

ACUTE PHASE PROTEINS AFTER ORTHOPAEDIC SURGERY

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SUMMARY

Introduction: Acute phase proteins (APP) are synthesised in the liver in response to tissue injury. The purpose of the study was to delineate the course of the circulating serum levels of various acute phase proteins after orthopaedic surgery and the effect of the length of the incision, transfusion, age and gender of the patient on these levels.

Patients and Method: C-reactive protein (CRP), alfa-1- acid glycoprotein, haptoglobin (HPT), transferrin, ceruloplasmin levels, and erythrocyte sedimentation rate (ESR) were determined preoperatively (Term I), and on the 1st (Term II), 3rd (Term III), 5th (Term IV), 7th (Term V), 14th (Term VI) days after orthopaedic surgery which included soft tissue operations –group A (n=30), open reduction and internal fixation –group B (n=11), amputations –group C (n=10). Analysis of the outputs was done by repeated measures of covariance analysis; $p < 0.05$ was considered significant. In CRP level measurement, since the variance was significantly large and the number of measurements was limited nonparametric Wilcoxon test was utilised.

Results: Infection or any other complication was not encountered in the study. CRP level changed between the terms in all operations ($p < 0.05$). There was no difference between HPT levels in terms in group A, but they varied in groups B and C ($p < 0.05$). In group A, and C there was no difference in ESR between the terms; in group B the mean level of ESR in term I was the lowest. Alfa-1-acid glycoprotein, Transferrin, Ceruloplasmin, Prealbumin levels were unchanged. CRP level was influenced by gender of the patient, and haptoglobin level by length of the incision. There was a positive correlation between the ESR and age of the patient and length of the surgical incision ($p < 0.01$).

Discussion: Knowledge about the natural course of ESR, and APP of which CRP and HPT are the most sensitive reactants may be helpful in the diagnosis of postoperative complications following orthopaedic surgery.

Key Words: *Acute Phase Proteins; Acute Phase Reactants; Postoperative Infection; Musculoskeletal Trauma; Orthopaedic Surgery.*

ÖZET

ORTOPEDİK CERRAHİDEN SONRA
AKUT FAZ PROTEİNLERİ

Giriş: Akut faz proteinleri (APP) doku zedelenmesine cevap olarak karaciğerde sentez edilir. Bu çalışmanın amacı, ortopedik cerrahiden sonra serum akut faz proteinlerinin seyrini ortaya koymak, ve insizyon boyunun, transfüzyon, yaş, ve cinsin etkisini ortaya koymaktır.

Hastalar ve Yöntem: C-reaktif protein (CRP), alfa-1- asid glikoprotein, haptoglobin (HPT), transferrin, seruloplasmin seviyeleri, ve eritrosit sedimentasyon oranı (ESO) yumuşak doku ameliyatları –grup A (n=30), açık redüksiyon and internal fiksasyon –grup B (n=11), ve amputasyonlar –group C (n=10) gibi ortopedik cerrahiden önce (Dönem I), ve sonraki 1. (Dönem II), 3. (Dönem III), 5. (Dönem IV), 7. (Dönem V), ve 14. (Dönem VI) günlerde değerlendirilmiştir. Verilerin analizi kovaryans analizinin tekrarlayan ölçümleriyle yapılmıştır; $p < 0.05$ anlamlı kabul edilmiştir. CRP seviyesi ölçümünde, varyans anlamlı derecede büyük olduğundan ve ölçüm sayısı sınırlı olduğu için nonparametrik Wilcoxon testi kullanılmıştır.

Bulgular: Çalışmada enfeksiyon veya herhangi başka bir komplikasyona rastlanmamıştır. CRP seviyesi tüm ameliyatlardan sonra her dönemde değişim göstermiştir ($p < 0.05$). HPT seviyesi grup

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A da dönemler arasında değişim göstermezken, grup B ve C arasında farklılık göstermiştir ($p < 0.05$). Grup A ve C de ESO dönemler arasında farklılık göstermezken, grup B de dönem I ortalaması en az seviyededeydi. Alfa-1-asid glikoprotein, Transferrin, Seruloplasmin, Prealbumin seviyeleri değişiklik göstermemiştir. CRP seviyesi hastanın cinsine göre değişiklik gösterirken, haptoglobulin seviyesi insizyon uzunluğundan etkilenmiştir. ESO ile yaş, insizyon uzunluğu arasında pozitif korrelasyon bulunmuştur ($p < 0.01$).

Tartışma: Eritrosit sedimentasyon oranı, ve CRP, HPT gibi hassas akut faz proteinlerinin doğal seyrinin bilinmesi ortopedik ameliyatlardan sonra gelişebilecek postoperatif komplikasyonların teşhisine yardımcı olabilecektir.

Anahtar Kelimeler: Akut Faz Proteinleri; Akut Faz Reaktantları; Postoperatif Enfeksiyon; Kas İskelet Sistemi Travması; Ortopedik Cerrahi.

INTRODUCTION

The early diagnosis of bacterial infection is still a formidable problem¹. The effects of the surgical procedure itself may mask septic complications. Postoperative fever, pain tachycardia, and leucocytosis are not definite indicators of sepsis. This difficulty leading to delay in diagnosis may cause high morbidity and mortality associated with postoperative sepsis. Thus, a method of early detection of sepsis would prevent serious complications^{1,2}.

A normal host response to bacterial infection is both local and systemic. The systemic response is mediated in part by the secretion of interleukin-1 from the macrophages attracted to the area and activated by local influences. A major function of interleukin-1 is to stimulate the production of acute phase proteins (APP) by hepatocytes^{2,3}.

Knowledge of the normal course of the acute phase proteins is essential prior to their employment as effective tools for early detection of infectious complications. The purpose of the study was to test the hypothesis that the circulating serum levels of acute phase proteins after orthopaedic surgery were altered. To achieve this, the samples were assessed on certain days determined in accordance with the time range set in the literature^{1,2,4}. As far as we are acquainted with the literature there is no study dealing with the change of parameters used in the surgical techniques employed in our study.

PATIENTS AND METHOD

Patients admitted to our department for three groups of orthopaedic surgery between October 2001 and December 2002 were included in the study. Namely, soft tissue operations (group A), open reduction internal fixation (group B), and amputations (group C). This selection represented groups with different degrees of tissue injury. Only patients with normal preoperative levels of C-reactive protein (CRP), alfa-1- acid glycoprotein, haptoglobulin (HPT), transferrin, ceruloplasmin, prealbumin and erythrocyte sedimentation rate (ESR) were included in the study. There were 30 patients (19 female, 11 male) in the soft tissue operations group with a mean age of 46 years (range 24-65 years). This group included lumbar discectomy, ganglion excision, carpal tunnel release and tendon transfer operations.

The open reduction internal fixation group consisted of 11 patients (6 female, 5 male) with a mean age of 35 years (range 12-69 years). There were six proximal femoral, and five tibial mid shaft closed fractures. Intramedullary nailing was performed in three patients, and internal fixation with plate and screws were done in eight.

The amputation group consisted of 10 patients with diabetes mellitus type II (4 female, 6 male) with a mean age of 56 years (range 34-69 years). Above the knee amputation was performed in four patients, and below the knee amputation in six patients.

The three types of operations were selected as they represented standardized and frequently performed orthopaedic surgery at our institution. Ganglion excision and carpal tunnel release were done under regional intravenous anesthesia, while the others were performed under general anesthesia. Prophylactic antibiotics were given to all patients for five days commencing at surgery.

The serial levels of C-reactive protein, alfa-1- acid glycoprotein, haptoglobulin, transferrin, ceruloplasmin, prealbumin and ESR were measured preoperatively (Term I), on the 1st (Term II), 3rd (Term III), 5th (Term IV), 7th (Term V), 14th (Term VI) days.

The samples were obtained at approximately the same time each morning. Whole blood samples were taken into standard sampling tubes. Serum samples were separated by centrifugation at 4000 rpm for 10 minutes. There were no infectious complications.

The levels of alfa-1-acid glycoprotein, ceruloplasmin, C-reactive protein, haptoglobulin, prealbumin and transferrin were determined immunoturbidometrically

at 340 nm. These were analysed by Cobas Integra 700 Biochemical Analyser (Roche Diagnostics GmbH, Mannheim, Germany). ESR was measured by Western Green method.

Factors that might influence the levels of APP and ESR such as age, gender, length of the surgical incision, transfusion were also recorded for all patients.

Statistical method: Analysis of the outputs was done by repeated measures of covariance analysis; $p < 0.05$ was considered significant. In CRP level measurement, since the variance was significantly large and the number of measurements was limited nonparametric Wilcoxon test was utilised.

RESULTS

A total of 51 patients underwent surgery with no complications. Preoperatively, all of the patients had normal levels of the acute phase proteins and ESR. The level of each APP and ESR at certain periods after each group of operation was documented.

C-reactive protein level was influenced by gender of the patient, males exhibiting increased levels, $p < 0.05$. In group A its level increased to the maximum point in term II, dropped to a lower level in term III, and reached to the preoperative level in term IV, stayed at this level in term V and reached to its lowest value in term VI. In group B, CRP level reached to its highest level in term II, decreased to a point above the preoperative level in term III, stayed around this level in term IV and V, but dropped to the preoperative level in term VI. In group C, CRP level continuously increased reaching to the maximum level in term VI. Pre and post operative values for each technique are depicted in Figure 1.

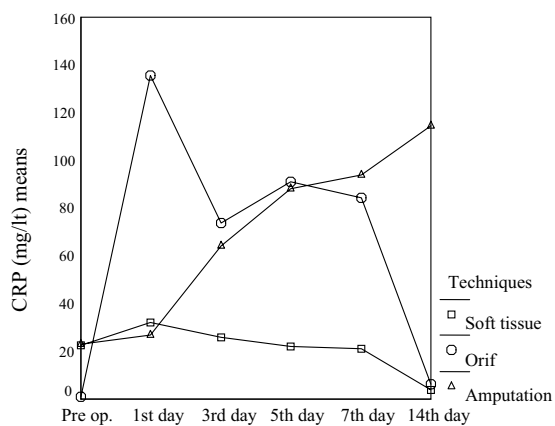


Fig. 1. CRP level means after soft tissue (Soft tissue), open reduction internal fixation (Orif), amputation (Amputation) operations.

Haptoglobin level was influenced by length of the incision, $p < 0.05$. As the length of the incisions increased it also caused an increase in HPT levels. In group A, there was no statistically significant difference between the mean values of the terms. In group B, the mean values of term I and II were not significantly different. Its level rose in term III, stood around this level in term IV and V, and dropped down to the initial level in term VI. In group C the mean values of terms I and II were not significantly different; dropped down in term III and started to rise in term IV reaching to its maximum level in terms V and VI. Pre and post operative levels pertaining to each technique are shown in Figure 2.

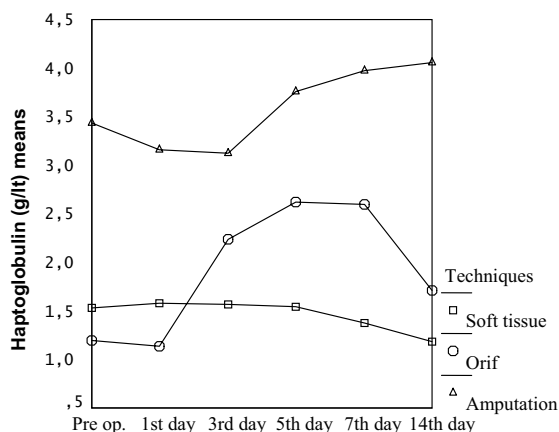


Fig. 2. Haptoglobin level means after soft tissue (Soft tissue), open reduction internal fixation (Orif), amputation (Amputation) operations.

Erythrocyte sedimentation rate was affected by age of the patient and length of the surgical incision $p < 0.01$. Older age and increased length of the incision caused an elevated ESR. In group A, and C there was no statistically significant difference in the ESR between the terms; in group B the mean level of ESR in term I was significantly lower than the ones in other terms $p < 0.01$. Pre and post operative values for each technique are displayed in Figure 3.

Transferrin, Ceruloplasmin, Prealbumin, alfa-1-acid glycoprotein levels were not influenced by age or gender of the patient, length of the incision, or transfusion. Their mean values which were unaffected by the surgical techniques were not altered significantly from one term to another.

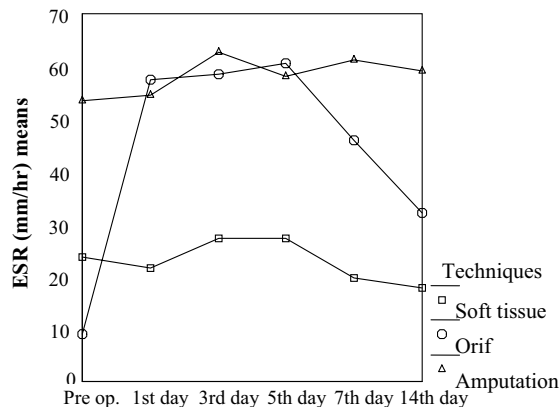


Fig. 3. ESR level means after soft tissue(Soft tissue), open reduction internal fixation (Orif), amputation (Amputation) operations.

DISCUSSION

Acute phase proteins are synthesized and released from the liver in response to different stimuli, a major one being tissue injury³. Inflammatory state that occurs after infection, injury, surgery, trauma, or other tissue necrosis causes release from leucocytes of proteolytic enzymes in tissue that must be neutralized by enzyme inhibitors to limit their extent of destruction. Scavenger proteins as haptoglobin, CRP help collect and transport debris and breakdown products to phagocytotic cells to process them and to conserve vital substances like iron. The elevation of APP is a response to interleukin-1 which is a chemical messenger released by macrophages attracted to the area of tissue injury and activated by local influences. The physiologic response involves induction of fever, catabolism of muscle, leucocytosis, and a shift in protein synthesis patterns with reduction in albumin production^{3,5}.

The measurement of any substance in the plasma depicts the net result of its synthesis and release in to the circulating blood, and degradation, binding and excretion of the substance from the plasma. Each APP has a specific function, affecting its rate of removal from the plasma. As a result some APP rises and others fall during the acute phase response^{2,3}.

CRP was discovered by Tillet and Francis^{3,6}. Later, Abernethy et al.³ characterised CRP as a protein and introduced the term acute phase sera to designate samples obtained from patients after acute infectious diseases. CRP was called an acute phase protein and this term was subsequently used for other proteins found in increased amounts in

acute phase sera^{7,8}. It is an unusual human protein in that its serum levels in normal subjects are extremely low, usually less than 1.0 mg/ dl^{1,3}. CRP appears to act as an opsonic substance binding to damaged phospholipid layers enhancing phagocytosis. It also suppresses differentiation of B lymphocytes with inhibition of antibody formation³.

Clinical reports indicate that CRP levels are elevated after various infectious processes, inflammation, trauma, and tissue damage at operation^{3,9-11}. Thelander et al.¹² reported that CRP levels increased after lumbar discectomy reaching peak levels in all 36 patients on the third day. C reactive protein levels usually dropped to normal in 5-14 days after surgery. No correlation was found between CRP response and age, gender and transfusion.

In their series of 50 lumbar disc surgeries, Jensen et al.¹³ found that CRP increased immediately, reached its maximum level on the second day and normalized within 6 days.

Yoon et al.⁴ stated that the highest values of CRP were recorded 2-3 days after the operation of long bone fractures which normalized by 3 weeks.

Scherer et al.¹⁴ in a study of 330 patients who had fracture treatment reported that the peak value of CRP was observed on the second postoperative day, and also depended on the region of trauma.

In our study the rise, decline and stabilization of CRP levels followed a similar course in groups A and B. However, in group C steady increase until the 14th day may be explained by the longer duration of wound healing in patients with diabetes¹⁵. There was a correlation between age of the patients and length of the incision in contrast to the aforementioned studies of CRP levels.

HPT is an alpha-2 globulin. Its major action in the plasma is to bind free haemoglobin. Most bacteria require free iron to proliferate actively. HPT functions as a natural bacteriostat by removing free iron from the plasma. HPT level increases in acute and chronic infections, malignancies and necrosis^{7,13}.

In their study of 12 patients with major abdominal trauma, Stahl³ showed a severe depression in the level of HPT on days 1 and 2. On the other hand elective general surgery operations inflicting fewer traumas caused increased synthesis. Although the operations in their series did not involve the musculoskeletal system they represented trauma to soft tissues. In our study increase in HPT levels on 3rd day in group B and 5th day onwards in

group C may be explained by the stimulatory effect of the trauma. The fall in the level on the third day in group C may be explained by the binding of large amounts of hemoglobin present after amputations. The stable level in group A may be due to the intermediary effect of the trauma which neither increases nor decreases.

Erythrocyte sedimentation rate is an uncertain indicator of postoperative infection because the decline to the preoperative value is variable¹³. Since it is influenced by fibrinogen concentration it could be considered as an indirect indicator of acute phase reaction. The shape and number of erythrocytes, immunoglobulin concentration, leucocytosis and some drugs modify ESR².

In their study of 20 lumbar discectomy, Jönsson et al.¹⁶ remarked that ESR reached its peak value on the 4th postoperative day and normalized after 2 weeks for majority of the patients.

Jensen et al.¹³ reported that ESR elevation occurred at the 6th day after lumbar disc surgery and was followed by a slow decrease. After 12 weeks some patients still had an elevated ESR.

Larsson et al.⁹, following serial measurements after uncomplicated elective orthopaedic surgery, determined that ESR reached to peak levels about five days after surgery followed by a slow and irregular decrease, remaining still elevated 42 days after surgery.

In our study, ESR in groups A and C did not exhibit any statistical difference. In group B it started to increase on the first post operative day and remained elevated for 14 days. Longer incision and a wider exposure required for open reduction and internal fixation may be incriminated for the increased ESR in group B.

Although in our study most frequently performed orthopaedic surgical procedures at our institution were included, obviously it did not cover all aspects of orthopaedic surgery. Thus further studies involving other set of operations are required. Although they were sufficient for statistical analysis, the number of the cases and samples obtained in each group could be enlarged and the levels determined after a longer period of follow up.

Our study was concerned with the physiological response of the acute phase proteins. The fluctuation of their levels in infected groups could provide a reliable means of diagnosis of infection.

In conclusion, deviation from the standard levels of APP and ESR, after surgical procedures warrant detailed investigation for postoperative complications.

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