Evaluation of C- reactive protein as a probable factor for cancer diagnosis

Safinaz Elshabrawy[1], Alyaa Farid[2], Mohamed El- Beddini[1], Ahmed Osman[2], Somaya El- Deeb[2]

Hematology Department, National Cancer Institute, Cairo University[1]
Zoology Department, Faculty of Science, Cairo University, Cairo, Egypt[2]
alyaafarid@yahoo.com

Abstract: C- reactive protein (CRP) is a definitive marker of inflammation produced and synthesized in the liver in response of interleukin-6 (IL-6). It was studied in 10 healthy individuals and 97 patients with different types of diseases including kidney failure (KF), cardiovascular disease (CVD), hepatocellular carcinoma (HCC), Non-Hodgkin lymphoma (NHL), lung cancer (L.C), colon and bladder carcinoma (C.C), ovary and cervix carcinoma (O.C) and breast cancer (B.C). Routine blood tests were assayed for the 107 studied cases such as some liver enzymes (aspartate aminotransferase (AST) and alanine aminotransferase (ALT)), some kidney function factors (urea and creatinine) and some tumor markers (alfafeto protein (AFP), carcinoembroyonic antigen (CEA), cancer antigens 19.9 (CA19.9), 15.3 (CA15.3) and 125 (CA125) pecific to the different studied types of cancers. This study examined the relationship between circulating levels of CRP and various parameters of blood analysis in addition to the level of various tumor markers. It was found that CRP is associated with both KF and CVD cases. The studied specific tumor markers were significantly high in HCC (AFP), NHL (CEA), L.C (CEA) and B.C (CA15.3) studied cases ($P<0.05$) but it showed no significance in the C.C (CA19.9) and O.C (CA125) studied cases. It was evident that CRP levels are closely related to CA19.9 and CA125 tumor markers in case of C.C and O.C, respectively.
[Safinaz Elshabrawy. **Evaluation of C- reactive protein as a probable factor for cancer diagnosis.** *Life Sci J* 2012;9(4):2796-2803] (ISSN:1097-8135). http://www.lifesciencesite.com. 411

Key words: C- reactive protein, acute phase proteins, inflammation, cancer, tumor markers.

1. Introduction

Cancer is one of the diseases which was found to be a major leading cause of death worldwide due to the late diagnosis of the disease. Hence, the early diagnosis of cancer plays a very important role in the management and the cure of the disease. The prognosis for many types of cancer is still poor for management and cure of the disease, so it has been of interest to find other parameters which could be more sensitive and could help in the early detection of cancer (Yasuda, 2006; Chang *et al.,* 2010).

C-reactive protein (CRP) is an acute phase protein synthesized by hepatocytes in the liver in response to interleukin-6 (IL-6) cytokine induction according to inflammatory process as a result of a host immune response. Although CRP is a nonspecific inflammatory marker associated with inflammatory diseases, it was flashed on to be an important marker for the early detection of abnormal conditions causing inflammatory action including, cancer, autoimmune diseases such as systemic lupus erythromatosus (SLE) (Szalai, 2004), kidney failure (KF) (Fox *et al.,* 2010; Hung *et al.,* 2011) and cardiovascular diseases (CVD) (Khreiss*et al.,* 2005).

Many common cancers develop as a consequence of years of chronic inflammation (Moss and Blaser, 2005). Chronic activation of the immune system by parasitic, viral and bacterial infections is associated with tumours at several sites (Il'yasova *et al.,* 2005), on the other hand, noninfectious chronic inflammation is also associated with several types of cancer (Hussain *et al.,* 2003).

The first findings of the association of the elevated levels of CRP with advanced cancer diseases were reported in 1985 (Zielinski *et al.,* 1985). CRP levels were investigated to might be a future prognostic biomarker in different malignancies such as Hodgkin lymphoma (Wieland *et al.,* 2003), colorectal cancer (Mazhar and Ngan, 2006) and ovarian cancer (McSorley *et al.,* 2007).

The aim of the present study is to investigate the role of the CRP as a probable factor for the early detection and diagnosis of some different types of cancer introducing another sensitive parameter for the early diagnosis of cancer that could help in the disease management and cure.

2. Material and Methods

1. The studied cases:

The total number of all studied cases was 107 individuals. The number per case was determined according to the availability. The individuals were chosen from National Cancer Institute (NCI) and Nasser Institute for research and treatment Hospital (NIH). A complete clinical history for each individual (age, sex and pathological examination) was obtained from the statistical department in NCI and NIH. According to the clinical history of each individual, the studied cases (males and females) were classified according to the clinical, pathological and radiological findings as follows (Table 1): Ten healthy individuals from both

sexes reported as free form any disease, 15 individuals were diagnosed as suffering from kidney failure (KF) disease, 9 individuals were diagnosed as having cardiovascular disease (CVD), 21 individuals having hepatocellular carcinoma (HCC), 9 individuals with lung cancer (L.C), 9 individuals with Non-Hodgkin lymphoma (NHL), 9 individuals with colon and/or urinary bladder cancer (C.C), 12 individuals (females) with breast cancer (B.C) and 13 individuals (females) with ovary and/or cervix cancer (O.C).

Table 1: Number of studied cases according to their clinical, pathological and radiological findings.

Studied cases	No. of individual
Healthy individuals (HI)	10
Kidney failure (KF) cases	15
Cardiovascular disease (CVD) cases	9
Hepatocellular carcinoma (HCC) cases	21
Lung cancer (L.C) cases	9
Non-Hodgkin lymphoma (NHL) cases	9
Colon and bladder cancer (C.C) cases	9
Breast cancer (B.C) cases	12
Ovary and cervix cancer (O.C) cases	13

2. Sample collection:

Venous blood samples were withdrawn in plain vacutainer tubes, two tubes of about 5 ml for each individual. Blood tubes were allowed to stand for 30 minutes (min) in water bath to clot then centrifuged at 3000 rpm for 5 min. The sera were collected and stored at -20 ^{O}C until use.

3. Parameters assessed:

3.1. C- reactive protein (CRP) assay:

CRP was measured according to nephlometric methods of analysis involving the reaction of CRP with the antibody bound to latex particle forming insoluble complexes (Okamura *et al.,* 1990; Ward *et al.,* 1999). The measurement of CRP was done using Minineph^{TM} Human C- reactive protein kit (The binding sit group Ltd, Birmingham, UK).

3.2. Assay of liver enzymes (aspartate aminotransferase (AST), alanine aminotransferase (ALT)):

AST and ALT were measured by kinetic method according to the International Federation of Clinical Chemistry (IFCC) (Bergmeyer *et al.,* 1986).

3.3. Assay of urea:

Urea was measured by urease-UV fixed rate (enzymatic method) (Tiffany *et al.,* 1972; Tietz, 1990).

3.4. Assay of creatinine:

Creatinine was measured by buffered kinetic Jaffé reaction without depolarization method (Bowers and Wong, 1980).

3.5. Assay of Lactate dehydrogenase (LDH):

LDH was measured by optimized test according to German Society of Clinical Chemistry (DGKC) (Recommendation of the German society of clinical chemistry, 1972).

3.6. Assay of creatine kinase (CK).

Creatine phosphokinase was measured by optimized UV- test according to IFCC and German Society of Clinical chemistry (DGKC) (Schumann *et al.,* 2002; Recommendations of the German society for clinical chemistry, 1977).

AST, ALT, urea, creatinine, LDH and CK were programmed to the automated Bechman system (Bechmansynchron CX®9 clinical system (Marca REG, USA)) according to the application sheets provided with the kits (Diasys diagnostic systems kits (GmbH, Germany)).

3.7. Assay for tumor markers:

Measured tumor markers:

Carcinoembryonic antigen (CEA): A follow up marker for various carcinomas (Lokich *et al.,* 1978; Khoo *et al.,* 1979).

Alfa-fetoprotein (AFP): A marker for HCC (Waldmann and Mclntire, 1974).

Cancer antigen 19.9 (CA 19.9): A colorectal, pancreatic and gastrointestinal tumor marker (Herlyn *et al.,* 1982).

Cancer antigen 15.3 (CA 15.3): Breast tumor marker (Gion *et al.,* 1991).

Cancer antigen 125 (CA 125): Ovarian tumor marker (Crombach *et al.,* 1985).

Tumor markers measurement is based on the Microparticle Enzyme Immunoassay (MEIA) technology (Gold and Freedman, 1964; US Department of Health and Human Services, 2007). Tumor markers kit (Abbott Diagnostics division kits (Finisklin business park, Sligo, Ireland)) was defined on the AxSYM automated analyzer system (Abbott AxSYM © system automated immunoassay analyzer (Abbott laboratories diagnostics division, Abbott park, IL6, Germany)) by the bar code specialized for the kit. Reaction vessels and matrix cells were loaded to the automated system preparing for the run.

4. Statistical analysis:

Collected data was analyzed by ANOVA test (analysis of variance test) using the statistical analysis systems (SAS) (2010) SAS program ver.9.1, SAS institute incorporation, cary, NC25713USA. The mean, frequency and standard error of the measured variables were calculated using the F-test and the data represented as mean±standard error with 95% confidence intervals. The significance of the studied parameters CRP, AST, ALT, urea and creatinine are reported as $P < 0.05$ referred as significant, $P< 0.01$ referred as highly significant and $P < 0.001$ referred as extremely significant. The relation between different tumor markers and the studied parameter CRP was measured using Pearson correlation coefficient for calculation the correlation coefficient factor r.

3. Results

Comparison of the mean level of each parameter assayed in all studied cases:

1. C- reactive protein (CRP):

The mean concentration level of the parameter of interest, the CRP, showed a high significant increase in all studied cases when compared with the HI cases but the highest mean concentration level of CRP was shown for patients with O.C (143.51 ± 41.2) followed by patients with CVD (128.75 ± 21.83), and the increased mean concentration level of CRP was shown to be associated with all cancer cases studied (HCC: 20.23 ± 4.73, L.C: 61.08 ± 19.59, NHL: 31.45 ±14.30, C.C: 29.14 ± 8.32, B.C: 15.02 ±4.34 and O.C: 143.51 ±41.2) which were significantly higher than those of the HI cases (Table 2).

2. Aspartate amino transferase

The mean level of the liver enzyme AST was shown to be significantly higher in patients with CVD (205.8 ± 69.03 U/l) and patients with HCC (57.53 ± 7.89 U/l), while it showed no significant difference in the other studied cases when compared with the HI cases. This indicates an association of the increased level of the AST with CVD and HCC diseases rather than the other studied diseases (KF, L.C, NHL, C.C, B.C and O.C) which showed no significant difference of the AST (Table 2).

3. Alanine amino transferase

The mean concentration level of ALT was shown to be significantly associated with patients with CVD syndromes (84.33 ± 36.99 U/l) and no significant association with the other studied cases (Table 2).

4. Urea and creatinine.

The kidney function parameters were assayed, the urea mean levels (132.80 ±9.67 and 109.22 ±16.19 mg/dl respectively) were shown to be associated with only the KF cases and CVD cases while these two parameters showed no significant association with the other studied diseases (HCC, L.C, NHL, C.C, B.C and O.C). Creatinine mean levels were also shown to be associated with KF and CVD patients but not associated with the other studied cases (HCC, L.C, NHL, C.C, B.C and O.C) (Table 2).

Table 2: Comparison of the mean level of each parameter in all studied cases.

Parameter	Cases studied								
	HI	KF	CVD	HCC	L.C	NHL	C.C	B.C	O.C
CRP (mg/L)	1.77 ±0.27	52.14 ±10.31***	128.74 ±21.83***	20.23 ±4.73**	61.08 ±19.59**	31.45 ±14.30*	29.14 ±8.32**	15.02 ±4.34*	143.51 ±41.20**
AST (U/l)	29.20 ±2.77	28.87 ±2.51	205.83 ±69.03**	57.52* ±7.89	27.44 ±2.52	29.77 ±1.99	28.66 ±3.07	29.41 ±3.42	34.67 ±5.44
ALT (U/l)	22.20 ±3.72	22.46 ±2.22	84.33* ±36.69	46.94 ±8.55	22.44 ±2.35	21.88 ±1.18	20.75 ±1.75	23.67 ±3.64	29.58 ±7.41
Urea (mg/dl)	31.60 ±3.16	132.80*** ±9.67	109.22*** ±16.19	34.04 ±2.36	28.66 ±4.01	34.33 ±2.68	33.44 ±4.02	27.33 ±2.54	40.00 ±9.93
Creatinine (mg/dl)	0.76 ±0.04	4.90*** ±0.63	3.03* ±0.85	0.89 ±0.04	0.84 ±0.07	0.76 ±0.04	0.81 ±0.04	0.71 ±0.04	1.13 ±0.25

CRP: C-reactive protein.
KF: kidney failure.
NHL:Non-Hodgkin lymphoma.
AST: Aspartate aminotransferase.
CVD: Cardiovascular disease.
C.C: Colon and bladder cancer.
ALT: Alanine aminotransferase.
HCC: Hepatocellular carcinoma.
B.C: Breast cancer.
HI: Healthy individuals.
L.C: Lung cancer.
O.C: Ovary and cervix cancer.
Significant: *P<0.05.
Highly significant: **P<0.01.
Extremly significant: ***P<0.001
Data are represented as mean ±standard error.

Relation between levels of C-reactive protein and various tumor markers:

The level of CRP for each case was compared with the levels of the different tumor markers in the different studied cases.

1. Hepatocellular carcinoma cases:

In the HCC cases (N=11), the CRP levels were shown not to be associated with the AFP tumor marker levels but it may be associated with the malignancy. The correlation coefficient value "r" studying the relation between the CRP and the AFP (r=-0.34) showed a weak reverse relationship between the levels of CRP and levels of AFP in patients with HCC (Fig. 1).

2. Lung cancer cases:

The increased CRP levels were also not associated with the levels of the tumor marker CEA which was shown to be within the normal range (up to 3.5 ng/ml) for patients (N=7) with L.C. So there was a weak reverse relationship between the CRP and the CEA (r= -0.48) in patients with L.C (Fig. 2).

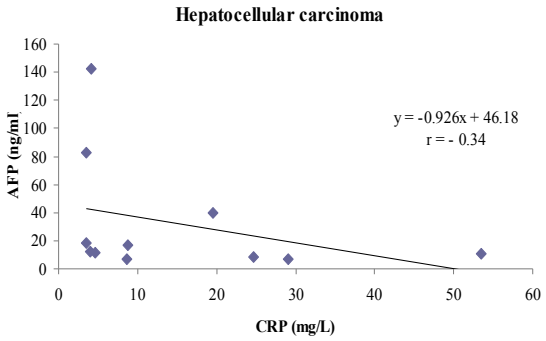

Figure1: The reverse relationship between C-reactive protein and alpha feto protein (AFP) tumor marker in patients with hepatocellular carcinoma.

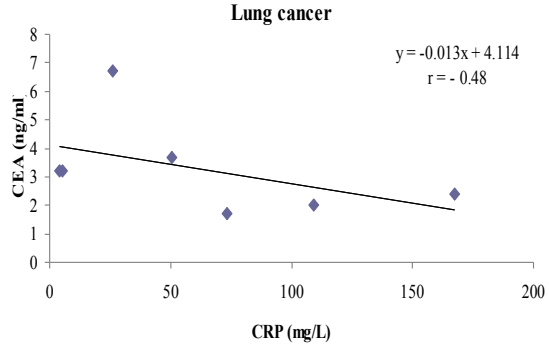

Figure 2: The reverse relationship between C-reactive protein and carcinoembroyinc antigen in patients with lung cancer.

4.3. Non-Hodgkin lymphoma cases:

The weak reverse relationship between the increased levels of CRP and CEA (r= -0.6) was revealed in NHL cases (N=7) (Fig. 3) where no association has been found between the CRP and CEA levels for the individuals studied in this case.

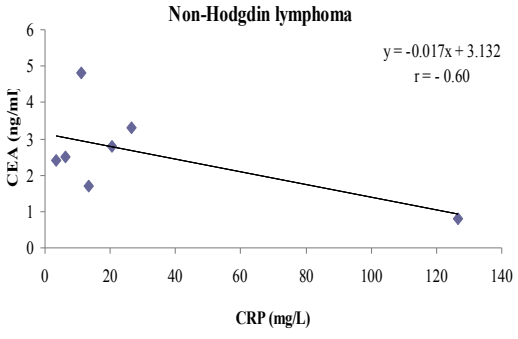

Figure 3: The reverse relationship between C-reactive protein and carcinoembryonic antigen in patients with non-Hodgkin lymphoma.

4.4. Colon and bladder cancer cases:

An association may have appeared between levels of CRP and CA19.9 tumor marker for the C.C cases (N=6). This association was supported by the strong proportional relationship revealed by the correlation coefficient factor r= 0.77 (Fig. 4).

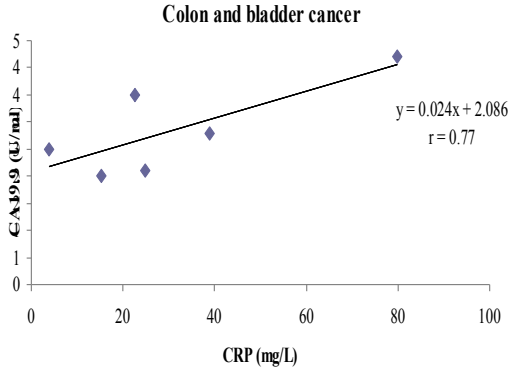

Figure 4: The proportional relationship between C-reactive protein (CRP) and cancer antigen 19.9 in patients with colon and bladder cancer.

4.5. Breast cancer cases:

In B.C cases (N=10), the CRP levels were also not related to the CA15.3 levels showing no association between the CRP and the tumor marker but there may be an association between the CRP and the breast malignancy. The studied correlation coefficient factor of the CRP and CA15.3 (r= -0.55) showed a weak reverse relationship between CRP and tumor marker CA15.3 levels (Fig.5).

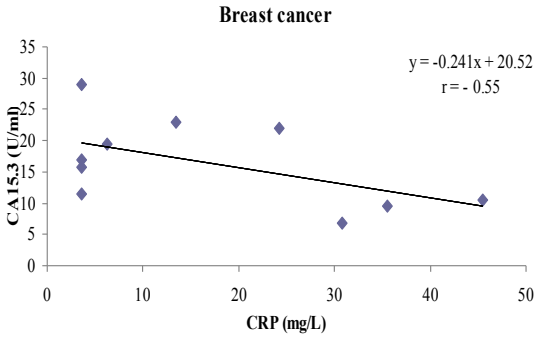

Figure 5: The reverse relationship between C-reactive protein and cancer antigen 15.3 in patients with breast cancer.

4.6. Ovary and cervix cases:

A proportional relationship has also been found between the CRP and CA125 levels for patients with O.C (N=9) (r= 0.48) but it is a weak relationship (Fig.

6), this is because the CRP levels were not associated with the levels of the CA125 demonstrating another evidence of non-association between the CRP levels and the level of the tumormarker but it may be associated with malignancy.

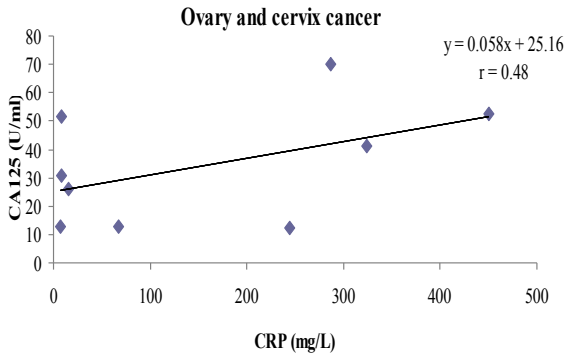

Figure 6: The proportional relationship between C-reactive protein and cancer antigen 125 in patients with ovary and cervix cancer.

4. Discussion:

Many of the common cancers are preceded by years of chronic inflammation. Tumor progression is always followed by development of acute phase protein response, that is chronic malignant disease involve changes in protein metabolism which result in production of acute phase proteins as a kind of immune response such as the production of CRP (Weinstein *et al.,* 1984; Stamatiadis *et al.,* 1992; Chung and Chang, 2003).

CRP is not only a marker of inflammation, it was also shown as a marker of activation of the immune system (Nauta *et al.,* 2003; Manfredi *et al.,* 2008).

Elevated levels of CRP were shown to be a predictive factor for different inflammatory diseases and infections and a predictive factor for increased risk of coronary events (Hage and Szalai, 2007), SLE (Szalai, 2004) and cancer risk (Allin *et al.,* 2009; Williams and Muddiman 2009) .

In the present study, there was a significant increase of CRP (P<0.0001) in patients with KF and this is in agreement with the study of Abraham and his colleagues, as they studied the levels of high sensitivity C-reactive protein (HsCRP) in Indian patients with CKD. They reported that the high HsCRP levels in Indian CKD patients indicate the high prevalence of inflammation in non-dialysis patients (Abraham *et al.,* 2009). The increased levels of CRP in the present study for the KF patients were also in accordance with the elevated concentrations of CRP in a heterogeneous population studied by Lobo and others, who stated that the elevated concentrations of serum CRP on ICU admission are correlated with an increased risk of organ failure and death (Lobo *et al.,* 2003).

The CRP levels in patients with CVD, during this study showed a significant increase (P<0.0001) and this was in agreement with Galante and others since, they found that CRP levels were higher in patients with aortic valvular stenosis than in controls (p = 0.0001) (Galante *et al.,* 2001).

Levels of AST, ALT, urea and creatinine in the present study also showed significant increase (P=0.0045, 0.0439, 0.0001 and 0.021, respectively) in CVD patients. The significant association of urea in CVD patient in this study was in accordance with Ostfeld and his coworkers who stated that elevated serum blood urea nitrogen (BUN) on admission was associated with an increased burden of coronary artery disease (CAD) on cardiac characterization during index hospitalization in patients who are presented with symptoms of unstable angina and without known cardiovascular disease suggesting that an elevated serum BUN in these subjects may predict a larger burden of CAD on cardiac characterization independent of creatinine clearance (Ostfeld *et al.,* 2010). Further study is warranted to explore this association. In addition in earlier studies it was documented that CRP is a strong, independent predictor of future myocardial infarction and stroke among apparently healthy asymptomatic men (Ridker *et al.,* 1997; 1998).

On the other hand elevated levels of CRP showed no association with the AFP during the study of the correlation between the CRP and AFP and this was demonstrated by the weak reverse correlation coefficient factor resulting from that correlation study (r=-0.34). This finding is in agreement with that of Lee and others since they found that the CRP levels correlated poorly with their corresponding AFP levels (r=0.0513). Although it was shown that CRP and AFP are not correlated, these markers seem to complement each other as out of 104 patients with HCC, CRP and AFP detected 78% and 80%, respectively (Lee *et al.,* 1989).

While AST was shown to be associated with HCC as its level significantly increases (P=0.0172). ALT was not statistically significantly different (P=0.051) but can be associated with the hepatic malignancy. The significant difference of CRP levels in HCC shows significant association of the elevated levels of CRP with the HCC. Thus, the significance of the elevated levels of CRP in patients with HCC cannot be neglected but it points to a new factor which may play a role in the early detection and diagnosis of the disease.

The current study showed an association of CRP levels with LC patients. CRP levels were shown to be

significantly increased (P <0.01) in patient with LC. These results are in accordance to those shown by Chaturvedi and his colleagues, since they stated that elevated CRP levels are associated with subsequently increased LC risk, suggesting an etiologic role for chronic pulmonary inflammation in lung carcinogenesis (Chaturvedi *et al.,* 2010).

The levels of the tumor marker CEA and levels of CRP in patients with LC in this study were shown not to be correlated and not associated and this was proved by the weak reverse correlation (r value -0.48) that appeared during this study.

As CRP levels were shown to be significantly increased in patients with LC (P=0.0036) while the other measured routine blood tests AST(P=0.6484), ALT (P =0.9533), urea (P=0.5700) and creatinine (P=0.3591) showed no significant association. The significantly elevated levels of CRP in this work also agreed with different studies on LC cases (Trichopoulos *et al.,* 2006; Allin *et al.,* 2009).

The present study showed significantly increased levels of CRP levels in patients with NHL (P < 0.05). That significant increase was also shown by Yildirim, and his colleagues, during their study when they measured the levels of ferritin and CRP in patients with NHL before and after treatment and they found a significant decrease in levels of ferritin and CRP following treatment when compared to pre-treatment measurement concluding that serum ferritin and CRP parameters may be used as tumor markers and may be indicators in the efficacy evaluation of treatment in NHL (Yildirim, *et al.,* 2009).

The correlation coefficient factor r (r=-0.60) studied for the correlation between the CRP and CEA in patients with NHL showed strong reverse relationship giving evidence of the probability that CRP can be used as a predictive factor for the presence of NHL in the absence of the role of the tumor marker CEA. Such a correlation was not available in previous reports.

The blood levels of AST, ALT, urea and creatinine showed no significant association in patients with NHL in the current work. Such a correlation was also not available in previous reports.

A strong proportional relationship (r=0.77) was found during the study of the relation between the CRP and the CA19.9 with CC. This was concurring with a previous study (Wong *et al.,* 2007), as they reported that CRP was associated with larger metastases size and elevated CA 19.9 level exploring the important role of CRP in the evaluation, detection and follow up of colon and bladder cancer

On the other hand, routine blood tests in the present study didn't show any significant association (AST, ALT, urea and creatinine). Pitifully there is no relevant information in the literature in case of CC patients with high CRP levels.

The correlation coefficient factor studied for the relationship between CRP and CA15.3 in B.C cases showed to be poorly correlated (r=-0.55) proving that the CRP is not associated with the tumor marker but may be with the malignancy. Such a correlation was not available in previous reports.

The studied CRP levels in patients with OC in the present study were shown to be significantly higher than that in HI cases. These results concur with the documented results of McSorley and others, who stated that ovarian cancer risk was positively associated with increasing CRP concentrations. They also proved that the risk of developing ovarian cancer among women in the highest third of the distribution of CRP compared with those in the lowest third produced evidence of and increasing risk with increasing concentration of CRP (McSorley *et al.,* 2007).

This present study showed weak proportional correlation between the CRP and CA125 (r=0.48) this is in correspondence with McSorley and others study who showed no correlation between CRP and CA125 (r=-0.02) (McSorley *et al.,* 2007) and Hefler's and coworkers study who found that serum CRP was not significantly correlated with serum CA 125 (r= 0.02) (Hefler *et al.,* 2008).

In conclusion, the present results suggest that (a) CRP levels are a more consistent indicator of cancer risk than some tumor markers, (b) the association between cancer incidence and CRP as an inflammatory marker may be tumor type specific (c) increased level of CRP may show a stronger association with risk of cancer incidence, recurrence, and death.

Corresponding author
Safinaz Elshabraw
Hematology Department, National Cancer Institute, Cairo University[1]
alyaafarid@yahoo.com

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10/10/2012