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Gene Expression For Interleukin -6 In Colonic Cancer Patients Induced The Cachexia Syndrome

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INTRODUCTION

Cancer cachexia is a syndrome characterized by a marked weight loss, anorexia and asthenia and anemia, accounting for at least 20% of deaths in neoplastic patients (Muscaritoli, 2006). The term (cachexia) is used in the literature with different definition and descriptions of clinical symptoms of a patient ,it has been shown that a loss of weight exceeding 10% of the stable pre illness weight is correlated to a worse prognosis (Bachmann , 2008).

Pancreatic cancer remains one of the most deadly tumor types, with the highest death to incidence ratio of all cancers, with a 5 year survival rate of less 5% and a death to incidence ratio of (0.99), pancreatic ductal adenocarcinoma is currently one of the most aggressive gastrointestinal carcinomas(Jemal ,2009). Recently, it was reported that IL-6 has important roles in cancer progression, including: proliferation, migration, and angiogenesis in several cancers, including colon cancer (Santer, 2010; Grivennikov**,** 2009**;** Liu , 2010)**.**

The cytokines are functional pleiotropy and redundancy that characteristic features, which include interleukins(Interferon's, colony-stimulating factors and many growth factors), cytokines are produced by many different cell types and often show overlapping activities regulating proliferation or differentiation ,depending on the type and development state of the target cells involved(Scheller,2010).

The Interlukin-6 is a multifunctional cytokine that has a central role in the regulation at inflammatory and immune response ,there are many of variety a cells can produced the IL-6 as primarily monocytes ,macrophages and several tumors cells, and it binds to the receptor, The IL-6 receptor has two forms; membrane –bound forms and soluble forms, There are many of tissues such as the liver and some immune system cells can the expression of the membrane –bound IL-6 receptor , and the soluble form at the IL-6 receptor is present in human sera at high concentrations ,It is generally believed that IL-6 is a trans-signaling in mainly dependent on the soluble of the IL-6 receptor (Gabers,2011;Rose ,2012).

Recently, it was reported that IL-6 has important roles in cancer progression, including: proliferation, migration, and angiogenesis in several cancers, including colon cancer (Santer, 2010; Grivennikov**,** 2009**;** Liu , 2010)**.**

Materials and Methods

Cell lines-

1.Colon cancer cell lines

Human colon adenocarcinoma cell line(Sw 480)1.1.

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Human colon adenocarcinoma (SW 480) was provided by Pasteur institute / IRAN. The cells produce carcinoembryonic antigen (CEA),transforming growth factor beta. The cells were obtained from a 51-years old Caucasian male. Passage No.100 of this cell line used in this study and the cells were maintained using RPMI-1640 medium.

2. Human colon adenocarcinoma cell line (LS 180)

Human colon aenocarcinoma (LS 180) was provided by Pasteur institute /IRAN. The cells produce carcinoembryonic antigen (CEA), IL-10 and IL-6 . The cells were obtained from a 58-years old Caucasian femal. Passage No:35 of this cell line used in this study and the cells were mainttated using RPMI-1640 medium.

Isolation of peripheral blood mononuclear cells (PBMCs) The peripheral blood mononuclear cells(PBMCs) were isolated from venous blood at cancer patients with cachexia and without cachexia and healthy sample as control .

Coculture Experiments of peripheral blood mononuclear cells with colon cancer cells

For Co culture experiments, LS-180(+) and SW-480(–)cancer cells lines. That many steps fowling:

●Were washed with PBS , diluted in complete medium ,counted adjusted to 2.4 x10(5) cell/ml for the Ls -180 and 2x10 (6) cell/ml for sw-480 and 1.5x10(6) cell /ml .

●And then 0.3 ml per insert were plated.

●The inserts were then transferred to the wells of companion plate containing the 0.7 ml PBMCs (peripheral blood mononuclear cells) and Co cultured for 24 hr and 48hr.

●For negative control , one insert was placed in 0.7 ml complete cell culture medium and one well with PBMCs stayed without insert.

●After 24 hr and 48hr ,supernatants were removed and stored at -80 C for further use.

●The cancer cells and the PBMCs were separately lyses with 300 µl of maganapure lysis buffer, vortexed and stored at -80 C for further analysis.

RNA Extraction

The mRNA extraction by the Total RNA mini kit (blood/cultural cell) from (Geneaid company , China).

Complementary DNA (cDNA) synthesis

RNA was then reverse transcribed into cDNA by use of moloney murine leukemia virus (MMLV) reverse transcriptase and random hexamer primer following the manufacturer's instructions from CinnaGen First Strand cDNA synthesis Kit / Sinaclon / IRAN . At the first, 1µg/µl of total RNA and 1 µl of the primer were added into a sterile RNase-free microcentrifuge tubes and were heated to 60 °C for 5 min to melt secondary structure in the RNA templates. Then, the tubes were chilled immediately on ice to prevent secondary form reforming. The tubes were briefly centrifuged and the solutions were collected. Subsequently, the following components in the indicated order were added: 4 µl of 5x reaction buffer, 2 µl of 10 mM dNTP mix and 1 µl of MMLV reverse transcriptase (200 units). Nuclease-free water was then added to final volume of 20 µl and the mixture were briefly centrifuged. The tubes were incubated for 10 min at 25 °C, heating for 60 min in 42 °C and eventually the reactions were terminated by heating at 70°C for 10 min. The reverse transcription reaction products were stored at -20 °C until further processing. These procedures were done using a Bio-Rad PCR machine.

quantitative reverse transcription –PCR for the Co culture analysis

The expression levels of IL-6 mRNA were quantified by qRT-PCR using SYBR Premix Ex

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Taq (Tli RNaseH plus) Takara company /Japan and the Light cycler qReal time PCR system (Roche). The PCR was performed in a 10 µl reactions system containing 1 µl of cDNA template, 5 µl of SYBR green reagents, 0.25pM of each of the primers : Forward primer sequence AAAGCAAAGAGGCACTG (Tm=54.2)

Reverse primer sequence TTTCACCAGGCAAGTCTCC(Tm=54.2)

And 4.25 µl of nuclease-free distilled water. The PCR conditions were 94 °C for 10 min followed by 45 cycles at 94 °C for 10 sec, 59 °C for 30 sec and 72 °C for 20 sec PCR product length is 108 bp . Relative IL-6 mRNA expression were calculated with the 2 ($2^{-\Delta\Delta CT}$ method (128), using GAPDH housekeeping gene :Forward primer sequence 5CACCATCTTCCAGGAGCGAG3

Reverse primer sequence 5TCACGCCACAGTTTCCCGGA3

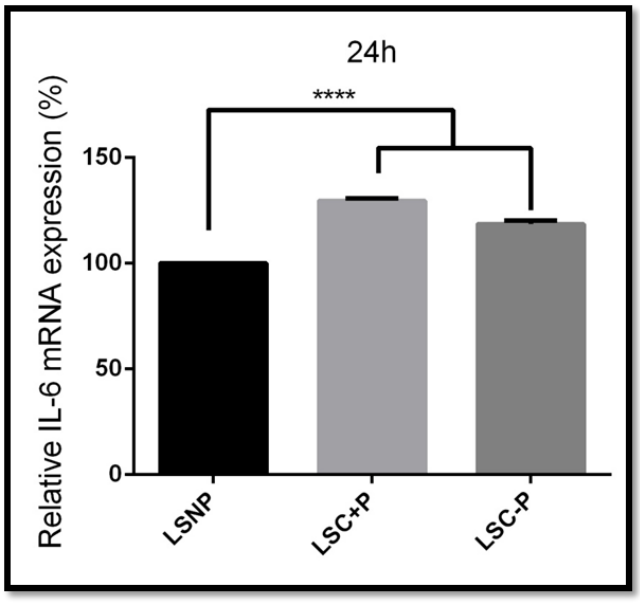
as an internal control.

Analysis of relative gene expression data

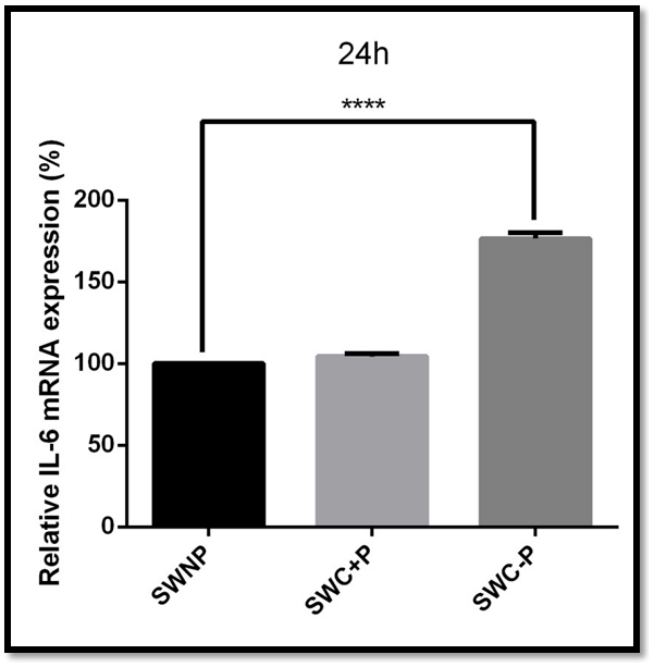
Relative gene expression quantification relates the PCR signal of the target transcript in a test group to that of another sample such as a control (calibrator). The 2 $^{-\Delta\Delta CT}$ method is a convenient way to determine the relative alterations in gene expression from qRT- PCR experiments.

Results

This study results shown that IL-6 gene expression in qRT-PCR for the mRNA extracted from the two types of colon cancer cell lines after Co-culture with peripheral blood mononuclear cells (PBMCs) from the colon cachectic cancer patients and non-cachectic cancer patients and with PBMCs from the normal samples at 24h and 48h . The IL-6 over expression is due in the types of colon cancer cell lines (LS-180)(that positive production of IL-6)with cachexia samples compare with same of samples Co-culture to another types of colon cancer cell lines (SW-480) (that negative production of IL-6) and the results shown that less expression for IL-6 in non-cachexia cancer patients in 24 h culturing Figure (1), (2).



Figure(1): the Levels of IL-6 expression at QRT-PCR from the cachectic , non-cachectic and normal peripheral blood mononuclear cells (PBMCs) in LS-180 cell lines at 24h.



Figure(2): The levels of IL-6 expression at qRT-PCR from the cachectic , non-cachectic and normal peripheral blood mononuclear cells (PBMCs) in SW-480 cell lines at 24h.

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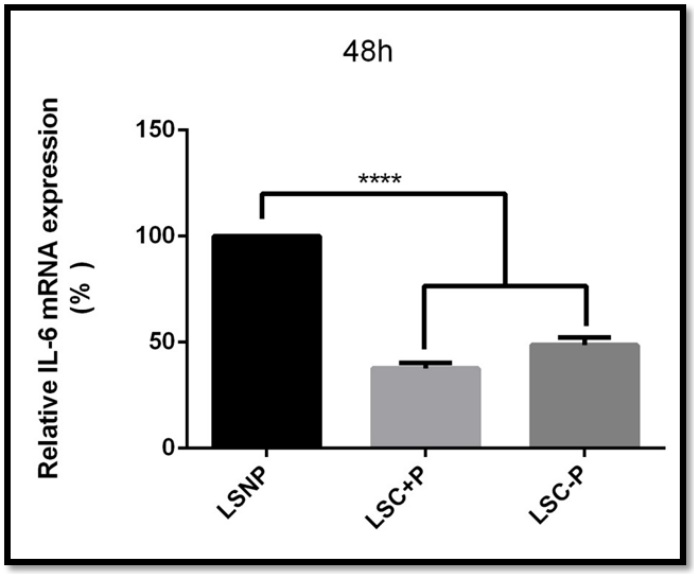
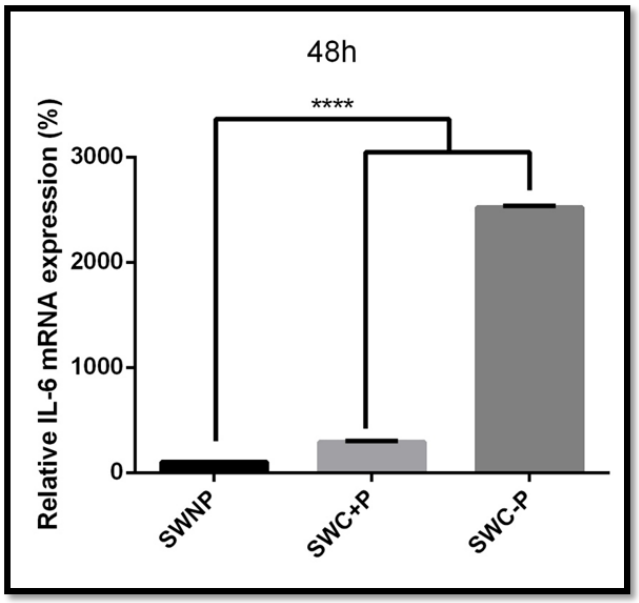
In the 48h Co-culturing that results shown decreased in levels of IL-6 expression at the cachectic cancer patients samples compare with the non-cachectic and normal samples Figure(3),(4).

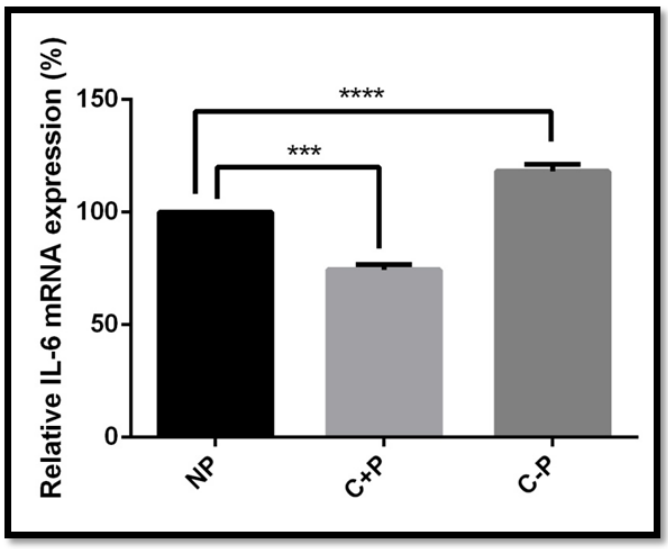


Figure(3): The levels of IL-6 expression at qRT-PCR from the cachectic , non-cachectic and normal peripheral blood mononuclear cells (PBMCs) in LS-180 cell lines at 48h.

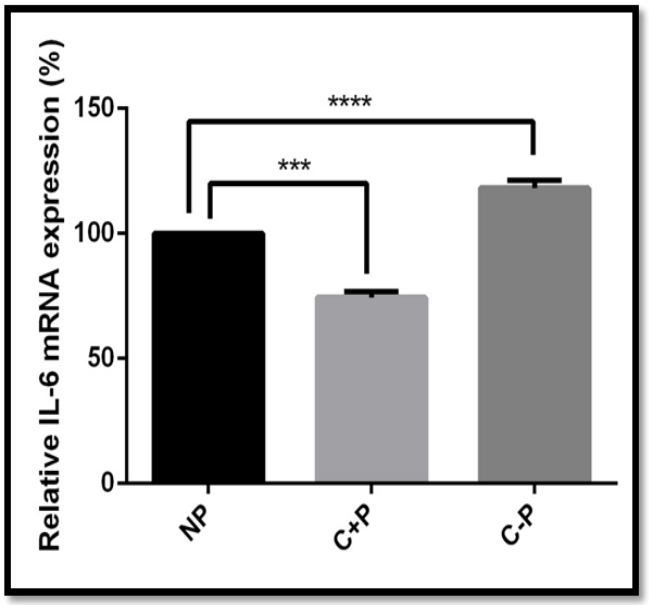


Figure(4): The levels of IL-6 expression at qRT-PCR from the cachectic , non-cachectic and normal peripheral blood mononuclear cells (PBMCs) in SW-480 cell lines at 48h.

While , the results of Co-culturing the PBMCs at cachectic cancer patients and non-cachectic and normal without the colon cancer cell lines shown the levels of IL-6 expression at decreased in cachectic samples compared with non-cachectic and normal at 24 h and 48h .Figure (5),(6).



Figure(5): The levels of IL-6 expression at qRT-PCR from the cachectic , non-cachectic and normal peripheral blood mononuclear cells (PBMCs) at 24h.



Figure(6): The levels of IL-6 expression at qRT-PCR from the cachectic , non-cachectic and normal peripheral blood mononuclear cells (PBMCs) at 48h.

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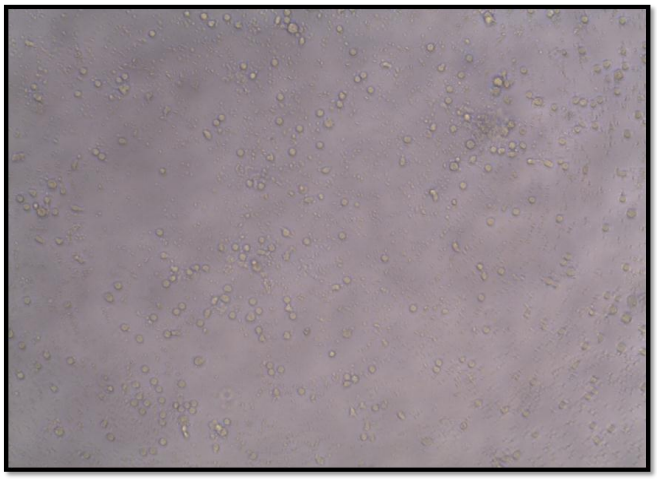
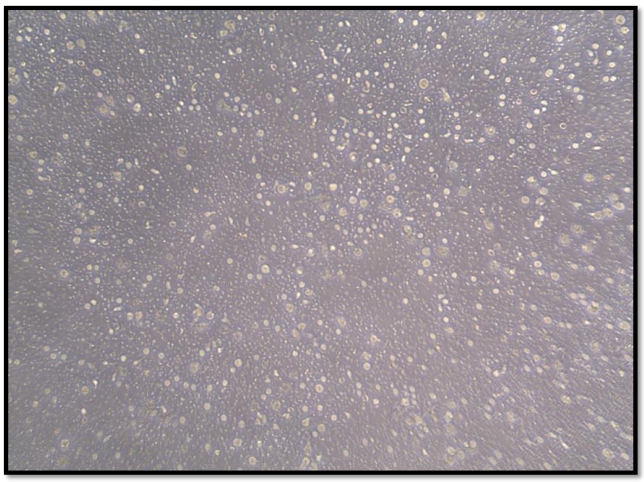
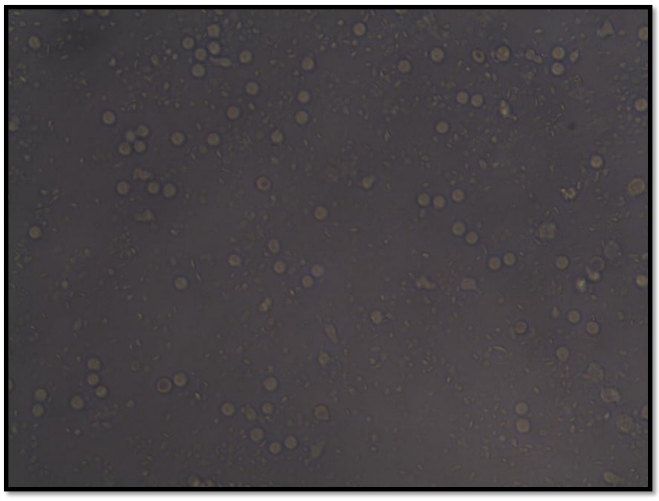


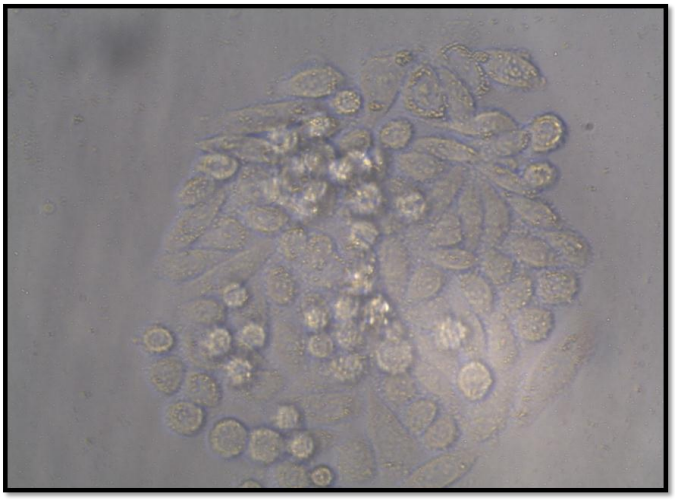
Picture (1): Show the LS-180 colon cancer cell lines Co-culture with the normal peripheral blood mononuclear cells at 24 h.(20X).



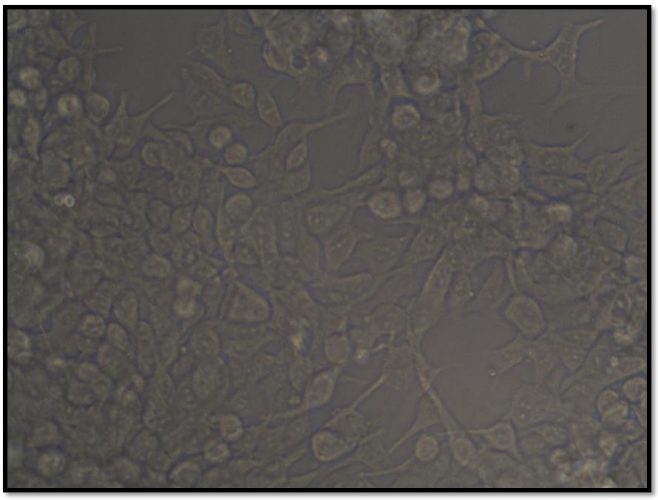
Picture(2): Show the LS-180 colon cancer cell lines Co-culture with the cachectic cancer patients peripheral blood mononuclear cells at 24 h.(20X).



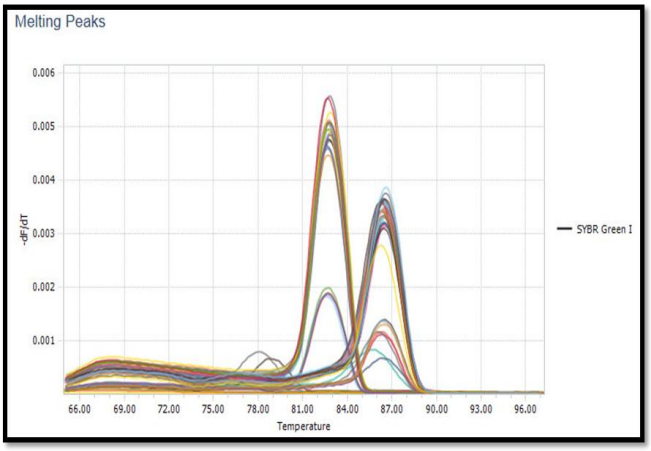
Pitcure (3): Show the LS-180 colon cancer cell lines Co-culture with the non-cachectic cancer patients peripheral blood mononuclear cells at 24 h.(100X).



Picture (4): The monolayer of SW-480 colon cancer cell lines .(40X)



Picture(5): The monolayer of LS-180 colon cancer cell lines .(40X)



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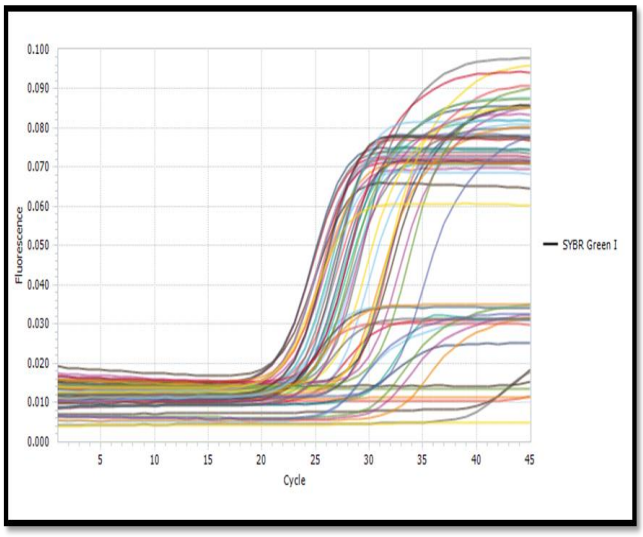
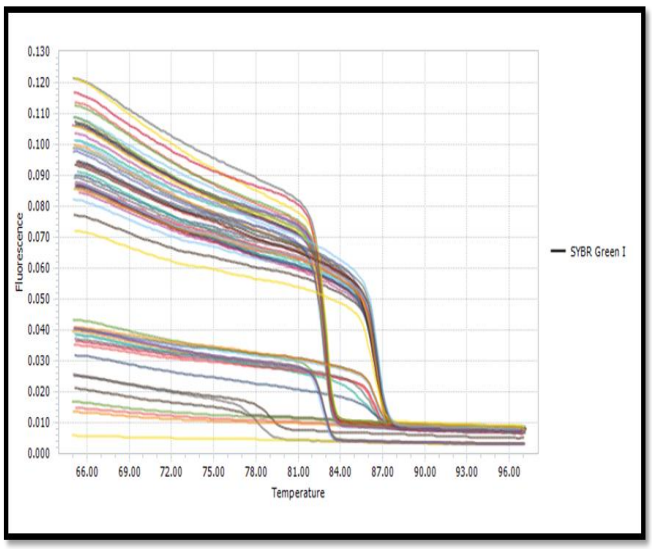
Figure(7): The melting peaks of qRT-PCR samples.



Figure(8): The Fluorescence peaks of qRT-PCR samples at 45 cycles .



Figure(9): The Fluorescence peaks of qRT-PCR samples at temperatures .

Discussion

The interlekin-6 have a several biologic activities initially described as a factor derived from fibroblasts and it is a polypeptide cytokine with antiviral activity (Weissenbach et al,1980).And it was found as a stimulating factor to B-cell (Muraguchi et al , 1985). In fact, various studies have shown that interleukin -6 (IL-6) is an important regulator have pathogenesis mainly through its effect on immune cell function (Neurah and Finotto, 2011). In 1992, Lahm et al were first study about the effecting of growth – promoting to IL-6 on colorectal cancer cell lines in vitro (Lahm et al , 1992). Galiza et al (2002) we found that increased expression of IL-6 from colorectal cancer patients and their compare with elevated in levels of IL-6 in serum in same patients.

In this present study show the gene expression for IL-6 were significantly over expression in colon cancer cachexia patients in cell line (LS-180) it Co-culture with peripheral blood mononuclear cells (PBMCs) compared with the results of the same gene expression in colon cancer non-cachexia patients in cell line (SW-480) with PBMCs for patients and normal peripheral blood mononuclear cells. And these experiments demonstrated that IL-6 promoter mediated transcription was not induced by cytokines in (SW-480) cells , whereas the IL-6 promoter was strongly activated in (LS-180) cells. This results agreement with Martignoni et al (2005) that IL-6 over expression in cachectic pancreatic patients by a Co-culture cancer cell lines T3M4 (IL-6 positive) and Panc-1 (IL-6 negative) and peripheral blood mononuclear cells obtained from donors and non-cachectic and cachectic pancreatic cancer patients.

Elias and Lentz (1990) ; Rola and Stankova (1992) demonstrated that lack of IL-6 gene expression in response to cytokines in some of carcinoma cell lines for did not the deficiency in the endogenous at IL-6 gene but because the IL-6 expression for regulating by post-transcriptional mechanism modulating mRNA stability.

Maximilian et al (2012) study of the correlation between the IL-6 expression with colorectal cancer (CRC) prognosis and patients with infection bowel diseases (IBD) by the increased the IL-6 expression and thought the role for IL-6 may be acting as a link between a chronic inflammation and tumor development.

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Belluco et al (2000) we study the correlation between the a polymorphism of the IL-6 promoter with serum levels to this cytokine from the colorectal cancer patients. Becker et al (2004) were shown that IL-6 secreted by lamina propria T cells and macrophages and may be also important for the cancer cachexia (CAC) developing.

Moreover, the increased IL-6 levels may be could for infiltration of tumors with IL-6 secreting inflammatory cells (Maximilian et al , 2012). Devita et al (2001) that study correlated the serum IL-6 levels in patients with gastrointestinal cancer with cancer stage, and suggest for the a suitable tumor marker to the IL-6 for monitoring the response to treatment of patients with gastrointestinal tract cancers.

Baba et al (1995) that demonstrated the growth and progression of gastrointestinal cancer cells at outocrine growth control by the granulocyte-colony stimulating factor (G-CSF) and established a human gastric cancer cell lines that produced a high level at IL-6 as well as excessive for granulocyte- macrophage colony stimulating factor (Gm-CSF) and G-CSF, therefore, there are various cytokines other than IL-6 be under the control of growth and progression at gastrointestinal cancer cells.

While, Matsuo et al (2003) suggest that in gastrointestinal cancer cell lines the IL-6 may be act in a paracrine growth control rather than an outocrine through the complex between the IL-6 gene and IL-6R gp130 that may be involved in certain the biological behaviors of these cancer cells.

Brekel et al (1995) study that circulating IL-6 levels were higher in weight losing with non-small cell lung carcinoma patients compared to weight stable cancer patients. And there reported to the IL-6 to be involved in early stages of cachexia (Tisdale ,2009;Scheede-Bergdahl,2012).

Iwase et al (2004) study showed IL-6 levels gradually increased during early stages of cachexia followed by rapid increase prior to death and it a conducted in patients with many of tumor types.

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