RABMS Journal of Research in Applied and Basic Medical Sciences

The serum level alterations of Acute-phase proteins, serum alpha-1-acid glycoprotein, haptoglobin and alpha-2-Macroglobulin in the Patients with Stage I of Multiple Myeloma

Sonya Mahabadi[1], Mohammad Hasan Khadem-Ansari*[2], Fatemeh Kheradmand[3], Youssef Rasmi[4]

[1] *MSc in Medical School University of Medical Sciences, Student Research Committee, University of Medical Sciences, Urmia, Iran*

[2] *Professor of Clinical Biochemistry, School of Medicine Department of Clinical Biochemistry, University of Medical Sciences, Urmia, Iran (Corresponding Author)*

[3] *Associate Professor of Clinical Biochemistry, School of Medicine Department of Clinical Biochemistry, University of Medical Sciences, Urmia, Iran*

[4] *Professor of Clinical Biochemistry, School of Medicine Department of Clinical Biochemistry, University of Medical Sciences, Urmia, Iran*

***Corresponding authors:** Mohammad Hassan Khadem-Ansari, **Address**: Urmia University of Medical Sciences, Urmia, Iran, **Email**: mhansari1@hotmail.com, **Tel**: +98 4432780803

Abstract

***Background & Aims*:** Acute phase proteins are mainly synthesized by the liver and macrophages in inflammatory conditions as a result of various traumas and malignancies. Increased levels of these proteins have been reported in many tumors like multiple myeloma as an important indicator for the prognosis of malignancies. We investigated the serum levels of acute phase proteins in multiple myeloma cancer patients in stage one due to the important role of these proteins in malignancies.

Materials & Methods: The serum levels of acute phase proteins (alpha 1-acid glycoprotein, haptoglobin and alpha-2-Macroglobulin) were measured in 30 patients with stage I of multiple myeloma and 30 healthy persons as the control group. The average age of population was 69 years. Measurement of the serum levels of proteins was done using Capillary zone electrophoresis and high resolution (HR). Total protein amounts were measured by biuret method, as well. The data were analyzed by SPSS method.

Results: The values obtained from results of the test and statistical calculations in the groups of study suggested that the serum levels of examined acute phase proteins decreased significantly in the patients compared to the control group. ($P<0.001$)

Conclusion: The results of this study indicated the reduction of serum alpha-1-acid glycoprotein, haptoglobin and alpha-2-Macroglobulin in stage I patients compared to the control group, which is inconsistent with previous studies. These proteins may be considered as specific prognostic and diagnostic factors in multiple myeloma stage I. However, further studies are needed to assess accurate results.

***Keywords*:** Multiple myeloma, Acute phase proteins, Alpha-1-acid glycoprotein, Haptoglobin and Alpha-2-Macroglobulin

Received 16 May 2019; accepted for publication 01 August 2019

Introduction

Multiple myeloma (MM) is the proliferation of malignant plasma cells, which is characterized by the accumulation of monotypic cells within the bone marrow (BM)(1). It causes about 1% of neoplastic diseases and 13% of hematological malignancies(2) . MM is the second most prevalent cancer in the world with an annual incidence of around 86,000 worldwide, which approximately covers 0.8% of all new cancer cases(3) and often older people with an average age of 65 years are affected by this disease(4).

The factors affecting progression of MM are not clearly identified(3). Clinically, MM patients represent one or more of the types of complications of this disease, including hypercalcemia, bone destruction, anemia, and kidney failure (5). Adhesion of multiple myeloma cells to bone marrow stromal cells produces cytokines such as Interleukine6(IL6)(6, 7) that spreads to the blood vessels and activates some receptors on the target cells and finally leads to changes in the concentration of some of the plasma proteins (8-10). In previous studies it has been noted that IL-6 is considered as an autocrine (11)/paracrine growth (12) factor for myeloma cells, as well as an important regulator in the acute-phase reactions (also called acute-phase responses) in humans(13-15). Acute phase proteins are synthesized and secreted primarily by liver cells and macrophages.

It should be noted that tumor tissues like inflammatory cells interfere with some of the serum proteins(16, 17). Alpha-1-acid glycoprotein (AAG), also known as Orosomucoid, is an acute phase protein found in human serum, which is mainly synthesized by the liver cells. The precise role of this protein in plasma is not well defined(18), but it plays an important role in suppressing the immune system like other acute phase proteins(19). The amount of AAG increases during acute phase reactions(20) and some cancers(21).

Haptoglobin (Hp) is another acute plasma phase protein, which is a serum tetrameric glycoprotein(20-22)and is often synthesized in the liver(23). This protein is bound to hemoglobin following hemolysis and prevents kidney damage and iron deficiency by removing hemoglobin from the circulatory(24), and also involves in the modernization of collagen which plays a role in tumor cell invasion, cell growth and metastasis(25). Its increased levels have been observed in some cancers, inflammations and traumatic conditions (26).

Moreover, Alpha-2 macroglobulin (α2M) is considered as an acute phase protein synthesized by the liver cells that is composed of four identical subunits acting as a protease inhibitor(27). The increased levels of this glycoprotein have been observed in some cancers, liver diseases and infections.

In recent years, the relationship between serum acute phase proteins and disease progression has been investigated in many cancers. However, the relationship between these proteins and MM is still not well understood. According to studies' reports, in patients with MM stage I, relatively small amounts of cancer cells spread in the body, the number of red blood cells (RBCs) and the amount of calcium in the blood are normal and the patient may have no clinical symptoms. On the other hand, due to the regulation of the cellular structures and biological functions of the cells by proteins, a wide selection of proteomic methods, including the level of proteins, provides valuable information for the diagnosis and treatment of various diseases, including cancer(28). Likewise, no study has been done on the serum levels of acute phase proteins specifically in the MM stage I. Therefore, finding new serum biomarkers can help discovering appropriate diagnosis and predictable response for MM stage I.

Materials and Methods

This case-control study was done on newly diagnosed 30 stage I MM patients who have not undergone any treatments or chemotherapy (mean age70, 22 men and 8 women).The control group consisted of 30 (mean age 70, 22 men and 8 women, with no history of drug use) and had the same attitudes with the patients in terms of smoking and sexual distribution, in Imam Khomeini Hospital, Urmia, West Azerbaijan province, Iran. The study procedures and protocols were approved by ethics committee of Urmia Medical University (faculty of medicine, department of

clinical biochemistry, Urmia, Iran). Written informed consents were obtained from all participants prior to the study. The patients were suspected of MM after undergoing clinical examination (e.g, CBC), bone marrow biopsy and radiography. After serum protein electrophoresis and immunotyping using the capillary zone method (Capillary Electrophoresis, Sebia, France), they were diagnosed as stage I MM patients. All patients having high gamma or high beta peaks (above 30%) and with the observation of kappa or lambda light chains with respect to IgA, IgM and IgG immunoglobulins. According to the international staging system, serum beta2-microglobulin levels were considered as less than 3.5 mg/L in stage I MM patients (Chemiluminescence, Diasorin, Liaison, Italy).

Five milliliters of blood samples were obtained from the patients and the control group after 12 hours of fasting. Blood samples were first placed at room temperature for 15 minutes to form a clot to separate the serum, and then centrifuged for 10 minutes with a centrifuge 4000 RPM (Hermle Z360 K, Japan). The serum samples were equally divided into 0.5-ml tubes. Also, 24-hour urine specimens were collected without adding any preservative agents. After mixing the urine specimens well, 10 ml of them was removed and then centrifuged for 5 minutes at 4000 RPM (Hermle Z360 K, Japan), and its supernatant was poured into plastic tubes. All specimens were kept in the freezer at −20°C until performing the tests. On the day of the test, the serum specimens were unfrozen and centrifuged to remove the possible fibrin clots.

In the present study, Sebia-France High Resolution (HR) was used according to the manufacturer's instructions. All alpha-1 glycoprotein, haptoglobin and alpha-2 macroglobulin proteins were measured using capillary zone electrophoresis based on HR method. HR Capillary is designed for human serum proteins in an alkaline buffer (PH 9.9) with a capillary system. The system performs all separation sequences to obtain protein profiles for both quantitative and qualitative analysis automatically. According to the capillary system, the amount of acute-phase proteins (APP) (in eight fractions) is shown as a curve, and its percentage is measured and displayed by the device. Then, the amount of each serum protein is calculated in grams per deciliter using the total amount of serum protein. The amounts of serum total proteins were then measured using Biuret test of Pars Azmoon kits and photometric apparatus (BioSystems BTS-330 model, Spain).

Data in our study were analyzed by using SPSS software, version 20(SPSS, Inc, Chicago, IL, USA). The average data of each group were expressed as Mean ± SD. The t-test parametric test was used to assess the significance of differences between two groups. The Mann-Whitney test was used for non-normal variables. Also, $P \leq 0.05$ was considered statistically significant.

Results

Table 1 shows the clinical profile and laboratory information of the MM patients and the control group. There was no significant difference regarding the mean age of the study participants between the two groups. The serum total protein levels increased in MM patients compared with the control group, due to an increase in immunoglobulins (P=0/0001), (Fig.1). As shown in Fig 1, the serum levels of AAG (0.23 ± 0.08) decreased significantly in patients' group in comparison with the control group (0.66 ± 0.07). Similarly, we found a significant decrease of haptoglobin and alpha-2 macroglobulin levels in patients' group (0.75 ± 0.71 vs. 1.07 ± 0.27 gr/dl, P=0/0001 and 0.54 ± 0.13 vs. 0.64 ± 0.12 gr/dl, P=0/0030, respectively) compared to the control one. Also, Fig.2 shows the electrophoresis of the patients with MM, which is related to the gamma fraction in the curve.

Table 1: clinical profile and laboratory information of MM patients and control group (mean ± SD)

Parameter	Patients (N = 30)	Controls(N = 30)	P-value
Age	Male 70.6±5.1	Male 71.1±7.7	0.45
	Female 68.8±8.2	Female 68.3±5.00	
Beta2microglobulin(mg/l)	1.7±0.53	1.69±0.47	0.88
Total protein(g/dl)	8.73±0.26	7.54±0.42	0.0001

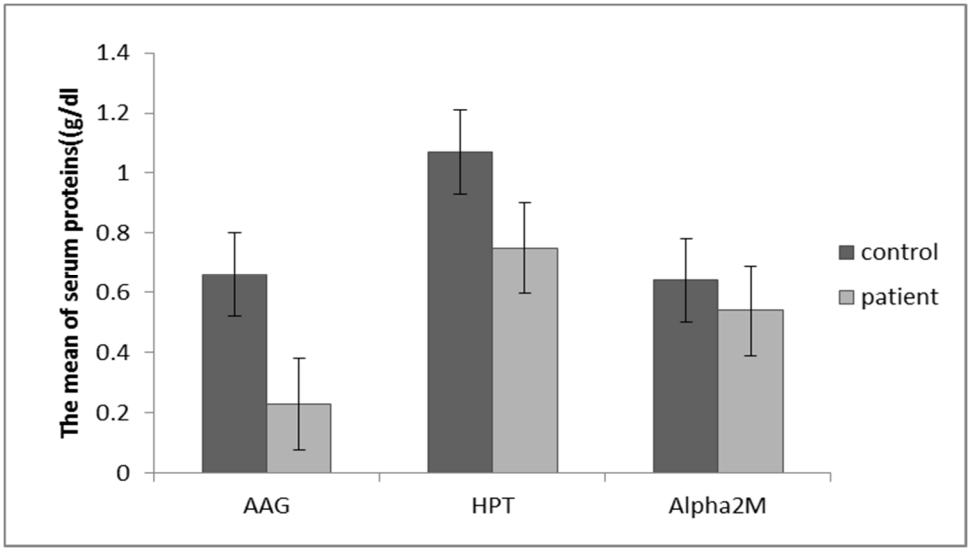
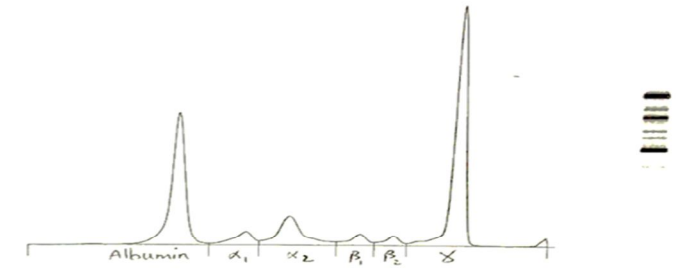


Figure 1: the mean serum proteins (alpha 1 acid glycoprotein (AAG), 2-haptoglobin (HPT) and 3-alpha2 macroglobulin (Alpha2M)) for the control group and the MM patients group, respectively.



Fractions	%	Ref. %	Ref. g/dl
Albumin	31.8	55.8 - 66.1	40.20 - 47.60
Alpha 1	4.7	2.9 - 4.9	2.10 - 3.50
Alpha 2	12.5	7.1 - 11.8	5.10 - 8.50
Beta 1	3.4	4.7 - 7.2	3.40 - 5.20
Beta 2	2.6	3.2 - 6.5	2.30 - 4.70
Gamma	45.0	11.1 - 18.8	8.00 - 13.50

Figure 2: Example of electrophoresis of a patient with MM stage 1

Discussion and Conclusion

Acute phase proteins are synthesized and secreted primarily by liver cells and macrophages. It has been reported that tumor cells have the ability to synthesize and secrete some of the acute phase proteins in some of the malignancies(16).

According to the results of our study, the serum levels of AAG, Hp and Alpha-2 macroglobulin in the patients with stage I of MM, decreased compared to the control group. In a study by Pelliniemi et al., the researchers found that the levels of AAG and alpha 1 antitrypsin increased in the patients with stages 1-3(29).

In another study with the similar results, the serum levels of IL6 and the acute phase proteins in the patients with MM stages 1-3 were determined and consequently came to this conclusion that the levels of AAG and Hp in these patients have increased as compared with the control group. It has been reported that IL6 is an important regulator of the acute phase proteins in MM patients which has increased in those compared to the control group (30-34). Since IL6 leads to the synthesis of acute-phase proteins in the liver of patients with MM as well as various cancers (35), it seems that tumor tissues interfere with the synthesis of some of the serum proteins, like inflammatory cells (16, 17), thus the levels of these proteins increase with the progression of the disease (33-36). However, in some malignancies, the levels of IL6 is normal or unidentifiable (37, 38).

The results of these studies are not consistent with the results of our study, which can be justified by several reasons: 1- In most studies the serum levels of plasma proteins were measured, along with the measurement of IL6 levels which is considered as a stimulant for secretion, but in our study, proteins might decrease in the amounts due to the low levels of IL6. 2- Many studies have been carried out on different types of the disease as well as in the post-treatment stages, but this study has focused on Stage I of MM, and as relatively small numbers of cancer cells have spread in the body, and the numbers of red blood cells and calcium levels are normal, therefore, it can be said that the kidney function of patients is normal and albumin excretion and proteinuria didn't happen as a result of renal impairment which leads to an increase in the levels of high molecular weight proteins, such as alpha2 macroglobulin in MM patients. 3- We know that a large number of RBCs are destroyed during tissue damage. After RBC destruction, hemoglobin is released. Hp binds to the hemoglobin preventing renal hemoglobin excretion. Therefore, as the tissue damage is higher, the hemoglobin levels increase and more Hp will be produced.

An increased Hp loss in the form of a complex with hemoglobin may be balanced by an increase in the production of serum Hp. Finally, it can be concluded that since this study has focused on type I and the onset of the disease, therefore, the levels of the Hp didn't increase. According to a research by Tabassum et al., the low levels of Hp were associated with an increase in the survival of the patients(39).

Furthermore, reduced levels of Hp may indicate conditions such as liver diseases, hematoma and anemia. Anemia is considered as a major clinical symptom of MM and it's treatment is one of the serious problems in today's medical world(15). In another study, the results showed that the levels of alpha 2 macroglobulin decreased in comparison with control group, and the levels of alpha1 anti-trypsin and Hp reduced compared to patients with MM- IgA type, while the amounts of these proteins increased in patients with MM-IgG type (40).

Therefore, it can be concluded that the differences in the results of this study might be due to the diversities of the synthesis of various classes of immunoglobulins, which may be involved in the stage of the MM and the level of serum protein. Additionally, various factors, such as the number of patients studied, differences in the sensitivities of measurement methods and disease-related parameters, could affect the results of the test. In summary, we have shown that the serum levels of AAG, Hp and alpha-2 macroglobulin decreased in the patients with stage I of MM. Acute phase proteins play an important role in the prognosis, diagnosis and treatment of various cancers. We reported the reduction of the amounts of these proteins in the stage I of MM for the first time, thus we could propose them as specific markers for the diagnosis and prognosis in MM stage I. However, more studies are needed to explore the association of the acute phase proteins' levels and the progression of MM.

Acknowledgements

The authors would like to thank members of the department of Clinical Biochemistry of Urmia University of Medical Sciences, Urmia, Iran. This study was supported by Urmia University of Medical Sciences.

References

1. Chng WJ, Lau LG, Yusof N, Mow BM. Targeted therapy in multiple myeloma. Cancer Control 2005;12(2):91-104.
2. Ms R. Podar k, Breitkreutz I, Richardson PG and Anderson kC: Multiple myeloma. Lancet 2009;374:324-39.
3. Becker N. Epidemiology of multiple myeloma. Multiple Myeloma: Springer; 2011. p. 25-35.
4. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA Cancer J Clin 2013;63(1):11-30.
5. Kumar S. Multiple myeloma–current issues and controversies. Cancer Treat Rev 2010;36:S3-S11.
6. Hideshima T, Mitsiades C, Tonon G, Richardson PG, Anderson KC. Understanding multiple myeloma pathogenesis in the bone marrow to identify new therapeutic targets. Nature Rev Cancer 2007;7(8):585.
7. Oancea M, Mani A, Hussein MA, Almasan A. Apoptosis of multiple myeloma. Int J Hematol 2004;80(3):224-31.
8. Trautwein C, Böker K, Manns M. Hepatocyte and immune system: acute phase reaction as a contribution to early defence mechanisms. Gut 1994;35(9):1163.
9. Ron D, Brasier AR, Habener JF. Transcriptional regulation of hepatic angiotensinogen gene expression by the acute-phase response. Mol Cell Endocrinol 1990;74(3):C97-C104.
10. Gruys E, Toussaint M, Niewold T, Koopmans S. Acute phase reaction and acute phase proteins. J Zhejiang Univ Sci 2005;6(11):1045.
11. Kawano M, Hirano T, Matsuda T, Taga T, Horii Y, Iwato K, et al. Autocrine generation and requirement of BSF-2/IL-6 for human multiple myelomas. Nature 1988;332(6159):83.
12. Bataille R, Jourdan M, Zhang X-G, Klein B. Serum levels of interleukin 6, a potent myeloma cell growth factor, as a reflect of disease severity in plasma cell dyscrasias. J Clin Invest 1989;84(6):2008-11.
13. Castell JV, Gómez-Lechón MJ, David M, Andus T, Geiger T, Trullenque R, et al. Interleukin-6 is the major regulator of acute phase protein synthesis in adult human hepatocytes. FEBS Lett 1989;242(2):237-9.
14. Biro L, Domján G, Falus A, Jakab L, Cseh K, Kalabay L, et al. Cytokine regulation of the acute-phase protein levels in multiple myeloma. Eur J Clin Invest 1998;28(8):679-86.
15. Victor M, Evgeniy H, Gergana T, Julia P, Vasil V, Borislav M, et al. Serum Hepcidin Levels in Multiple Myeloma. Clin Laboratory 2017;63(7):1273-7.
16. Hey E, editor Hyperglycaemia and the very preterm baby. Seminars in Fetal and Neonatal Medicine. Elsevier; 2005.
17. Lee J-W, Kim H-S. Endogenous retrovirus HERV-I LTR family in primates: sequences, phylogeny, and evolution. Arch Virol 2006;151(8):1651-8.
18. Fournier T, Medjoubi-N N, Porquet D. Alpha-1-acid glycoprotein. Biochim Biophys Acta 2000;1482(1–2):157–71.
19. Colombo S, Buclin T, Décosterd LA, Telenti A, Furrer H, Lee BL, et al. Orosomucoid (α1-acid glycoprotein) plasma concentration and genetic variants: Effects on human immunodeficiency virus protease inhibitor clearance and cellular accumulation. Clin Pharmacol Ther 2006;80(4):307-18.
20. Shah A, Singh H, Sachdev V, Lee J, Yotsukura S, Salgia R, et al. Differential serum level of specific haptoglobin isoforms in small cell lung cancer. Current proteomics 2010;7(1):49-56.
21. Duché J-C, Urien S, Simon N, Malaurie E, Monnet I, Barré J. Expression of the genetic variants of human alpha-1-acid glycoprotein in cancer. Clin Biochem 2000;33(3):197-202.
22. Kurosky A, Barnett DR, Lee T-H, Touchstone B, Hay RE, Arnott MS, et al. Covalent structure of human haptoglobin: a serine protease homolog. Proc Natl Acad Sci 1980;77(6):3388-92.
23. Ahmed N, Barker G, Oliva K, Hoffmann P, Riley C, Reeve S, et al. Proteomic-based identification of haptoglobin-1 precursor as a novel circulating biomarker of ovarian cancer. Br J Cancer 2004;91(1):129.

24. Sadrzadeh SH, Bozorgmehr J. Haptoglobin phenotypes in health and disorders. Pathol Patterns Rev 2004;121(suppl_1):S97-S104.
25. Abdullah M, Schultz H, Kähler D, Branscheid D, Dalhoff K, Zabel P, et al. Expression of the acute phase protein haptoglobin in human lung cancer and tumor-free lung tissues. Pathol Res Pract 2009;205(9):639-47.
26. Wang F, Huang W, Li A. Serum haptoglobin suppresses T-lymphocyte functions following burns. Chinese medical sciences journal= Chung-kuo i hsueh k'o hsueh tsa chih 1996;11(3):180-3.
27. Barrett AJ, Starkey PM. The interaction of α2-macroglobulin with proteinases. Characteristics and specificity of the reaction, and a hypothesis concerning its molecular mechanism. Biochem J 1973;133(4):709-24.
28. Hamrita B, Chahed K, Trimeche M, Guillier CL, Hammann P, Chaïeb A, et al. Proteomics-based identification of α1-antitrypsin and haptoglobin precursors as novel serum markers in infiltrating ductal breast carcinomas. Clin Chim Acta 2009;404(2):111-8.
29. Pelliniemi T-T, Irjala K, Mattila K, Pulkki K, Rajamaki A, Tienhaara A, et al. Immunoreactive interleukin-6 and acute phase proteins as prognostic factors in multiple myeloma. Finnish Leukemia Group. Blood 1995;85(3):765-71.
30. Zhao C, Annamalai L, Guo C, Kothandaraman N, Koh SCL, Zhang H, et al. Circulating haptoglobin is an independent prognostic factor in the sera of patients with epithelial ovarian cancer. Neoplasia 2007;9(1):1-7.
31. Solakidi S, Dessypris A, Stathopoulos GP, Androulakis G, Sekeris CE. Tumour-associated trypsin inhibitor, carcinoembryonic antigen and acute-phase reactant proteins CRP and α1-antitrypsin in patients with gastrointestinal malignancies. Clin Biochem 2004;37(1):56-60.
32. Zeyad J, Abu-Awad AM, Sharara AM, Khader YS. The importance of alpha-1 antitrypsin (α1-AT) and neopterin serum levels in the evaluation of nonsmall cell lung and prostate cancer patients. Neuroendocrinol Letters 2010;31(1).
33. Li Y, Krowka MJ, Qi Y, Katzmann JA, Song Y, Li Y, et al. Alpha1-antitrypsin deficiency carriers, serum alpha 1-antitrypsin concentration, and non-small cell lung cancer survival. J Thorac Oncol 2011;6(2):291-5.
34. San JM, Corrales A, Alberca I, Vicente V, Lopez AB. Acute phase reactant proteins in differential diagnosis of monoclonal gammopathy. Neoplasma 1983;30(1):57-62.
35. Castell JV, Gómez-lechón MJ, David M, Fabra R, Trullenque R, Heinrich PC. Acute-phase response of human hepatocytes: regulation of acute-phase protein synthesis by interleukin-6. Hepatology 1990;12(5):1179-86.
36. Thompson DK, Haddow JE, Smith DE, Ritchie RF. Elevated serum acute phase protein levels as predictors of disseminated breast cancer. Cancer 1983;51(11):2100-4.
37. Famularo G, Giacomelli R, Di SG, Sacchetti S, Tonietti G. Cytokine production in patients with monoclonal gammapathies. J Clin Lab Immunol 1991;34(2):63-9.
38. Ballester O, Corrado C, Moscinski L, Bruno S, Burgess J. Clinical significance of serum interleukin-6 (IL-6) levels in patients with multiple myeloma (MM). Proceedings of ASCO; 1992.
39. Tabassum U, Reddy O, Mukherjee G. Elevated serum haptoglobin is associated with clinical outcome in triple-negative breast cancer patients. Asian Pac J Cancer Prev 2012;13(9):4541-4.
40. San Miguel J, Vicente V, Battle J, Hernandez F, Lopez BA. Acute phase reactants and clinical stages in multiple myeloma. Neoplasma 1981;28(3):333.