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Niveles de ICAM-1, P-Selectina, Leucotrieno B4 y Mieloperoxidasa en pacientes con trasplante hepático.

ICAM-1, P-Selectin, Leucotriene B4, and Myeloperoxidase levels in patients with Liver Transplantation.

Cordero-Pérez, P.a, Torres-González, L.a, Camara-Lemarroy, C.a, Cepeda-de la Garza, M.a, De Luna-Saldivar, M.a, Zapata-Chavira, H.b, Cura-Esquivel, I.a, Casillas-Ramirez A.d, Pérez-Rodríguez, E.b, Escobedo-Villarreal, M.b, Rivas-Estilla, A.M.c, Muñoz-Espinosa, L.E.a.b

a.Liver Unit, Department of Internal Medicine. b.Department of Transplants. c.Department of Biochemistry and Molecular Biology; School of Medicine and University Hospital "Dr. José E. González"; Universidad Autónoma de Nuevo León d Hospital Regional de Alta Especialidad de Ciudad Victoria "Vicentenario 2010". Ave. Gonzalitos 235 Mitras Centro C.P.64460, Monterrey, Nuevo León, México.

Resumen

Objetivo: Determinar las concentraciones séricas de las moléculas de adhesión intracelular 1 (ICAM-1), P-selectina, leucotrieno B4 (LTB 4), y mieloperoxidasa (MPO) en pacientes con trasplante hepático ortotópico (THO). Materiales y métodos: ICAM-1, P-selectina, LTB 4, y los niveles de MPO se determinaron en suero de pacientes con THO por ensayo inmunoenzimático. Resultados: ICAM-1, P-selectina y LTB 4 estuvieron mas elevados en el suero de los receptores con THO en comparación con los controles sanos. Además, ICAM-1 y los niveles de LTB 4 fueron significativamente mayores en los receptores con alteración de los niveles de transaminasas, en comparación con aquellos con valores normales de transaminasas. Conclusiones: Algunos marcadores inflamatorios parecen estar asociados con la función del injerto en los receptores de THO. La vigilancia inmunológica con marcadores inflamatorios en suero puede ser útil para la clínica.

Palabras clave: inflamación, moléculas de adhesión, trasplante de hígado.

Abstract

Aim: To determine the serum concentrations of inter-cellular adhesion molecule 1 (ICAM-1), P-selectin, leukotriene B4 (LTB4), and myeloperoxidase (MPO) in patients with orthotopic liver transplantation (OLT). Materials and methods: ICAM-1, P-selectin, LTB4, and MPO levels were determined in serum from patients with OLT by enzyme-linked immunosorbent assay. Results: ICAM-1, P-selectin and LTB4 were elevated in the serum of OLT recipients compared with normal controls. Additionally, ICAM-1and

LTB4 levels were significantly higher in recipients with altered transaminase levels, compared to those with normal transaminase values. Conclusions: Some inflammatory markers seem to be associated with graft function in OLT recipients. Immunological monitoring with serum inflammatory markers might be of clinical use.

Keywords: adhesion molecules, inflammation, liver transplantation.

I. Introduction.

Liver transplantation (LT) is considered the only therapeutic option for end-stage liver disease (1). During the early postoperative period after orthotopic LT (OLT), there is an increase in circulating inflammatory cytokines and adhesion molecules, probably induced by ischemia/reperfusion. However, the clinical predictive value of the determination of these alterations remains unclear (2,3) and few studies have been concentrated on the evaluation of cytokine or adhesion molecule profiles at later time periods after LT. Recently, it has been shown that alterations in inflammatory mediator and adhesion molecule levels, among other immunological alterations, persist many months after LT in clinically stable patients (4). Inflammatory mediators and adhesion molecules are known to play many important roles in graft rejection and function. The release of circulating adhesion molecules, such as inter-cellular adhesion molecule 1 (ICAM-1), is a prominent feature of graft rejection after LT, participating in the activation and infiltration of effector cells (5).

A controversial topic is the question of how neutrophils actually accumulate within the liver. The classical theory argues that the increased expression of adhesion molecules, such as ICAM-1 and P-selectin, plays a key role in neutrophil collection and the subsequent liver damage associated with I/R. By contrast, it has also been reported that neutrophil accumulation in the liver after I/R occurs independently of the upregulation of either ICAM-1 or P-selectin. This alternative theory proposed that mechanical factors such as active vasoconstriction, vascular cell swelling and injury, and reduced membrane flexibility after activation of neutrophils are involved in trapping these leukocytes into the sinusoids (6).

Neutrophils are considered important effectors of graft rejection, and increased serum myeloperoxidase (MPO) levels, an enzyme of neutrophilic granulocytes, have been shown also to be associated with rejection or infection after LT (7). Other inflammatory mediators, such as leukotrienes, have also been implicated in the processes of graft injury and rejection after LT in experimental studies (8,9).

In acute inflammation, such as that observed following limb I/R, de novo LTB4 generation has been associated with PMN activation and distant tissue injury (10). MPO is an enzyme stored in azurophilic granules of polymorphonuclear neutrophils and macrophages, and released into extracellular fluid in the setting of inflammatory process. MPO is involved in acute and chronic inflammatory diseases (11).

It would be of great interest to find immunological markers that might predict the clinical course of patients that underwent LT. In this study, our aim was to determine the serum concentrations of ICAM-1, P-selectin, MPO and leukotriene B4 (LTB4), 6 months after patients that had underwent OLT, and compare them to those from a group of normal healthy subjects. We also wanted to see if these values had any association with clinical parameters, such as liver function tests, with particulate emphasis on aspartate transaminase (AST),alanine transaminase (ALT) and gamma glutamyl transpeptidase (GGT), which have been shown to be useful as predictive factors for survival in LT patients (12).

II. Materials

Serum concentrations of ICAM-1, P-selectin, LTB4 and MPO.

Serum ICAM-1 (Chemicon International), P-selectin (Biosource), LTB4 (Cayman Chemical) and MPO (Assay Designs) levels were measured by enzyme-linked immunosorbent assay according to the manufacturer´s instructions (ELISA ASYS Hitech GmbH Expert Plus UV G020151).

III.Methods

Population and Experimental Design

The study was carried out from October 2005 to May 2007. Approval was obtained from the local ethics committees and the study was conducted in accordance with the Helsinki Declaration. All patients provided consent prior to their inclusion in the study. The study population included: A)

13 patients with OLT (>6 months post-transplant; 6 women and 7 men, 21 to 71-years-old); and B) a control group of 13 healthy subjects (control group, CG) without liver disease (4 women and 9 men between 22 and 63-years-old). OLT patients were selected irrespective of etiology, and immunosupression was based on cyclosporin-sirolimus-MM-steroid combinations. We carried out liver function tests and determinations of MPO, LTB4, ICAM-1 and P-selectin in all subjects, and both groups were compared. Additionally, group OLT was divided into subgroups, consisting of patients with normal transaminase values (OLT1) and patients with altered transaminase values (OLT2).

Liver Function Tests

Five milliliters of blood were obtained, coagulated at room temperature, then centrifuged at 3000 rpm for 10 minutes, separated into aliquots and stored at -80°C. Liver function tests (LFT) were measured by dry chemistry according to the manufacturer's instructions (System Vitros Chemistry DT60II and DTSCII by Johnson and Johnson).

Statistical Analysis

Results are expressed as a mean±standard deviation (SD). Statistical comparisons between the groups were done using an unpaired two-tailed Student´s test or ANOVA test with multiple comparisons with Tukey *ad hoc* test where appropriate. A *p*-value less than 0.05 was considered statistically significant. All statistical analyses were performed using SPSS v8.0 software.

IV. Results

Liver function tests

Statistically significant higher concentrations of the following enzymes was found in group OLT compared to CG: AST (p=0.001), ALT (p=0.004), ALP (p=0.03), GGT (p=0.02) and LD (p=0.01). There was no difference in age between groups.

Inflammatory parameters

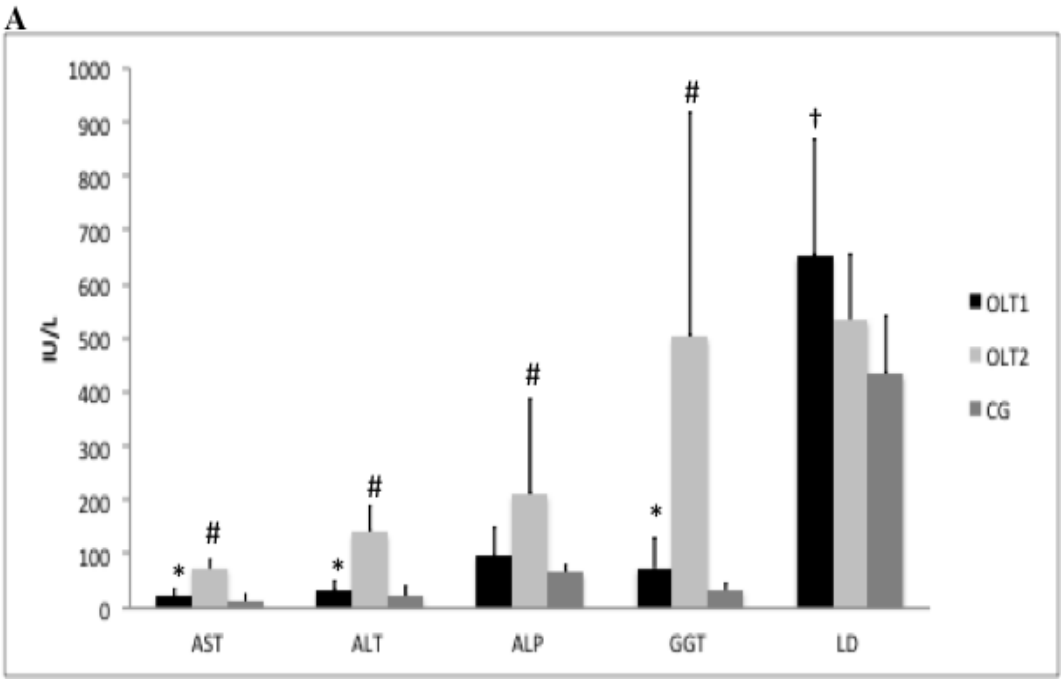
ICAM-1, P-selectin and LTB4 serum levels were significantly higher in OLT group compared to CG group. ICAM-1: OLT (51.69 ± 29.60 ng/mL) *vs* CG (24.22±9.58 ng/mL) (p=0.004). P-selectin: OLT (14.7±6.86 ng/mL) *vs* CG (7.59 ± 3.75 ng/mL) (p=0.003). LTB4: OLT (5.95 ± 0.18 pg/mL) *vs* CG (5.86 ± 0.06 pg/mL)(p=0.044). We found no difference in serum MPO concentrations between groups.

Variable	Group OLT (n=13)	Control group (n=13)	*P*
Age (years)	51 ± 12	49 ± 10	0.613
AST (IU L-1)	48 ± 30	14 ± 13	0.001
ALT (IU L-1)	82 ± 66	23 ± 18	0.004
ALP (IU L-1)	150 ± 135	67 ± 14	0.037
GGT (IU L-1)	272 ± 353	35 ± 14	0.023

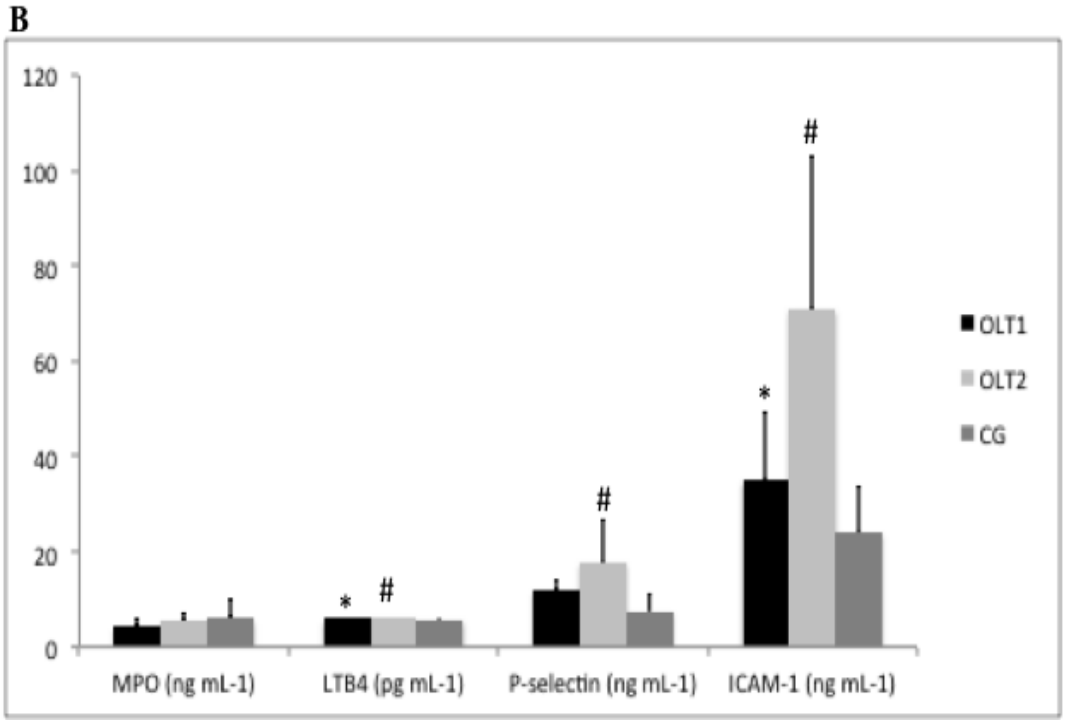
LD (IU L-1)	600 ± 184	435 ± 108	0.010
TB (mg dL-1)	0.15 ± 0.19	0.11 ± 0.03	0.405
MPO (ng mL-1)	4.76 ± 1.87	5.89 ± 4.17	0.382
LTB4 (pg mL-1)	5.95 ± 0.18	5.86 ± 0.06	0.044
P-selectin (ng mL-1	14.70 ± 6.87	7.59 ± 3.75	0.003
ICAM-1 (ng mL-1)	51.69 ± 29.6	24.22 ± 9.58	0.004

Table 1. Analysis of liver enzymes and inflammatory mediators of patients with OLT and controls

Values are the mean ± SD; AST, aspartate transaminase; ALT, alanine transaminase; ALP, alkaline phosphatase; GGT, gamma glutamiltranspeptidase; LD: lactate dehydrogenase; TB: total bilirubin; MPO: myeloperoxidase ; LTB4:leukotriene B4 ; ICAM-1: inter cellular adhesion molecule-1 ; IL-1b: interleukin-1beta; P: P-value.



Graphic 1. A.)Analysis of liver enzymes according to altered transaminases in patients with OLT and controls. B.) Analysis of liver inflammatory mediators according to altered transaminases in patients with OLT and controls.



Values are the mean ± SD; AST, aspartate transaminase; ALT, alanine transaminase; ALP, alkaline phosphatase; GGT, gamma glutamiltranspeptidase; LD: lactate dehydrogenase; TB: total bilirubin; MPO: myeloperoxidase ; LTB4:leukotriene B4 ; ICAM-1: inter cellular adhesion molecule-1 ; IL-1b: interleukin-1beta; * OLT-1 vs OLT-2, # OLT-2 vs control group, † OLT-1 vs control group; P: ANOVA one way, P-value.

OLT Subgroups

When OLT patients were divided into subgroups according to LFT alterations, the following grouping characteristics were obtained: group OLT2 had significantly higher serum concentrations of the following enzymes compared to OLT1: AST (p<0.001), ALT (p<0.001), and GGT (p=0.002). There was a trend toward higher ALP levels as well (p=0.08). There was no difference in age between the subgroups, and no difference in enzyme levels between OLT1 group and CG.

Additionally, patients in group OLT2 had significantly higher serum concentrations of LTB4 and ICAM-1 compared to group OLT1. ICAM-1: OLT1 (35.16 ± 14.34 ng/mL) *vs OLT2* (70.98± 32.07 ng/mL) (p=0.004). LTB4: OLT1 (5.89 ± 0.20 pg/mL) *vs* OLT2 (6.06 ± 0.11 pg/mL)(p=0.037). These values were not significantly different from those in CG. There was no difference in MPO levels between subgroups, or between any subgroup and CG. P-selectin levels in CG were significantly lower than in OLT2 (17.88 ± 9.25 pg/mL) but there was only a trend towards higher levels in OLT2 compared to OLT1 (11.97 ± 2.02 pg/mL)(p=0.1).

V. Discussion

It is known that inflammatory mediators and adhesion molecules participate in graft rejection and are also elevated after infection, and may reflect the immunological activity during post-transplantation. Their measurement in serum has been proposed as a way to monitor the immunological state, in order to detect rejection, infection or function, and molecules such as ICAM-1 have been shown to be of value during the early postoperative period (13,14). Indeed, elevated serum levels of adhesion molecules such as ICAM-1are closely associated to the initial stages of hepatic allograft rejection, probably mediating alterations in endothelial function and leukocyte activation (15,16). Additionally, adhesion molecules are up regulated in the liver during all kinds of inflammatory reactions, and play an important role in liver function (17). However, little is known of the role of circulating adhesion molecules in later stages of the post-transplant period. In our study, we found elevated levels of ICAM-1 and P-selectin in OLT recipients when compared to healthy controls, and although we did not associate adhesion molecule levels with rejection, we did find that elevated levels of ICAM-1 were present in OLT patients with altered LFT.

Although leukocytes are thought to be the main cellular mediators of rejection, neutrophils are also known to play an important role, and neutrophil infiltrates are a common pathological finding in liver biopsies during rejection (18). A recent study showed that MPO, an enzyme marker of neutrophil activity, could also be used to assess OLT recipients for the presence of early infection or rejection (7). However, we did not find differences in MPO levels between OLT recipients and healthy subjects, nor an association of MPO and LFT. This could be due to the lack of established cases of rejection in our patients.

There are plenty of experimental animal studies that have implicated leukotrienes in the immune response after liver transplantation. Local expression of lypooxygenase is increased in liver grafts, suggesting alterations in leukotriene synthesis that might lead to tissue and systemic injury (19). Plasma levels of LTB4 have been found to be closely correlated to rejection, suggesting an important role for this molecule in the immunological response to the allograft (8), and LTB4 inhibition has also been shown to have a beneficial effect on liver allografts (20). Moreover, the biological half life of LTB4 is know to depend on adequate liver function (21). In our study, we found elevated LTB4 levels in OLT recipients with altered LFT. This might reflect both liver inflammation and inadequate LTB4 metabolism.

VI. Conclusions

In conclusion, our results show that patients who months before underwent OLT have altered LFT and elevated serum concentrations of ICAM-1, P-selectin and LTB4 when compared to normal healthy subjects. We also found that when OLT patients are divided into those with or without LFT alterations, elevated levels of ICAM-1and

LTB4 are present in patients who have altered LFT. This suggests an association between a persisting alteration in the serum levels of these mediators and impaired liver function or ongoing liver inflammation. Given that these mediators are known to play a role in graft rejection, their measurement could turn out to be of clinical value. However, larger studies, as well as associations between serum concentrations of these mediators and clinical outcome, instances of rejection or infection are needed in order to establish a precise indication.

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