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Original Article

Induction of acute phase in response to tacrolimus induced hepatotoxicity

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Abstract
Activation of inflammatory pathways may contribute to the beginning and progression of many complications in the body. Current study was aimed to evaluate the status of acute phase response and inflammation by examining the alterations in the level of acute phase proteins due to the toxic effect of an immunosuppressive drug (tacrolimus). Aqueous suspension of tacrolimus powder (3 mg/ml) was orally given to four experimental groups of wistar rats. Control group was provided with normal drinking water and dissections were done after 6, 12, 24 and 48 h of tacrolimus dose. Densitometric analysis revealed considerable elevated level of some positive acute phase proteins, specifically C-reactive protein, haptoglobin and ceruloplasmin. While albumin and transferrin levels were found to be low as compared to control animals. The results obtained in this study revealed that tacrolimus resulted in the onset of acute phase response in experimental animals owing to its toxic potential.
Keywords: Acute phase response, hepatotoxicity, immunosuppression, proteins, SDS-PAGE, tacrolimus

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INTRODUCTION

Among the assorted general responses against injury or infection, the acute phase response is a complex systemic early hours defense system incited by any mediator that directs to loss of the integrity of the tissue (Malik *et al.*, 2011; Sheikh *et al.*, 2007). APR is aimed to limit the environs of damage on one aspect and to eliminate, or at least isolate, the damaging agent on the opposite aspect (Sheikh *et al.*, 2007). The APR is thought to be initiated, regulated and amplified by a complex activated cytokine cascade. Pro-inflammatory cytokines, such as tumor necrosis factor α, interleukin-l and interleukin-6, induce a cascade of other mediators which act to up regulate acute phase proteins gene expression in the liver (Sheikh *et al.*, 2006). Within a few hours after infection the pattern of protein synthesis by the liver is drastically altered. Therefore one of the major characteristics of the APR is the modulation of hepatic protein synthesis resulting in a rapid increase or decrease of a distinct subset of plasma proteins known as acute phase proteins (APPs). Serum proteins whose concentration changed at least 25% in response to inflammation are termed as APPs. In view of that, APPs are the sensitive markers for estimating the grade of inflammation (Gruys *et al.*, 2006). During the last few decades there has been increasing interests in the possibility that transplantation may be an effective technique for the treatment of various diseases (Bakari *et al.*, 2012). Generally organ transplantation has become a formidable respite for patients with end stage organ failure and transplantation medication plot is one of the most challenging and complex area of modern medicine system (Elizabeth, 2008). Major limitation for organ transplantation is organ rejection, which is an adaptive immune response caused by the activation of T-cells. To overcome this problem, immunosuppression is mediated in the patients with transplanted organs for suppressing the immunity to make the transplant stabilized in the body (Kanamoto *et al.*, 2011). Tacrolimus (previously known as FK 506) is an immunosuppressive drug that is widely used both as a primary immunosuppressive agent and as rescue therapy in patients undergoing organ transplantation (Paterson and Singh, 1997). In current medical setting, tacrolimus is preferred to other conventional drugs such as Cyclosporine A (CSA) owing to its high potency

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to suppress the T-cell activation along with its fibro genetic and hemodynamic effects (Al-Harbi *et al.*, 2014) but at the same time tacrolimus is also reported as the reason of many secondary complications in the body (Plosker and Foster, 2000). It is well known that the immunosuppressive effect of tacrolimus appears to depend on calcineurin inhibition. As a result of calcineurin inhibition, tacrolimus alters multiple biochemical processes in a variety of cells besides (Dumont, 2000; Haas and Mayer, 1997; Plosker and Foster, 2000). The current experimental work was carried out to evaluate the status of APR and inflammation by examining the alterations in the level of APPs owing to the toxic effect of tacrolimus.

MATERIAL AND METHODS

Animals

Male Wistar rats (*Rattus norvegicus*) of twelve to fourteen weeks of age and 250 ± 25g of body weight were used in this study. The rats used in this study were from the breeding colony at the Department of Zoology, University of the Punjab, Lahore. They all were caged in a fully aerated and clean environment at room temperature 23-25°C. All the experimental animals were supplied with the normal Rat Chow (20% crude proteins) and fresh water *ad libitum.* Nine animals were used as control (-ve control) and thirty six as experimental (nine for each time point).

Dose preparation & administration

Animals were divided into five equal sized groups: one group is control and the other four groups of experimental animals. Dose of tacrolimus was prepared by diluting 3mg of tacrolimus powder in 1ml of distilled water and vortex for 30 seconds to make homogenous suspension. Aqueous suspension of tacrolimus was given to experimental animals orally (using feeding catheter) and animals were dissected 6, 12, 24 and 48h after suspension administration.

Blood sampling and processing

All the animals were anesthetized by using equal ratio of ketamine plus pyrogen free water mixture intraperitonealy. Dissections were done in aseptic maintained conditions to draw the blood through direct cardiac puncture by using sterilized disposable syringes (Becton Dickinson, Private Ltd.). 5ml blood of each animal was collected in clotting factor free vacutainers for serum separation. Blood samples were centrifuged at 4000rpm for 20 minutes after 2-3 h incubation at room temperature. After that serum was collected in new labeled eppendorf cups and was stored at -20°C, till further use.

SDS-PAGE for evaluation of serum proteins variations

Serum protein profiling was measured through Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) by using the Bio-Rad Mini-Protean® tetra cell gel apparatus. 1 mm in thickness, discontinuous gel composed of 12% resolving gel and 5% stacking gel, was used to resolve low molecular weight proteins and electrophoresis was carried out according to the method of Laemmli (Laemmli, 1970). Fermentas PageRuler™ unstained protein ladder # SM0661 was used as standard. The gels were run in 0.1% SDS, 250 mM glycine, 25 mM Tris, pH 8.0 at 65 V awaiting the dye get to the resolving gel, after that at 120 V until the dye reached the base of the resolving gel. Proteins were stained using Coomassie Brilliant Blue R-250 and destained in 70:50:1000 acetic acid:methanol:water (v:v:v). The gel was scanned, analyzed and the densitograms were drawn. Electrophoretically alienated protein fractions were quantified by using Total Lab Quant v11.5.

RESULTS

In our present study protein marker purchased from Fermentas used as a reference standard to identify different proteins on the basis of their molecular size. Difference bands present in the gel and variations in the normalized volume of all sera samples against molecular weight are shown in Fig. 1. The comparative analysis of serum protein profile showed an increasing trend in CRP level as compared to control group. Haptoglobin (38.57 KDa) level was unchanged after 6h but an increasing trend in the level of this APP was observed after 12 and 48h of treatment. Another positive APP, Ceruloplasmin showed elevated density after 12 and 48h in comparison to control group. *Antithrombin* III which is a single chain glycoprotein with a *molecular weight* of 52.24KDA was found to be decline after 12 and 24h time point. Albumin level was initially found

to be increase at 12h time point but at 48h a significant decline in albumin level was observed. trasferrin (76.37 KDa) level decrease at 12, 24 and 48 h as compared to control (Fig. 2).

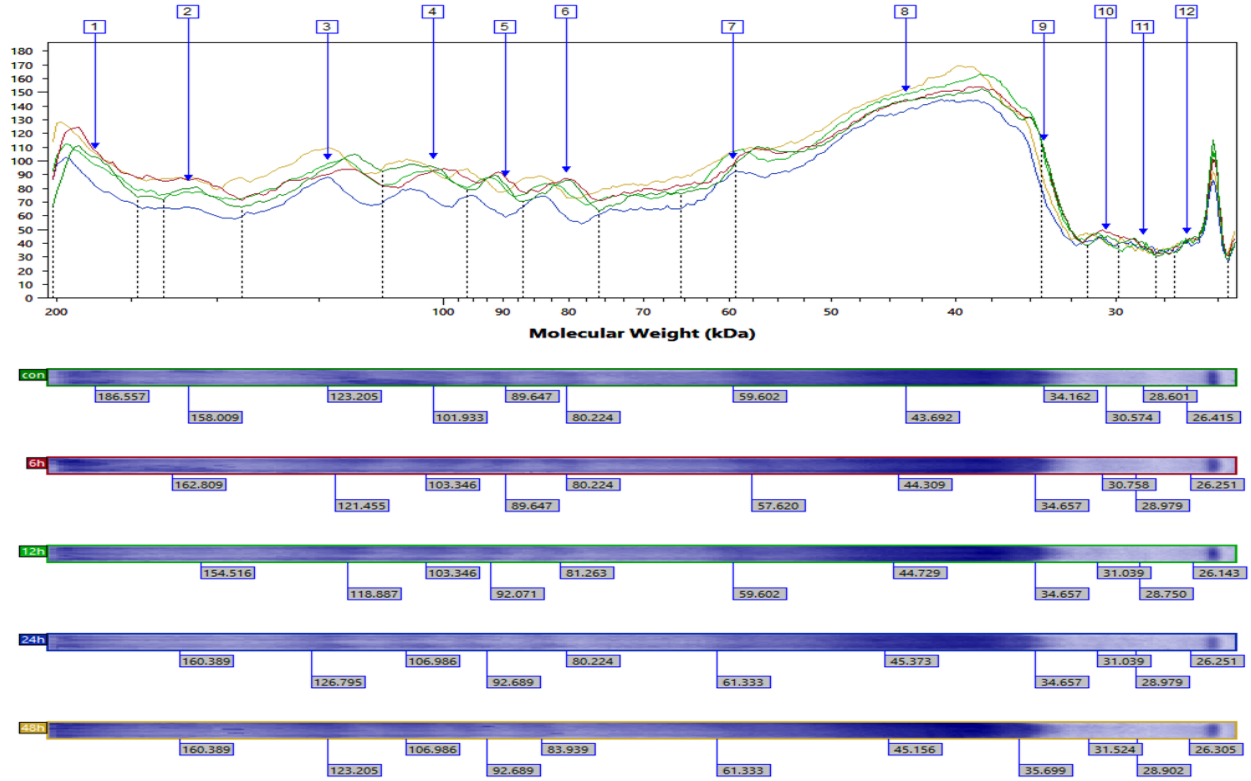

Figure 1: Representation of all lanes present in the gel. Electropherogram showing the variations in the normalized volume of all serum samples against molecular weight (KDa).

DISCUSSION

The comparative study of serum protein profile indicate different levels of variations in different serum proteins (Akhtar *et al.*, 2014). APPs are defined as proteins whose serum concentration changed at least 25% in response to APR and inflammation. APPs are alienated in to positive APPs and negative APPs. Cytokines are mainly involved in activating APR (Polepalle *et al.*, 2015). In our study density of CRP, haptoglobin and ceruloplasmin was found to be increased as compared to control group, consequently showing the onset of APR. CRP is closely linked to IL-6 which is an important pro-inflammatory cytokines (Gruys *et al.*, 2006). Within 24 to 48h after acute tissue damage, CRP level rise in serum or plasma (Yoshihiro *et al.*, 2003). CRP level associated with increased risk of atherosclerosis and many other complications (Effie *et al.*, 2006). In most reports, tacrolimus hepatotoxocity has been characterized by elevated level of hepatocellular enzymes, either alone or with minimal cholestasis and hyperbilirubinemia (Taniai *et al.*, 2008). Increased in the level of haptoglobin, as revealed in this study, is an outcome of tacrolimus induced cell damage which affected synthetic capacity of the liver. Recent studies reported that serum level of cruloplasmin increased in both acute and chronic conditions as observed in periodontitis patients (Harshavardhana *et al.*, 2013). So, ceruloplasmin is also categorized as acute phase reactant. Ceruloplasmin have an effect on the iron uptake into the cells due to its ability to change the iron ferrous form into ferric form. Changes in serum ceruloplasmin are often accomplished by alterations in serum iron level (Akhtar *et al.*, 2014). Major serum iron transport proteins, transferrin and albumin are shown to be decreased at 24 and 48h time point. Malnutrition and inflammation both lessen albumin concentration by slowing its rate of synthesis (Karas *et al.*, 2015). It appears that

two major down regulators of these acute phase reactants are IL-6 and TNF-α. IL-6 and TNF-α create an environment having trouble in protein, lipid and carbohydrate metabolism (Emami and Zaerin, 2015).

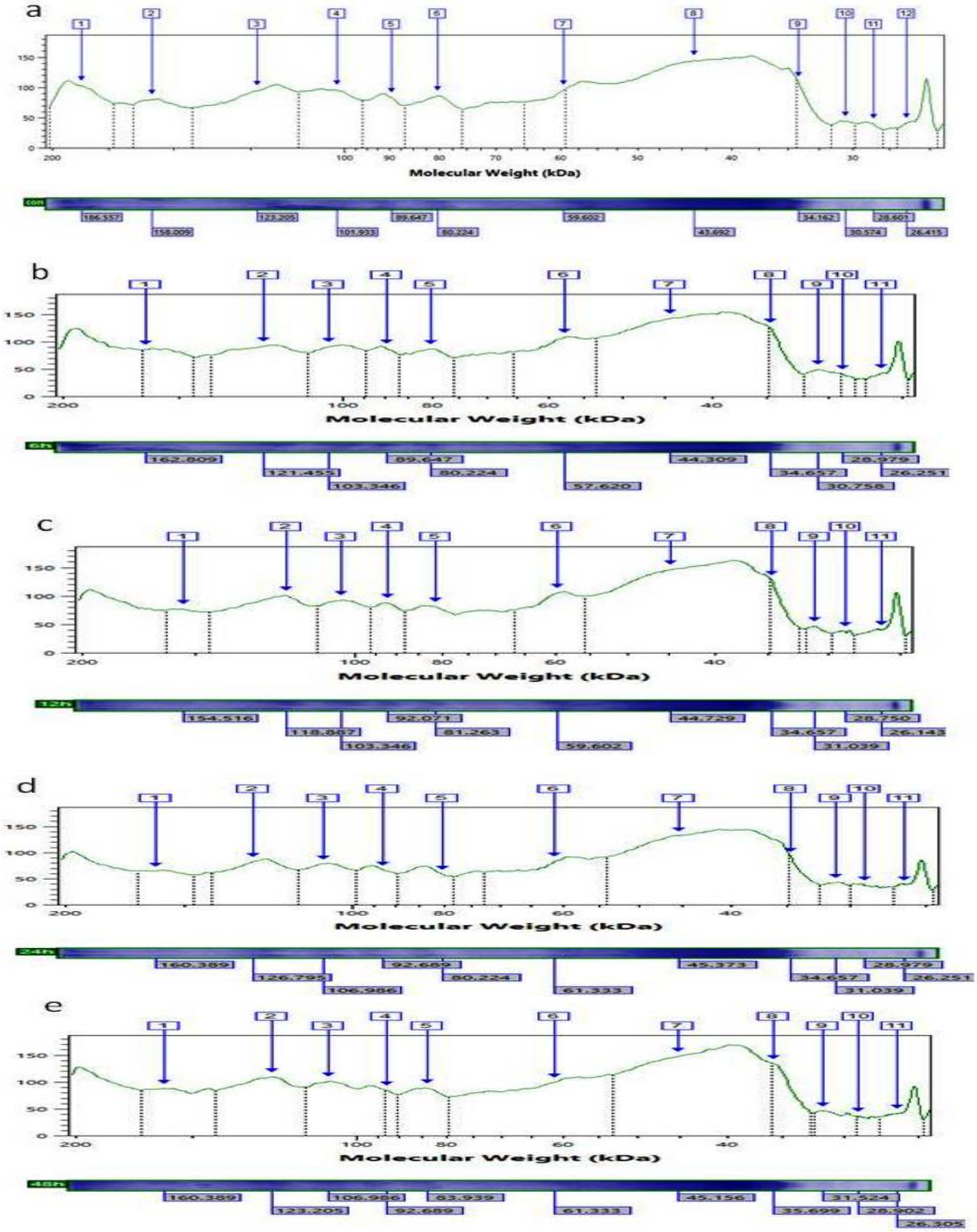

Figure 2: One-dimensional SDS-PAGE and densitometric comparison of serum proteins level after 6h (b), 12h (c), 24h (d) and 48h (e) of tacrolimus dose in comparison to control group (a)

CONCLUSION

Taken together these findings, we can conclude that tacrolimus resulted in the onset of acute phase response owing to its toxic potential. Tacrolimus induced inflammation changed the serum level of different acute phase proteins. Tacrolimus must be used with some other drugs to minimize its toxic effects or some more efficient drugs to be discovered with minimum side effects. We necessitate novel strategies for a healthier management of transplant receiver.

REFERENCES

AKHTAR, T., SHEIKH, N., ABBASI, M.H. AND ABBAS, A.S., 2014. Effects of Nerium oleander leaves extract against Thioacetamide induced Liver injury. *Biologia*, **60**(1): 37-41.

AL-HARBI, N.O., IMAM, F., AL-HARBI, M.M., IQBAL, M., NADEEM, A., SAYED-AHMED, M.M., ALABIDY, A.D. AND ALMUKHALLAFI, A.F., 2014. Olmesartan attenuates tacrolimus-induced biochemical and ultrastructural changes in rat kidney tissue. *Biomed. Res Int*, **2014**: 607246.

BAKARI, A.A., ABBO JIMETA, U.S., ABUBAKAR, M.A., ALHASSAN, S.U. AND NWANKWO, E.A., 2012. Organ Transplantation: Legal, Ethical and Islamic Perspective in Nigeria. *Niger. J Surg.*, **18**(2): 53-60.

DUMONT, F.J., 2000. FK506, an immunosuppressant targeting calcineurin function. *Curr. Med. Chem.*, **7**(7): 731-748.

EFFIE, I., TANNAZ, M. AND ANNA, D., 2006. Effect of periodontal treatment on serum C-reactive protein levels: a systematic review and meta-analysis. *Journal of Periodontology*, **77**(10): 1635-1642.

ELIZABETH, I., 2008. Mechanism of cellular rejection in transplantation. *Pediatric Nephrology*, **25**(1): 61-74.

EMAMI, A.M. AND ZAERIN, O., 2015. Role of Serum Interleukin 6, Albumin and C-Reactive Protein in COPD Patients. *Tanaffos.*, **14**(2): 134-140.

GRUYS, E., TOUSSAINT, M.J., NIEWOLD, T.A., KOOPMANS, S.J., VAN, D.E. AND MELOEN, R.H., 2006. Monitoring health by values of acute phase proteins. *Acta Histochem.*, **108**(3): 229-232.

HAAS, M. AND MAYER, G., 1997. Cyclosporin A-associated hypertension--pathomechanisms and clinical consequences. *Nephrol. Dial. Transplant.*, **12**(3): 395-398.

HARSHAVARDHANA, B., RATH, S.K. AND MUKHERJEE, M., 2013. Evaluation of serum ceruloplasmin in aggressive and chronic periodontitis patients. *J Indian Soc. Periodontol.*, **17**(3): 333-337.

KANAMOTO, M., IMURA, S., MORINE, Y., IKEMOTO, T., MORI, H., ARAKAWA, Y., HANAOKA, J., TOKUNAGA, T., SUGIMOTO, K., NISHI, M. AND SHIMADA, M., 2011. De novo lung cancer diagnosed 32 months after liver transplantation: Report of a case. *Surgery Today*, **41**(9): 1280-1283.

KARAS, P.L., GOH, S.L. AND DHITAL, K., 2015. Is low serum albumin associated with postoperative complications in patients undergoing cardiac surgery? *Interact. Cardiovasc. Thorac. Surg.*, **21**(6): 777-786.

LAEMMLI, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**(5259): 680-685.

MALIK, I.A., NAZ, N., SHEIKH, N., KHAN, S., MORICONI, F., BLASCHKE, M. AND RAMADORI, G., 2011. Comparison of changes in gene expression of transferrin receptor-1 and other iron-regulatory proteins in rat liver and brain during acute-phase response. *Cell Tissue Res*, **344**(2): 299-312.

PATERSON, D.L. AND SINGH, N., 1997. Interactions between tacrolimus and antimicrobial agents. *Clin Infect. Dis*, **25**(6): 1430-1440.

PLOSKER, G.L. AND FOSTER, R.H., 2000. Tacrolimus: a further update of its pharmacology and therapeutic use in the management of organ transplantation. *Drugs*, **59**(2): 323-389.

POLEPALLE, T., MOOGALA, S., BOGGARAPU, S., PESALA, D.S. AND PALAGI, F.B., 2015. Acute Phase Proteins and Their Role in Periodontitis: A Review. *J Clin. Diagn. Res*, **9**(11): ZE01-ZE05.

SHEIKH, N., DUDAS, J. AND RAMADORI, G., 2007. Changes of gene expression of iron regulatory proteins during turpentine oil-induced acute-phase response in the rat. *Lab Invest*, **87**(7): 713-725.

SHEIKH, N., TRON, K., DUDAS, J. AND RAMADORI, G., 2006. Cytokine-induced neutrophil chemoattractant-1 is released by the noninjured liver in a rat acute-phase model. *Lab Invest*, **86**(8): 800-814.

TANIAI, N., AKIMARU, K., ISHIKAWA, Y., KANADA, T., KAKINUMA, D., MIZUGUCHI, Y., MAMADA, Y., YOSHIDA, H. AND TAJIRI, T., 2008. Hepatotoxicity caused by both tacrolimus and cyclosporine after living donor liver transplantation. *J Nippon Med. Sch*, **75**(3): 187-191.

YOSHIHIRO, L., FUSANORI, N., YOSHIHIKO, S., KAZU, T., MIKINAO, K., SHOGO, T. AND YOJI, M., 2003. Antimicrobial Periodontal Treatment Decreases Serum C-Reactive Protein, Tumor Necrosis Factor-Alpha, But Not Adiponectin Levels in Patients with Chronic Periodontitis. *Journal of Periodontology*, **74**(8): 1231-1236.