample t-test used for comparison between each two groups. Categorical data were described as count and percentage. Chi-square test or fisher exact test was used to estimate the association between variables. The lower level of accepted statistical significant difference is bellow or equal to 0.05. Correlation of coefficient used for the estimation of correlation between studied variables [133] .

Chapter Three

Results & Discussion

Results & Discussion:

Table 3-1 and figures 3-1, 3-2, 3-3, and 3-4 show the levels of IL-17 , IL-17RA , vitamin D and CEA, concentration respectively in sera of patients males and females with colorectal cancer before and after three weeks first and second dose of chemotherapy and healthy control .

In this Table it was found highly significant increasing of IL-17 male and female before taking the dose of chemotherapy (G1) than other groups and high significant level of IL-17RA in male patient to the same group (G1) . However, the female patients whose taking the second dose of chemotherapy (G3) it be high significant increase than other groups.

A higher IL-17 and IL-17RA serum levels in patients without taking chemotherapy dose than control groups and after taking the dose of chemotherapy groups may be due to that proinflammatory cytokines . They are considered to be associated with CRC and that interleukin and its receptor which are one of the most important cytokines , promotes angiogenesis and tumor growth , and attracts neutrophils to the site of inflammation and enhances the inflammatory cascade . [75]

The IL-17RA signaling was required for outgrowth of aberrant crypt foci (ACF) into colonic tumors during the early phase of CRC development. The immune modulator of (IL-17A) could inhibit tumor progression and sensitize established tumors to chemotherapy . So the numbers of Th17 cells and expression of (IL-17) are increased in human cancer and it increased to indicate poor prognosis. [134]

The main source of IL-17 in CRC tumors and adjacent tissues is $CD4^+$ Th17 immune cells [135,50] , while the (IL-17) binds to a heterodimer receptor comprising an (IL-17ARA) and (IL-17RC) chain and the (IL-17) R receptor is expressed on a variety of cells, such as hematopoietic , fibroblastic and epithelial cells [56,136] . The increased level of that receptor of IL-17 signaling on trans formed colonic epitheral cells is sunfficent to promote CRC development . [135]

Table (3-1) IL-17 concentration and some biochemical parameters in sera of male and female patients groups with colorectal cancer and healthy control .

	Parameters	Patients group			Control group (No. 30)	*P* value					
		G1	G2	G3	G4	G1 vs. G2	G1 vs. G3	G1 vs. G4	G2 vs. G3	G2 vs. G4	G3 vs. G4
Male Patients group (No. 48)	IL-17 (pg/mL)	117.21 ± 10.575	83.51 ± 7.621	69.45 ± 8.712	35.13 ± 1.495	h s	h s	h s	h s	S	Ns
	IL-17RA (pg/mL)	292.31 ± 18.021	195.03 ± 13.882	139.26 ± 8.90	155.15 ± 7.554	h s	h s	h s	h s	Ns	Ns
	Vitamin D (ng/mL)	11.08 ± 3.4988	27.07 ± 9.267	34.06 ± 11.388	46.04 ± 1.565	h s	h s	h s	h s	h s	h s
	CEA (ng/mL)	5.62 ± 0.351	3.97 ± 0.214	5.50 ± 0.387	3.47 ± 0.142	S	Ns	S	h s	Ns	S
	Acid phosphatase (U/L)	10.16 ± 1.071	10.83 ± 2.047	9.09 ± 1.197	7.96 ± 0.601	S	h s	h s	h s	h s	h s
	Magnesium (mg/dl)	7.74 ± 1.897	6.86 ± 1.439	6.25 ± 1.152	2.05± 0.421	S	h s	h s	S	h s	h s
	Phosphorus (mg/dl)	8.98 ± 1.721	8.43 ± 1.564	7.74 ± 1.207	5.07 ± 1.625	h s	h s	h s	h s	h s	h s
	BMI (Kg/m^2)	26.62 ± 4.603	27.28 ± 4.268	27.55 ± 3.529	28.05 ± 5.671	Ns	Ns	Ns	Ns	Ns	Ns
Female Patients group (No. 40)	IL-17 (pg/mL)	160.90 ± 9.783	157.63 ± 11.0699	86.65 ± 5.948	35.17 ± 1.325	Ns	h s	h s	h s	h s	h s
	IL-17RA (pg/mL)	266.55 ± 17.405	210.03 ± 7.859	279.90 ± 18.452	184.18 ± 18.418	h s	h s	S	h s	Ns	S
	Vitamin D (ng/mL)	10.58± 2.224	13.30 ± 1.781	14.90 ± 2.969	41.45 ± 1.180	h s	h s	h s	h s	h s	h s
	CEA (ng/mL)	6.03 ± 0.866	6.81 ± 0.857	8.25 ± 0.819	2.78 ± 0.131	h s	h s	Ns	h s	S	h s
	Acid phosphatase (U/L)	10.97 ± 2.467	10.61 ± 1.139	8.99 ± 1.258	6.26 ± 0.157	Ns	h s	h s	h s	h s	h s
	Magnesium (mg/dl)	8.97 ± 1.027	6.18 ± 1.312	4.74 ± 0.833	2.32 ± 0.753	h s	h s	h s	h s	h s	h s
	Phosphorus (mg/dl)	9.29 ± 0.827	8.93 ± 0.895	8.71 ± 0.831	4.11 ± 0.531	Ns	h s	h s	Ns	h s	h s
	BMI (Kg/m^2)	30.19 ± 7.125	29.1 ± 6.646	29.18 ± 7.188	27.31 ± 4.164	S	S	h s	Ns	h s	S

G1 = patients group before taking dose of chemotherapy
G2 = patients group after taking first dose of chemotherapy
G2 = patients group after taking second dose of chemotherapy
G4 = healthy control

S = Significant (*P* value ≤ 0.05)
Ns = Non significant (*P* value > 0.05)
h s = high significant (*P* value ≤ 0.001)

Chemotherapy remains an important treatment option for patients diagnosed at an advanced stage , and a large proportion of patients with cancer relapse and develop drug resistance , the chemotherapy usually induced tumor microenvironment remodeling to sustain the cellular hierarchy of the tumor by secreting cytokine [137] . While other study

show that decrease of (IL-17) and (IL-17RA) in sera of the same patients after taking chemotherapy dose due to the chemotherapy drugs inhabit the IL-17 and (IL-17RA) .

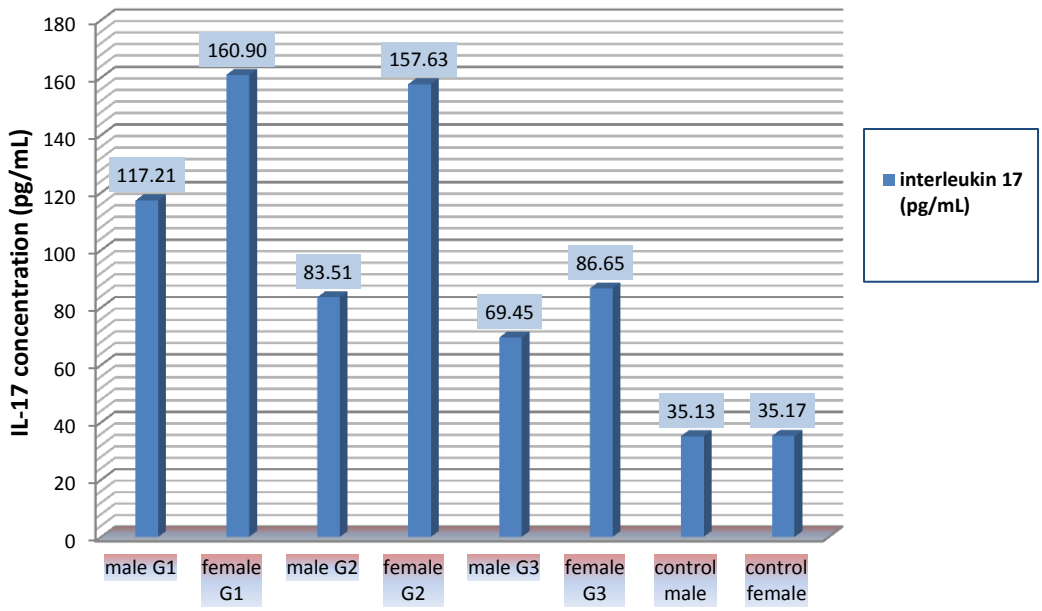

Figure (3-1): IL-17 concentration (pg/mL) in sera of male and female patients groups with colorectal cancer and healthy control

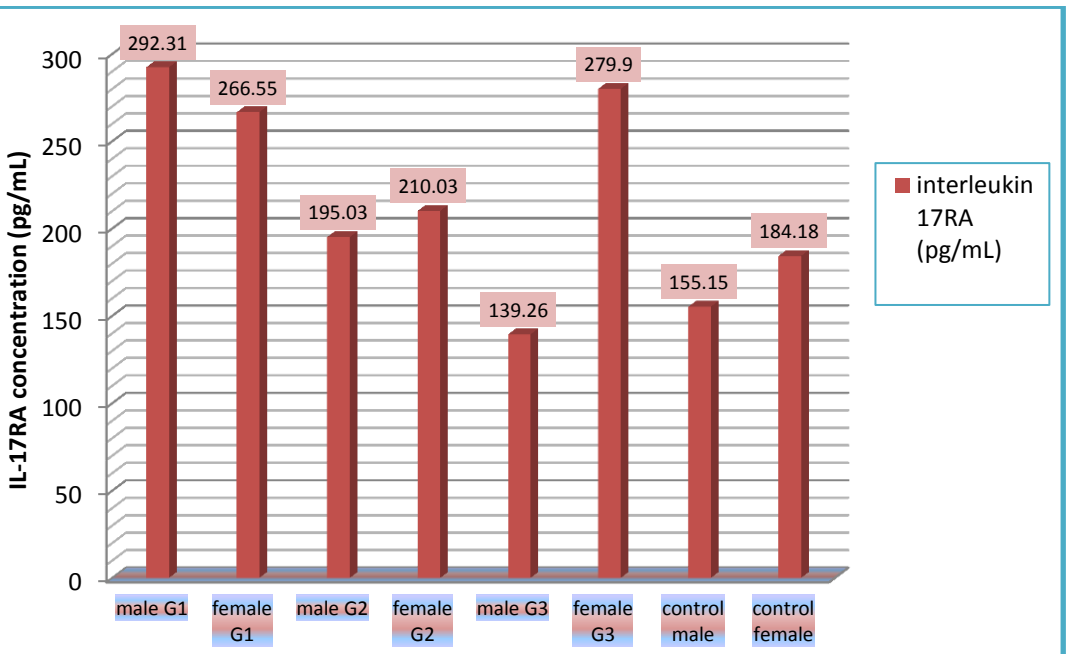

Figure (3-2): IL-17RA concentration (pg/mL) in sera of male and female patients groups with colorectal cancer and healthy control

The result in Table 3-1 and Figures 3-3, 3,4 showed the levels concentration for vitamin D and CEA in male and female before and after taking the dose of chemotherapy and healthy control group.

In the recent study, we observed a highly decrease in vitamin D for group one (G1) in male and female patient before taking a dose of chemotherapy than other groups , so a highly significant increase in CEA for male and female patients of G1 than other groups.

A highly significant decrease of vitamin D in male and female patients before taking a dose of chemotherapy as compression with healthy control and after taking a dose of chemotherapy. Recent studies suggest that a much broader range of biological functions of vitamin D, including potential of anti neoplastic effects colon cancer mortality rates in US. Vitamin D can be an effective way to reduce cancer incidence and mortality[138,,139]. Vitamin D from the skin and diet is activated to calcitrol by two cytochrome pusomediated hydroxylation steps . The response of cancer cells to calcitrol depends not only on vitamin D. Vitamin D receptor (VDR) but on the intra-cellular concentration of calcitrol as well[140]. Vitamin D receptor is present in most cell of the human body and in well or moderately differentiated colorectal cancer tissues[141]. Also in the same tables the levels of vitamin D increased after taking a dose of chemotherapy. This is due to the patient's after taking chemotherapy dose taking also supplementation of vitamin D.

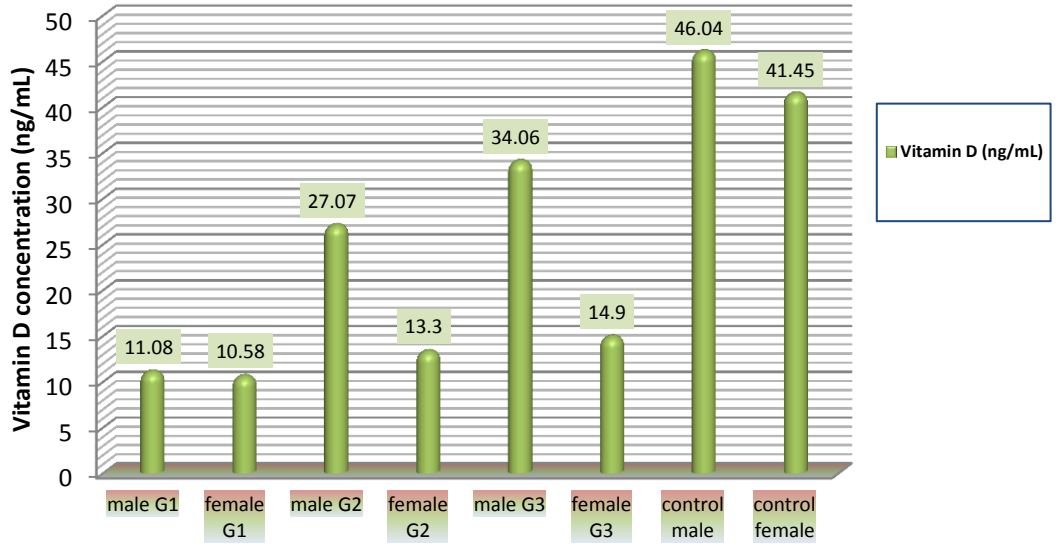

Figure (3-3): Vitamin D concentration (ng/mL) in sera of male and female patients groups with colorectal cancer and healthy control

Table 3-1 and figure 3-4 showed a highly significant levels in CEA for males and females patients before and after taking a dose of chemotherapy when compared with control group.

Carcinoembryonic antigen (CEA) was first described when they identified as antigen that was present in both fetal colon and colon adenocarcinoma but that appeared to be absent from healthy[142].

So poorly significant levels in CEA for control healthy than patient male and female due to the protein was detected in only cancer and embryonic tissue. It was given the name carcinoembryonic antigen or (CEA)[143]. So the use of CEA as a markers in colorectal cancer. The results study of table 1-3 showed the significant decrease in CEA after taking dose of chemotherapy in male and female patients and a highly significant increase after taking the second dose of chemotherapy.

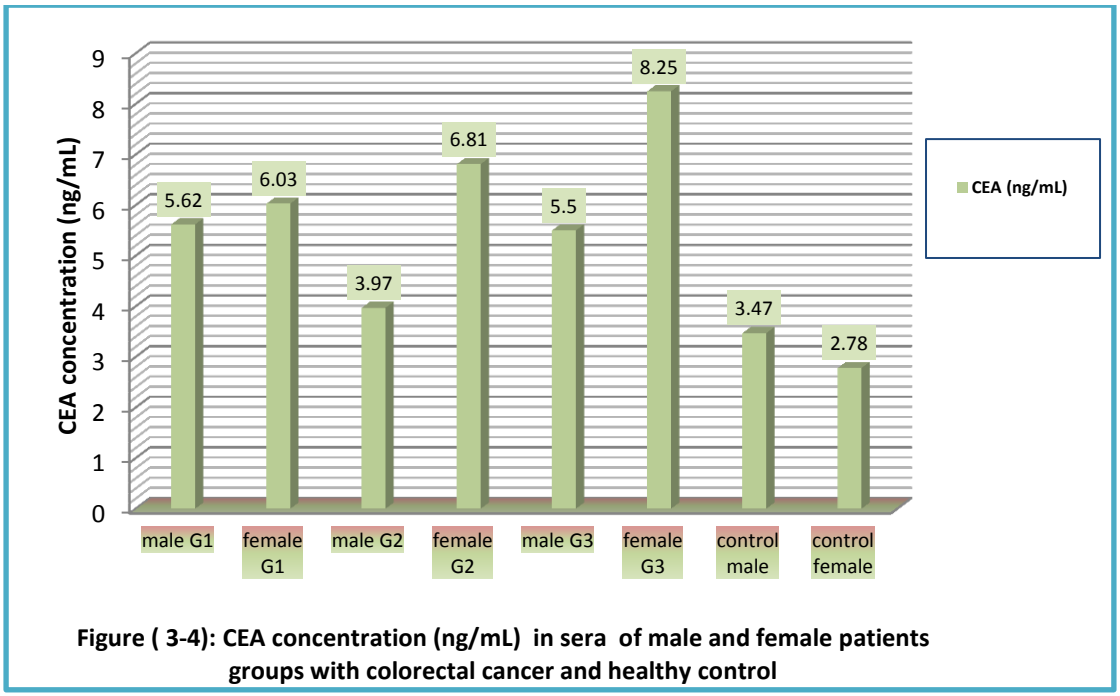

Figure (3-4): CEA concentration (ng/mL) in sera of male and female patients groups with colorectal cancer and healthy control

Table 3-1 and Figures 3-5, 3-6, 3-7 also show the levels of acid phosphatase , magnesium and phosphors . There were a highly significant increase in levels of acid phosphatase, magnesium, and phosphors.

Acid phosphatase in table 3-1 and figure 3-5 show a significant elevated levels in sera of patients with (CRC) as compered with healthy control in males and females .

Acid phosphatase are usually associated with carcinoma of the prostate however primary bone tumors and the elevations of acid phosphatase in patients with carcinoma of the colon have been reported [144].

All patients with CRC examination of the prostate revealed no prostatic abnormalities which might explain this elevation of acid phosphatase[144]. This elevated of acid phosphatase may be due to the therapy dose[145].

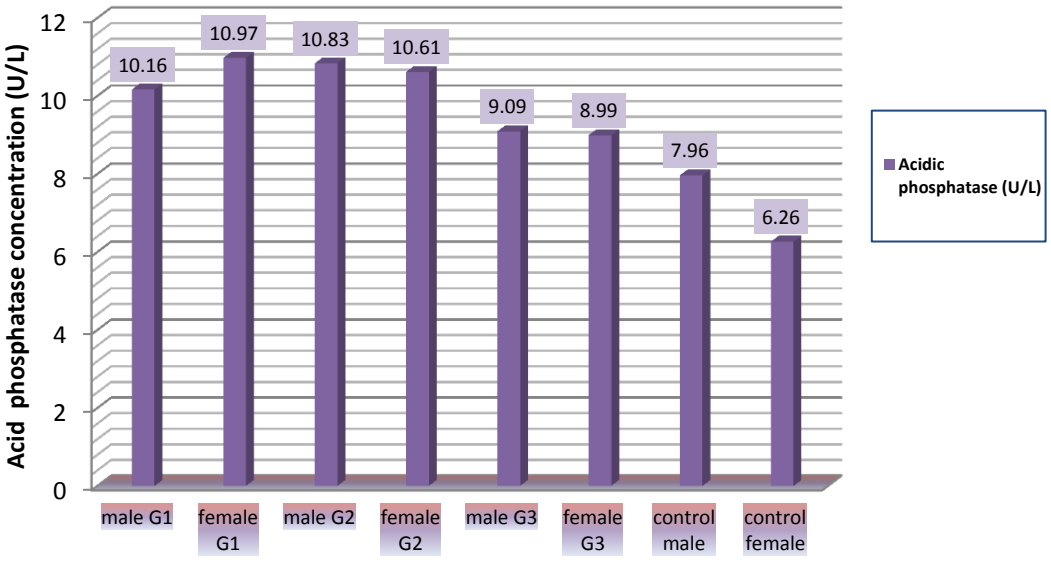

Figure (3-5): Acid phosphatase concentration (U/L) in sera of male and female patients groups with colorectal cancer and healthy control

On the other hands, table 3-1 and figure 3-6 show an elevated of magnesium in patients with colorectal cancer after and before taking chemotherapy dose as compared with control group.

Magnesium acts as a second messenger, and actives a vast array of enzymes[146], and it is play an essential role in DNA repair, cell differentiation and proliferation, apoptosis and angiogenesis[147]. So , the low blood of Mg levels was associated with high grade cancer[148]. There was an association between low intake of magnesium and the risk of colon cancer[149]. So, the concentration of Mg in patients of cancer

increased after and before taking chemotherapy dose than control groups . This may be due to in taking of supplementation of Mg.

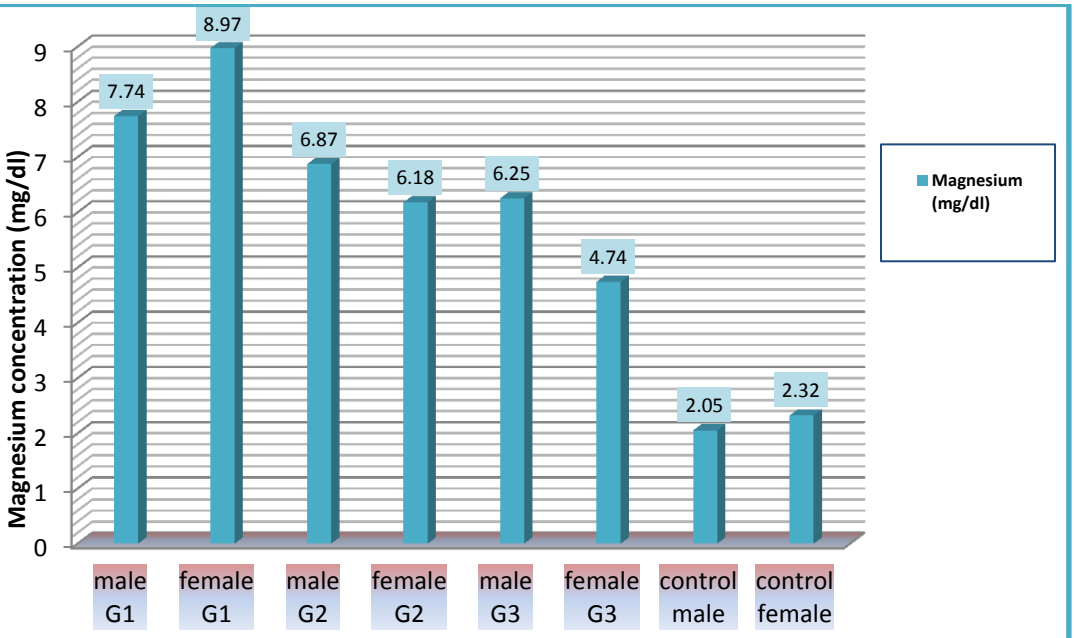

Figure (3-6): Magnesium concentration (mg/dl) in sera of male and female patients groups with colorectal cancer and healthy control

Table 3-1 and figure 3-7 showed an elevated of phosphors in patients with colorectal cancer after and before taking chemotherapy dose as compared with control group.

The hyperphosphatemia in general is associated with several clinical situation trauma, bone fractures and inflammation. Inorganic phosphors is a vital component of nucleotides membrane phospholipids and phosphorylated intermediates in cellular signaling and cancer cells that proliferate rapidly, require a highly amount of ribosome and other p-rich RNA components that are necessary to manufacture proteins[(150)].

The cancer cell showed the require a high amount of P in order to maintain their high growth rate and to proliferate as well as to metastasive[(120, 151)].

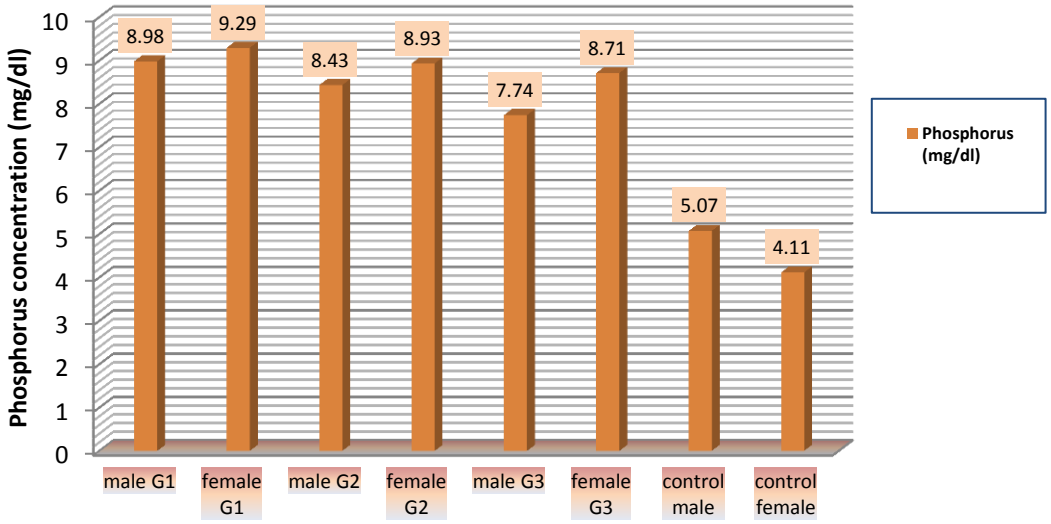

Figure (3-7): Phosphorus concentration (mg/dl) in sera of male and female patients groups with colorectal cancer and healthy control

Table 3-1 and figure 3-8 showed a non significant decrease of BMI in the first group of CRC patients as compared with second, third and fourth group in male patients, while there was significant increase in the first group in female patients with CRC as compared with the other groups. All patients male and female were low BMI. Previous studies found that over weight (obesity) leads to worse (CRC) prognosis [152].

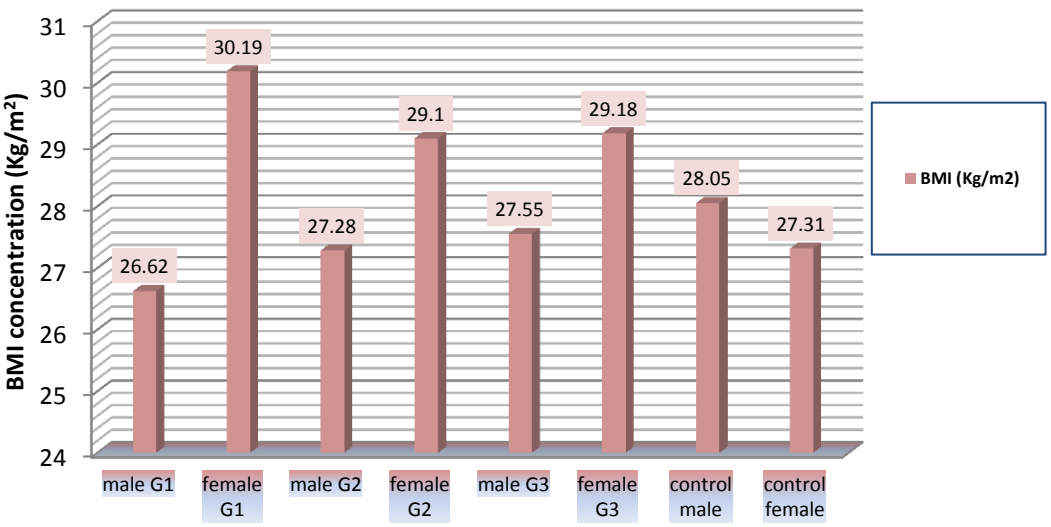

Figure (3-8): BMI concentration (Kg/m²) in sera of male and female patients groups with colorectal cancer and healthy control

Tables 3-2, 3-3, 3-4 showed the comparison study of IL-17 concentration and some biochemical parameters in male and female patient before and after taking chemotherapy dose .

Table (3-2): IL-17 concentration and some biochemical parameters in sera of male and female patients with colorectal cancer before taking the dose of chemotherapy.

Parameters	Groups		*P* value
	B1M (No. 48)	B1F (No. 40)	B1M vs. B1F
IL-17 (pg/mL)	117.21 ± 10.58	160.90 ± 9.78	S
IL-17RA (pg/mL)	292.31 ± 18.02	266.55 ± 17.40	Ns
Vitamin D (ng/mL)	11.08 ± 3.50	10.58± 2.22	Ns
CEA (ng/mL)	5.62 ± 0.35	6.03 ± 0.87	Ns
Acid phosphatase (U/L)	10.16 ± 1.07	10.97 ± 2.47	S
Magnesium (mg/dl)	7.74 ± 1.90	8.97± 1.03	h s
Phosphorus (mg/dl)	8.98 ± 1.72	9.29 ± 0.83	Ns
BMI (Kg/m^2)	26.62 ± 4.60	30.19 ± 7.12	h s

B1M = Male patients before taking the dose of chemotherapy
B1F = Female patients before taking the dose of chemotherapy

S = Significant (*P* value ≤ 0.05)
Ns = Non significant (*P* value > 0.05)
h s = high significant (*P* value ≤ 0.001)

Table (3-3): IL-17 concentration and some biochemical parameters in sera of male and female patients with colorectal cancer after taking first dose of chemotherapy.

Parameters	Groups		P value
	A1M (No. 48)	A1F (No. 40)	B1M vs. B1F
IL-17 (pg/mL)	83.51 ± 7.62	157.63 ± 11.07	h s
IL-17RA (pg/mL)	195.03 ± 13.88	210.03 ± 7.86	Ns
Vitamin D (ng/mL)	27.07 ± 9.27	13.30 ± 1.78	h s
CEA (ng/mL)	3.97 ± 0.21	6.81 ± 0.86	S
Acid phosphatase (U/L)	10.83 ± 2.05	10.61 ± 1.14	Ns
Magnesium (mg/dl)	6.86 ± 1.44	6.18± 1.31	S
Phosphorus (mg/dl)	8.43 ± 1.56	8.93 ± 0.89	Ns
BMI (Kg/m^2)	27.28 ± 4.27	29.1 ± 6.65	Ns

A1M = Male patients after taking first dose of chemotherapy
A1F = Female patients after taking first dose of chemotherapy

S = Significant (*P* value ≤ 0.05)
Ns = Non significant (*P* value > 0.05)
h s = high significant (*P* value ≤ 0.001)

Table (3-4) IL-17 concentration and some biochemical parameters in sera of male and female patients with colorectal cancer after taking second dose of chemotherapy.

Parameters	Groups		P value
	A2M (No. 48)	A2F (No. 40)	B1M vs. B1F
IL-17 (pg/mL)	69.45 ± 87.11	86.65 ± 59.48	N s
IL-17RA (pg/mL)	139.26 ± 89.00	279.90 ± 18.45	h s
Vitamin D (ng/mL)	34.06 ± 11.45	14.90 ± 2.97	h s
CEA (ng/mL)	5.50 ± 0.39	8.25 ± 0.82	S
Acid phosphatase (U/L)	9.09 ± 1.20	8.99 ± 1.26	N s
Magnesium (mg/dl)	6.25 ± 1.152	4.74 ± 0.83	h s
Phosphorus (mg/dl)	7.74 ± 1.21	8.71 ± 0.83	h s
BMI (Kg/m^2)	27.55 ± 3.53	29.18 ± 7.19	N s

A2M = Male patients after taking second dose of chemotherapy
A2F = Female patients after taking second dose of chemotherapy

S = Significant (*P* value ≤ 0.05)
Ns = Non significant (*P* value > 0.05)
h s = high significant (*P* value ≤ 0.001)

In the same tables found in recent study a significant levels of IL-17 in female patients before taking chemotherapy dose than male as show in figure 3-9. A high concentration levels of IL-17 also shown in female patients than male after taking the first dose of chemotherapy as show in figure 3-10. A low non significant levels of IL-17 in male patients than female after taking the second dose of chemotherapy as show in figure 3-11.

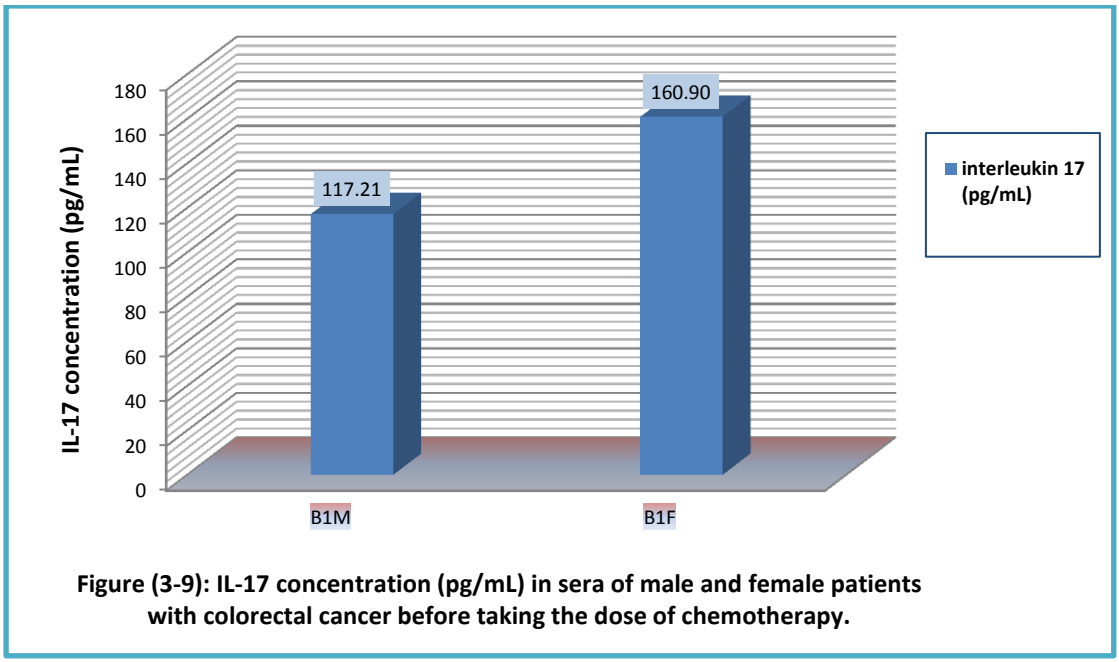

Figure (3-9): IL-17 concentration (pg/mL) in sera of male and female patients with colorectal cancer before taking the dose of chemotherapy.

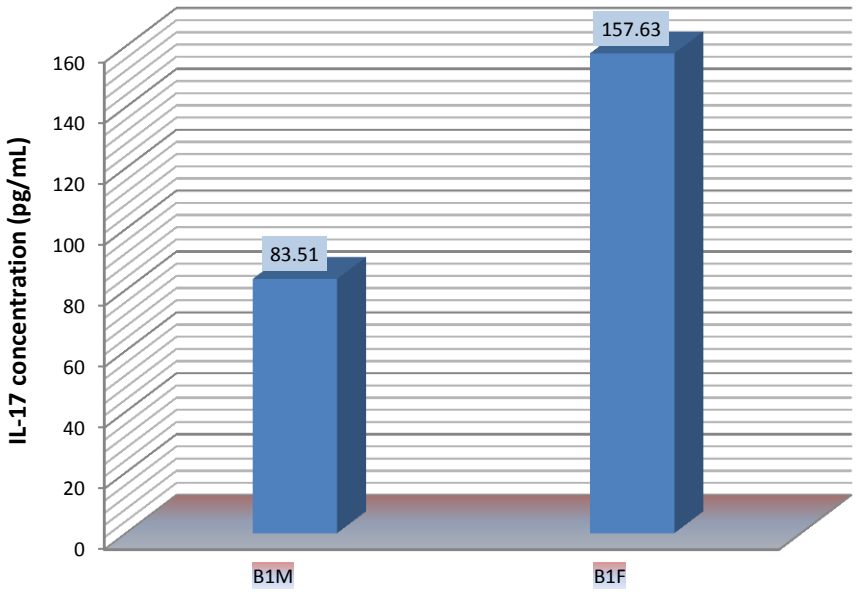

Figure (3-10): IL-17 concentration (pg/mL) in sera of male and female patients with colorectal cancer after taking first dose of chemotherapy.

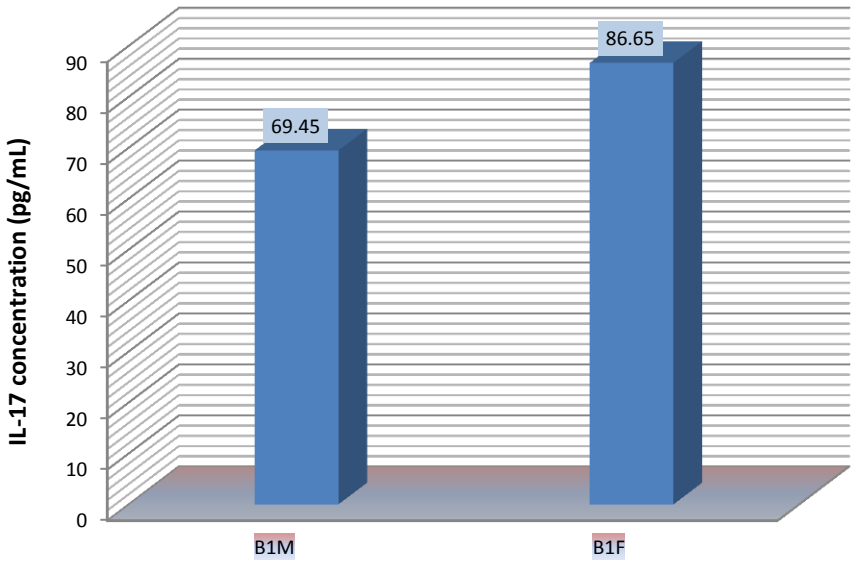

Figure (3-11): IL-17 concentration (pg/mL) in sera of male and female patients with colorectal cancer after taking second dose of chemotherapy.

In the same tables also found in recent study a non significant levels of IL-17RA in male patients than female before and after taking the first dose of chemotherapy as show in figures 3-12 and 3-13 respectively, and high significant level in IL-17RA after taking the second dose of chemotherapy as show in figure 3-14.

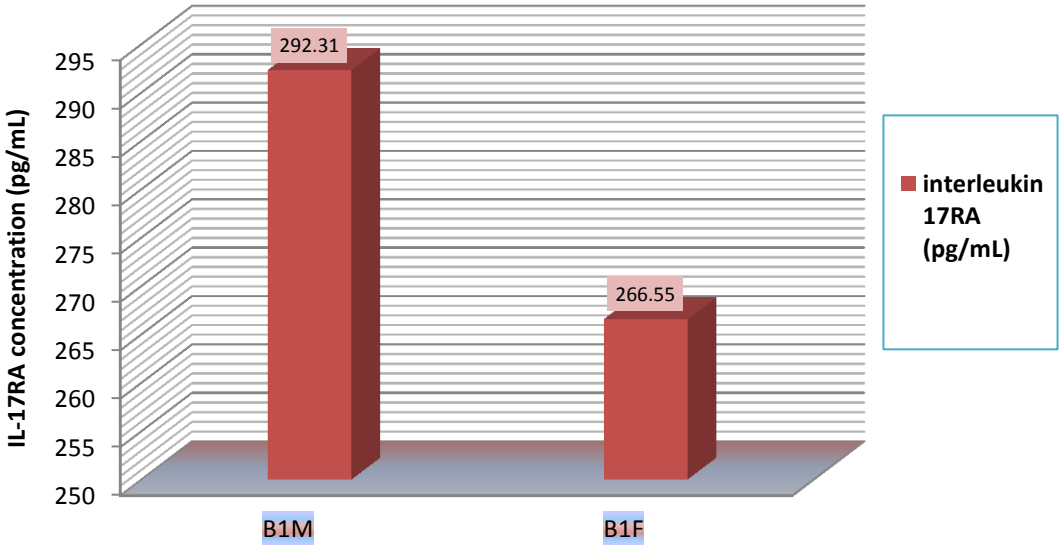

Figure (3-12): IL-17RA concentration (pg/mL) in sera of male and female patients with colorectal cancer before taking the dose of chemotherapy.

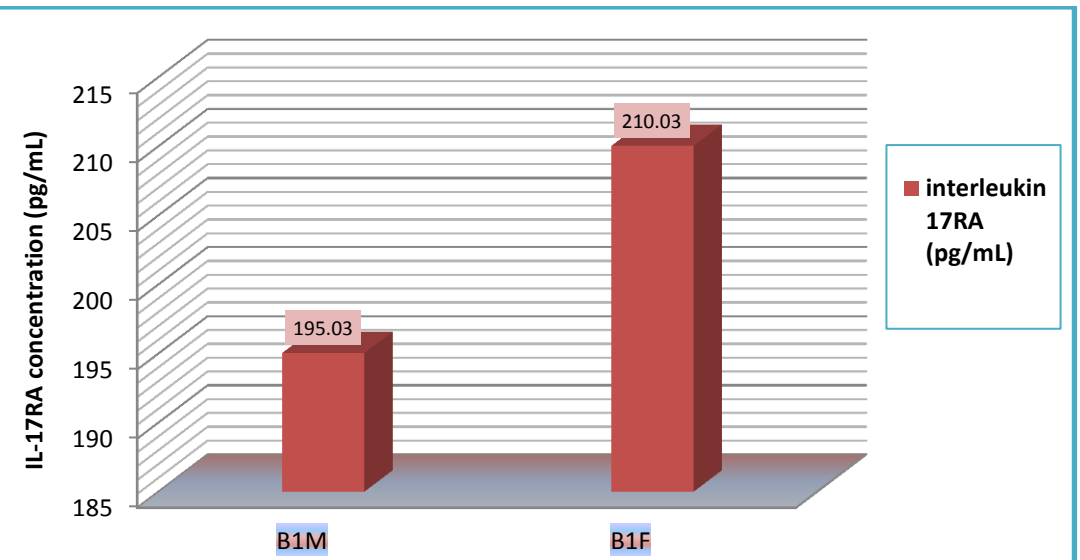

Figure (3-13): IL-17RA concentration (pg/mL) the sera of male and female patients with colorectal cancer after taking first dose of chemotherapy.

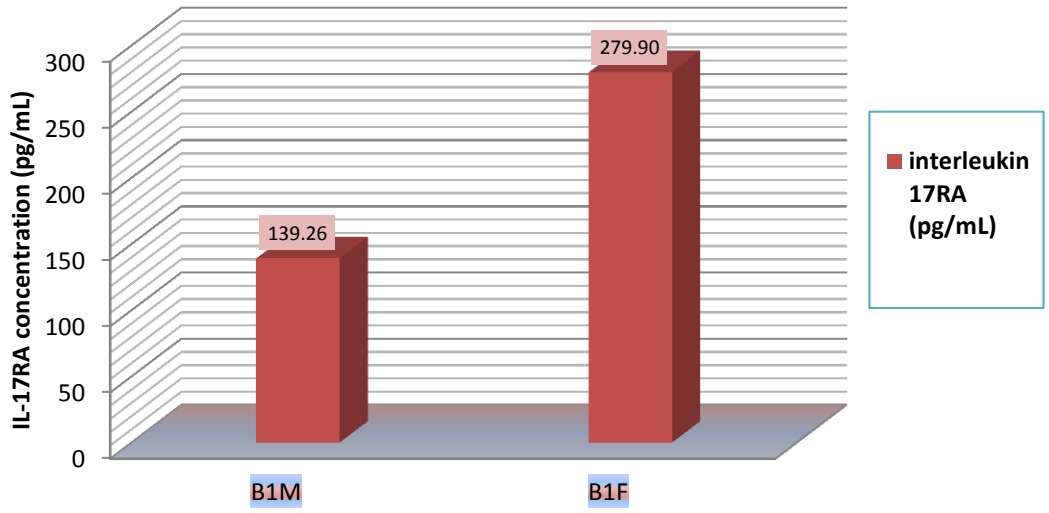

Figure (3-14): IL-17RA concentration (pg/mL) in sera of male and female patients with colorectal cancer after taking second dose of chemotherapy.

In the same table, male patients with colorectal cancer show a non significant increasing levels of vitamin D than female patients before chemotherapy dose as show in figure 3-15. A highly significant increase levels of vitamin D than female patients after taking the first and second chemotherapy dose as show in figures 3-16 and 3-17 respectively.

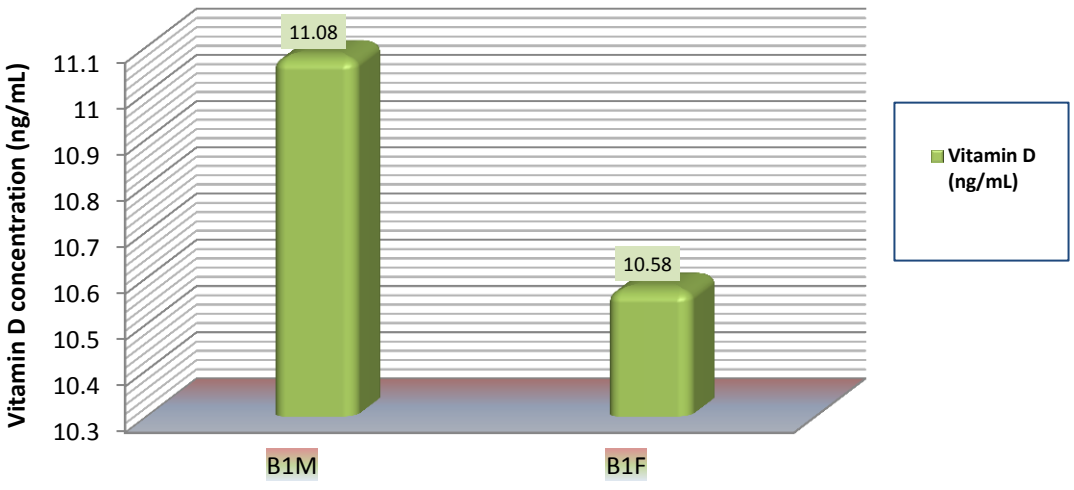

Figure (3-15): Vitamin D concentration (ng/mL) in sera of male and female patients with colorectal cancer before taking the dose of chemotherapy.

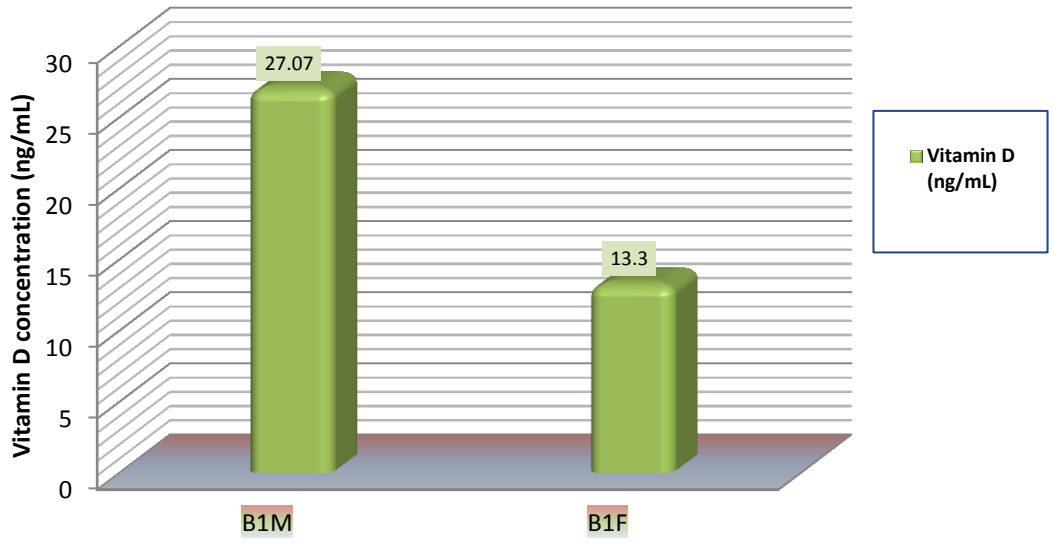

Figure (3-16): Vitamin D concentration (ng/mL) in sera of male and female patients with colorectal cancer after taking first dose of chemotherapy.

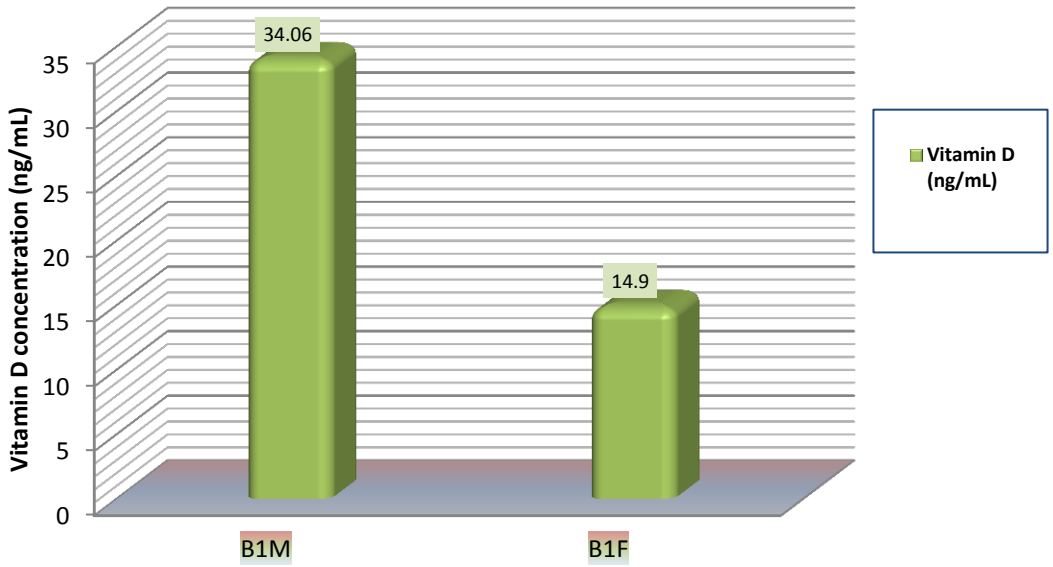

Figure (3-17): Vitamin D concentration (ng/mL) in sera of male and female patients with colorectal cancer after taking second dose of chemotherapy.

The comparison study between male and female show a non significant difference in CEA in male and female patients before taking chemotherapy dose as shown in figure 3-18 . A significant increase in female patients than male after taking the first and second chemotherapy dose for the same groups of patients as shown in figures 3-19 and 3-20 respectively.

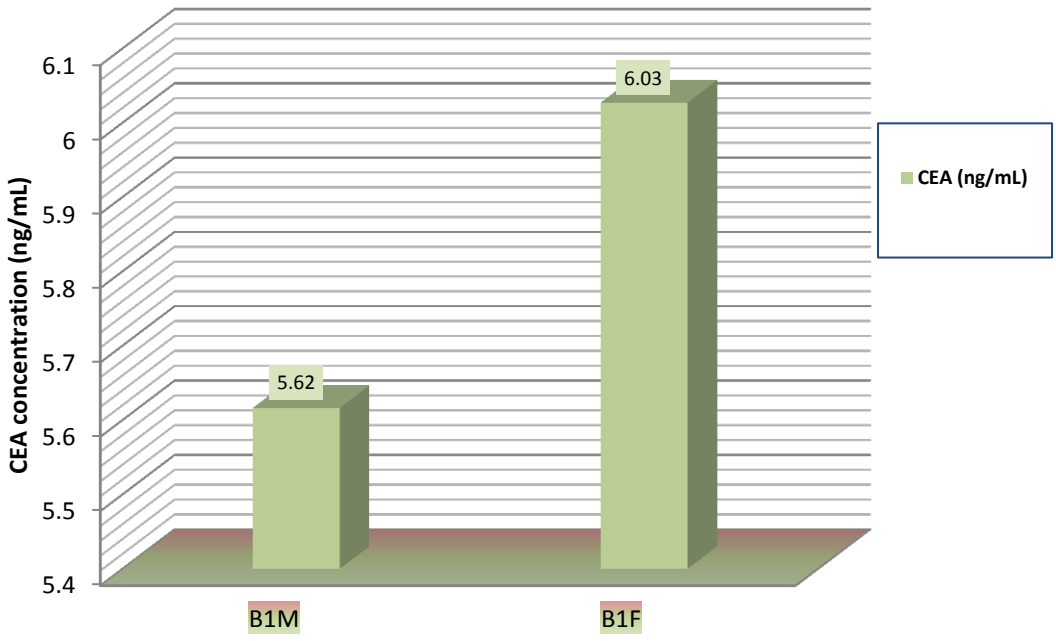

Figure (3-18): CEA concentration (ng/mL) in sera of male and female patients with colorectal cancer before taking the dose of chemotherapy.

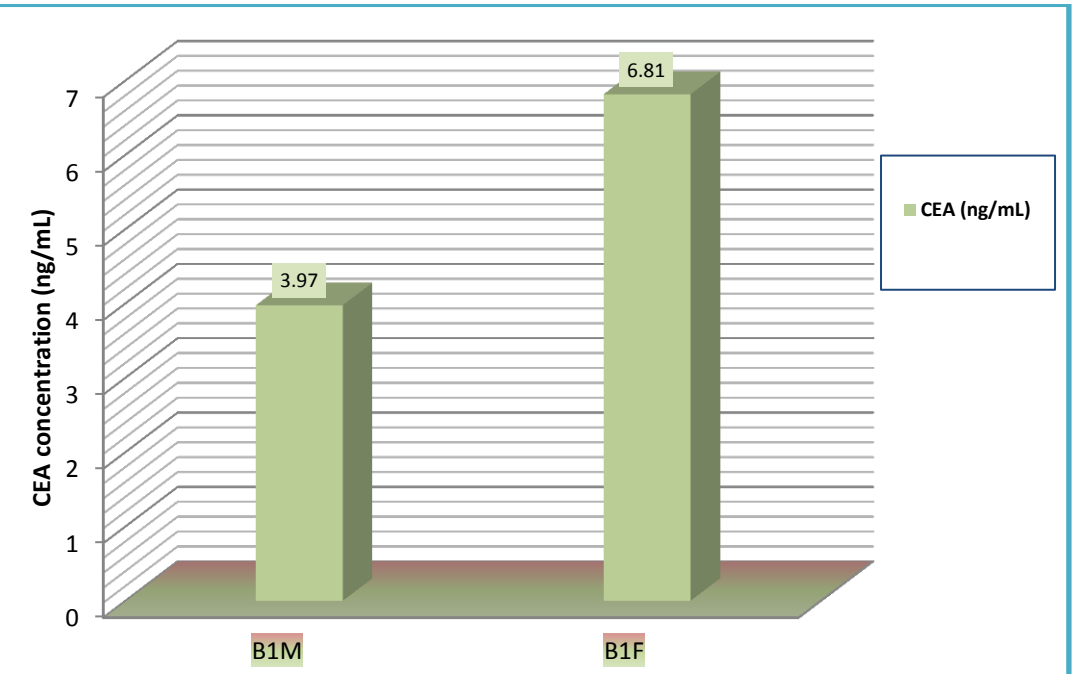

Figure (3-19): CEA concentration (ng/mL) in sera of male and female patients with colorectal cancer after taking first dose of chemotherapy.

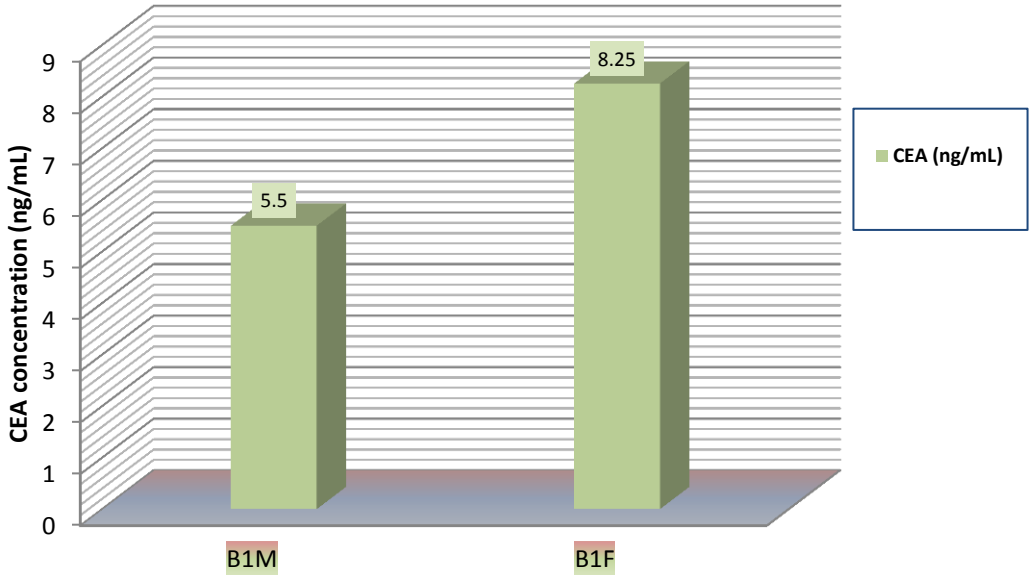

Figure (3-20): CEA concentration (ng/mL) in sera of male and female patients with colorectal cancer after taking second dose of chemotherapy.

In this study it was found a significant increase acid phosphatase in female patients than male before taking chemotherapy dose as shown in figure 3-21, A non significant difference in male and female patients after taking the first and second chemotherapy dose for the same groups of patients as shown in figures 3-22 and 3-23 respectively.

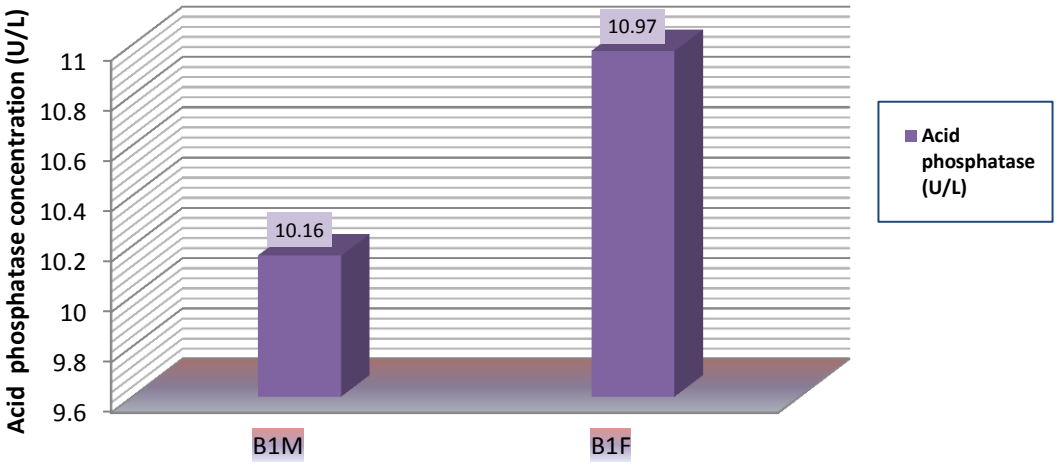

Figure (3-21): Acid phosphatase concentration (U/L) in sera of male and female patients with colorectal cancer before taking the dose of chemotherapy.

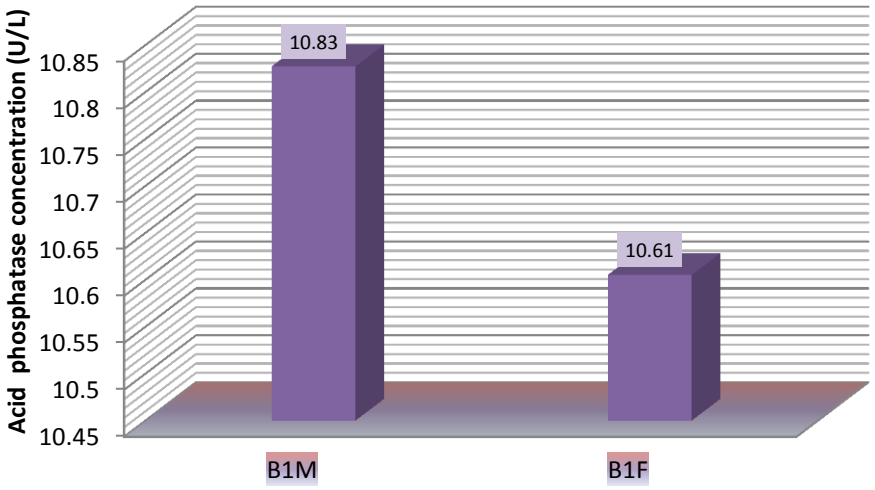

Figure (3-22): Acid phosphatase concentration (U/L) in sera of male and female patients with colorectal cancer after taking first dose of chemotherapy.

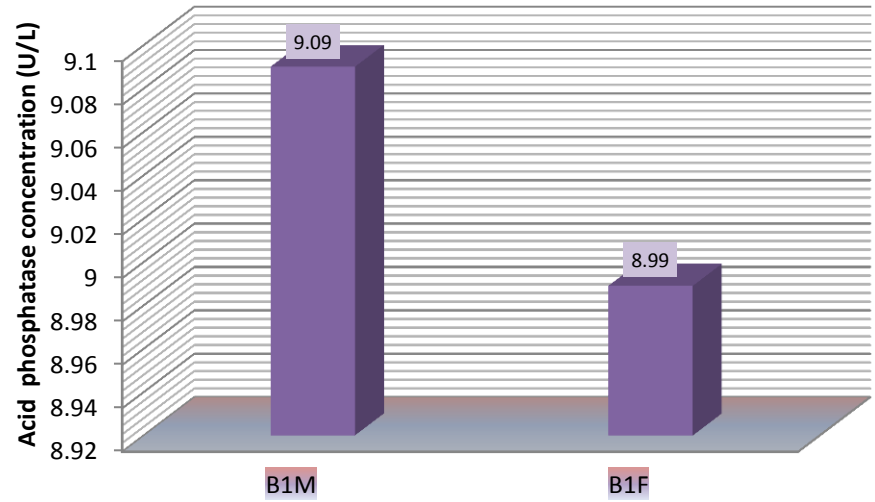

Figure (3-23): Acid phosphatase concentration (U/L) in sera of male and female patients with colorectal cancer after taking second dose of chemotherapy.

Tables 3-2, 3-3 and 3-4 showed a highly significant increase in magnesium in female patients than male before taking chemotherapy dose as shown in figure 3-24. While the male patients of CRC show a significant increasing levels of magnesium than female patients after taking the first chemotherapy dose as shown in figure 3-25, and a significant increase in male patients than female after taking the second chemotherapy dose as shown in figure 3-26.

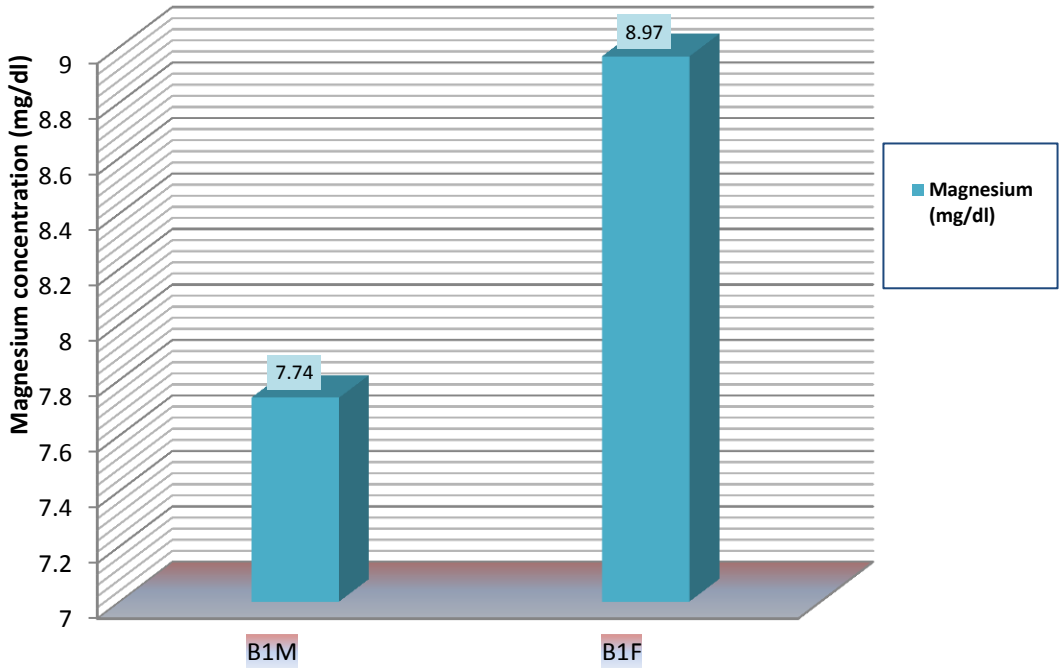

Figure (3-24): Magnesium concentration (mg/dl) in sera of male and female patients with colorectal cancer before taking the dose of chemotherapy.

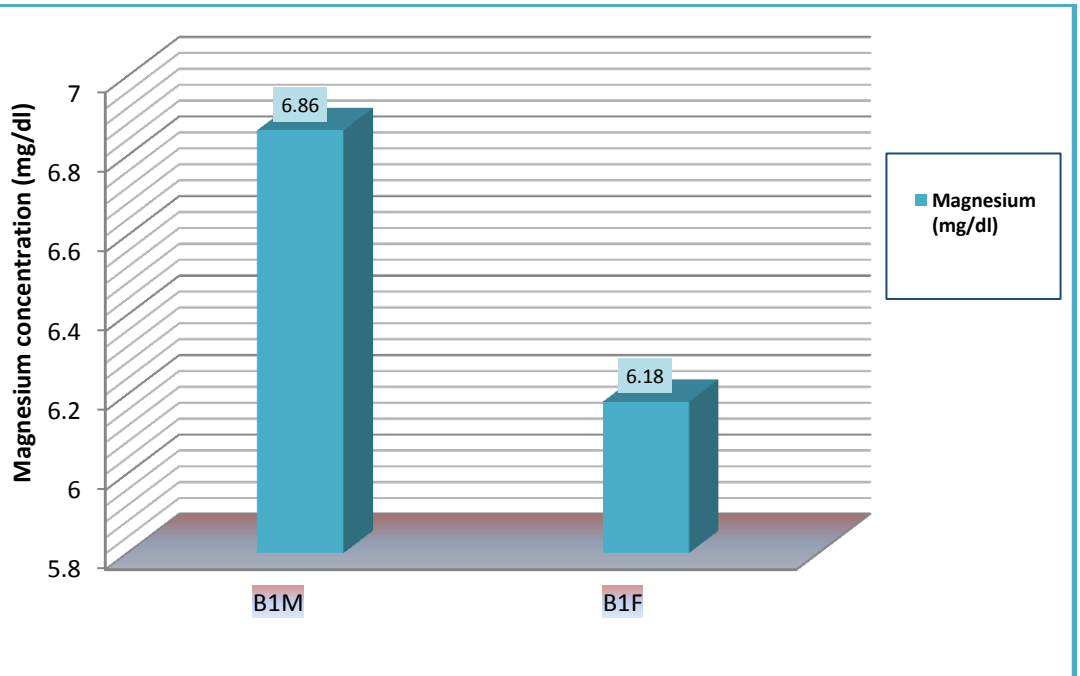

Figure (3-25): Magnesium concentration (mg/dl) in sera of male and female patients with colorectal cancer after taking first dose of chemotherapy.

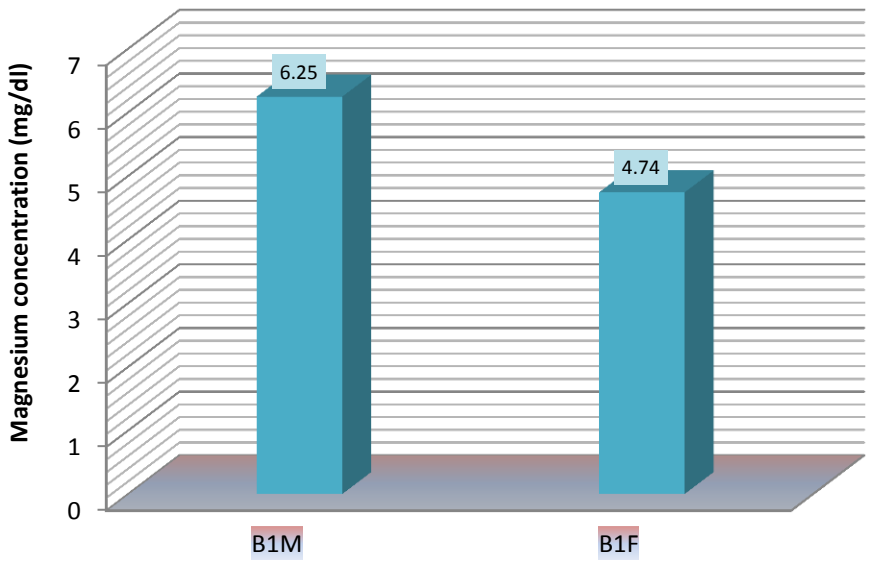

Figure (3-26): Magnesium concentration (mg/dl) in sera of male and female patients with colorectal cancer after taking second dose of chemotherapy.

A non significant increase in phosphorus in female patients when compered with male before and after taking the first chemotherapy dose for the same groups of patients as shown in figures 3-27 and 3-28 respectively. While a highly significant increase in female patient was found compered with male after taking the second chemotherapy dose for the same groups of patients as shown in figure 3-29.

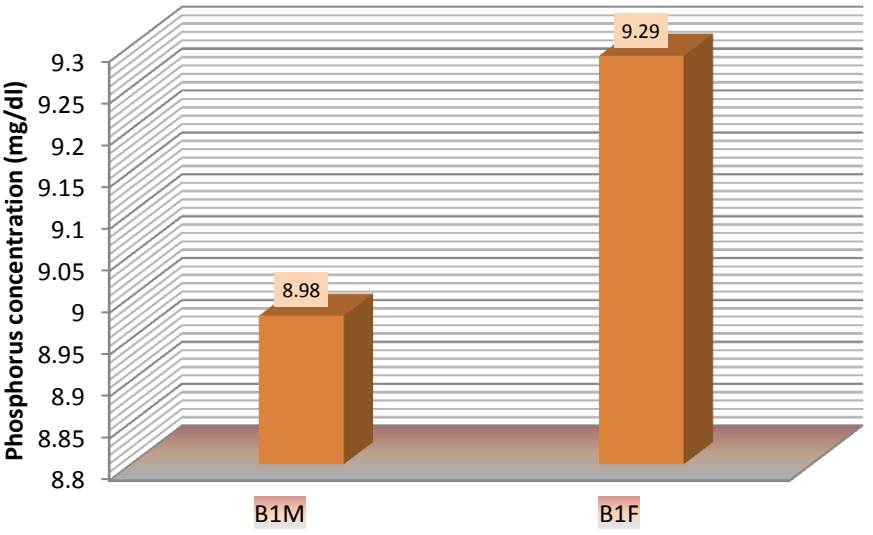

Figure (3-27): Phosphorus concentration (mg/dl) in sera of male and female patients with colorectal cancer before taking the dose of chemotherapy.

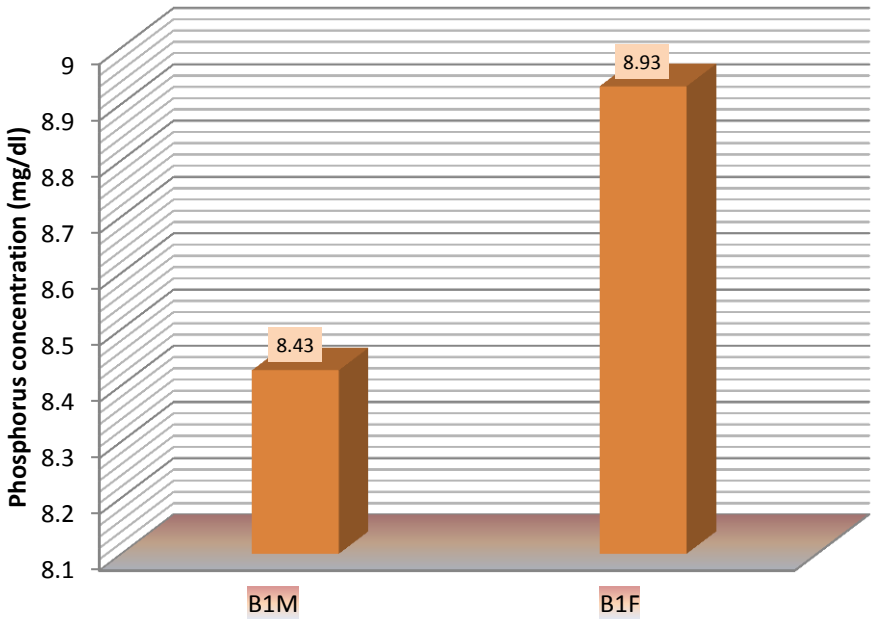

Figure (3-28): Phosphorus concentration (mg/dl) in sera of male and female patients with colorectal cancer after taking first dose of chemotherapy.

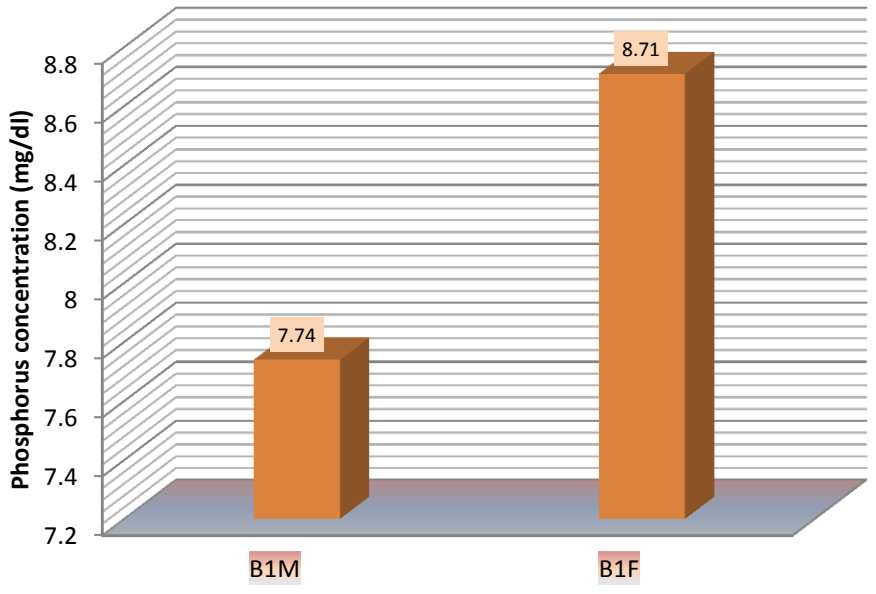

Figure (3-29): Phosphorus concentration (mg/dl) in sera of male and female patients with colorectal cancer after taking second dose of chemotherapy.

In the same tables also show the comparison study of BMI in male and female patient before and after taking chemotherapy dose.

A highly significant increase of BMI in female patients was found compered with male before taking chemotherapy dose as show in figure 3-30, and a non significant difference in male and female patients after taking the first chemotherapy dose for the same groups of patients as show in figure 3-31. As well as we found a non significant increase in female patient than male after taking the second chemotherapy dose for the same groups of patients as show in figure 3-32.

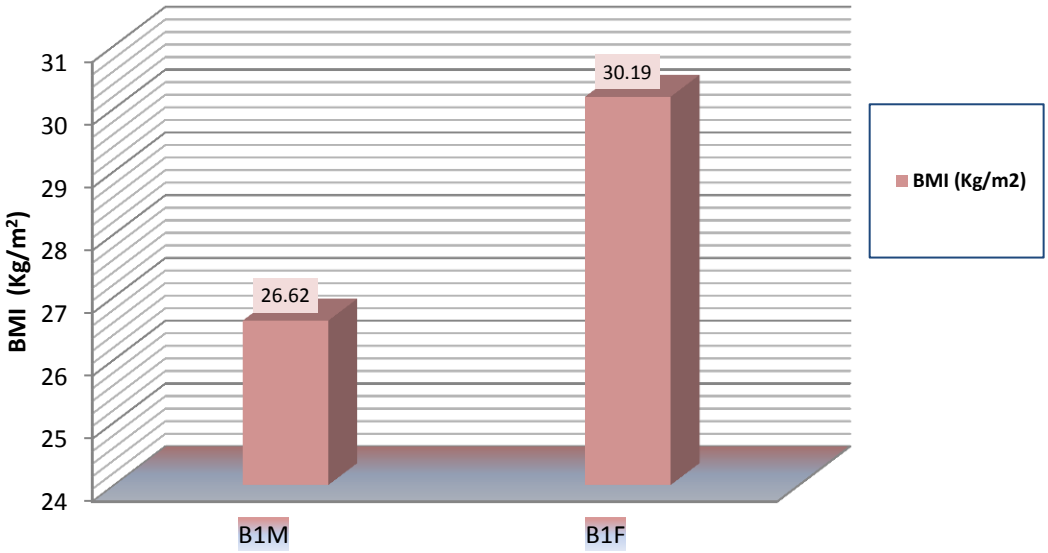

Figure (3-30): BMI (Kg/m²) in male and female patients with colorectal cancer before taking the dose of chemotherapy.

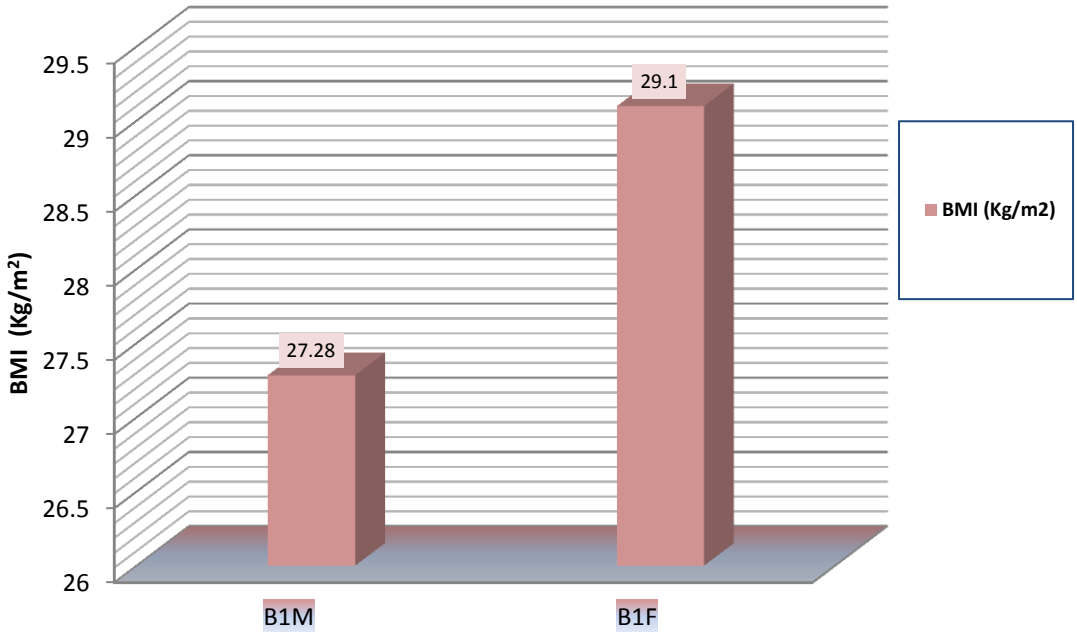

Figure (3-31): BMI (Kg/m²) in male and female patients with colorectal cancer after taking first dose of chemotherapy.

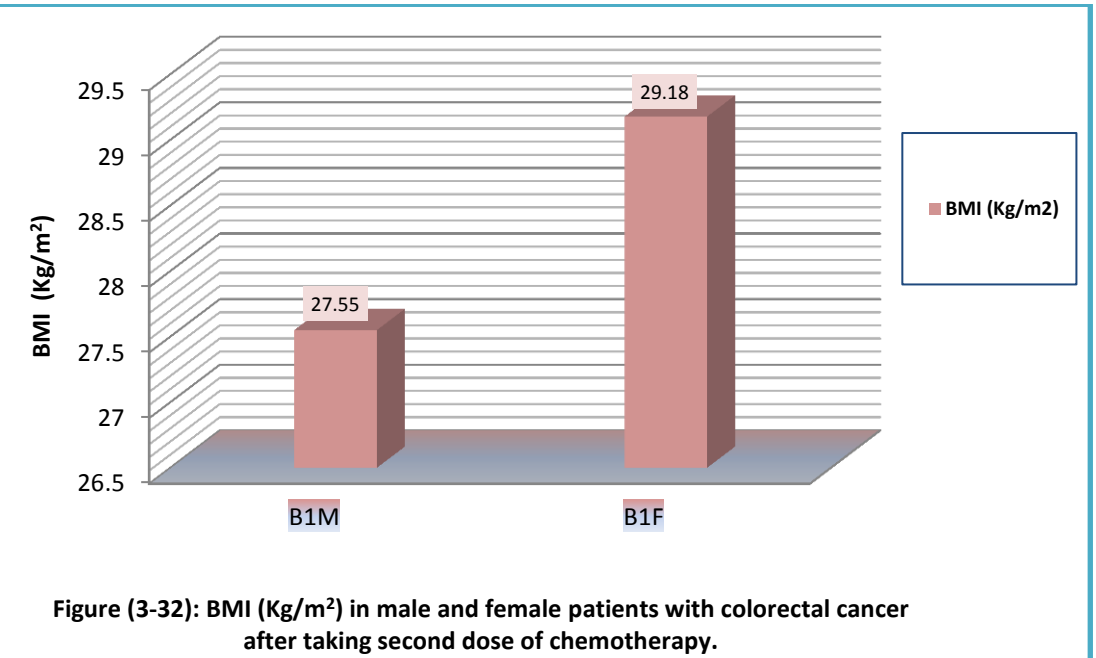

Figure (3-32): BMI (Kg/m²) in male and female patients with colorectal cancer after taking second dose of chemotherapy.

The results in table 3-5 and figures 3-33 , 3-34 , 3-35 , 3-36 , 3-37 , 3-38 , 3-39 , 3-40 , 3-41 , 3-42, 3-43 , 3-44 , 3-45 was shown the correlation between IL-17& IL-17RA with (Vitamin D, CEA, acid phosphatase, magnesium, phosphorus and BMI) in male patients before taking the dose of chemotherapy (G1).

Table (3-5): Correlations between IL-17 and some bio-chemical parameters in sera of CRC patients male before taking the dose of chemotherapy (G1).

Parameters		IL-17	IL-17RA
IL-17RA	r	0.272	
	P	0.062 Ns	
Vitamin D	r	0.100	0.199
	P	0.497 Ns	0.175 Ns
CEA	r	0.265	- 0.726
	P	0.050 S	0.000 h s
Acid phosphatase	r	-0.154	0.388
	P	0.297 Ns	0.006 h s
Magnesium	r	-0.298	0.086
	P	0.040 S	0.559 Ns
Phosphorus	r	-0.536	-0.128
	P	0.000 h s	0.384 Ns
BMI	r	-0.186	0.184
	P	0.205 Ns	0.210 Ns

S = Significant (*P* value ≤ 0.05)
Ns = Non significant (*P* value > 0.05)
h s = high significant (*P* value ≤ 0.001)

In this table a non significant negative (- ve) correlation between (IL-17& BMI) , and a non significant positive (+ ve) correlation between (IL-17& IL-17RA) , and a non significant positive (+ ve) correlation between (IL-17 & vitamin D) . A significant positive (+ ve) correlation

also shown between (IL-17 & CEA) , and non significant negative (- ve) correlation between (IL-17& Acid phosphatase) , and a significant negative (- ve) correlation between (IL-17 & Magnesium) . Also there was a high significant negative (- ve) correlation between (IL-17 & Phosphorus) before taking dose of chemotherapy.

In this table also found a non significant positive (+ ve) correlation between (IL-17RA & BMI), and a non significant positive (+ ve) correlation between (IL-17RA & Vitamin D), and a high significant negative (- ve) correlation between (IL-17RA & CEA). However, a high significant positive (+ ve) correlation was found between (IL-17RA & Acid phosphatase), and a non significant positive (+ ve) correlation between (IL-17RA & Magnesium). A non significant negative (- ve) correlation also shown between (IL-17RA & Phosphorus) before taking dose of chemotherapy.

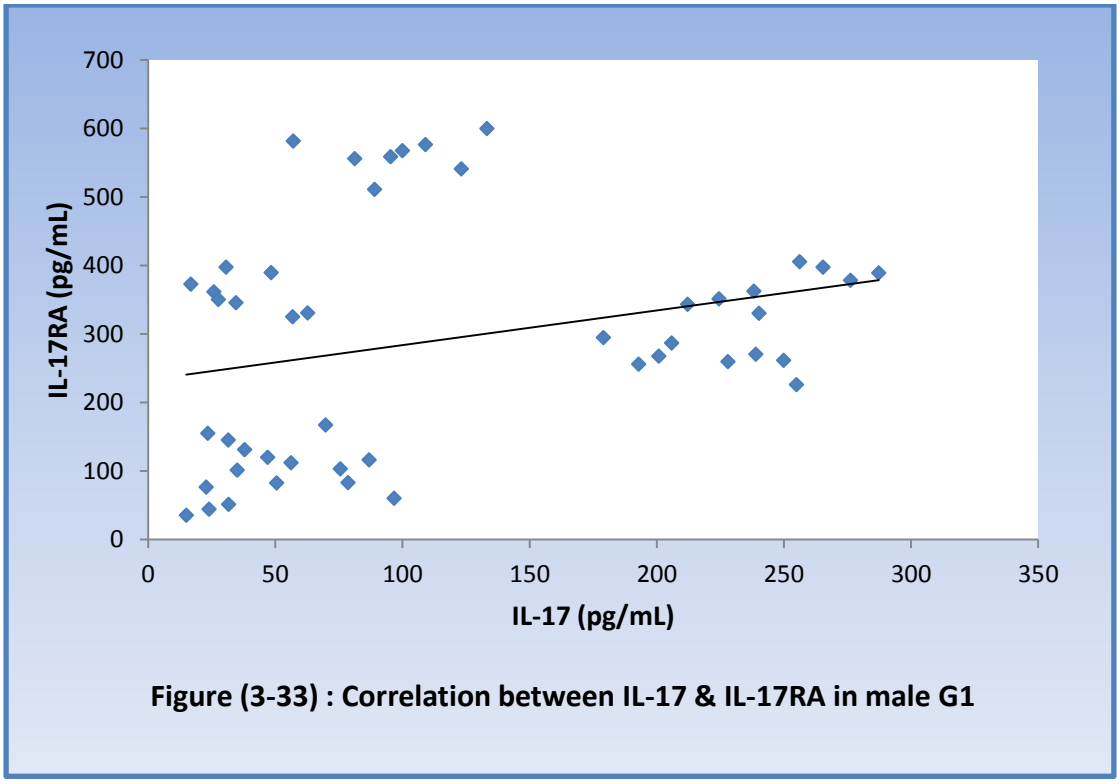

Figure (3-33) : Correlation between IL-17 & IL-17RA in male G1

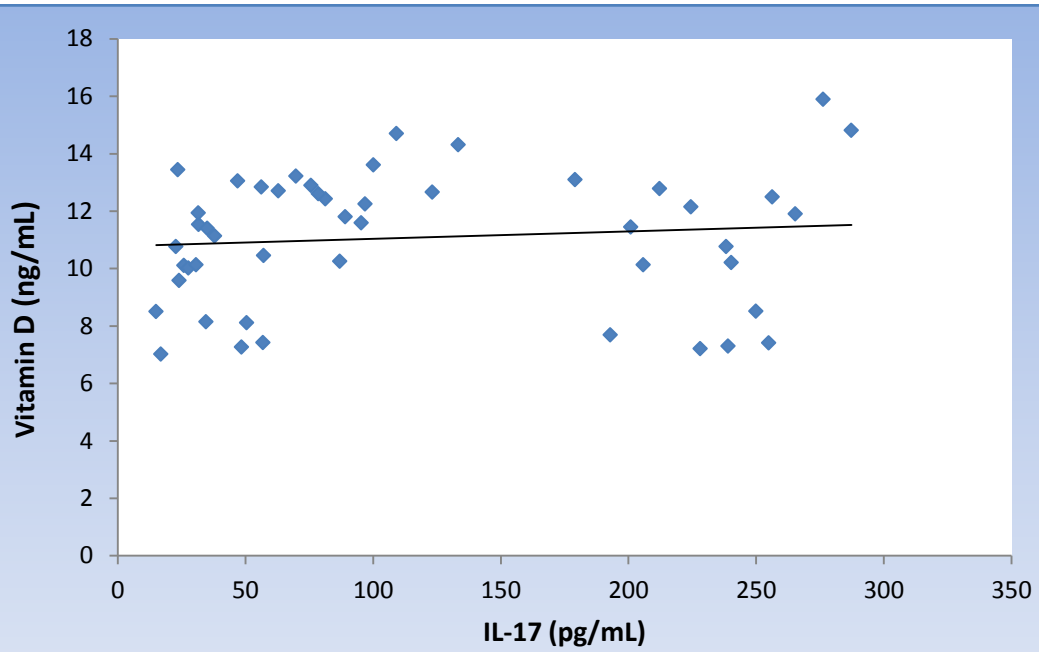

Figure (3-34) : Correlation between IL-17 & Vitamin D in male G1

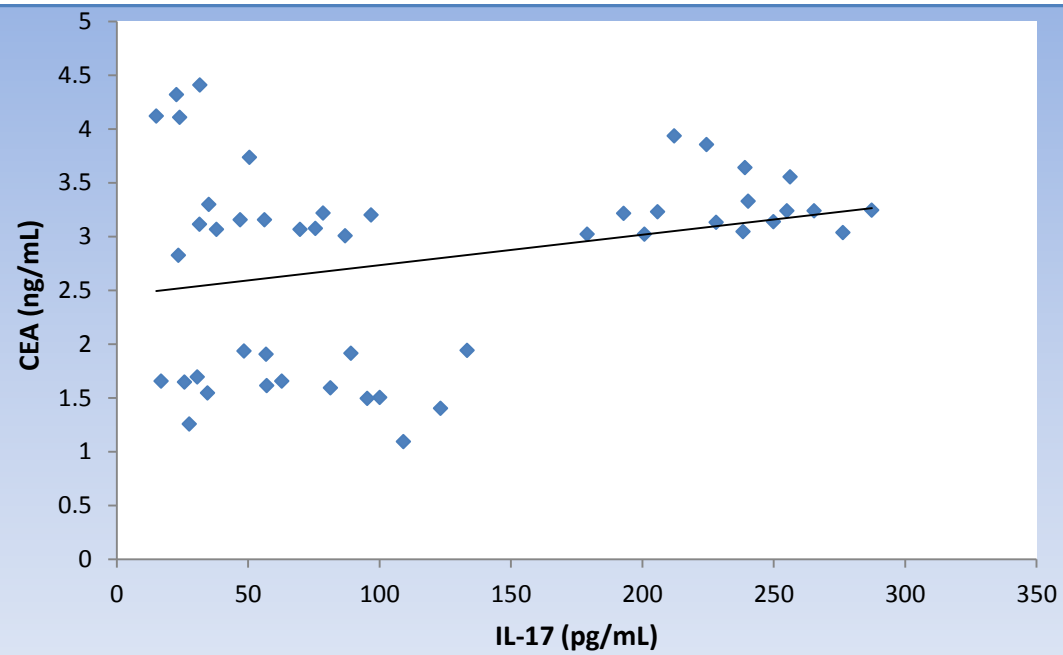

Figure (3-35): Correlation between IL-17 and CEA in male G1

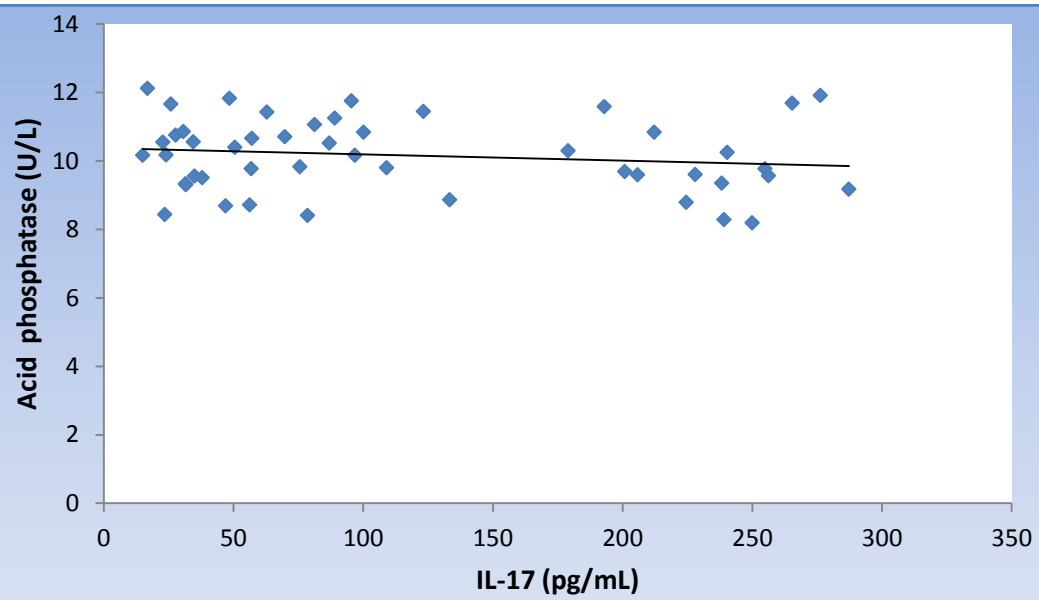

Figure (3-36): Correlation between IL-17 & Acid phosphatase in male G1

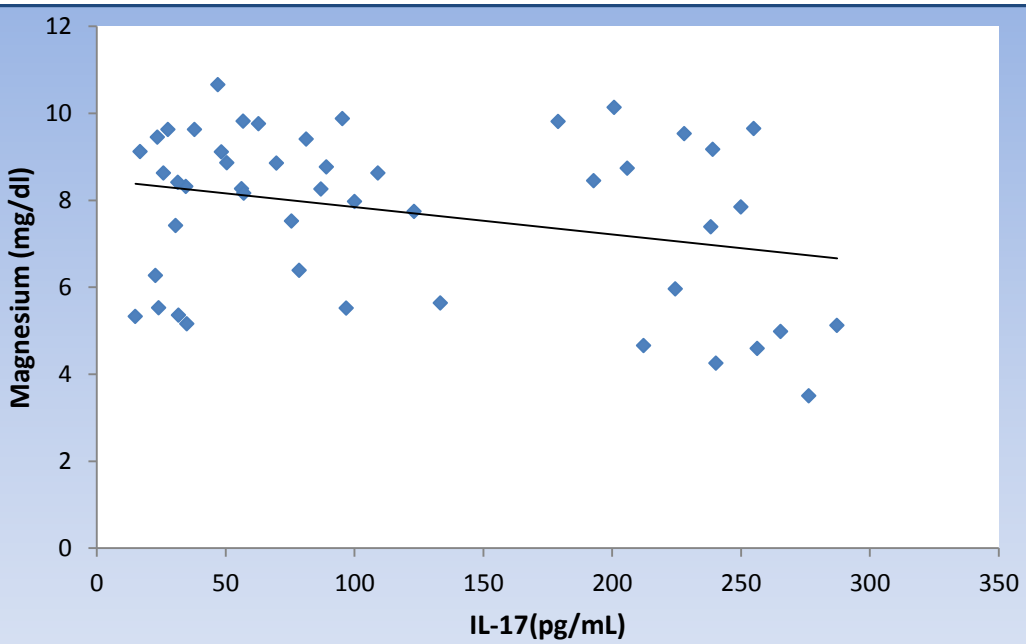

Figure (3-37): Correlation between IL-17 and Magnesium in male G1

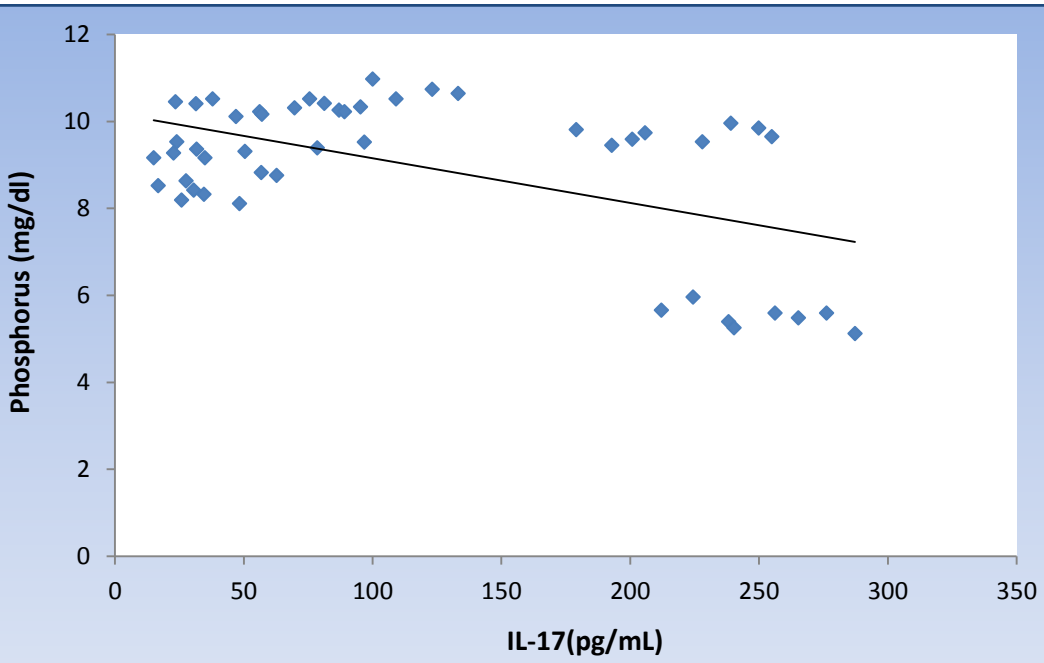

Figure (3-38): Correlation between IL-17 and Phosphorus in male G1

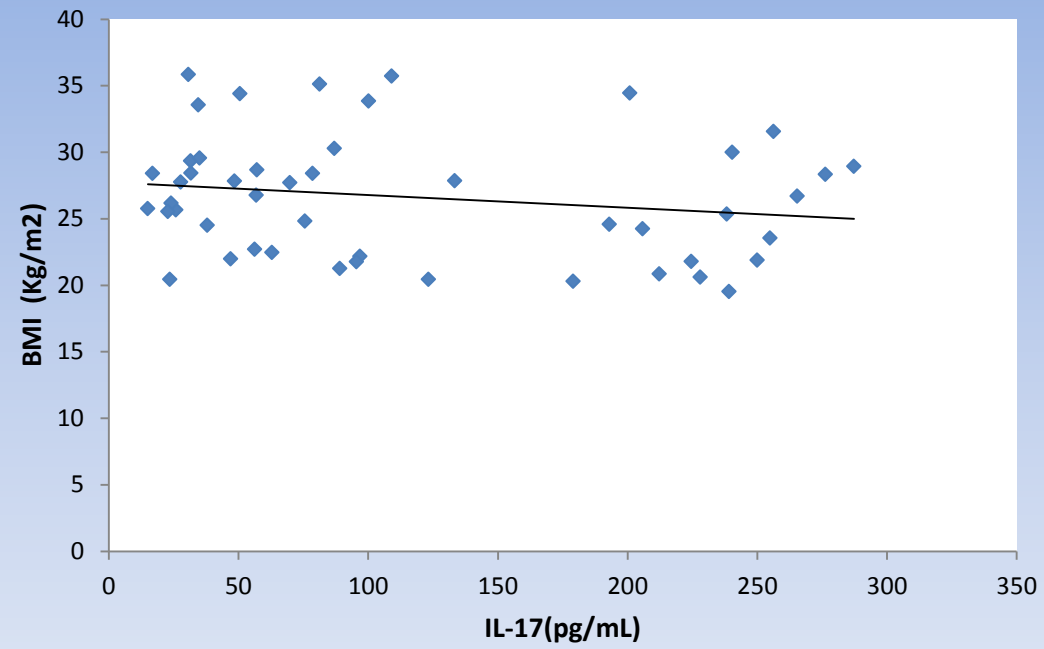

Figure (3-39): Correlation between IL-17 and BMI in male G1

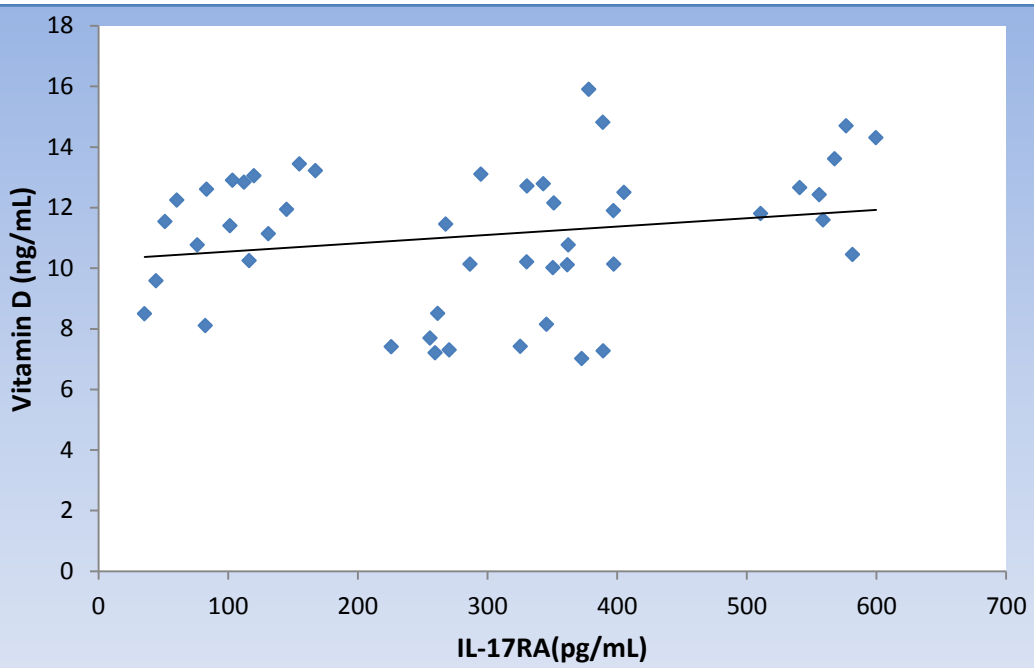

Figure (3-40) : Correlation between IL-17RA & Vitamin D in male G1

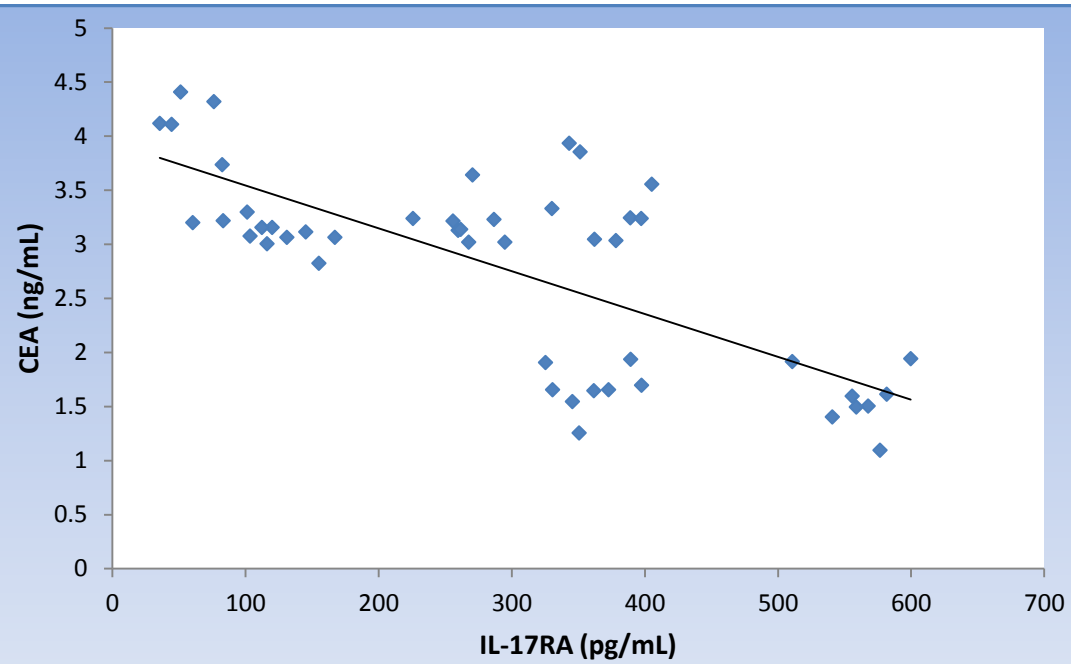

Figure (3-41): Correlation between IL-17RA and CEA in male G1

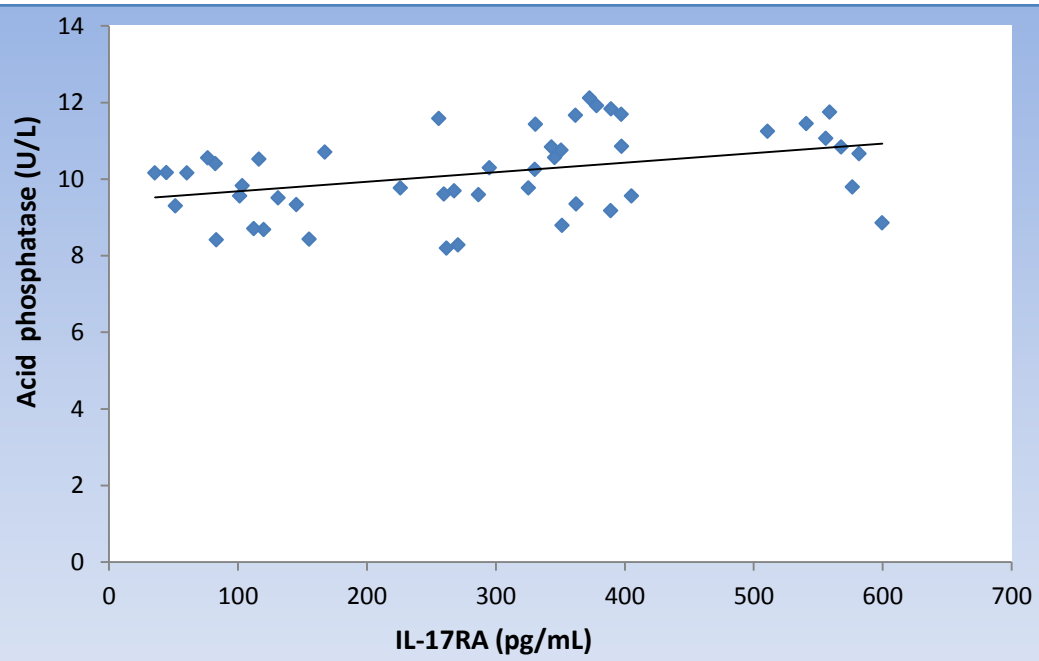

Figure (3-42): Correlation between IL-17RA and Acid phosphatase in male G1

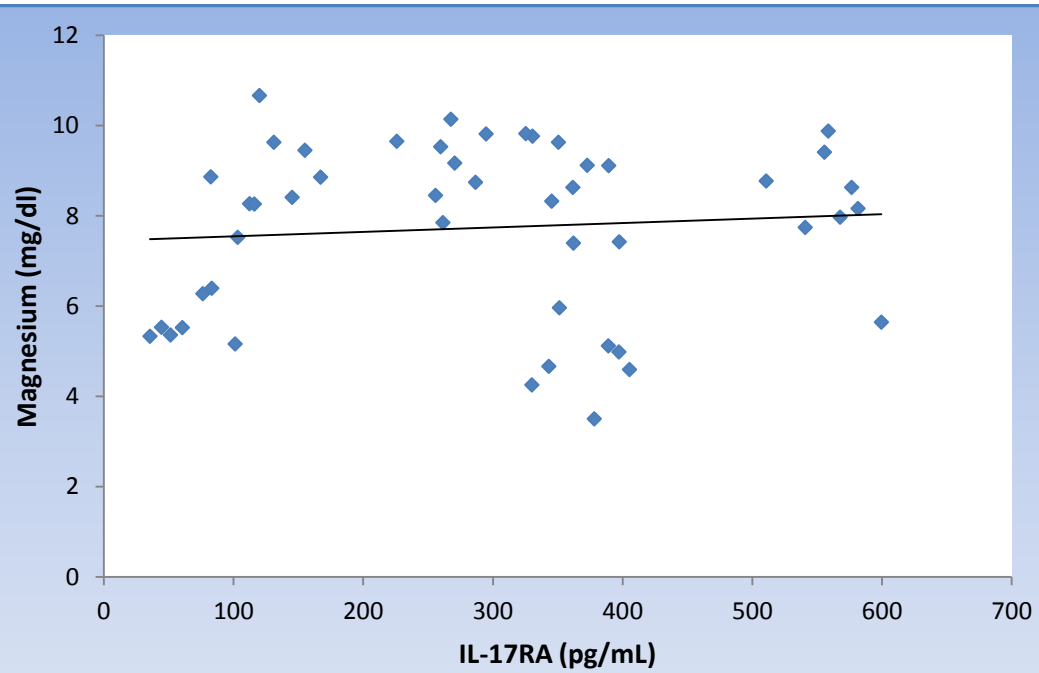

Figure (3-43): Correlation between IL-17RA and Magnesium in male G1

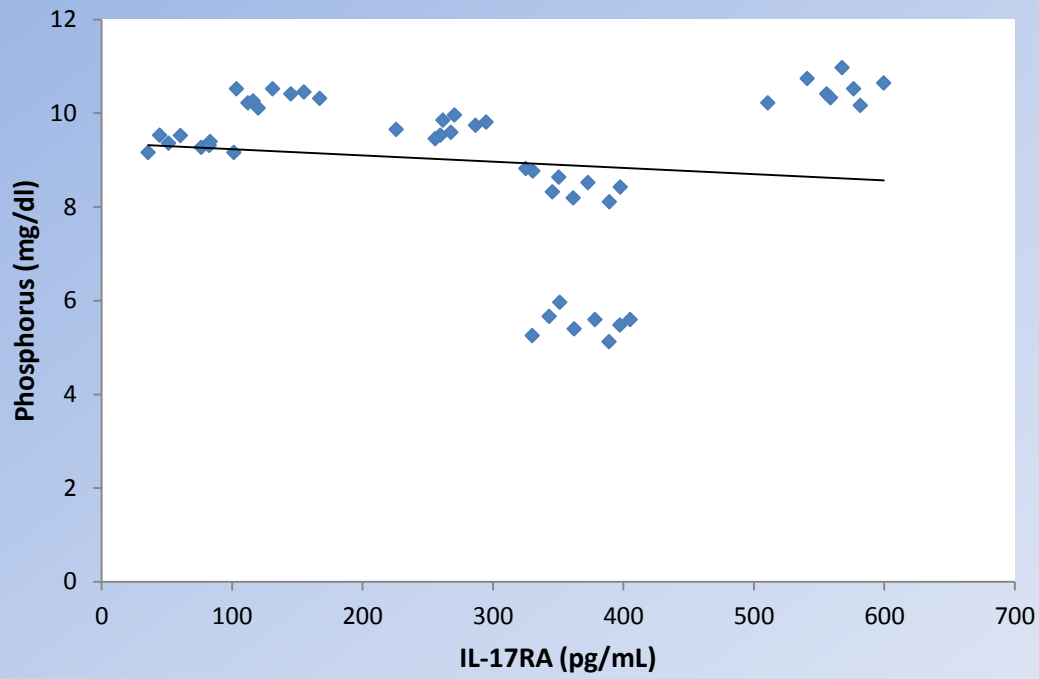

Figure (3-44): Correlation between IL-17RA and Phosphorus in male G1

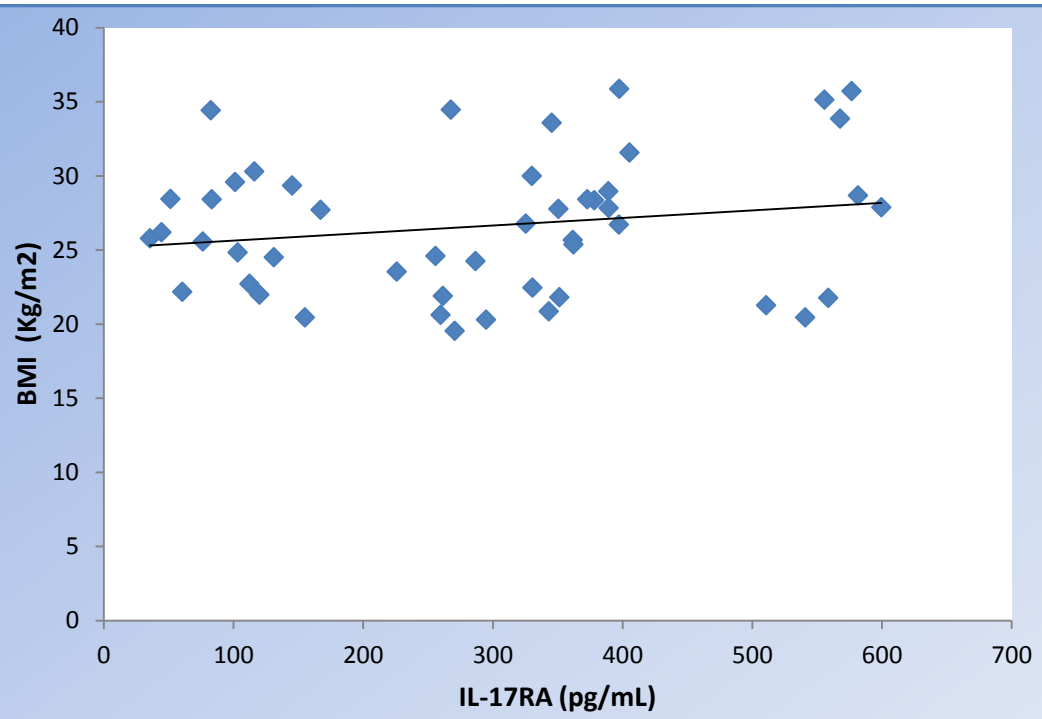

Figure (3-45): Correlation between IL-17RA and BMI in male G1

The results in table 3-6 and figures 3-46 , 3-47 , 3-48 , 3-49 , 3-50 , 3-51, 3-52 , 3-53 , 3-54 , 3-55 , 3-56 , 3-57 , 3-58 show the correlation between IL-17& IL-17RA with (Vitamin D, CEA, acid phosphatase, magnesium, phosphorus and BMI) in female patients before taking the dose of chemotherapy (G1).

Table (3-6): Correlations between IL-17 and some bio-chemical parameters in sera of CRC patients female before taking the dose of chemotherapy (G1).

Parameters		IL-17	IL-17RA
IL-17RA	r	-0.358	
	P	0.024 S	
Vitamin D	r	0.134	-0.243
	P	0.409 Ns	0.131 Ns
CEA	r	0.480	-0.679
	P	0.002 h s	0.000 h s
Acid phosphatase	r	-0.178	0.726
	P	0.273 Ns	0.000 h s
Magnesium	r	0.056	0.412
	P	0.731 Ns	0.008 hs
Phosphorus	r	-0.218	0.402
	P	0.176 Ns	0.010 S
BMI	r	-0.151	0.095
	P	0.353 Ns	0.560 Ns

S = Significant (*P* value ≤ 0.05)
Ns = Non significant (*P* value > 0.05)
h s = high significant (*P* value ≤ 0.001)

In this table, a non significant negative (- ve) correlation between (IL-17& BMI), and a significant negative (- ve) correlation between (IL-17& IL-17RA), and non significant positive (+ ve) correlation between (IL-17 & vitamin D). However, a high significant positive (+ ve)

correlation shown between (IL-17 & CEA) , and non significant negative (- ve) correlation between (IL-17& Acid phosphatase) , and a non significant positive (+ ve) correlation between (IL-17 & magnesium) . A non significant negative (- ve) correlation also shown between (IL-17 & phosphorus) before taking dose of chemotherapy.

In this table a non significant positive (+ ve) correlation also shown between (IL-17RA & BMI), and a non significant negative (- ve) correlation between (IL-17RA & vitamin D). However, a high significant negative (- ve) correlation was shown between (IL-17RA & CEA), and a high significant positive (+ ve) correlation between (IL-17RA & acid phosphatase). A high significant positive (+ ve) correlation also shown between (IL-17RA & magnesium), and a significant positive (+ ve) correlation between (IL-17RA & phosphorus) before taking dose of chemotherapy.

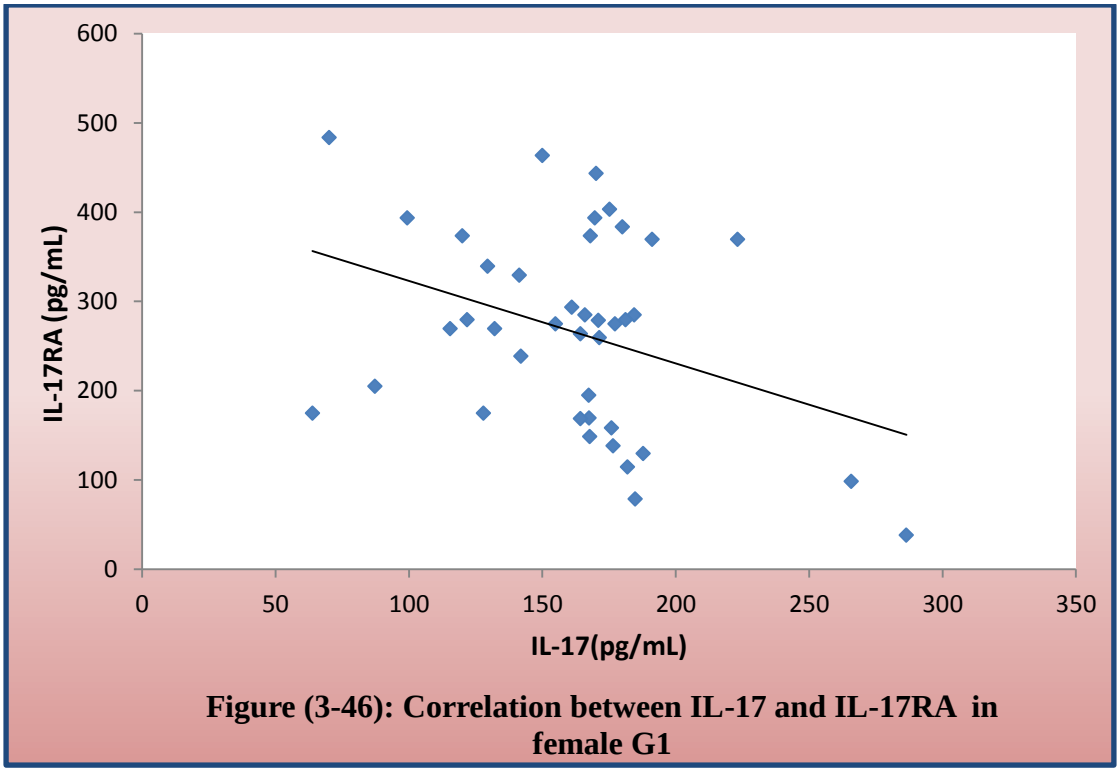

Figure (3-46): Correlation between IL-17 and IL-17RA in female G1

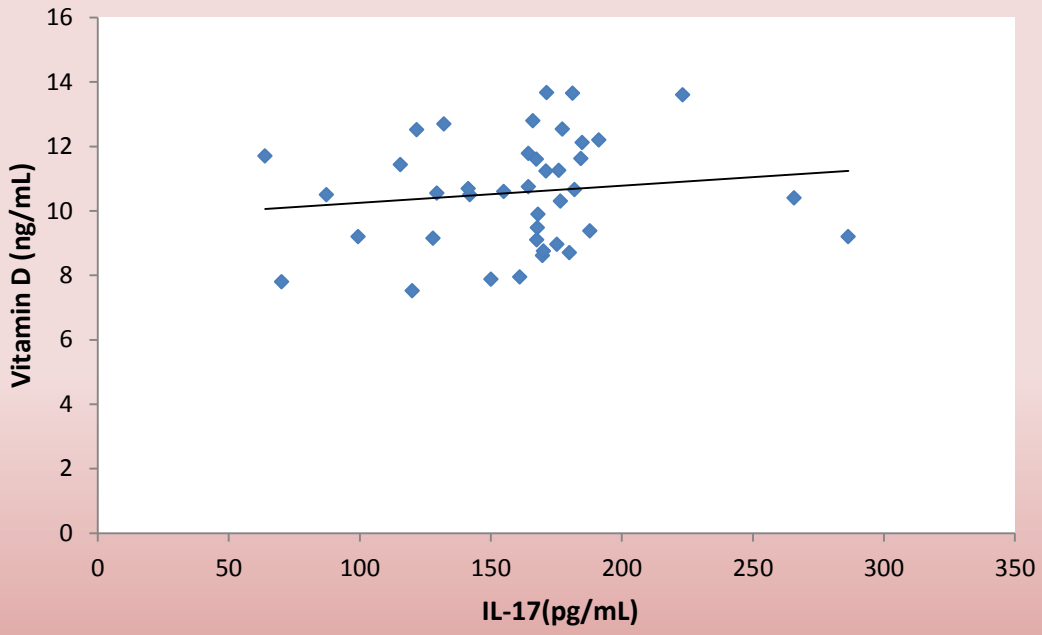

Figure (3-47) : Correlation between IL-17 & Vitamin D in female G1

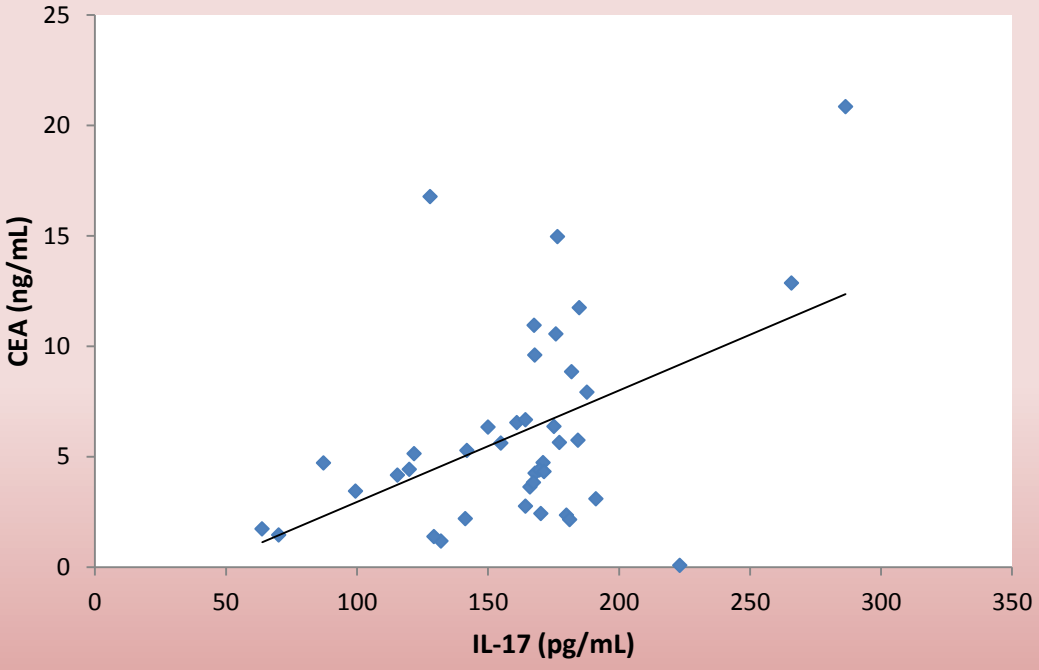

Figure (3-48): Correlation between IL-17 and CEA in female G1

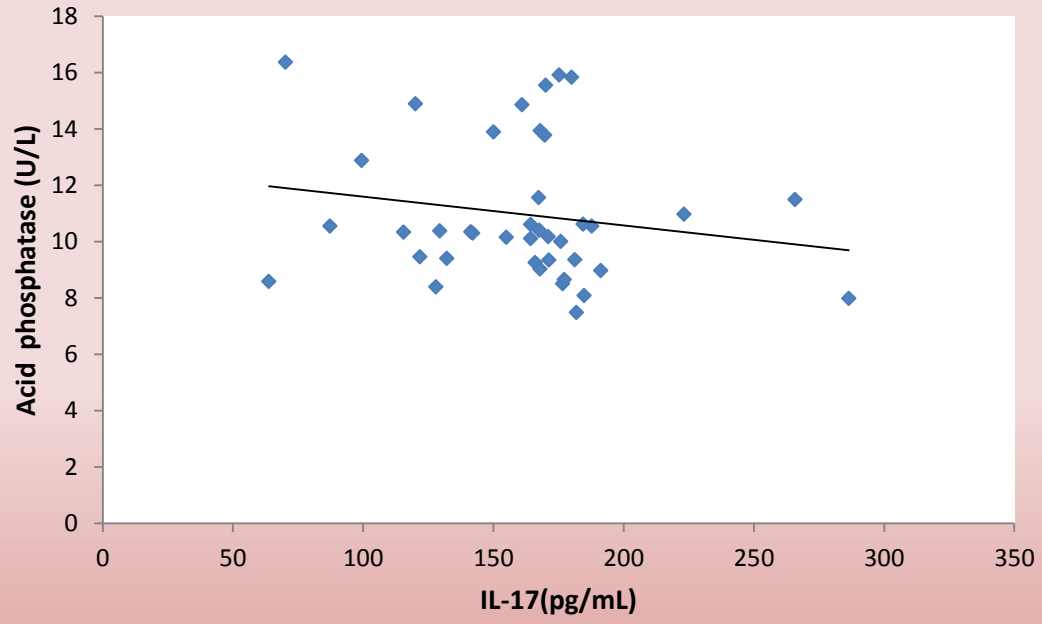

Figure (3-49): Correlation between IL-17 and Acid phosphatase in female G1

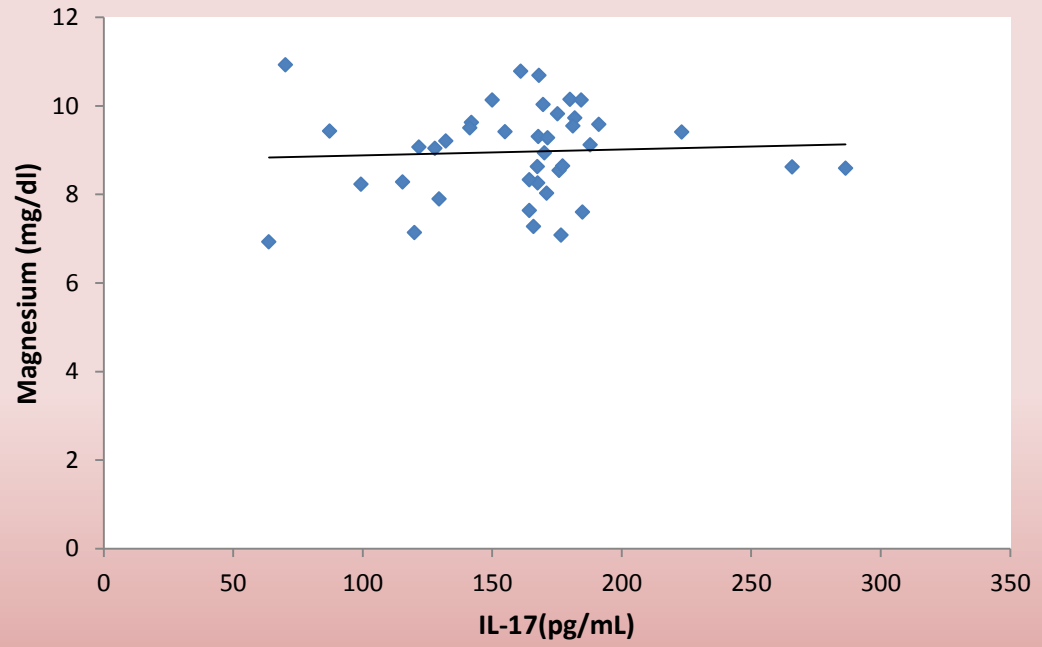

Figure (3-50): Correlation between IL-17 and Magnesium in female G1

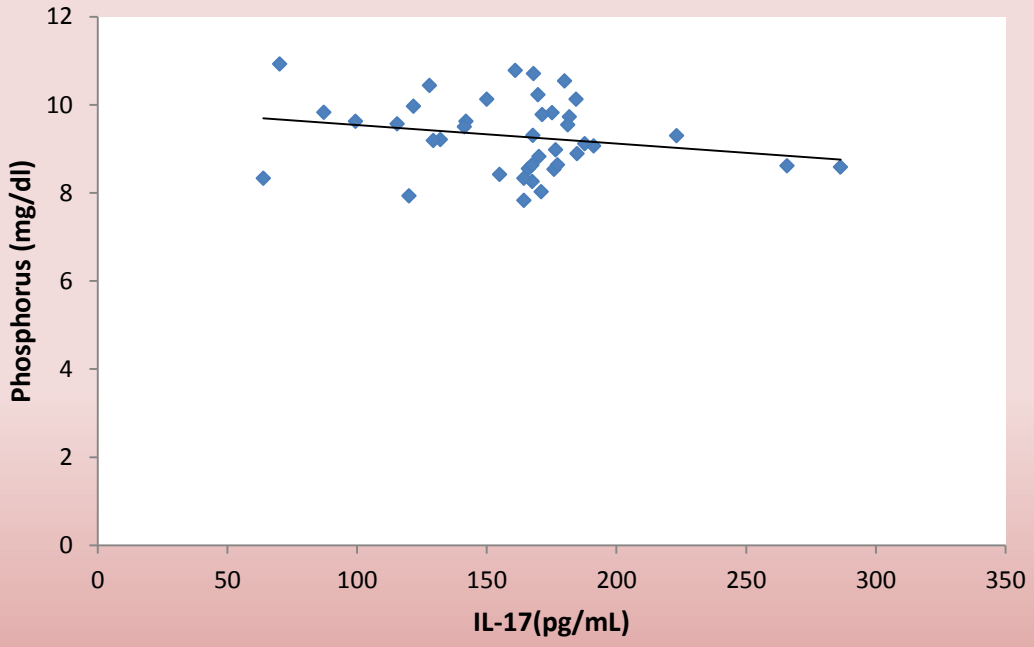

Figure (3-51): Correlation between IL-17 and Phosphorus in female G1

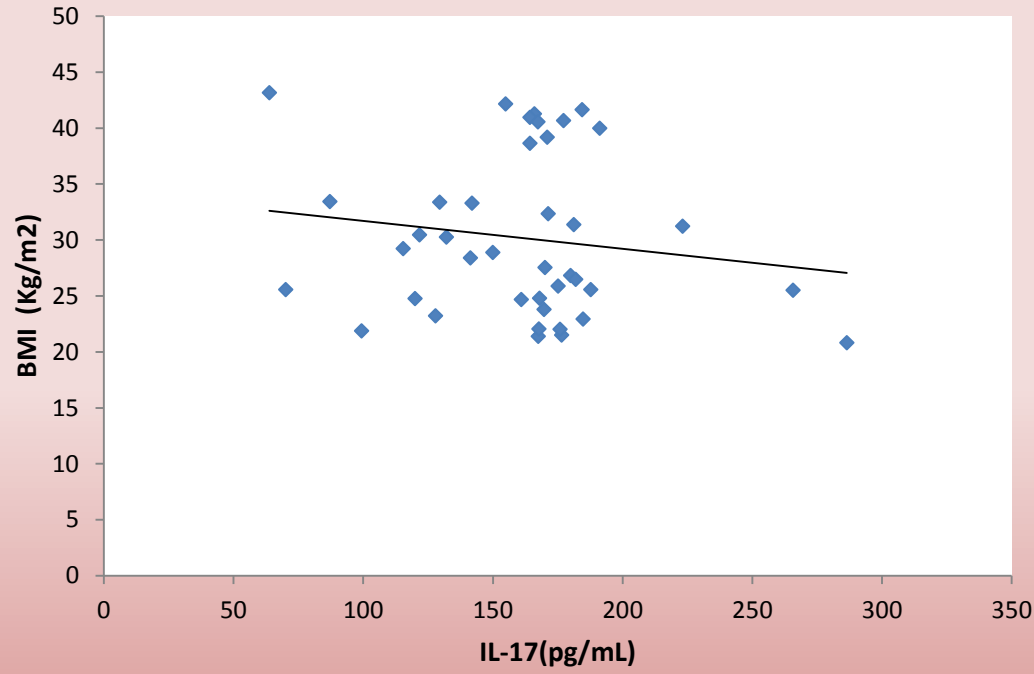

Figure (3-52): Correlation between IL-17 and BMI in female G1

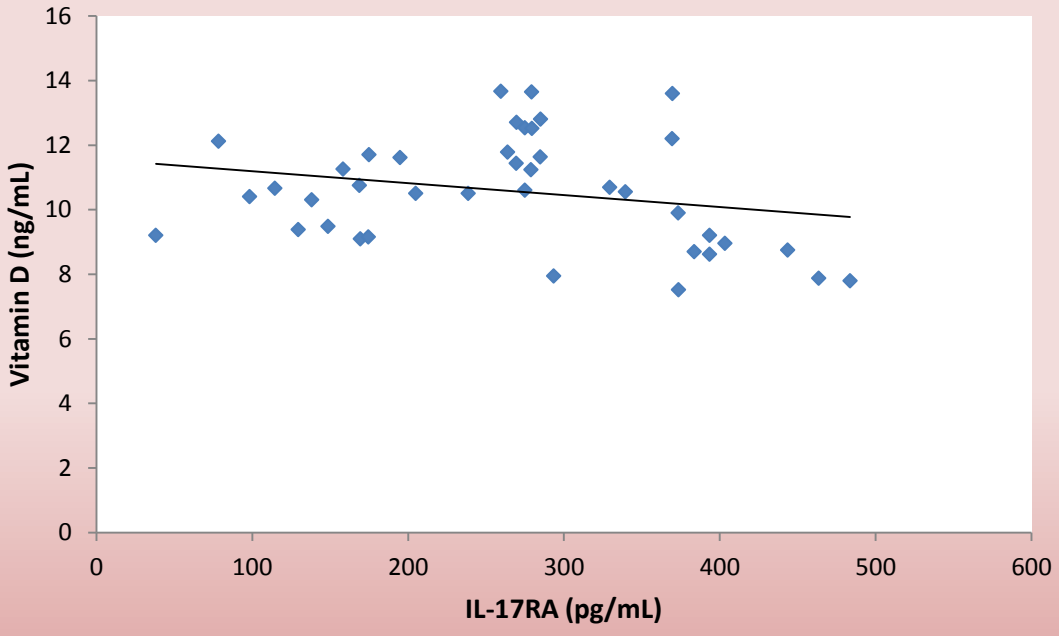

Figure (3-53) : Correlation between IL-17RA & Vitamin D in female G1

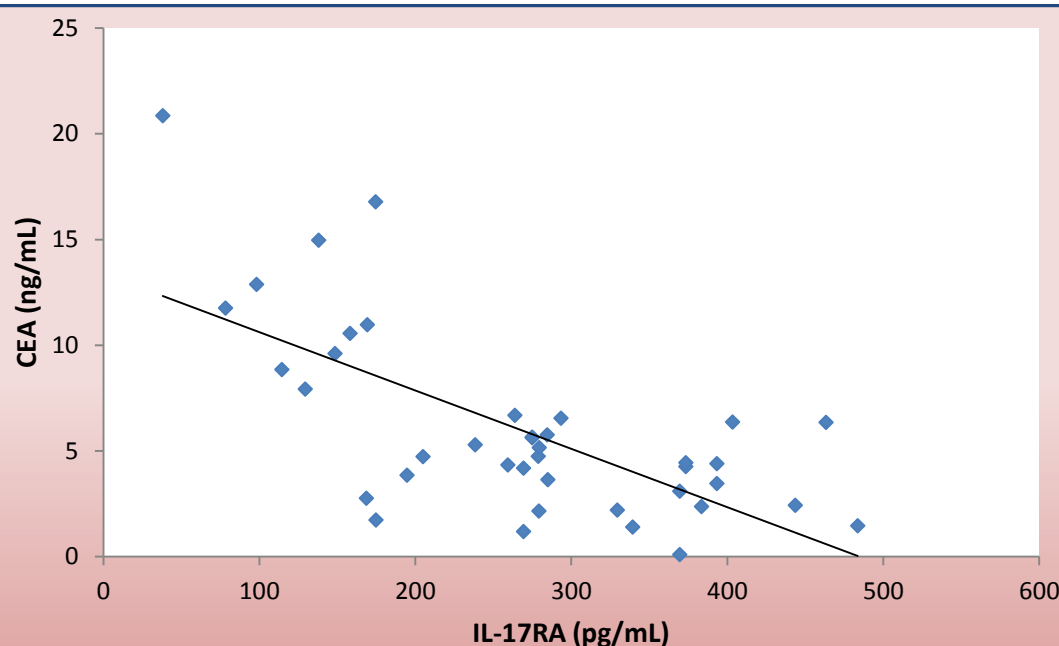

Figure (3-54): Correlation between IL-17RA & CEA in female G1

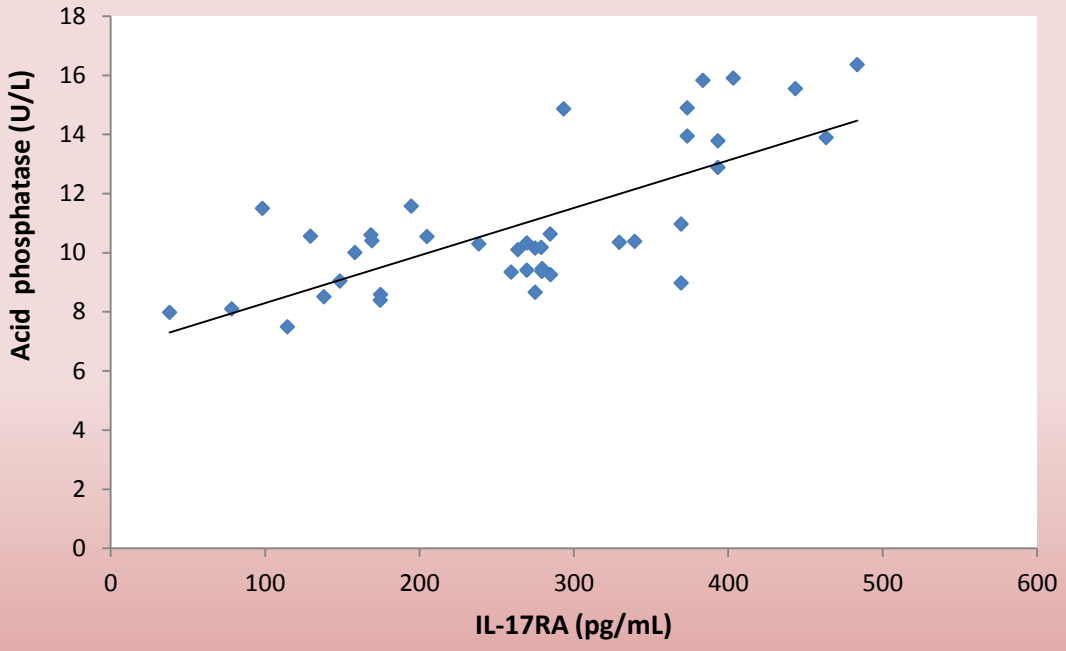

Figure (3-55): Correlation between IL-17RA & Acid phosphatase in female G1

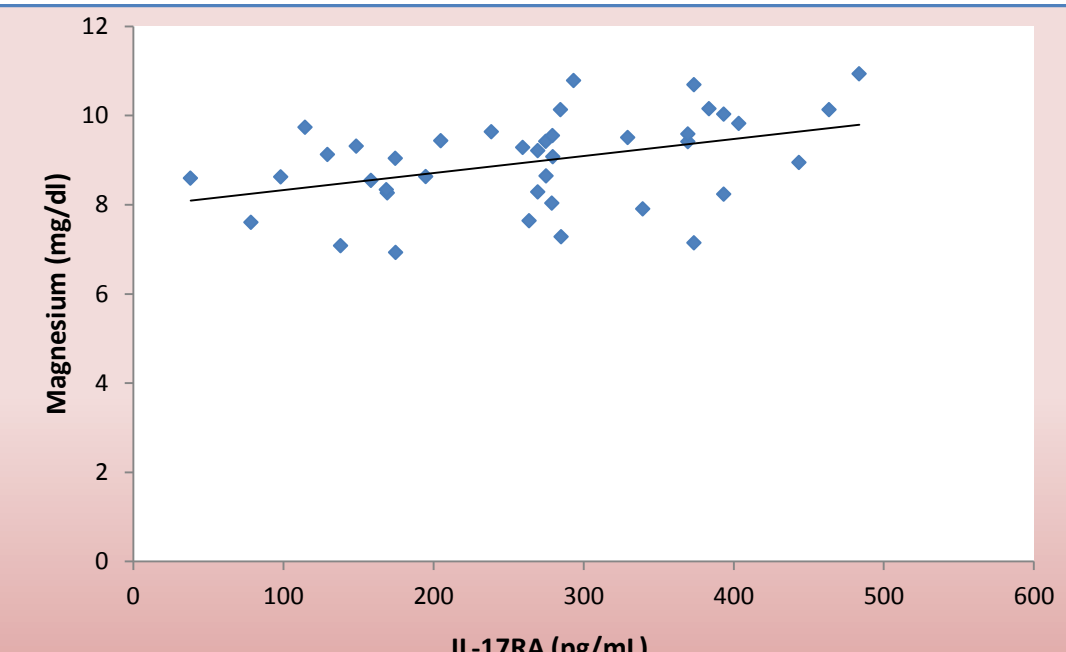

Figure (3-56): Correlation between IL-17RA & Magnesium in female G1

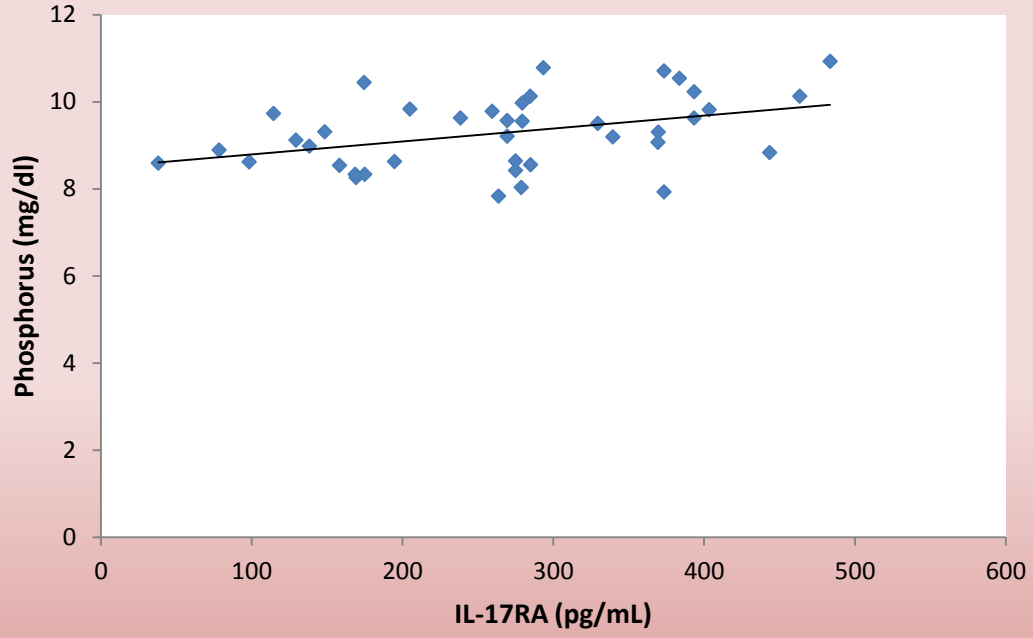

Figure (3-57): Correlation between IL-17RA & Phosphorus in female G1

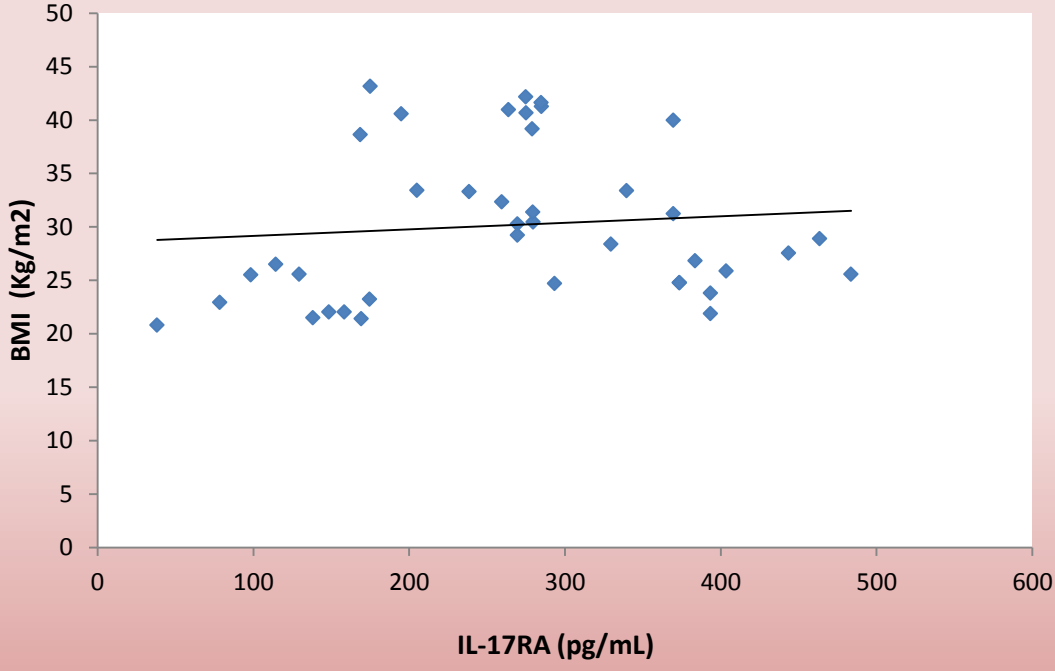

Figure (3-58): Correlation between IL-17RA and BMI in female G1

The results in table 3-7 and figures 3-59 , 3-60 , 3-61 , 3-62 , 3-63 , 3-64 , 3-65 , 3-66 , 3-67 , 3-68 3-69 , 3-70 , 3-71 show the correlation between IL-17& IL-17RA with (Vitamin D, CEA, acid phosphatase, magnesium, phosphorus and BMI) in male patients after taking first dose of chemotherapy (G2).

Table(3-7): Correlations between IL-17 and some bio-chemical parameters in sera of CRC patients male after taking first dose of chemotherapy (G2).

Parameters		IL-17	IL-17RA
IL-17RA	r	0.374	
	P	0.009 h s	
Vitamin D	r	0.192	0.174
	P	0.190 Ns	0.236 Ns
CEA	r	0.103	-0.622
	P	0.485 Ns	0.000 h s
Acid phosphatase	r	-0.447	0.107
	P	0.001 h s	0.468 Ns
Magnesium	r	0.285	-0.137
	P	0.050 S	0.352 Ns
Phosphorus	r	-0.820	-0.134
	P	0.000 h s	0.363 Ns
BMI	r	-0.146	0.251
	P	0.321 Ns	0.085 Ns

S = Significant (*P* value ≤ 0.05)
Ns = Non significant (*P* value > 0.05)
h s = high significant (*P* value ≤ 0.001)

In this table a non significant negative (- ve) correlation between (IL-17& BMI), and a high significant positive (+ ve) correlation between (IL-17& IL-17RA) , and non significant positive (+ ve) correlation between (IL-17 & Vitamin D) . A non significant positive (+ ve)

correlation was shown between (IL-17 & CEA) , and a high significant negative (- ve) correlation between (IL-17& Acid phosphatase) . However, a significant positive (+ ve) correlation was shown between (IL-17 & Magnesium) , and a high significant negative (- ve) correlation between (IL-17 & Phosphorus) after taking first dose of chemotherapy.

In this table a non significant positive (+ ve) correlation also shown between (IL-17RA & BMI), and a non significant positive (+ ve) correlation between (IL-17RA & vitamin D). However, a high significant negative (- ve) correlation was shown between (IL-17RA & CEA), and a non significant positive (+ ve) correlation between (IL-17RA & Acid phosphatase), and a non significant negative (- ve) correlation between (IL-17RA & magnesium). A non significant negative (- ve) correlation was shown between (IL-17RA & phosphorus) after taking first dose of chemotherapy.

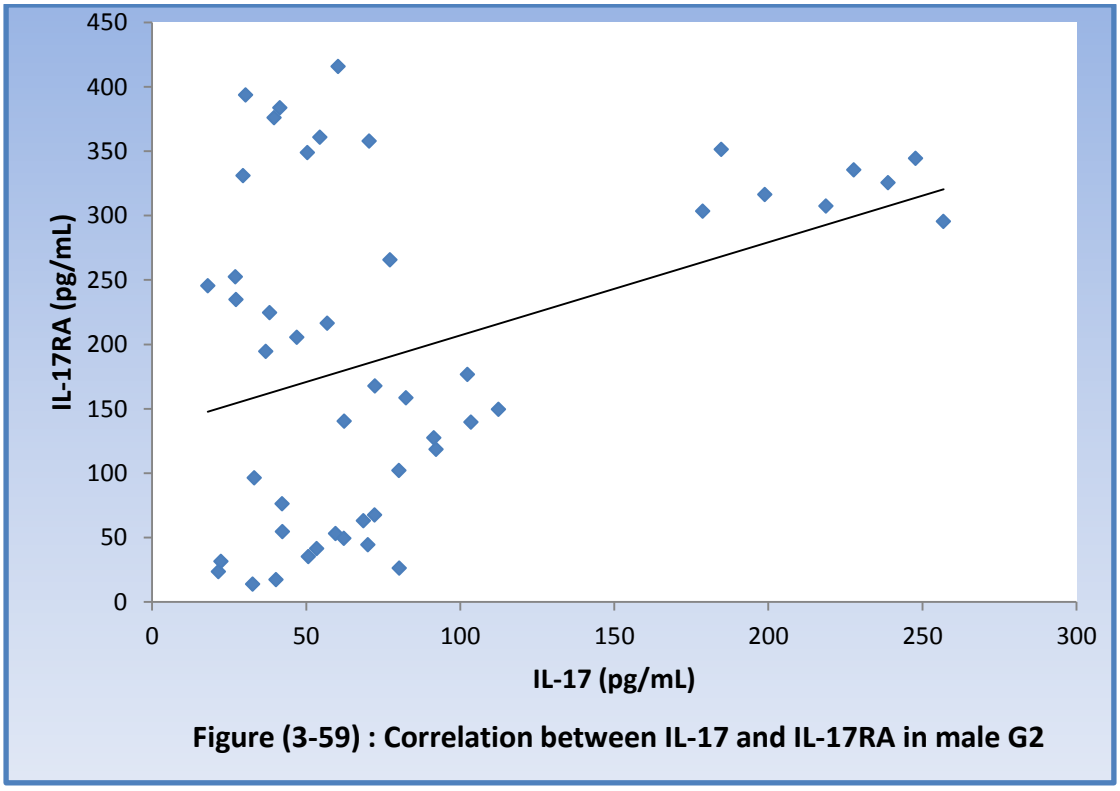

Figure (3-59) : Correlation between IL-17 and IL-17RA in male G2

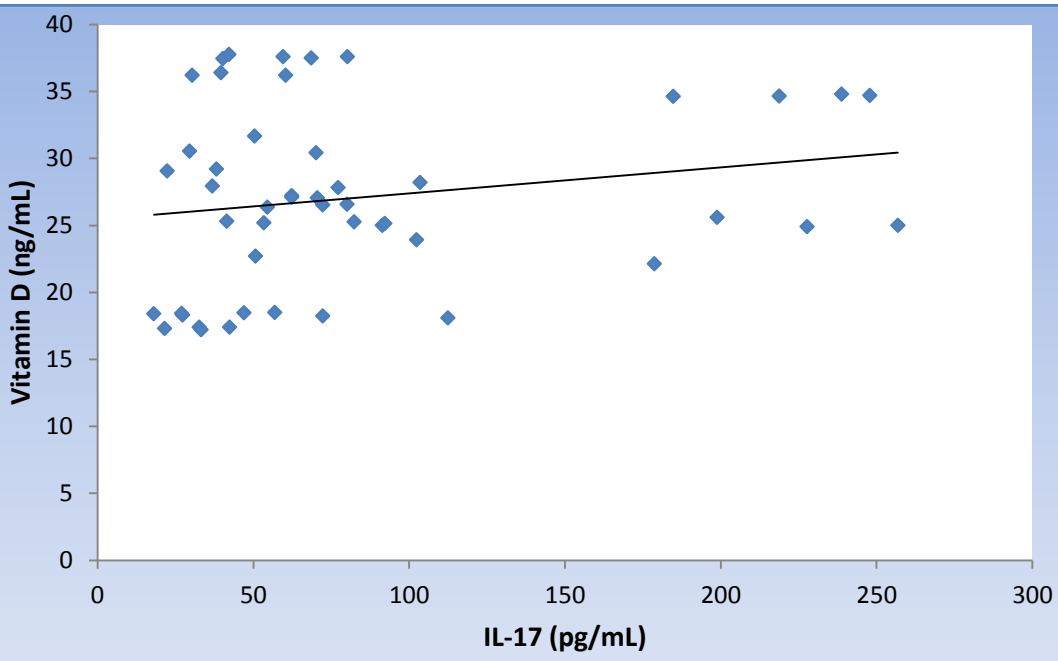

Figure (3-60): Correlation between IL-17 and Vitamin D in male G2

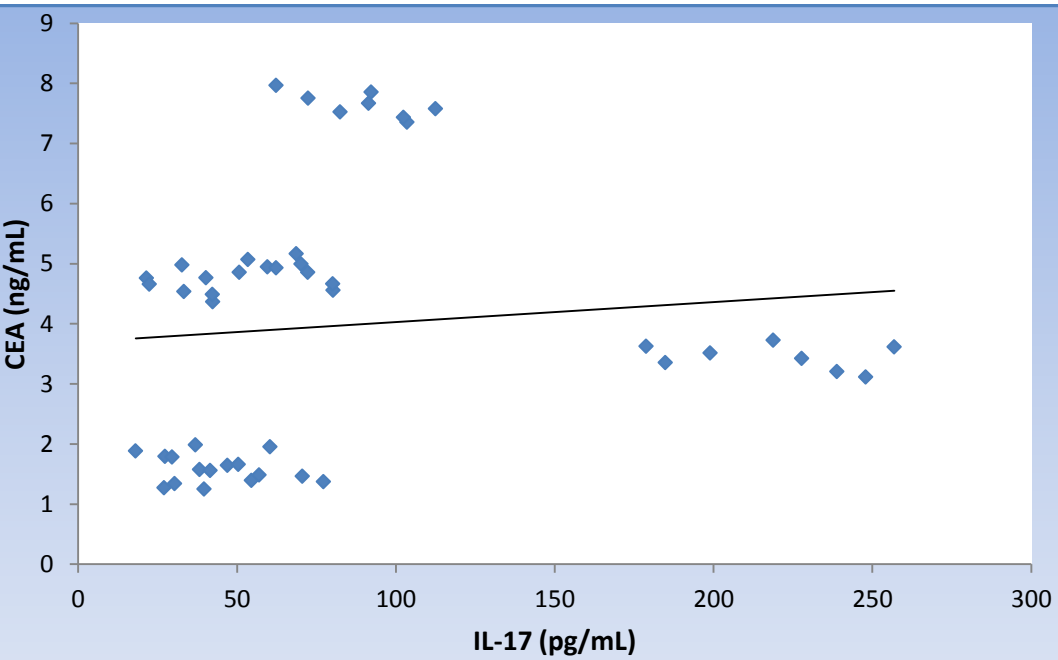

Figure (3-61): Correlation between IL-17 and CEA in male G2

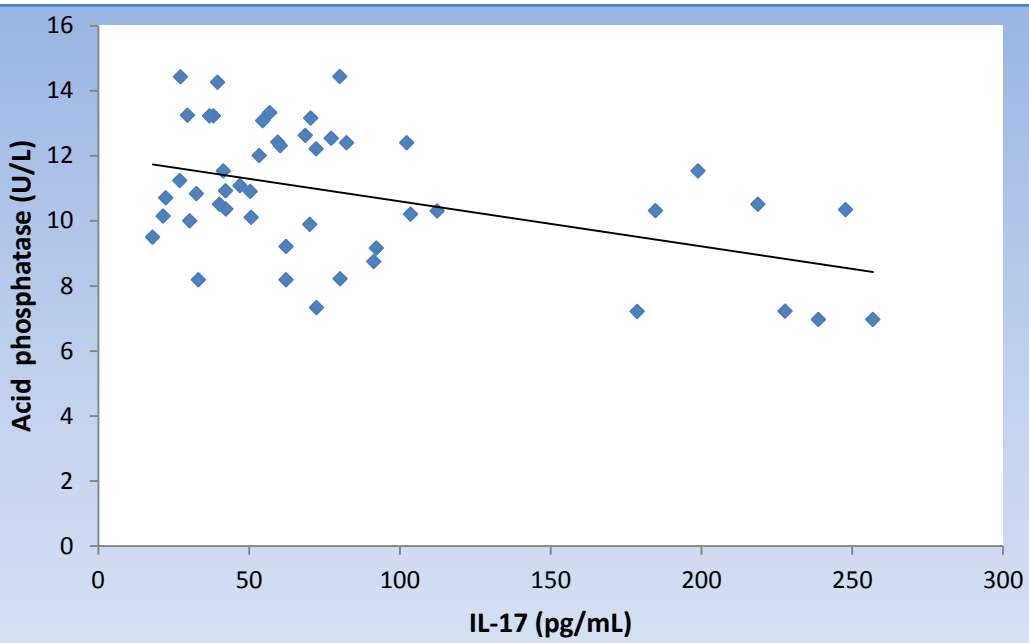

Figure (3-62) : Correlation between IL-17 and Acid phosphatase in male G2

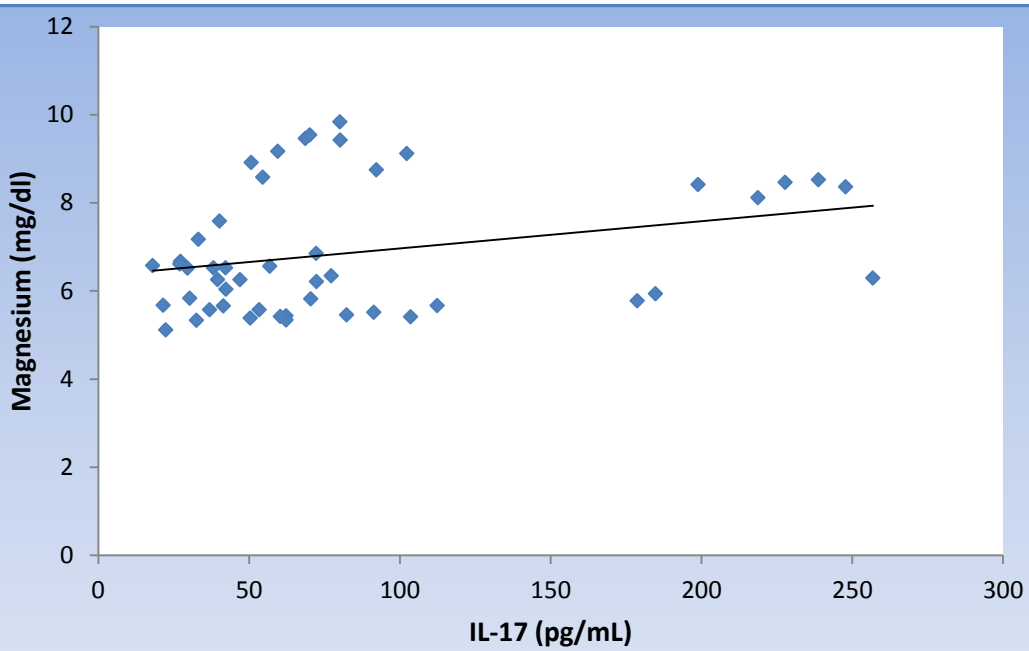

Figure (3-63) : Correlation between IL-17 and Magnesium in male G2

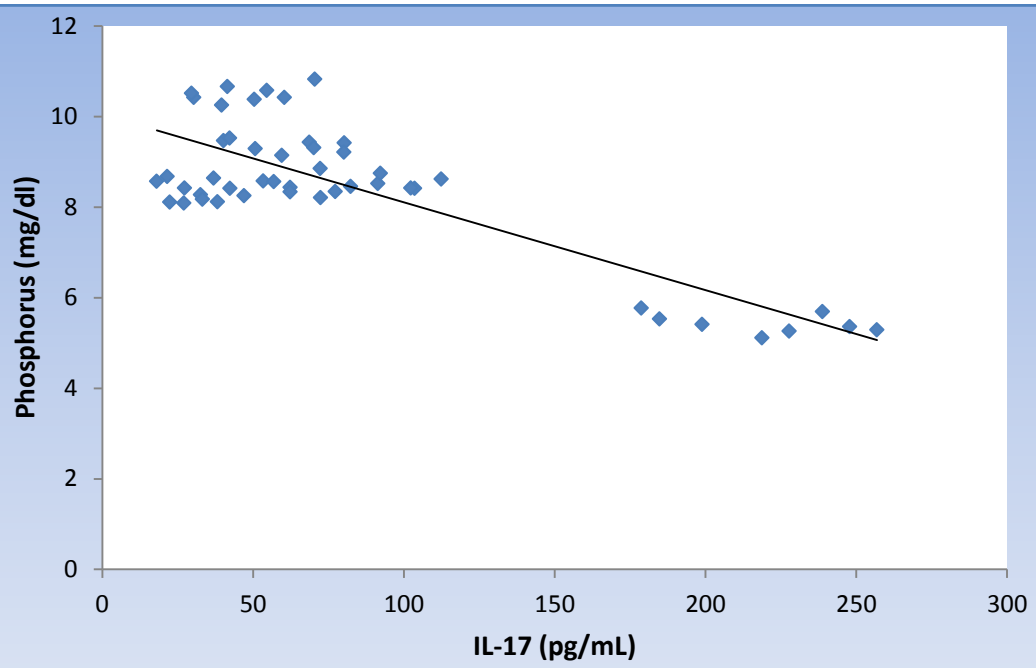

Figure (3-64) : Correlation between IL-17 and Phosphorus in male G2

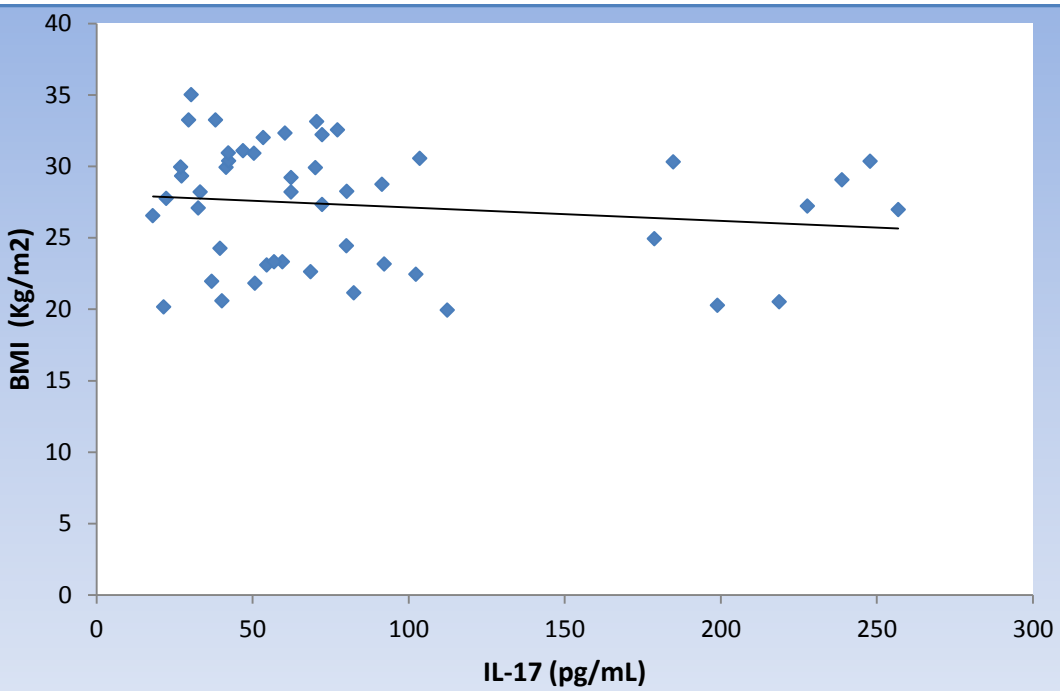

Figure (3-65): Correlation between IL-17 and BMI in male G2

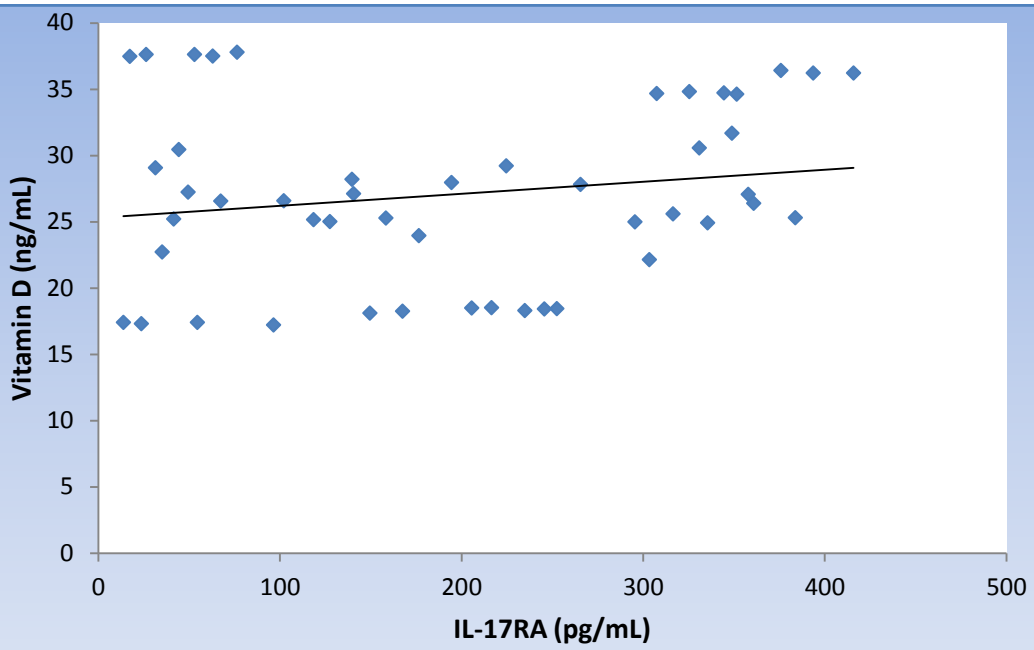

Figure (3-66): Correlation between IL-17RA and Vitamin D in male G2

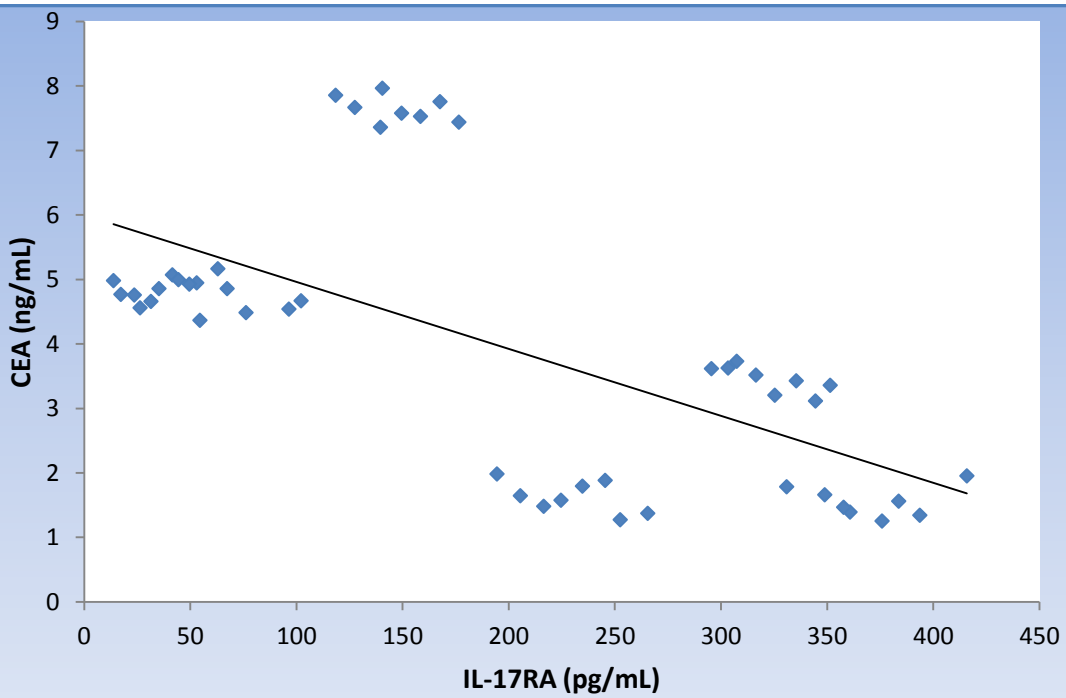

Figure (3-67) : Correlation between IL-17RA and CEA in male G2

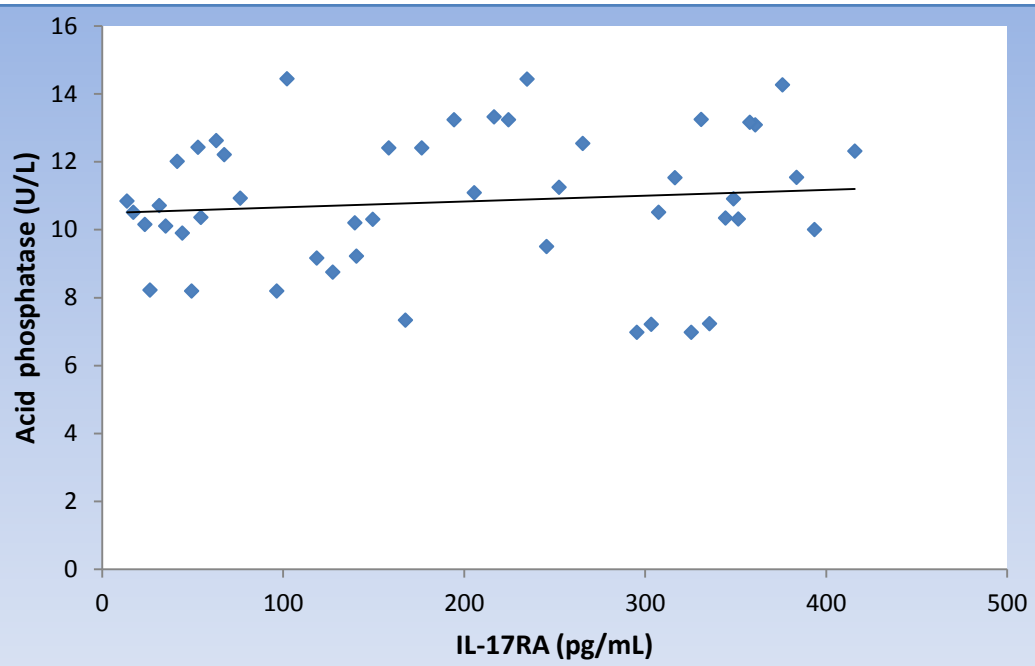

Figure (3-68) : Correlation between IL-17RA and Acid phosphatase in male G2

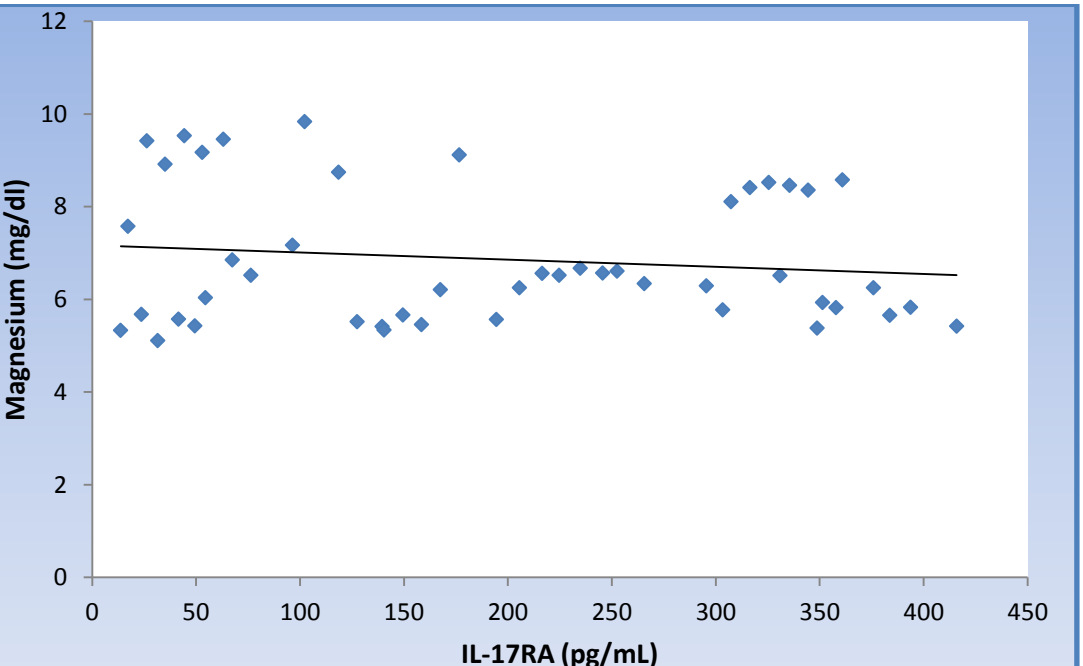

Figure (3-69) : Correlation between IL-17RA and Magnesium in male G2

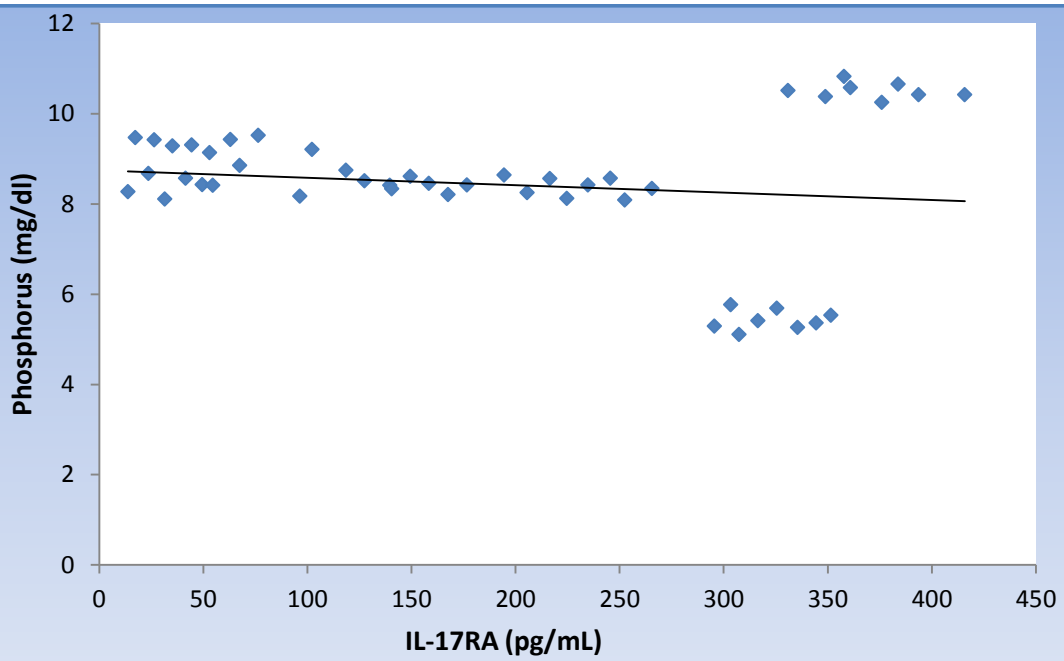

Figure (3-70) : Correlation between IL-17RA and Phosphorus in male G2

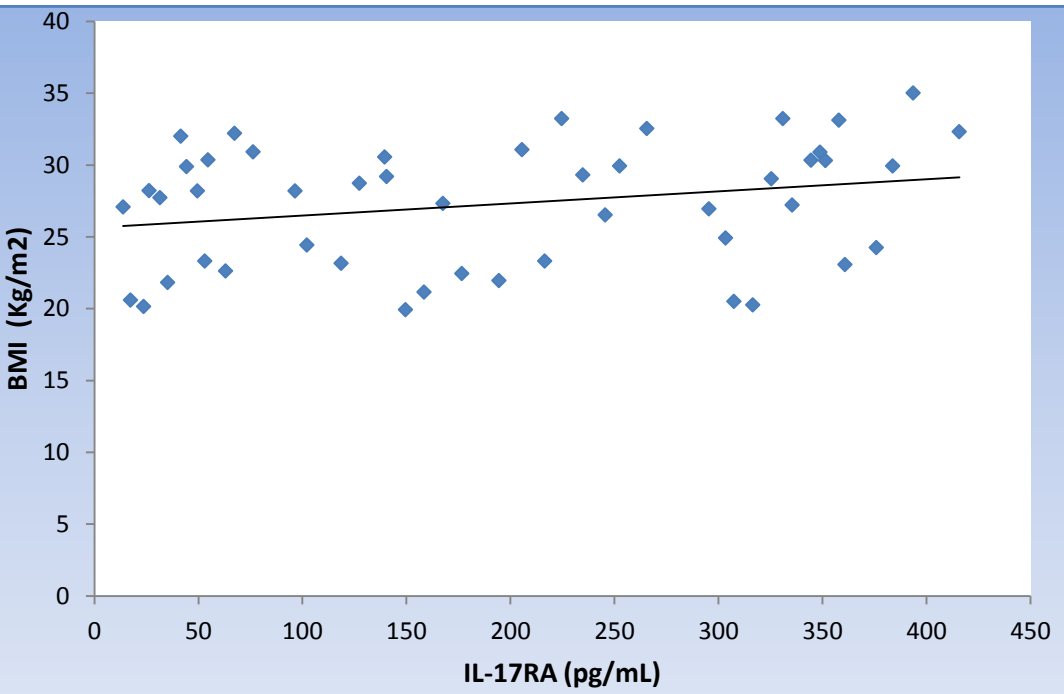

Figure (3-71): Correlation between IL-17RA and BMI in male G2

The results in table 3-8 and figures 3-72, 3-73, 3-74, 3-75 , 3-76 , 3-77 , 3-78 , 3-79 , 3-80, 3-81 3-82, 3-83, 3-84 show the correlation between IL-17& IL-17RA with (Vitamin D, CEA, acid phosphatase, magnesium, phosphorus and BMI) in female patients after taking first dose of chemotherapy (G2).

Table(3-8): Correlations between IL-17 and some bio-chemical parameters in sera of CRC patients female after taking first dose of chemotherapy (G2).

Parameters		IL-17	IL-17RA
IL-17RA	r	-0.670	
	P	0.000 h s	
Vitamin D	r	0.616	-0.571
	P	0.000 h s	0.000 h s
CEA	r	0.589	-0.776
	P	0.000 h s	0.000 h s
Acid phosphatase	r	0.075	-0.280
	P	0.647 Ns	0.080 Ns
Magnesium	r	-0.403	0.272
	P	0.010 S	0.089 Ns
Phosphorus	r	-0.163	-0.002
	P	0.315 Ns	0.992 Ns
BMI	r	-0.440	0.478
	P	0.004 h s	0.002 h s

S = Significant (*P* value ≤ 0.05)
Ns = Non significant (*P* value > 0.05)
h s = high significant (*P* value ≤ 0.001)

In this table a high significant negative (-ve) correlation was found between (IL-17& BMI), and a high significant negative (-ve) correlation between (IL-17& IL-17RA), and high significant positive (+ve) correlation between (IL-17 & vitamin D). A high significant positive

(+ve) correlation also was shown between (IL-17 & CEA) , and a non significant positive (+ve) correlation between (IL-17& acid phosphatase), and a significant negative (-ve) correlation between (IL-17 & magnesium). However, a non significant negative (-ve) correlation was shown between (IL-17 & phosphorus) after taking first dose of chemotherapy.

In this table there was a high significant positive (+ve) correlation between (IL-17RA & BMI), and a high significant negative (-ve) correlation between (IL-17RA & vitamin D). A high significant negative (-ve) correlation also was found between (IL-17RA & CEA) , and a non significant negative (-ve) correlation between (IL-17RA & acid phosphatase). However, A non significant positive (+ve) correlation was found between (IL-17RA & magnesium), and a non significant negative (-ve) correlation between (IL-17RA & phosphorus) after taking first dose of chemotherapy.

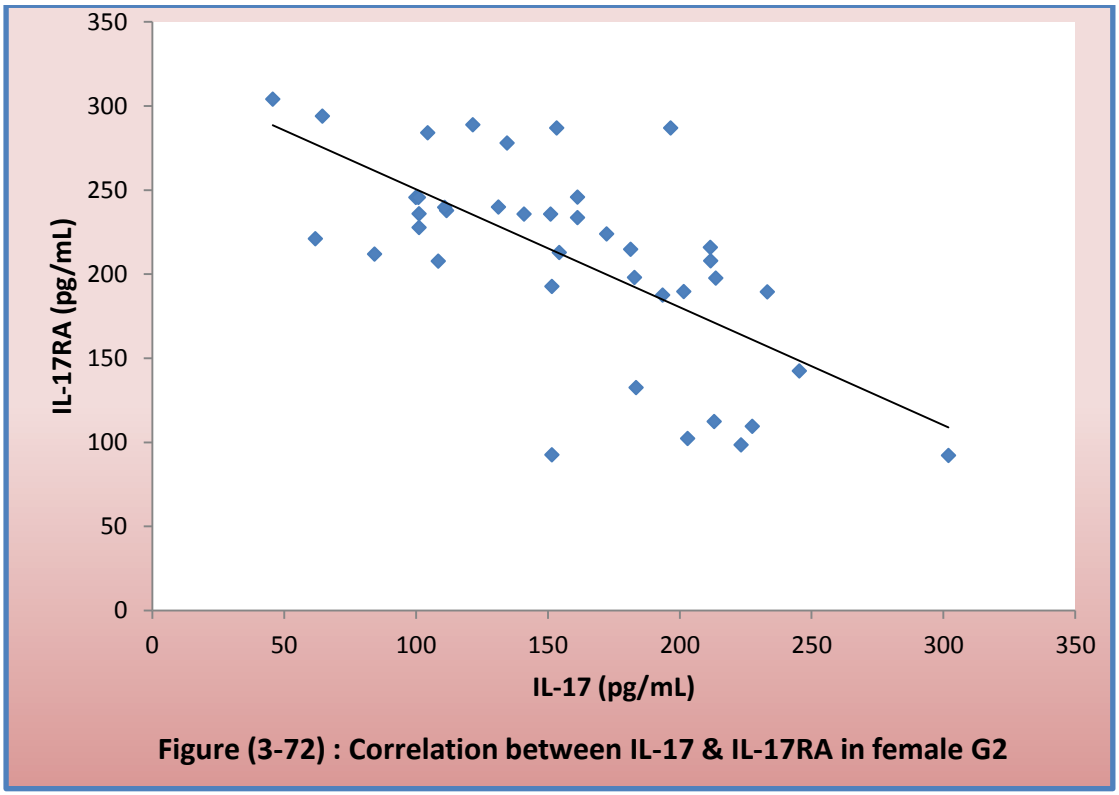

Figure (3-72) : Correlation between IL-17 & IL-17RA in female G2

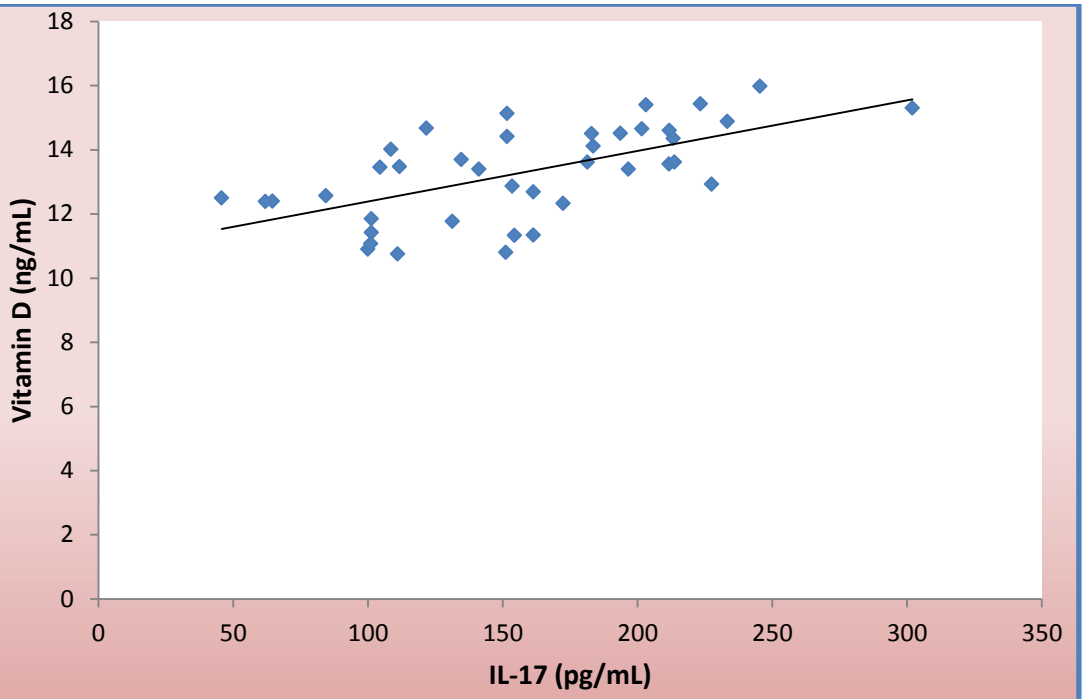

Figure (3-73): Correlation between IL-17 and Vitamin D in female G2

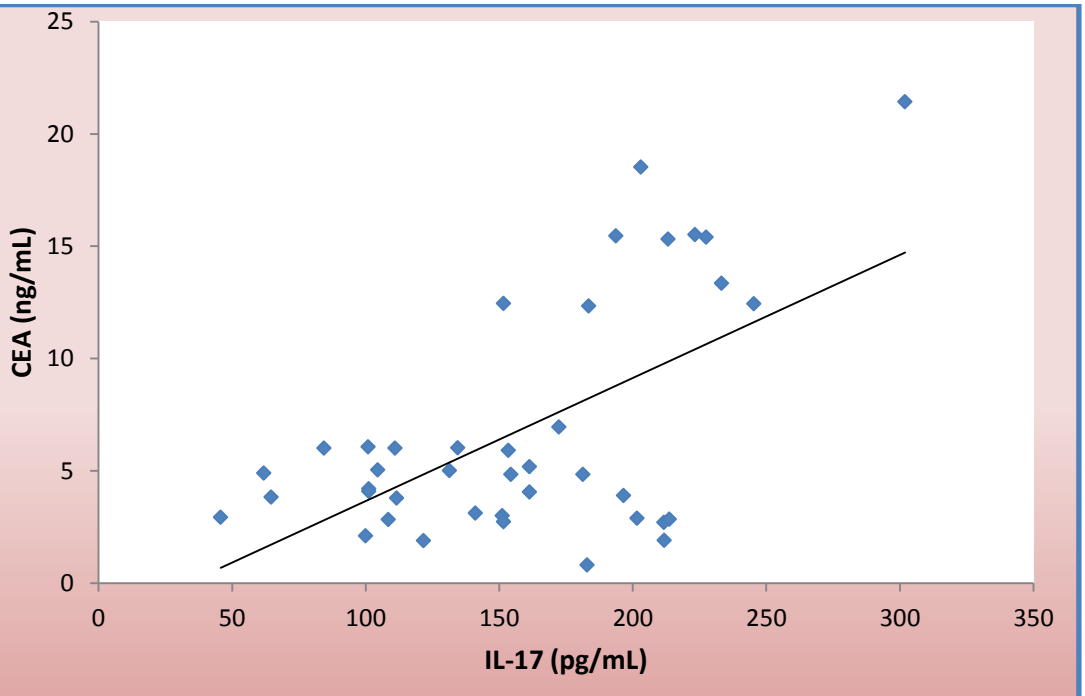

Figure (3-74): Correlation between IL-17 and CEA in female G2

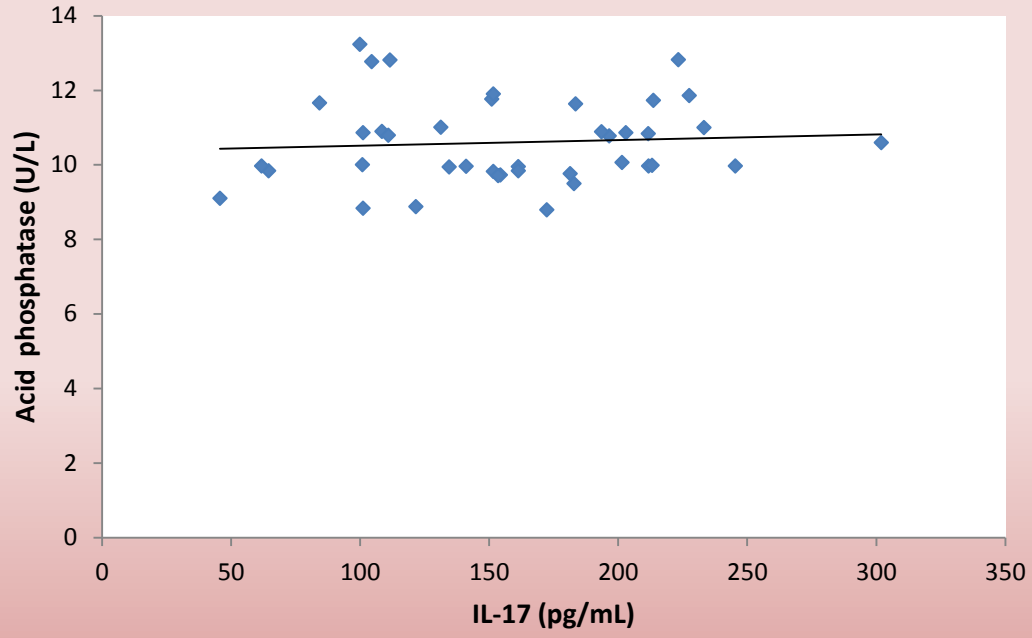

Figure (3-75): Correlation between IL-17 and Acid phosphatase in female G2

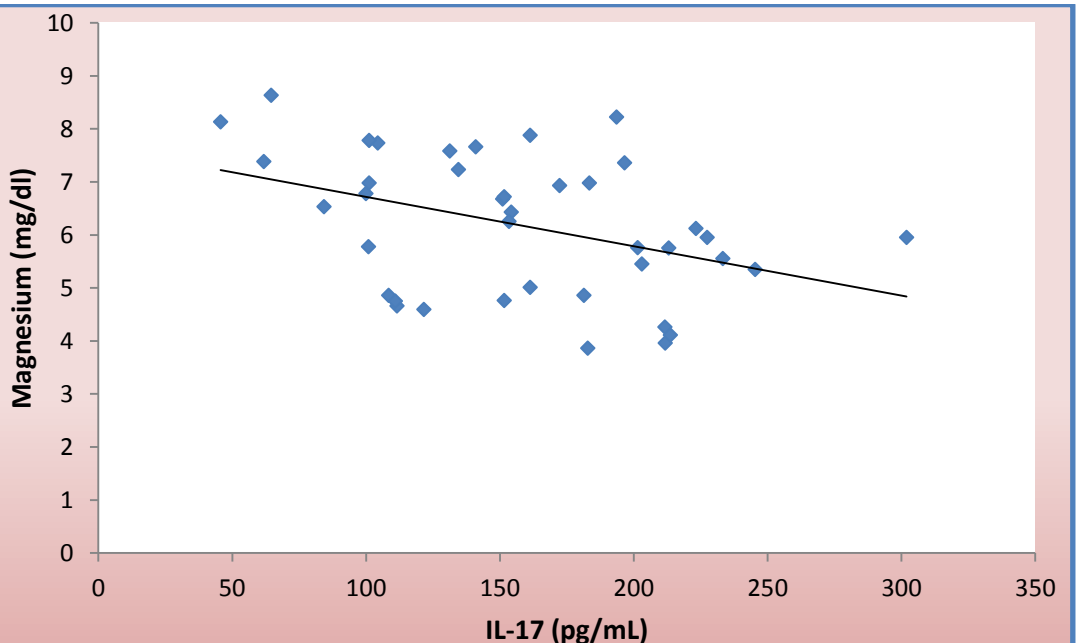

Figure (3-76): Correlation between IL-17 and Magnesium in female G2

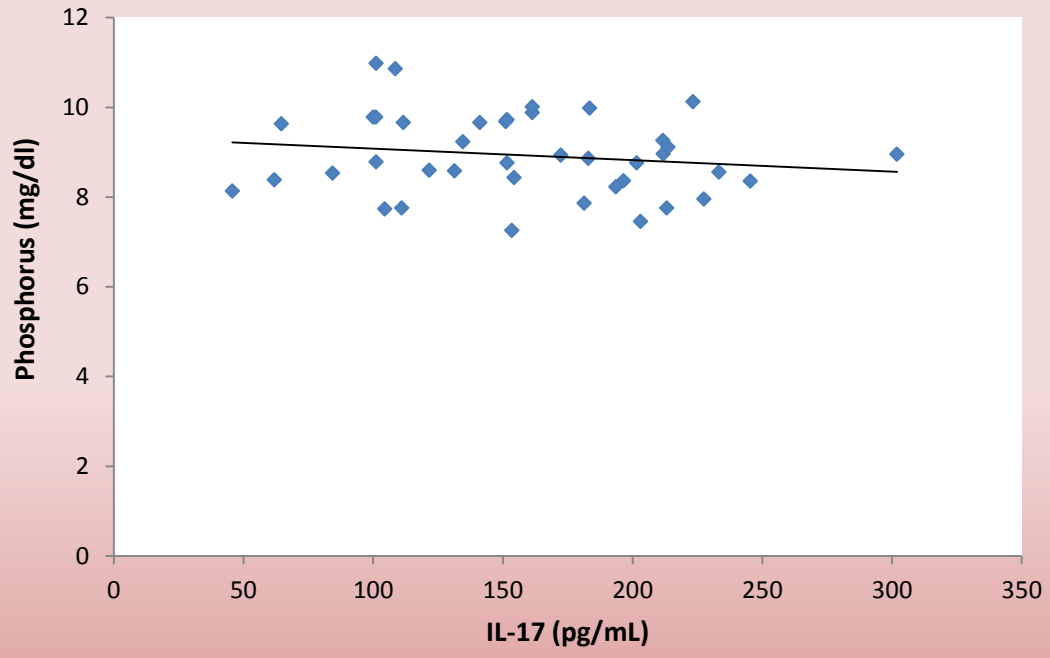

Figure (3-77): Correlation between IL-17 and Phosphorus in female G2

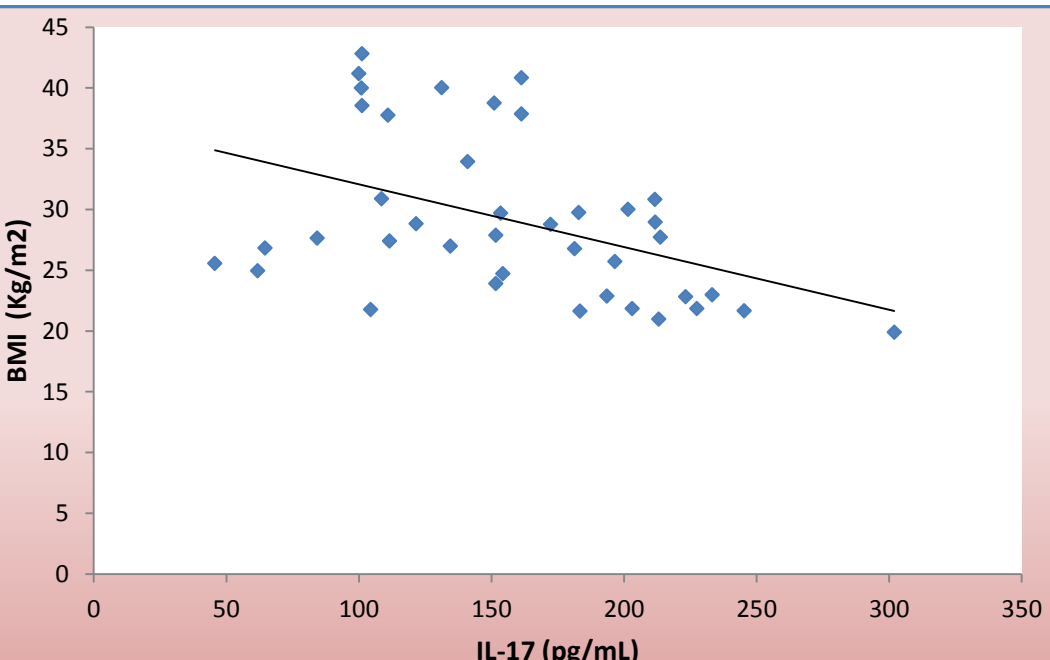

Figure (3-78): Correlation between IL-17 and BMI in female G2

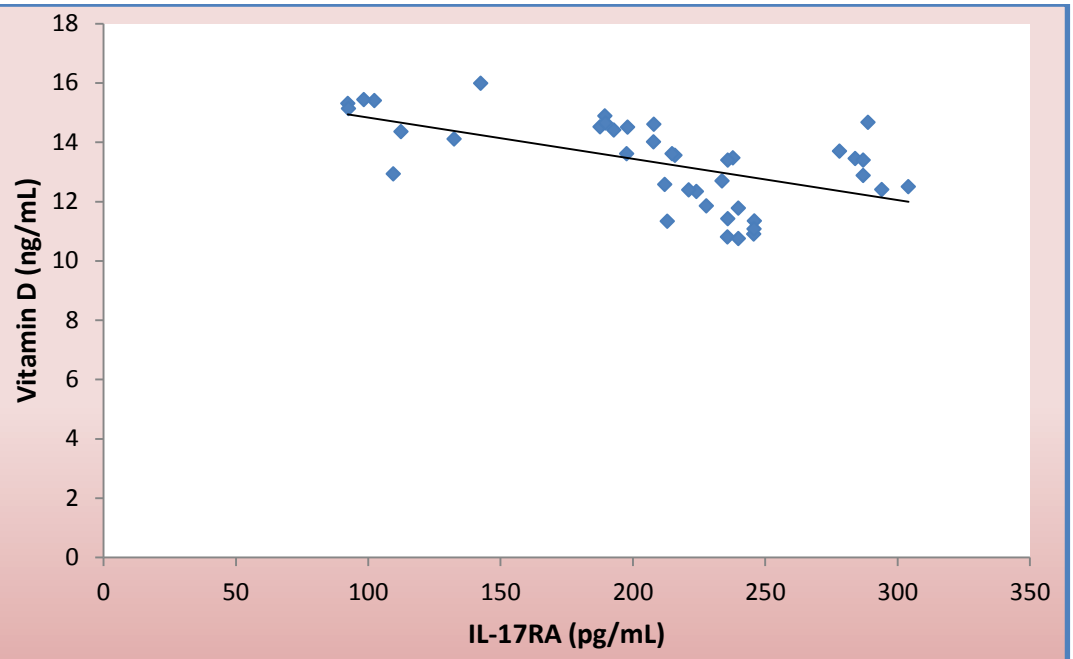

Figure (3-79): Correlation between IL-17RA and Vitamin D in female G2

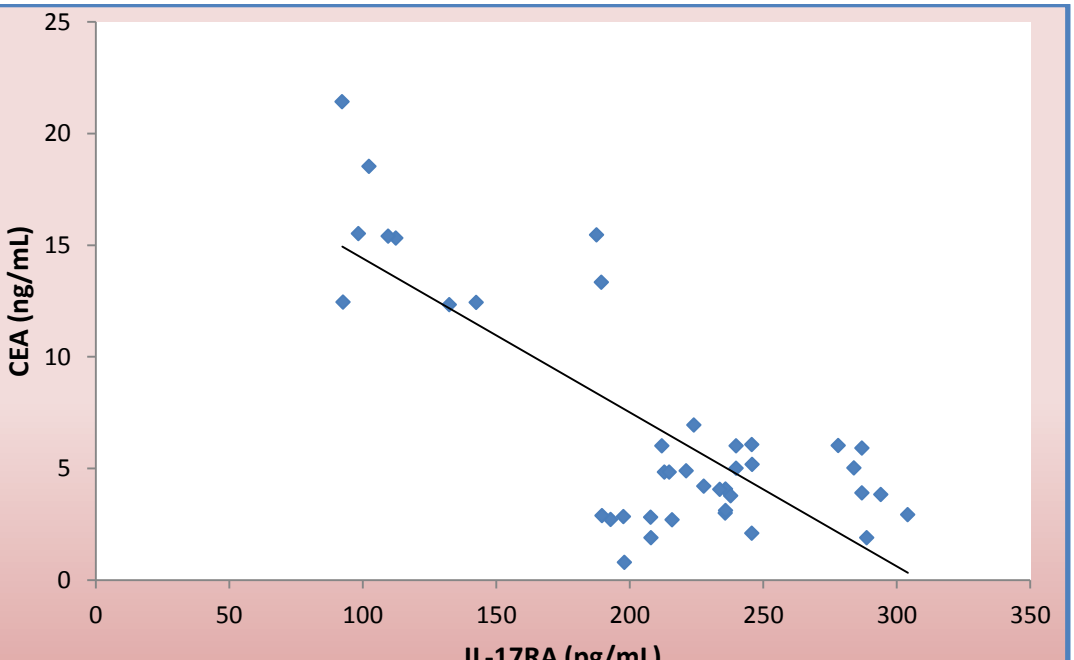

Figure (3-80): Correlation between IL-17RA and CEA in female G2

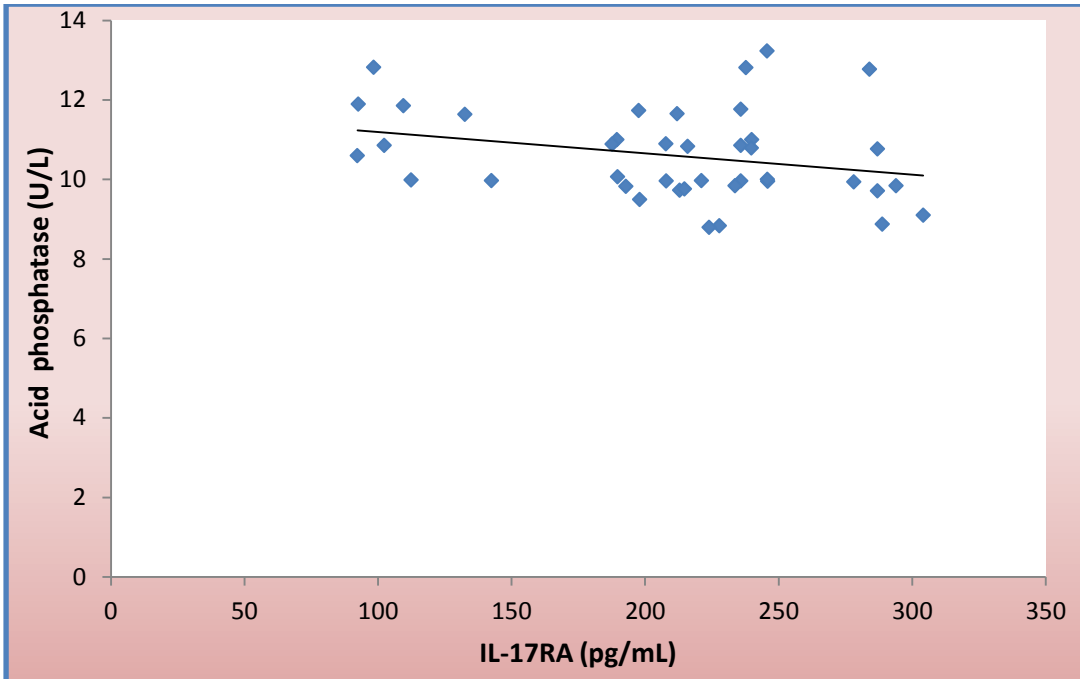

Figure (3-81): Correlation between IL-17RA and Acid phosphatase in female G2

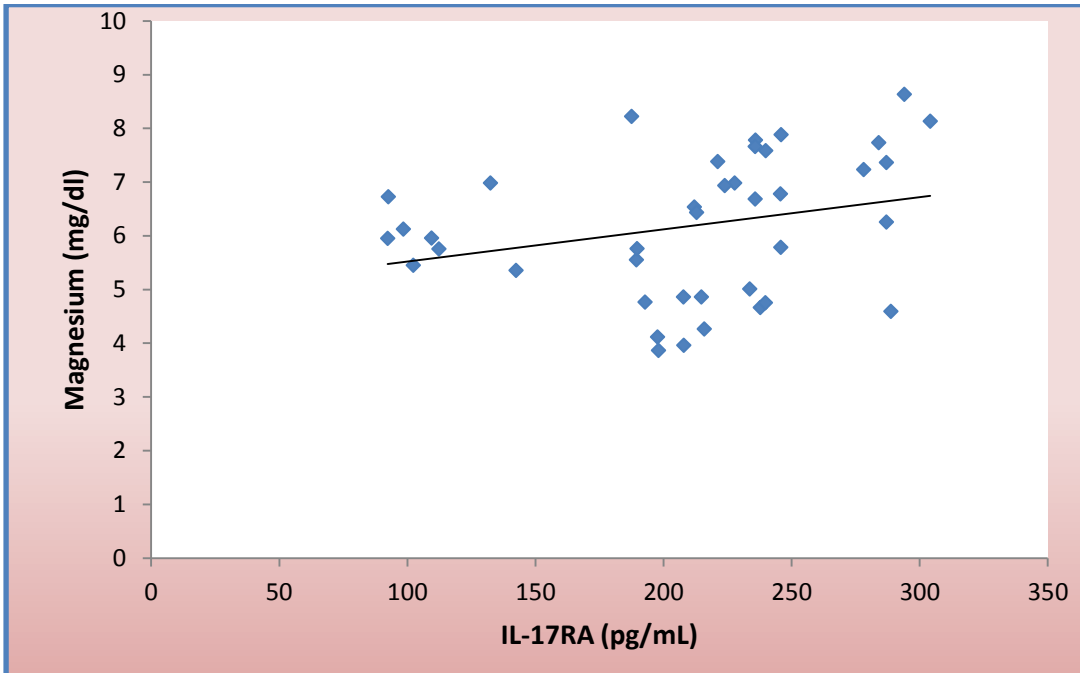

Figure (3-82): Correlation between IL-17RA and Magnesium in female G2

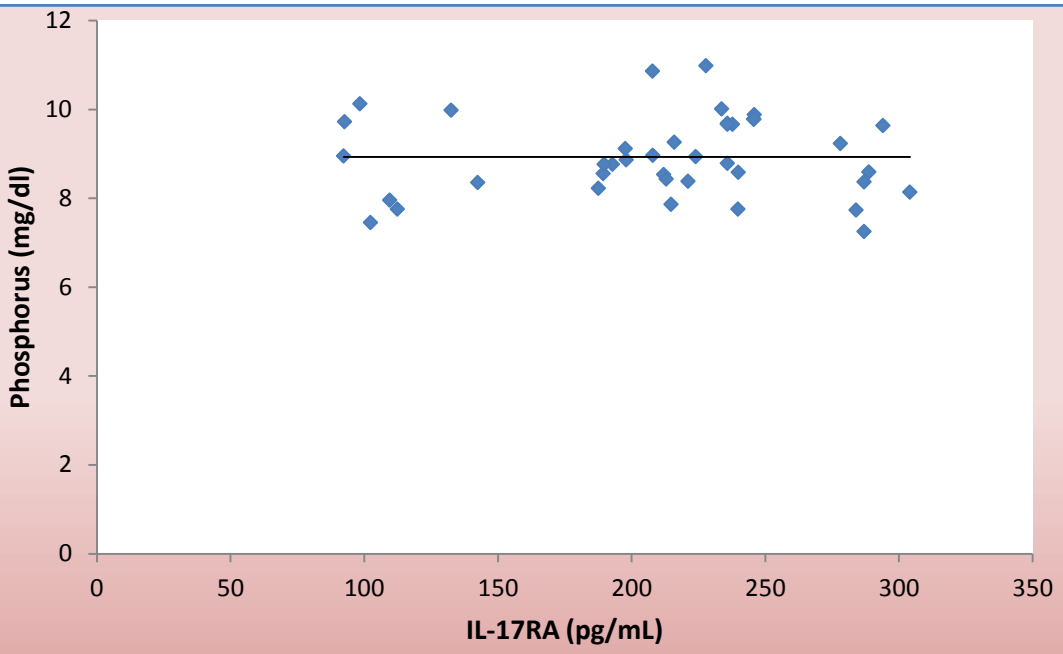

Figure (83): Correlation between IL-17RA and Phosphorus in female G2

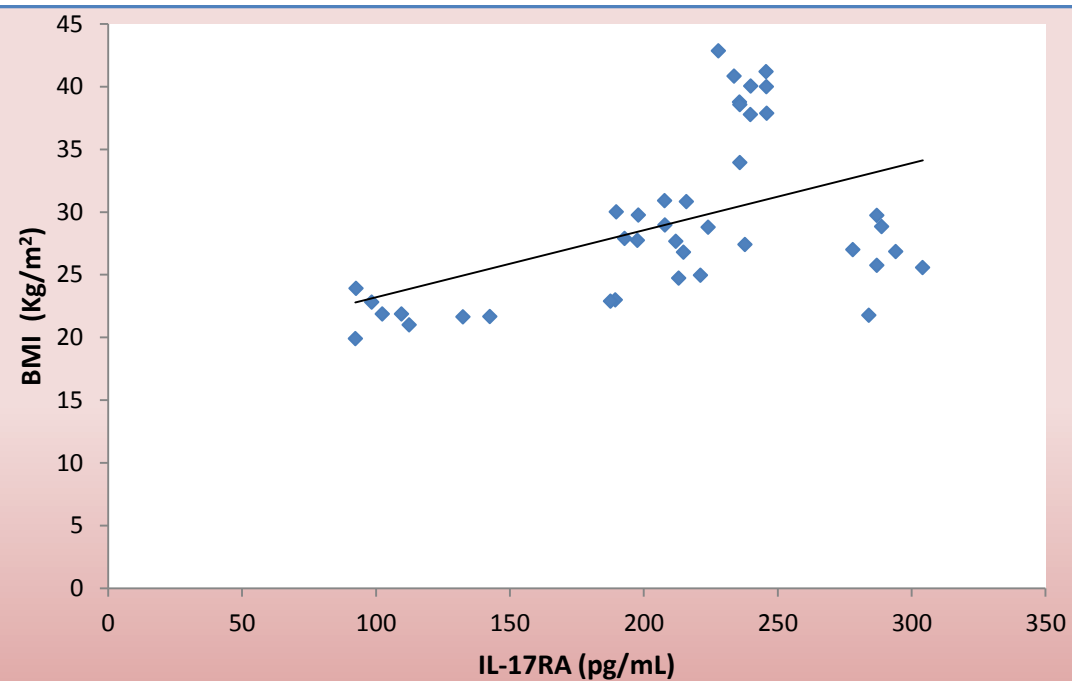

Figure (3-84): Correlation between IL-17RA and BMI in female G2

The results in table 3-9 and figures 3-85 , 3-86 , 3-87 , 3-88 , 3-89 , 3-90 , 3-91 , 3-92 , 3-93 , 3-94, 3-95 , 3-96 , 3-97 show the correlation between IL-17& IL-17RA with (Vitamin D, CEA, acid phosphatase, magnesium, phosphorus and BMI) in male patients after taking second dose of chemotherapy (G3).

Table(3-9): Correlations between IL-17 and some bio-chemical parameters in sera of CRC patients male after taking second dose of chemotherapy (G3).

Parameters		IL-17	IL-17RA
IL-17RA	r	0.464	
	P	0.001 h s	
Vitamin D	r	0.509	0.124
	P	0.000 h s	0.402 Ns
CEA	r	-0.227	-0.398
	P	0.121 Ns	0.005 h s
Acid phosphatase	r	-0.102	0.192
	P	0.489 Ns	0.190 Ns
Magnesium	r	-0.092	-0.101
	P	0.532 Ns	0.496 Ns
Phosphorus	r	-0.776	-0.152
	P	0.000 h s	0.301 Ns
BMI	r	-0.124	0.284
	P	0.402 Ns	0.050 S

S = Significant (*P* value ≤ 0.05)
Ns = Non significant (*P* value > 0.05)
h s = high significant (*P* value ≤ 0.001)

In this table a non significant negative (-ve) correlation between (IL-17& BMI), and a high significant positive (+ve) correlation between (IL-17& IL-17RA). A high significant positive (+ve) correlation also

found between (IL-17 & vitamin D), and a non significant negative (-ve) correlation between (IL-17 & CEA), and a non significant negative (-ve) correlation between (IL-17& acid phosphatase), and a non significant negative (-ve) correlation between (IL-17 & magnesium). However, a high significant negative (-ve) correlation was shown between (IL-17 & phosphorus) after taking second dose of chemotherapy.

In this table there was a significant positive (+ve) correlation between (IL-17RA & BMI), and a non significant positive (+ve) correlation between (IL-17RA & vitamin D). However, a high significant negative (-ve) correlation was found between (IL-17RA & CEA), and a non significant positive (+ve) correlation between (IL-17RA & acid phosphatase), and a non significant negative (-ve) correlation between (IL-17RA & magnesium). A non significant negative (-ve) correlation also was shown between (IL-17RA & phosphorus) after taking second dose of chemotherapy.

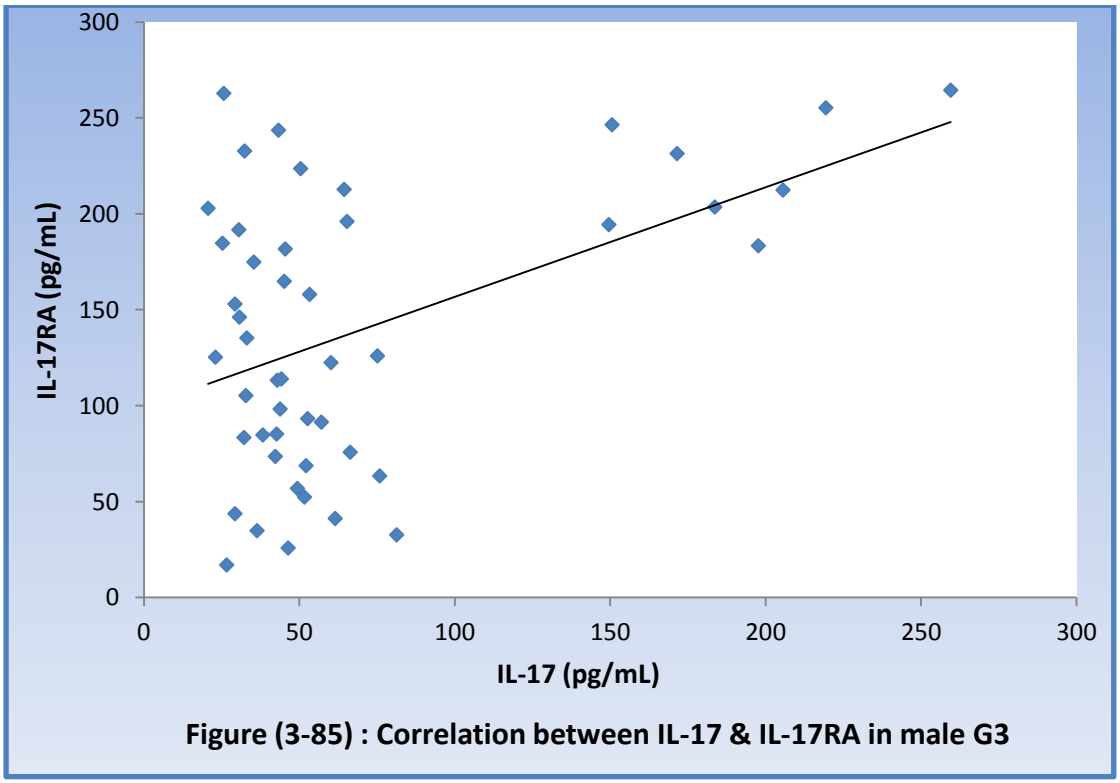

Figure (3-85) : Correlation between IL-17 & IL-17RA in male G3

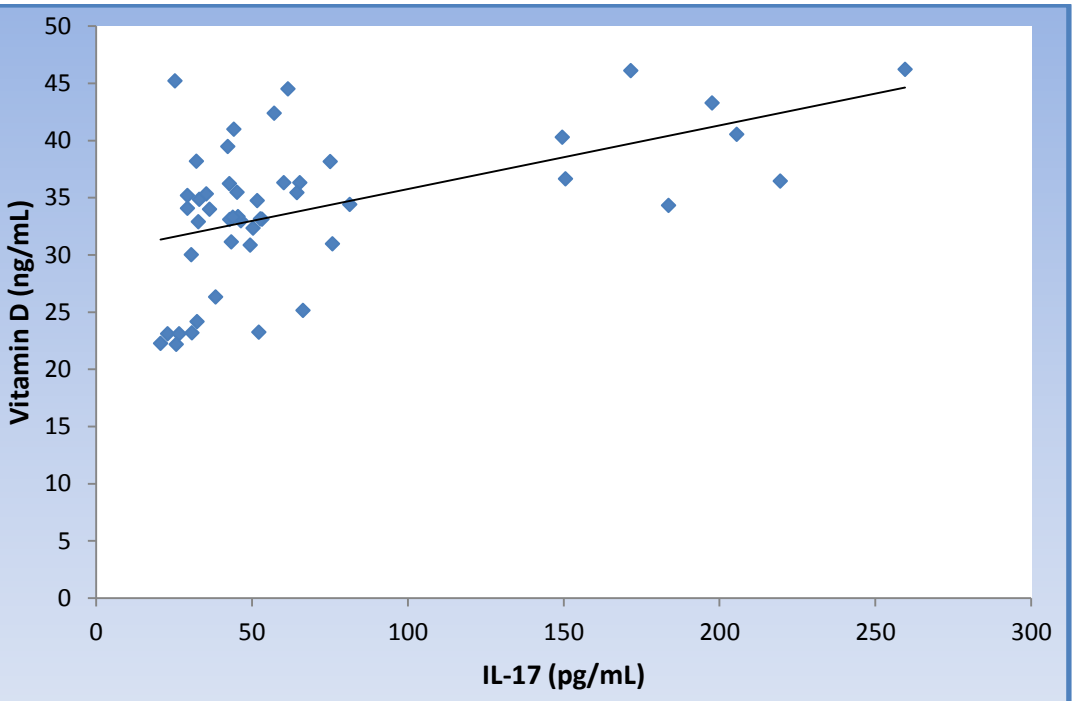

Figure (3-86) : Correlation between IL-17 and Vitamin D in male G3

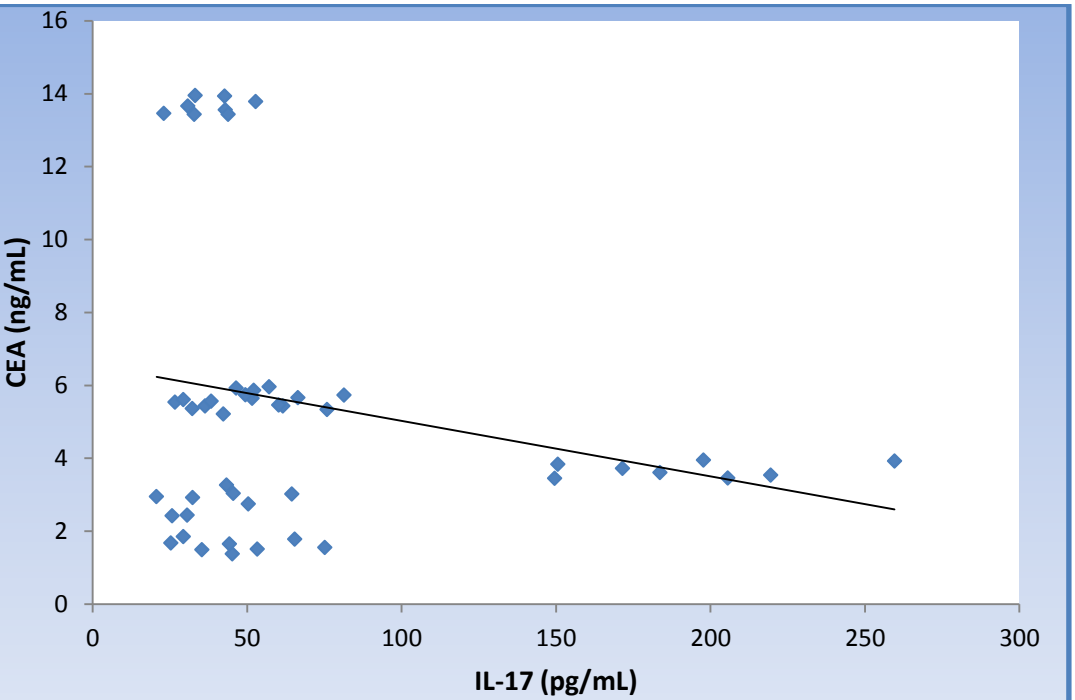

Figure (3-87) : Correlation between IL-17 and CEA in male G3

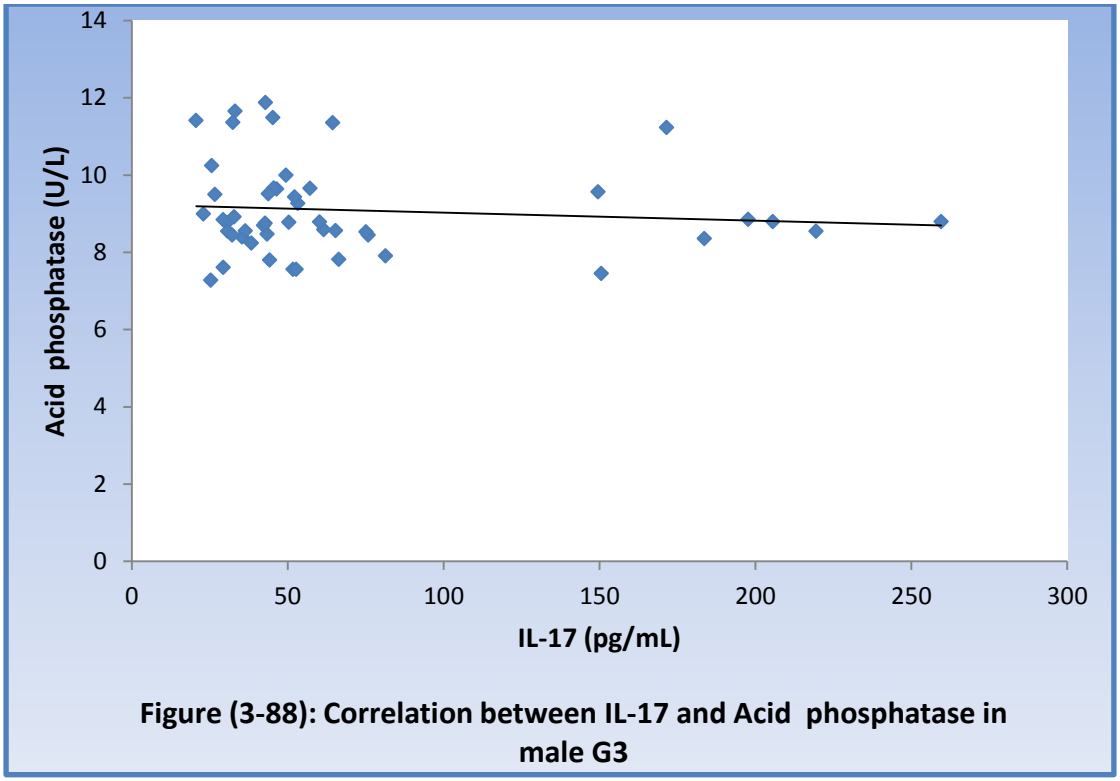

Figure (3-88): Correlation between IL-17 and Acid phosphatase in male G3

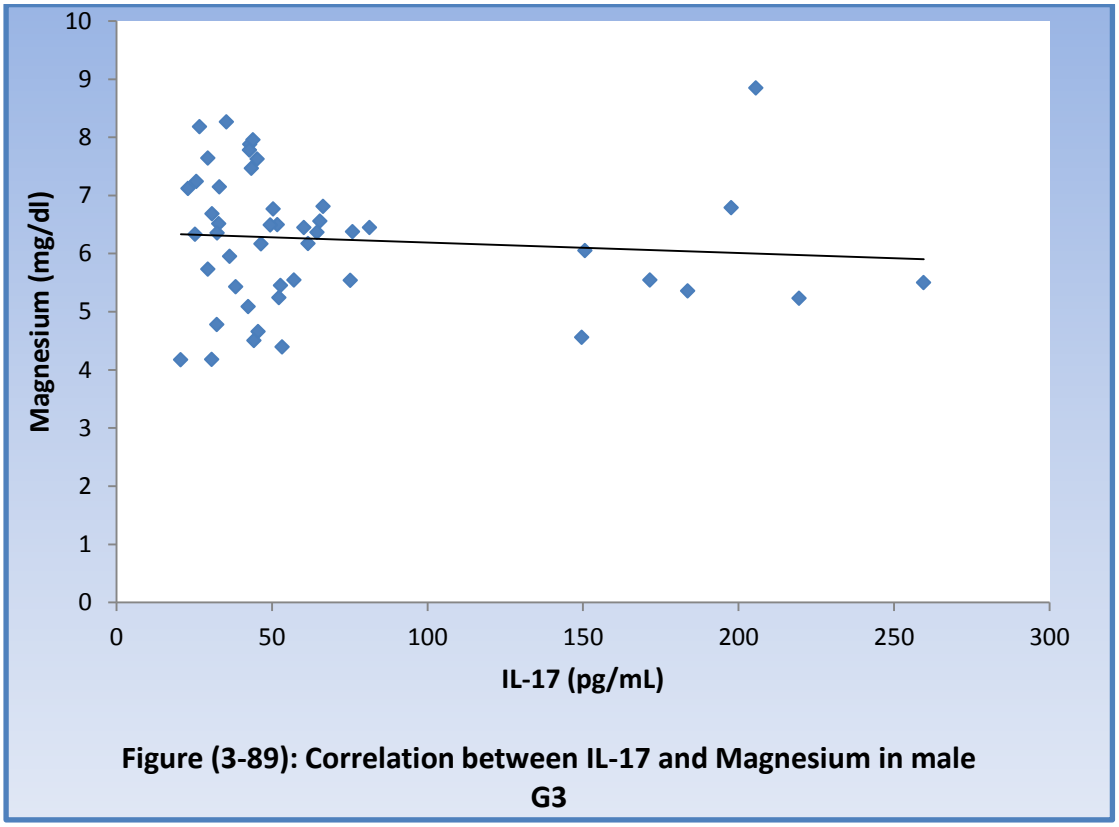

Figure (3-89): Correlation between IL-17 and Magnesium in male G3

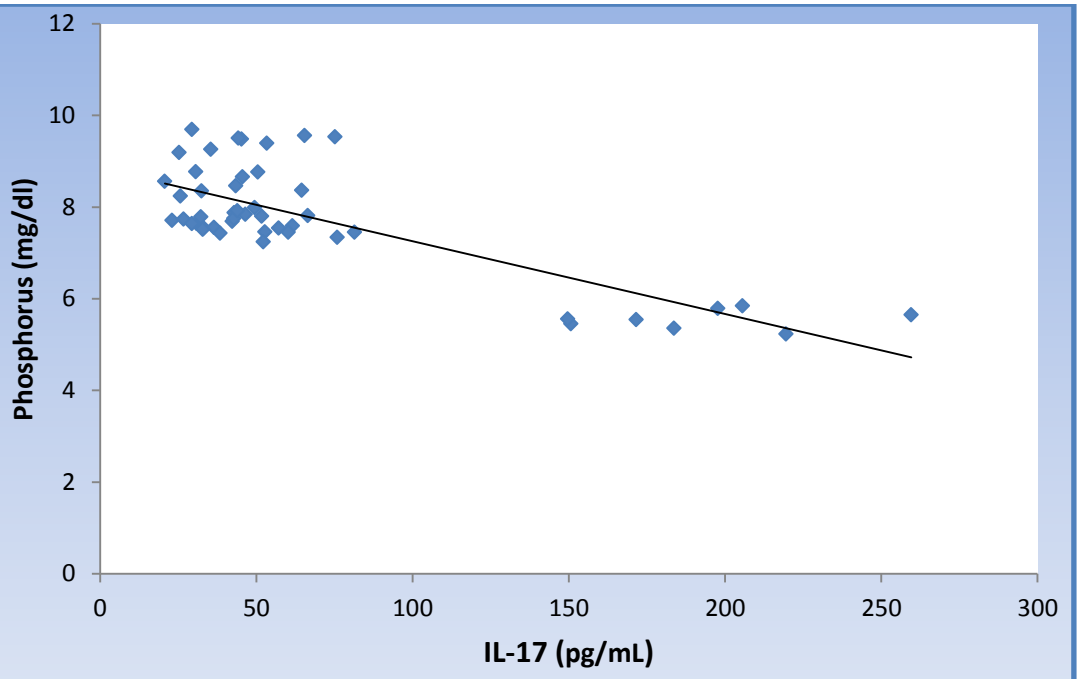

Figure (3-90) : Correlation between IL-17 and Phosphorus in male G3

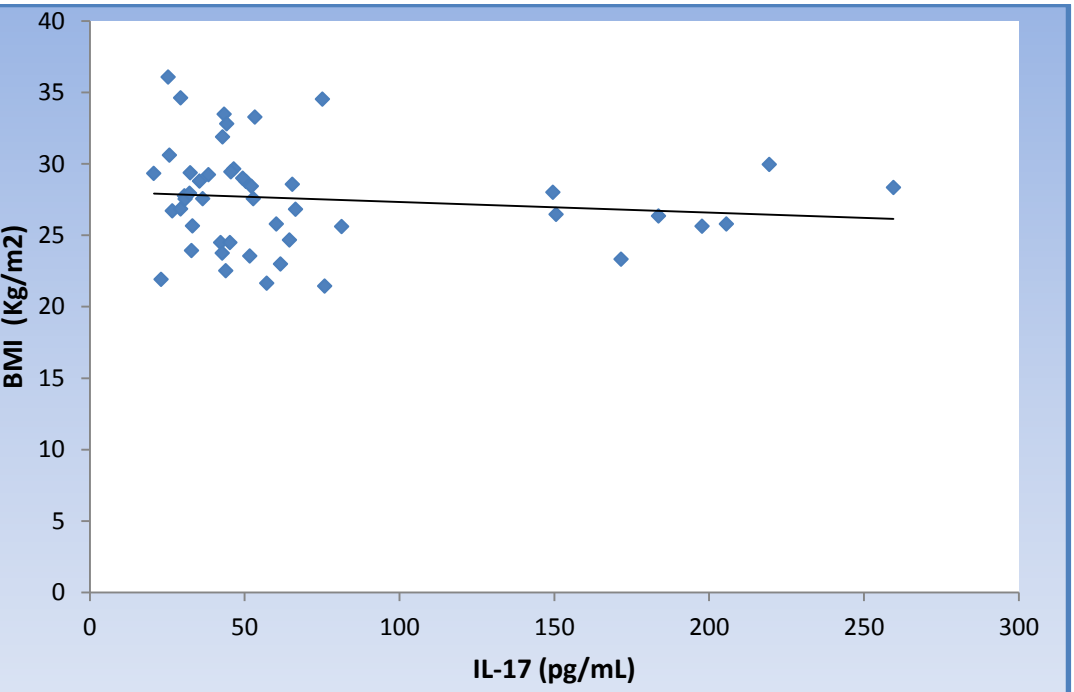

Figure (3-91): Correlation between IL-17 and BMI in male G3

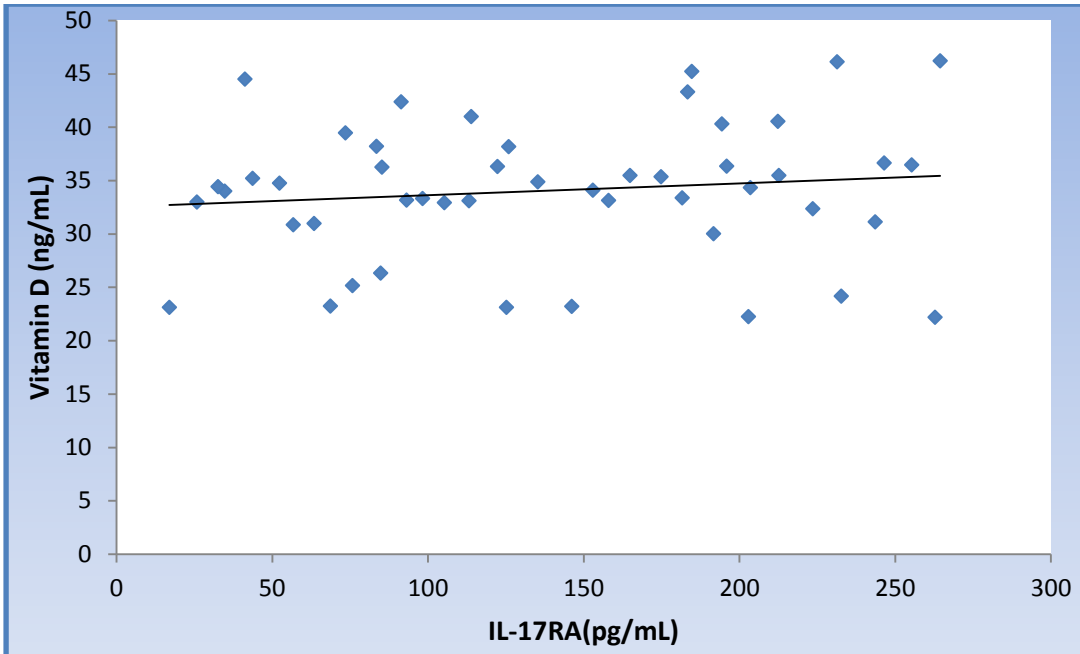

Figure (3-92) : Correlation between IL-17RA and Vitamin D in male G3

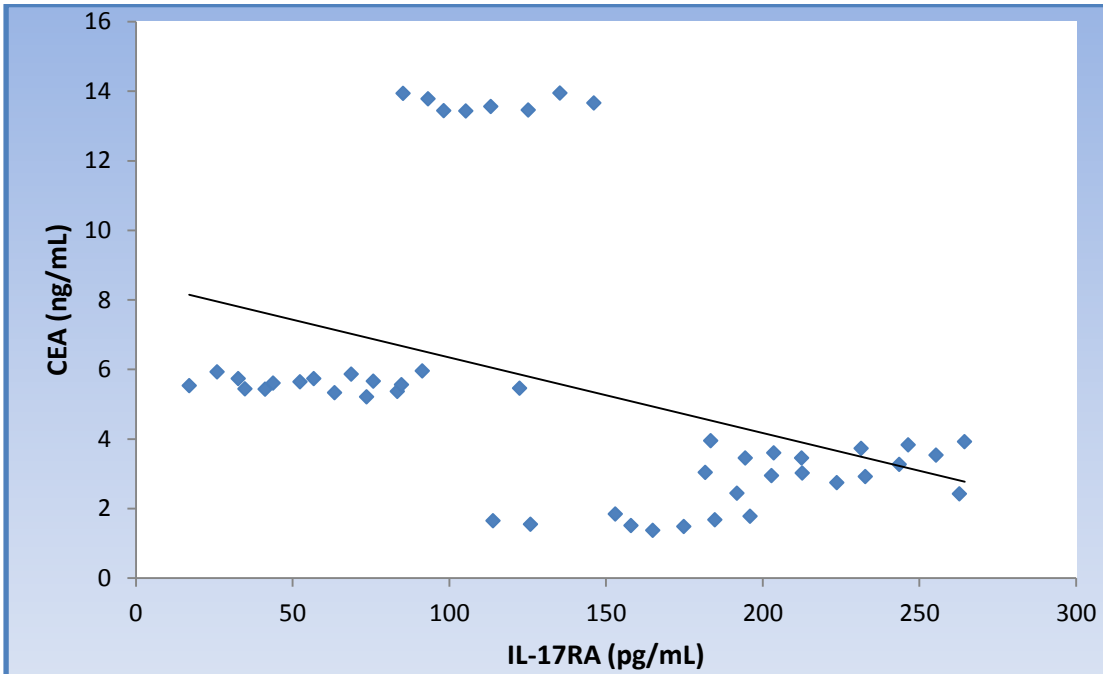

Figure (3-93) : Correlation between IL-17RA and CEA in male G3

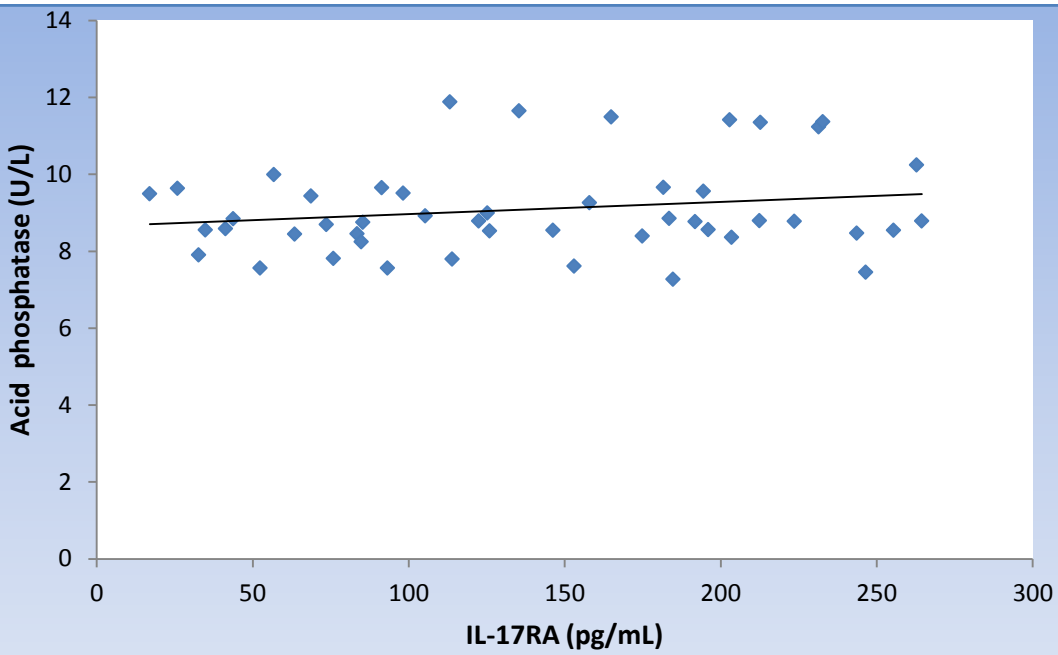

Figure (3-94): Correlation between IL-17RA and Acid phosphatase in male G3

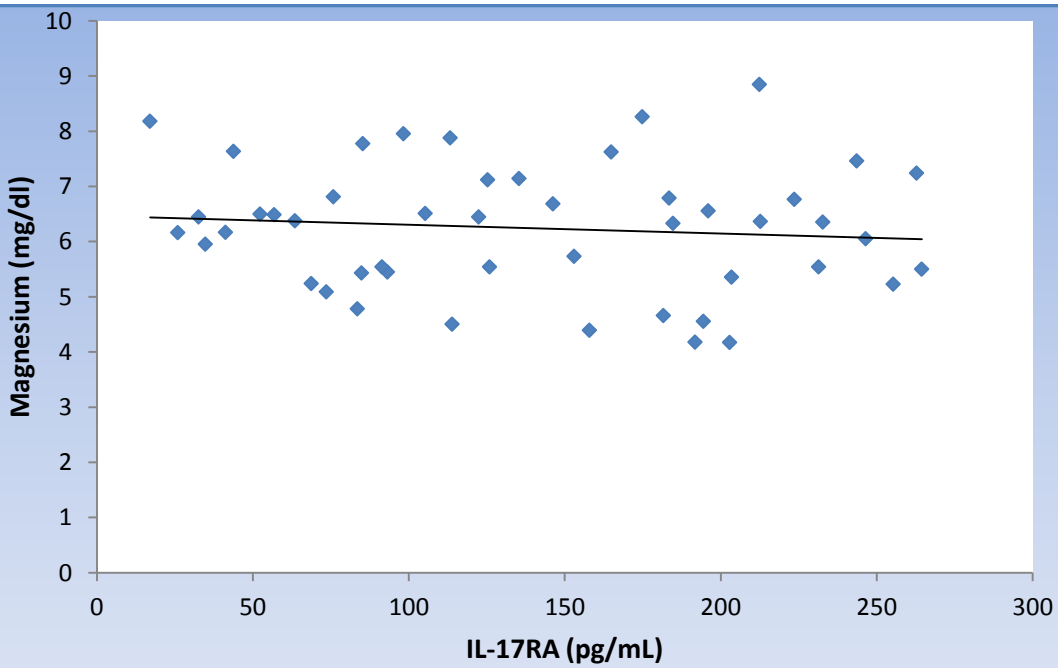

Figure (3-95): Correlation between IL-17RA and Magnesium in male G3

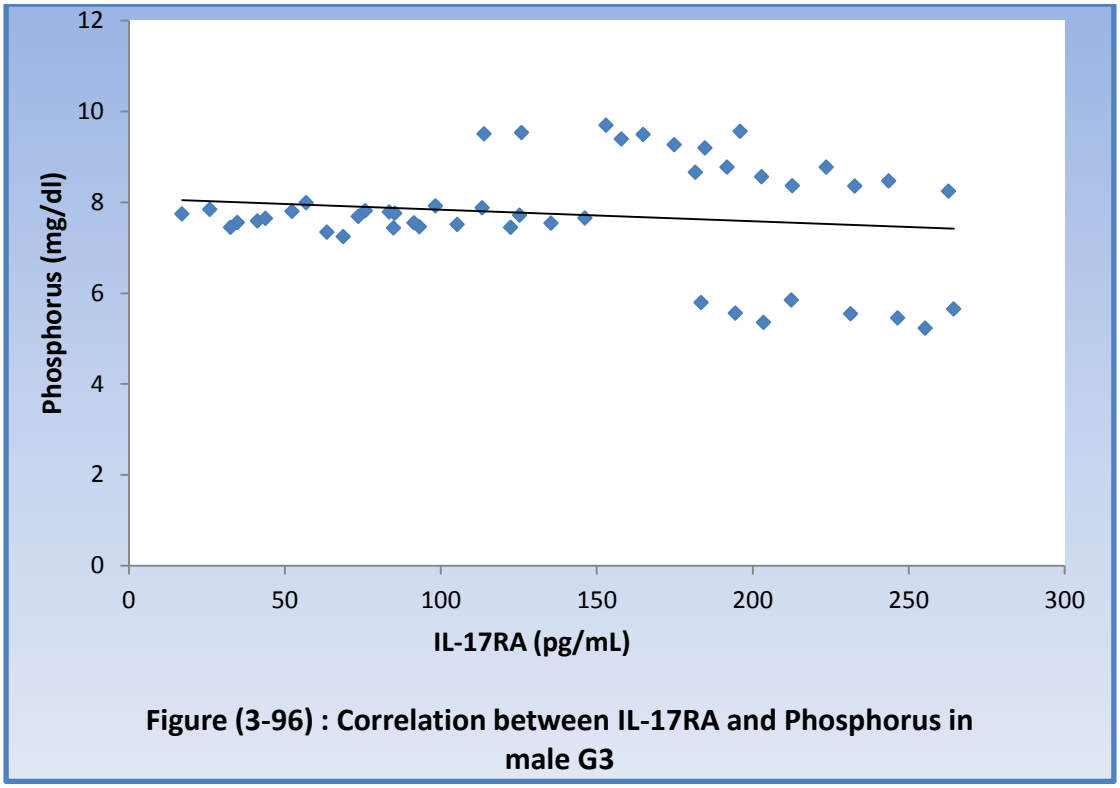

Figure (3-96) : Correlation between IL-17RA and Phosphorus in male G3

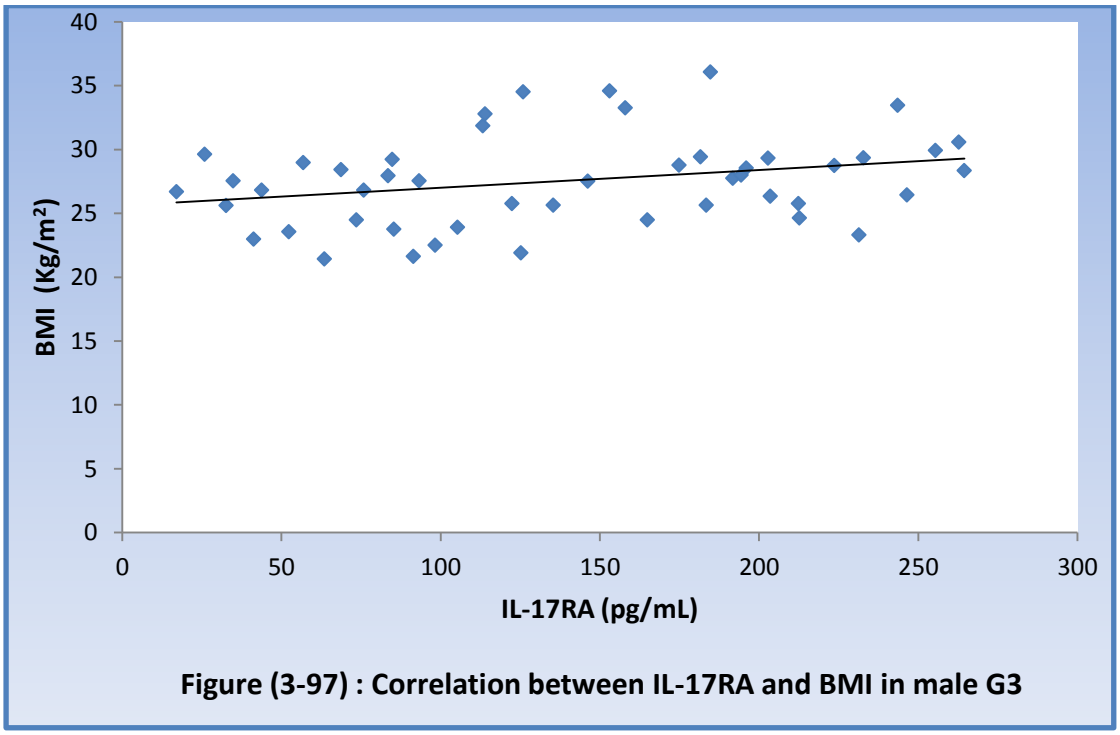

Figure (3-97) : Correlation between IL-17RA and BMI in male G3

The results in table 3-10 and figures 3-98 , 3-99 , 3-100 , 3-101 , 3-102 , 3-103 , 3-104 , 3-105 , 3-106 , 3-107 , 3-108 , 3-109 , 3-110 show the correlation between IL-17& IL-17RA with (Vitamin D, CEA, acid phosphatase, magnesium, phosphorus and BMI) in female patients after taking second dose of chemotherapy (G3).

Table(3-10): Correlations between IL-17 and some bio-chemical parameters in sera of CRC patients female after taking second dose of chemotherapy (G3).

Parameters		IL-17	IL-17RA
IL-17RA	r	-0.199	
	P	0.218 Ns	
Vitamin D	r	-0.382	-0.003
	P	0.015 S	0.983 Ns
CEA	r	0.239	-0.734
	P	0.137 Ns	0.000 h s
Acid phosphatase	r	0.192	0.222
	P	0.235 Ns	0.168 Ns
Magnesium	r	-0.394	0.058
	P	0.012 S	0.723 Ns
Phosphorus	r	0.180	0.061
	P	0.265 Ns	0.710 Ns
BMI	r	-0.002	0.022
	P	0.993 Ns	0.895 Ns

S = Significant (*P* value ≤ 0.05)
Ns = Non significant (*P* value > 0.05)
h s = high significant (*P* value ≤ 0.001)

In this table, there was a non significant negative (-ve) correlation between (IL-17& BMI), and a non significant negative (-ve) correlation between (IL-17& IL-17RA), and a significant negative (-ve) correlation between (IL-17 & vitamin D). However, a non significant positive (+ve) correlation was shown between (IL-17 & CEA), and a non significant positive (+ve) correlation between (IL-17& acid phosphatase). A significant negative (-ve) correlation also was shown between (IL-17 & magnesium), and a non significant positive (+ve) correlation between (IL-17 & phosphorus) after taking second dose of chemotherapy.

In this table we found a non significant positive (+ve) correlation was also found between (IL-17RA & BMI), and a non significant negative (-ve) correlation between (IL-17RA & vitamin D). However, a high significant negative (-ve) correlation was shown between (IL-17RA & CEA), and a non significant positive (+ve) correlation between (IL-17RA & acid phosphatase). A non significant positive (+ve) correlation also was shown between (IL-17RA & magnesium) , and a non significant positive (+ve) correlation between (IL-17RA & phosphorus) after taking first dose of chemotherapy.

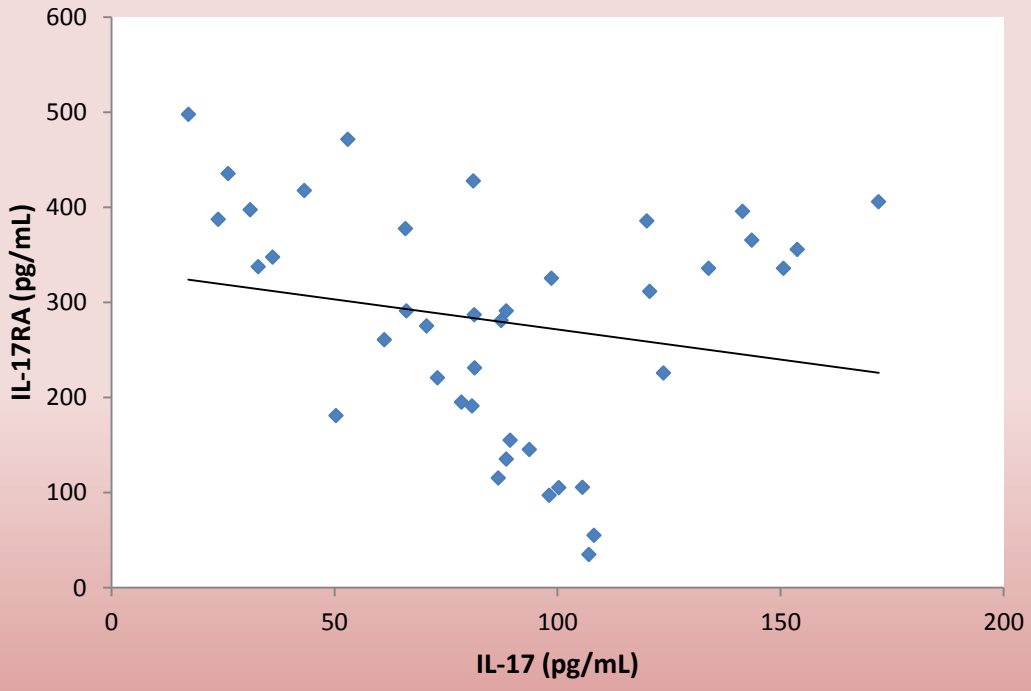

Figure (3-98) : Correlation between IL-17 & IL-17RA in female G3

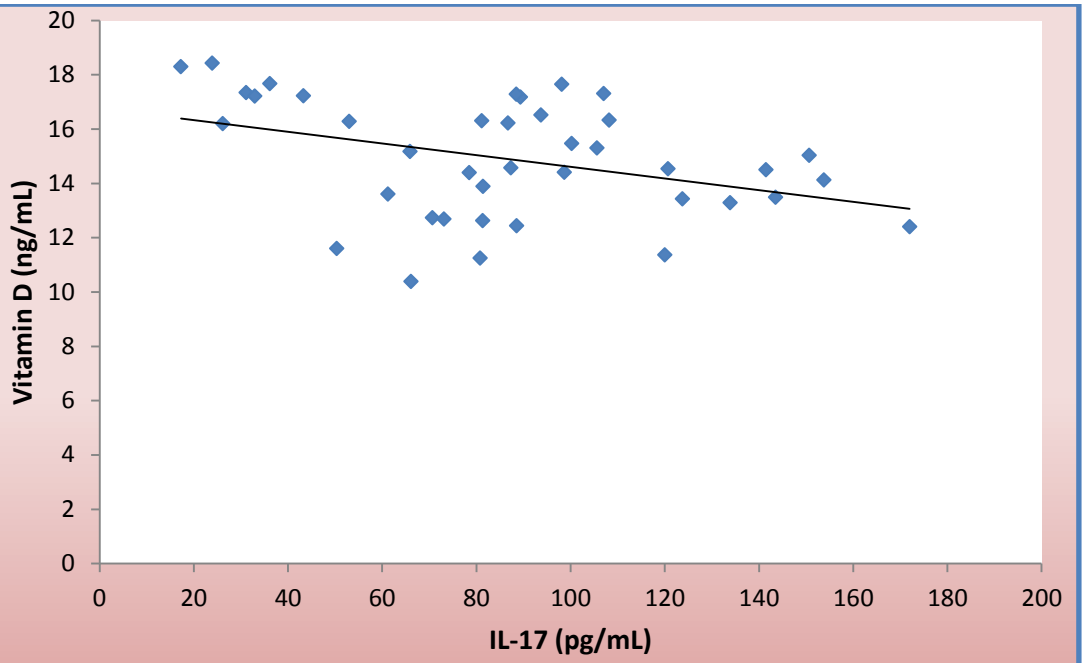

Figure (3-99) : Correlation between IL-17 and Vitamin D in female G3

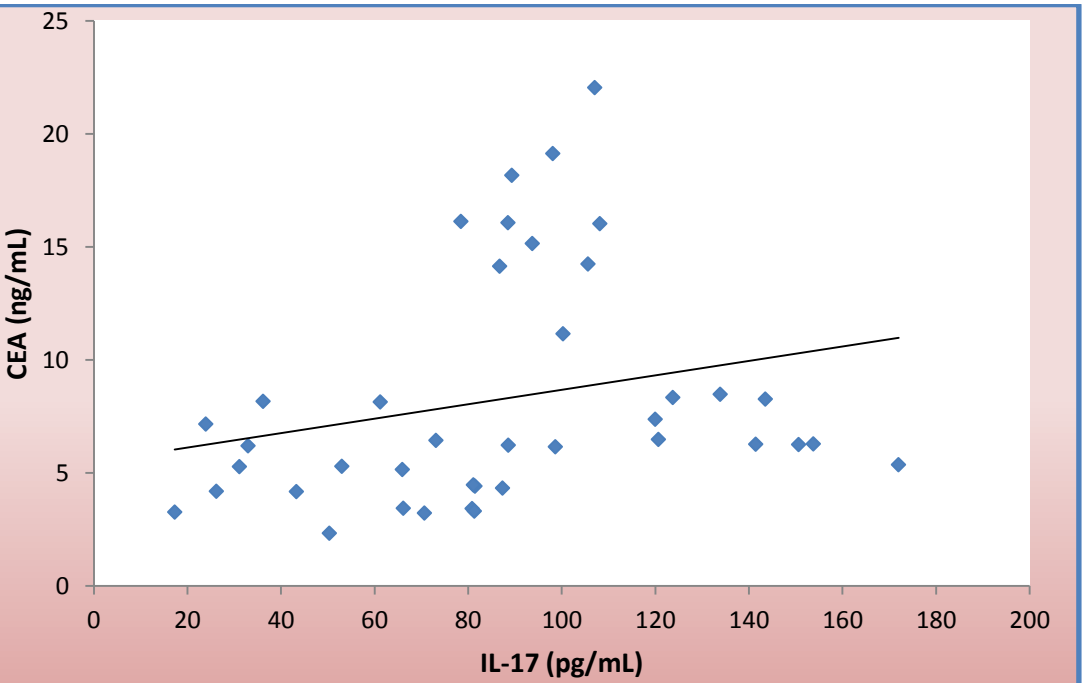

Figure (3-100): Correlation between IL-17 and CEA in female G3

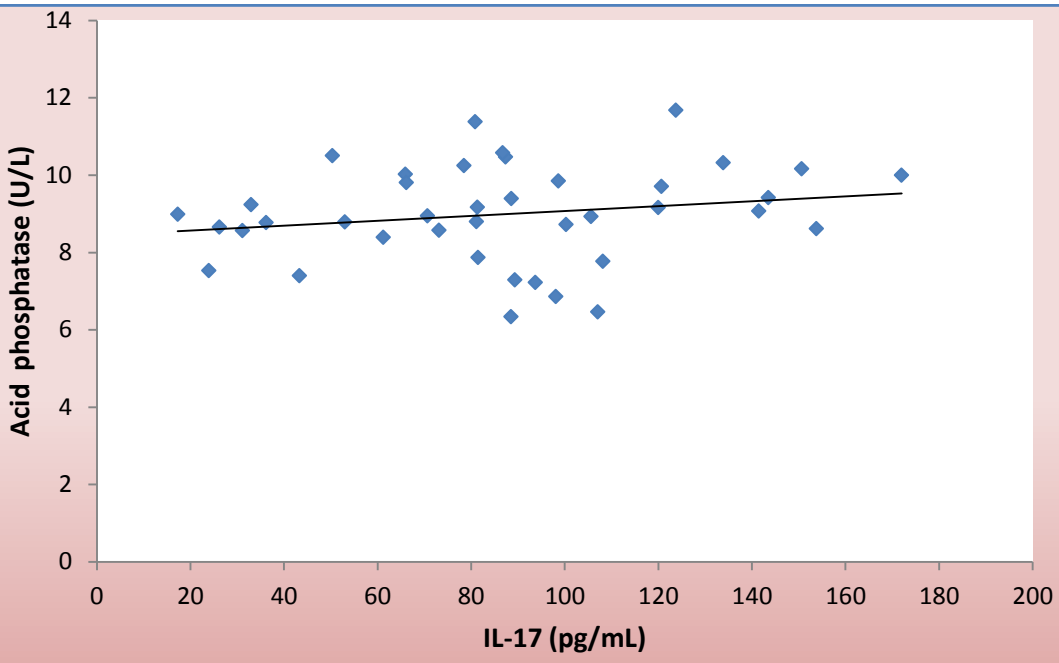

Figure (3-101): Correlation between IL-17 and Acid phosphatase in female G3

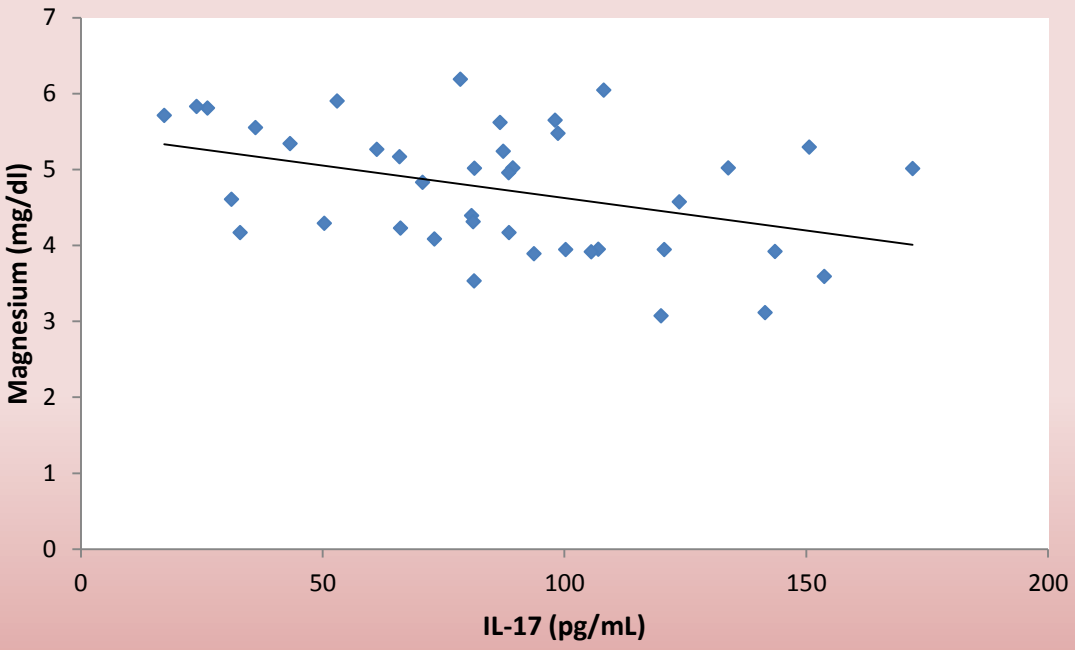

Figure (3-102) : Correlation between IL-17 and Magnesium in female G3

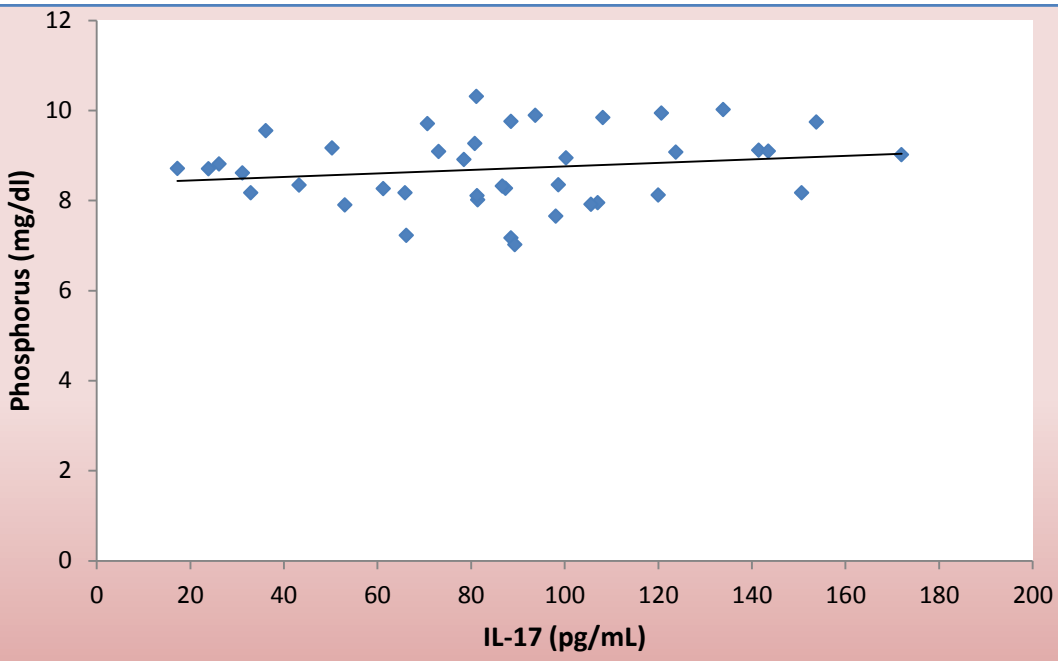

Figure (3-103): Correlation between IL-17 and Phosphorus in female G3

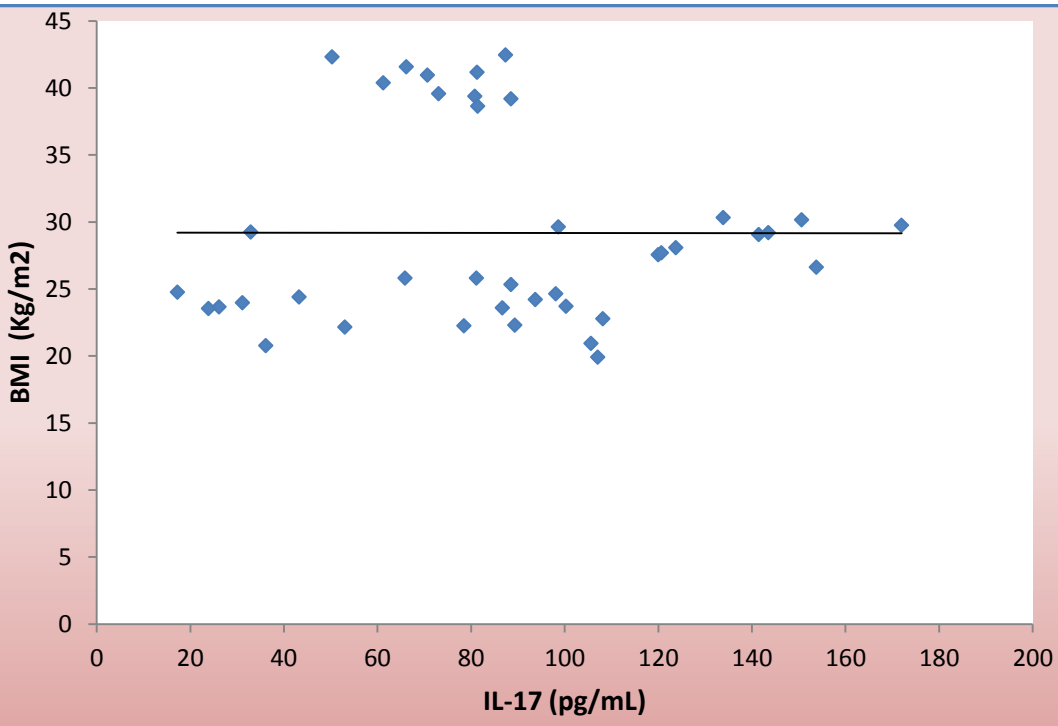

Figure (3-104): Correlation between IL-17 and BMI in female G3

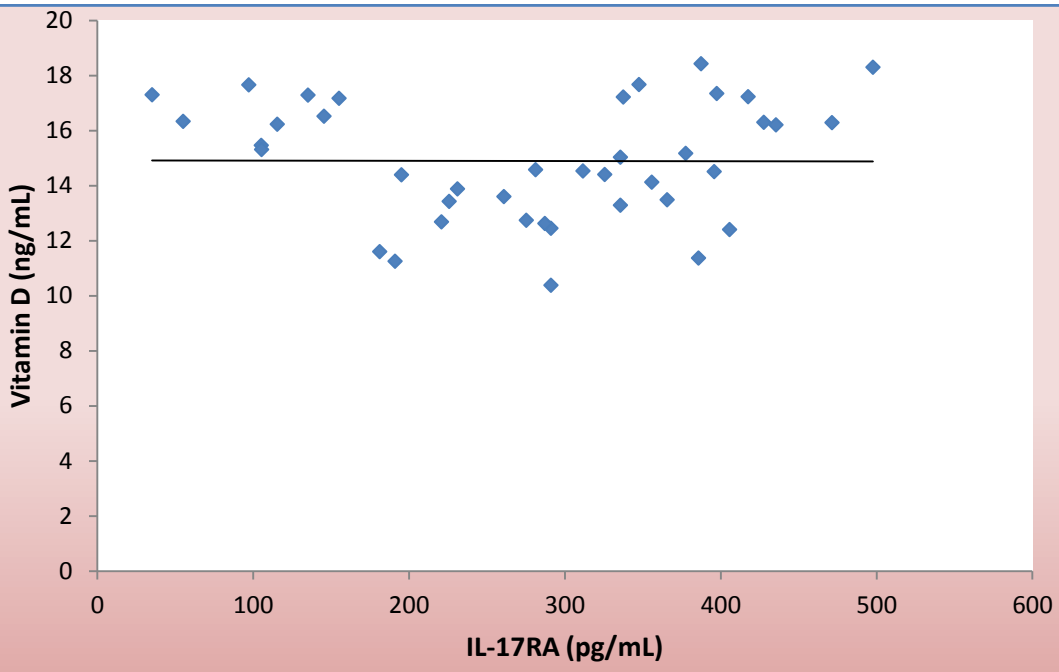

Figure (3-105) : Correlation between IL-17RA and Vitamin D in female G3

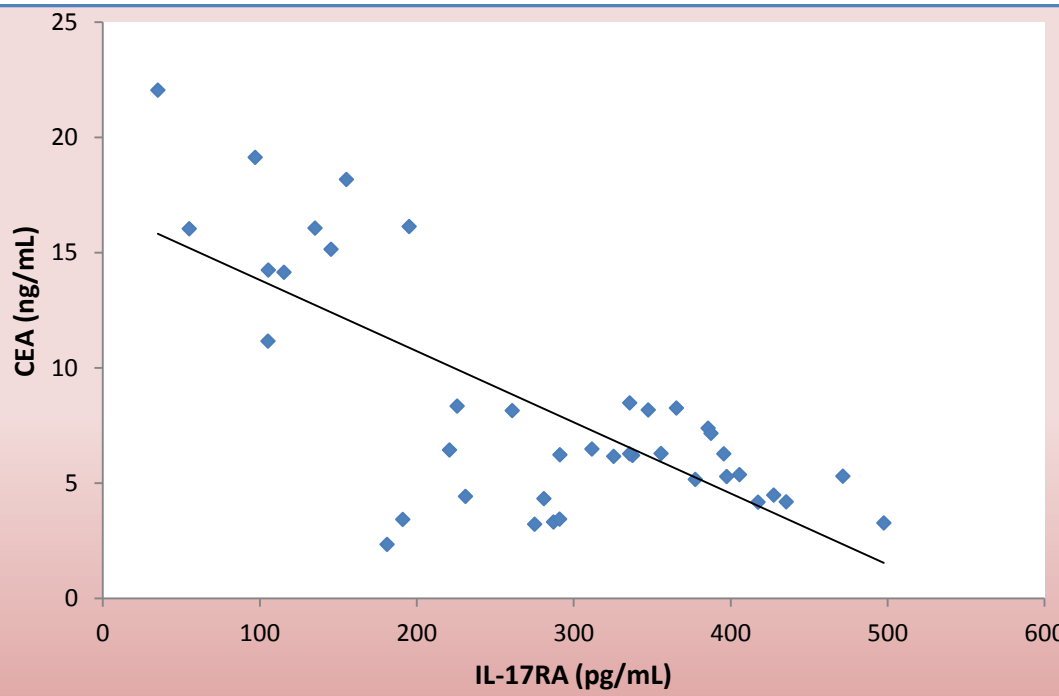

Figure (3-106) : Correlation between IL-17RA and CEA in female G3

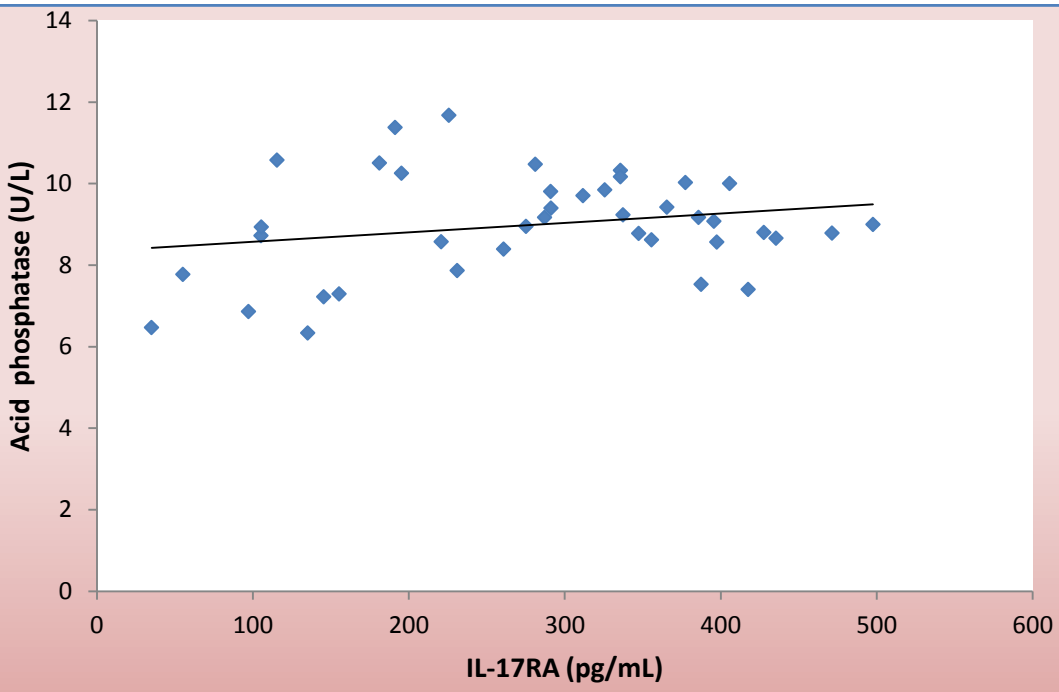

Figure (3-107): Correlation between IL-17RA and Acid phosphatase in female G3

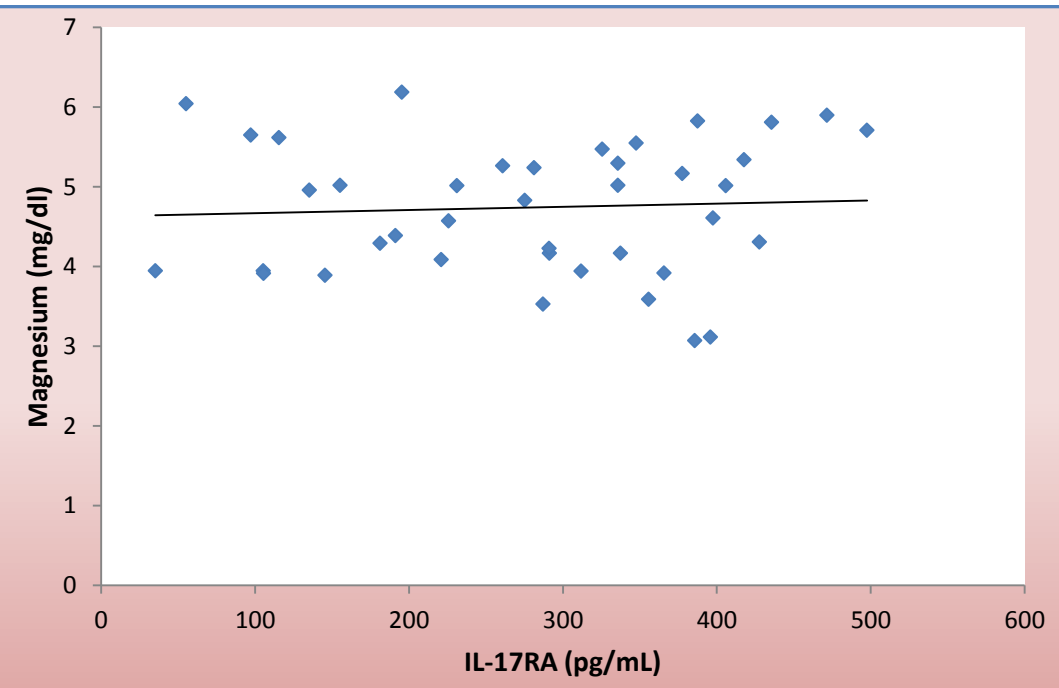

Figure (3-108) : Correlation between IL-17RA and Magnesium in female G3

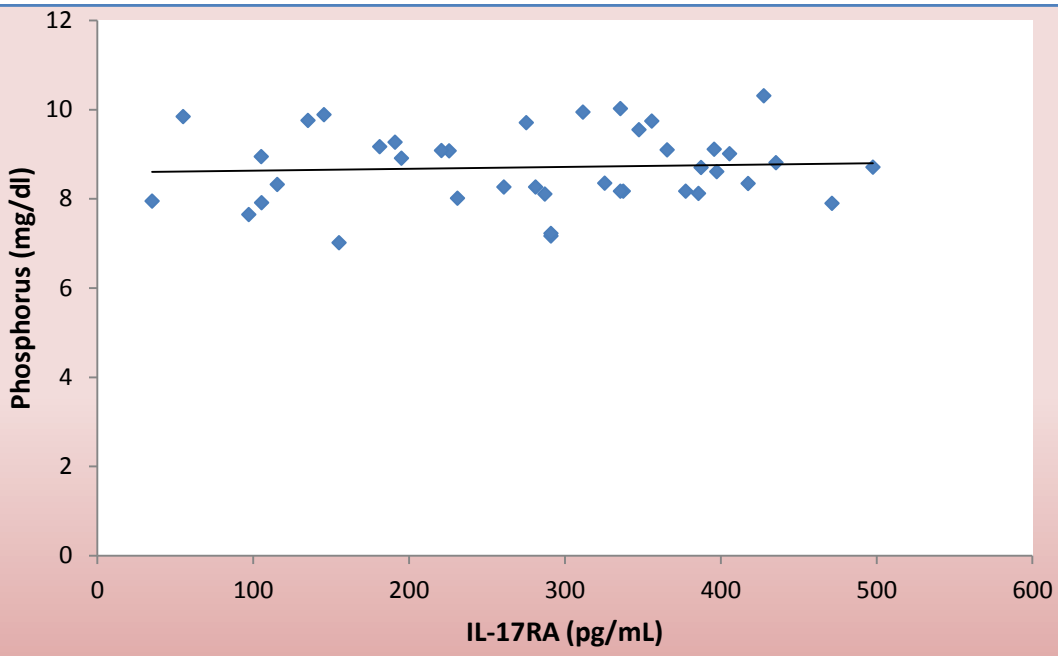

Figure (3-109): Correlation between IL-17RA and Phosphorus in female G3

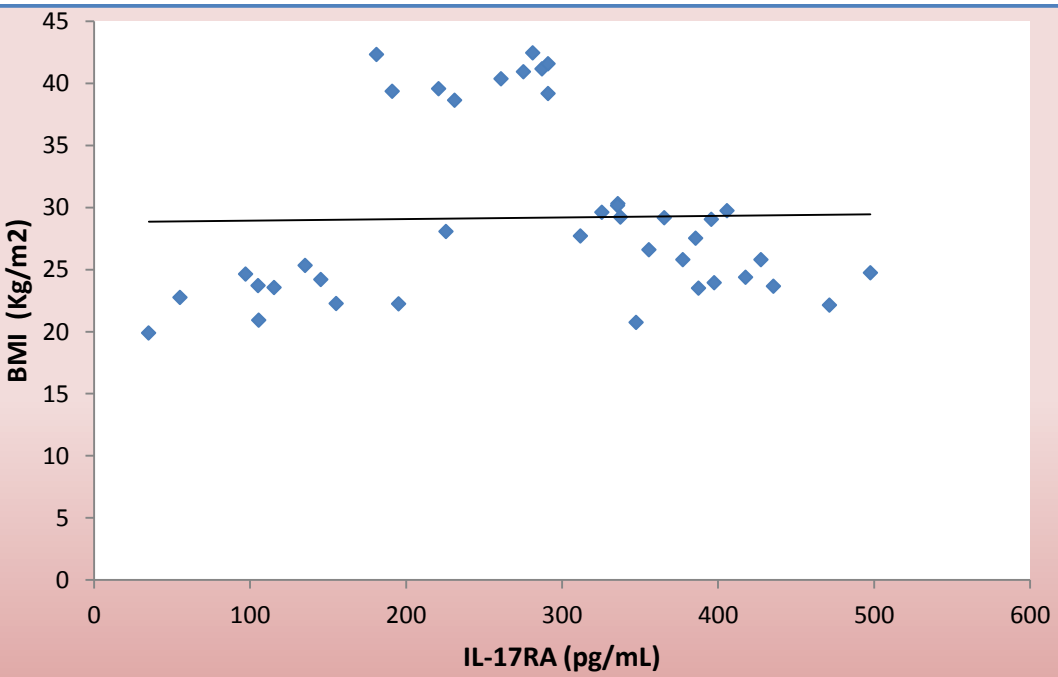

Figure (3-110): Correlation between IL-17RA and BMI in female G3

Chapter Four
Conclusion
&
Future Study

Conclusion:

1. A highly significant increasing of IL-17 male and female before taking the dose of chemotherapy (G1) than other groups. Result indicate that IL-17 could be used as another parameter in these patients.
2. A significantly high levels of IL-17RA in male patients to the same group (G1) while the female patients whose taking the second dose of chemotherapy (G3) it be high significant increase than other groups.
3. Male patients in G1 have a significant increase in CEA, phosphatase acid, magnesium and phosphorus.
4. Iraqi female patients in group G2 had a significant increase in BMI and a significant increase in IL-17, IL-17RA, whereas there was a highly significant increase in G2 in female patients.
5. There was non significant decrease in BMI and high significant decrease in vitamin D in G1 of Iraqi male patients .
6. High significant decrease of vitamin D in G1 of Iraqi female patients.
7. A high significant increase in BMI, magnesium and a significant increase in IL-17, Acid phosphatase was found in G1 female (B1F) compared with the same male group (B1M) . However, there was a non significant decrease in IL-17RA, Vitamin D, in Iraqi female patients (B1F) and a non significant increase in phosphorus in the same patients as a compared with Iraqi male patients (B1M).
8. The data in recent study in G2 as a comparative study (male and female) we show a high significant increase in IL-17 and non significant increase in BMI, IL-17RA, CEA and phosphorus in Iraqi female patients A1F and high significant decreasing in Magnesium in A1F group than A1M .
9. The results in recent study in G3 also showed a high significant increase in IL-17RA and phosphorus and significant increase in CEA while there was a high significant decrease in vitamin D in Iraqi female patients A2F as a compared with Iraqi male patients A2M .
10. A high significant negative (-ve) correlation was found between (IL-17RA and CEA) and between (IL-17 and phosphorus). A significant positive (+ve) and negative (-ve) correlation also was

shown between (IL-17 and CEA), (IL-17 and magnesium) respectively in the patients male G1 before taking chemotherapy dose . However, in the same groups of female patients there was a high significant positive (+ve) correlation between (IL-17 & CEA), (IL-17RA & acid phosphatase), (IL-17RA & magnesium) . A high significant negative (-ve) correlation also was found between (IL-17RA & CEA).

11. The correlation result in Iraqi male G1 show a high significant negative (-ve) correlation between (IL-17RA & CEA), (IL-17 & acid phosphatase) and (IL-17 & phosphorus) . However, there was a high significant positive (+ve) correlation between (IL-17 & IL-17RA) , while in female patients G2 we find in the recent study there was a high significant negative (-ve) correlation between IL-17 and(BMI, IL-17RA) and between IL-17RA and (CEA, vitamin D).
12. In male patients as G3 we find a high significant negative (-ve) correlation between (IL-17RA & CEA) and (IL-17 & phosphorus) and high significant positive (+ve) correlation between (IL-17 & IL-17RA). While in female patient in the same group we find a high significant negative (-ve) correlation between (IL-17RA & CEA).

Future study :

1- Study of IL-17 levels and its receptors for CRC patients with DMII.
2- The IL-17 and his receptors in CRC patients can be measured with renal failure.
3- Study of IL-18 levels and its receptors for CRC patients.
4- Study IL-17 and its receptors in CRC patients can be measured with hypothyroidism and hyperthyroidism.
5- Study some adipokines such as (retinol binding protein 4, obestatin) for CRC patients .
6- Study the correlation between vitamin D and vitamins (A, E) for CRC patients.

Appendixes

Study protocol:

Code No.: Date:

Name: Age: Occupation:

Weight: Height:

History:

- Duration of symptoms: New: Old:
- Medical history:
- Surgical history:
- Drug user: Injection : Tab:
- Habits:
- Alcohol ingestion:
- Cigarette smoking:

Parameters to be measured:

1. BMI
2. interleukin-17
3. IL-17RA
4. Vitamin D
5. CEA
6. Acidic phosphatase
7. Magnesium
8. Phosphorus

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الخلاصة:

سرطان القولون والمستقيم هو ثالث أكثر أنواع السرطان تشخيصًا عند الذكور والثاني في الإناث ، وهو أحد الأمراض غير المتجانسة.

تم أخذ عينات من الدم الوريدي من 48 من الذكور و 40 من الإناث الذين يعانون من مرض CRC تمثل المجموعة الاولى (G1) المرضى الذين لم يأخذوا جرعة العلاج الكيميائي ، المجموعة الثانية (G2) نفس المرضى ولكن بعد تناولهم جرعة العلاج الكيميائي الأولى ، في حين أن المجموعة الثالثة (G3) هم نفس المرضى الذين في (G1) و (G2) ولكن بعد أخذهم جرعة العلاج الكيميائي الثانية. والمجموعة الرابعة (G4) تتكون من (30 ذكور و 30 إناث) كمجموعة سيطرة للأصحاء العراقيين. تم تحليل جميع عينات الدم لـ (IL-17) و (IL-17RA) وفيتامين D و CEA و ACP والمغنيسيوم ومؤشر كتلة الجسم والفوسفور.

تظهر النتائج في هذه الدراسة :

- زيادة معنوية في IL-17، IL-17RA، CEA ، ACP ، المغنيسيوم والفوسفور في المجموعة الاولى G1 للمرضى الذكور.
- زيادة ملحوظة في مؤشر كتلة الجسم وزيادة معنوية كبيرة في IL-17، IL-17RA في المجموعة الاولى G1 للمرضى العراقيات أكثر من المجموعات الأخرى . بينما كانت هناك زيادة معنوية كبيرة في المجموعة الثانية G2 للمرضى الإناث.
- انخفاض غير ملحوظ في مؤشر كتلة الجسم وانخفاض كبير في فيتامين (د) في G1 من المرضى الذكور العراقيين.
- انخفاض معنوي كبير في فيتامين (د) في المجموعة الاولى G1 للمرضى العراقيات.
- كانت هناك زيادة معنوية كبيرة في مؤشر كتلة الجسم ، والمغنيسيوم وزيادة ملحوظة في IL-17 ، ACP في المجموعة الاولى G1 الأنثوية (B1F) مقارنة مع نفس المجموعة من الذكور (B1M) بينما كان هناك انخفاض غير ملحوظ في IL- 17RA ، فيتامين (د) في المرضى العراقيات (B1F) وزيادة غير ملحوظة في الفوسفور في نفس المرضى بالمقارنة مع المرضى الذكور العراقيين (B1M).
- تظهر النتائج في المجموعة الثانية G2 كدراسة مقارنة (ذكور وإناث) زيادة معنوية كبيرة في IL-17 وزيادة غير ملحوظة في مؤشر كتلة الجسم ، IL-17RA ، CEA والفوسفور في المرضى الإناث العراقيات A1F وانخفاض معنوي كبير في المغنيسيوم في مجموعة A1F بالمقارنة مع A1M.
- كما أظهرت البيانات في الدراسة الحديثة التي أجريت في المجموعة الثالثة G3 زيادة معنوية كبيرة في IL-17RA والفوسفور وزيادة معنوية في CEA بينما كان هناك انخفاض معنوي كبير في فيتامين D في مرضى النساء العراقيات A2F بالمقارنة مع المرضى الذكور العراقيين A2M.
- كان هناك ارتباط معنوي سلبي كبير بين (IL-17RA و CEA) وبين (IL-17 والفوسفور) ، وارتباط معنوي ملحوظ إيجابي وسلبي على التوالي بين (IL-17 و

الخلاصة

CEA) و (IL-17 والمغنيسيوم) في المرضى الذكور G1 قبل تناول جرعة العلاج الكيميائي ، بينما في نفس المجموعات من المرضى الإناث كان هناك ارتباط معنوي إيجابي كبير بين (IL-17 و CEA) ، (IL- 17RA و ACP) ، (IL-17RA والمغنيسيوم) وارتباط معنوي سلبي كبير بين (IL-17RA و CEA).

- في المرضى الذكور في G1 ، نجد ارتباط معنوي سلبي كبير بين (CEA و IL-17RA) و (ACP و IL-17) و(IL-17 و phosphorus) ، بينما كان هناك ارتباط معنوي ايجابي كبير بين (IL-17 و IL-17RA) ، بينما في المرضى الإناث G2 وجدنا في الدراسة الحديثة أن هناك علاقة معنوية سلبية كبيرة بين IL-17 و (BMI، IL-17RA) وبين IL-17RA و (CEA ، فيتامين د).
- تظهر العلاقة الترابطية بين الذكور العراقيين G3 وجود علاقة سلبية كبيرة بين (IL-17RA و CEA) و (IL-17 و phosphorus) وارتباط معنوي إيجابي كبير بين (IL-17 و IL17RA) ، بينما في المريضات من نفس المجموعة ، نجد ارتباط معنوي سلبي كبير بين (IL-17RA و CEA).

إن النتائج المستخلصة في علاقة IL-17 و IL-17RA في مصل سرطان القولون والمستقيم بين الذكور والإناث العراقيين قبل وبعد تناول جرعة العلاج الكيميائي تشير إلى أن IL-17 و IL-17RA قد يكون علامة جيدة للورم لتشخيص سرطان القولون والمستقيم .

بسم الله الرحمن الرحيم

(فَسُبْحَانَ الَّذِي بِيَدِهِ مَلَكُوتُ كُلِّ شَيْءٍ وَإِلَيْهِ تُرْجَعُونَ)

يس: (٨٣)

جامعة بغداد
وزارة التعليم العالي والبحث العلمي
كلية التربية/ ابن الهيثم للعلوم الصرفة
قسم الكيمياء

دراسة العلاقة بين الانترليوكين-١٧ و الانترليوكين المستقبل-١٧ر وغيرها من العوامل الكيموحيوية لمرضى عراقيين مصابين بسرطان القولون والمستقيم

رسالة مقدمة الى
كلية التربية (ابن الهيثم) – جامعة بغداد كجزء من متطلبات نيل درجة الماجستير في الكيمياء الحياتية

من قبل

مزهر نصيف مسلم

بكالوريوس كيمياء كلية التربية (ابن الهيثم) للعلوم الصرفة / جامعة بغداد ٢٠٠٢

بإشراف
استاذ مساعد د. أنوار فاروق الطائي

١٤٤١ هـ ٢٠١٩ م