EXPERIMENTAL STUDY

Are the cytokines and chemokines important for the early diagnosis of mesenteric ischemia?

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ABSTRACT

BACKGROUND: Acute mesenteric ischemia (AMI) has very high mortality and morbidity rates, and the most important factor in the prognosis of AMI is the duration of ischemia. This study aims to evaluate the changes of these markers according to the ischemia duration and possible use of cytokines and chemokines in the early diagnosis of AMI.

METHODS: Twenty-one male Sprague–Dawley rats were divided into three equal groups. The Superior Mesenteric Artery and Superior Mesenteric Vein were tied tightly and exposed to ischemia for 2 h for Group 1 and 6 h for Group 2. There was no intervention for Group 3, and it was selected as a control group. Intracardiac blood samples were collected after 2 h in Group 1 and 6 h in Groups 2 and 3. The IL-1α, 1β, 6, 10, 12p70, 17A, 18, 33, CXCL1/KC, CCL2/MCP-1, GM-CSF, IFN-γ, and TNF-α were measured using flow cytometry.

RESULTS: Significant differences were observed between the groups in IFN-γ, CXCL1, MCP1, TNF-α, and IL-6 parameters. In the correlation analyses performed according to the mesenteric ischemia time, a very strong correlation was observed in CXCL1, as well as a strong level for MCP-1, TNF-α, and IL-6. Furthermore, a moderate level of correlation was found in IFN-γ, IL-10, and IL-18.

CONCLUSION: The increased levels of CXCL1, MCP-1, TNF-α, and IL-6, which had a high correlation with the duration of ischemia in patients with intestinal ischemia, may help clinicians with diagnoses and treatment decision-making.

Keywords: Acute mesenteric ischemia; chemokine; cytokine.

INTRODUCTION

Acute mesenteric ischemia (AMI) is a disease with a low incidence but very high mortality and morbidity rates.[1] The most important factor in AMI's prognosis is ischemia's duration.[2] With the deterioration of intestinal blood flow, deterioration in the intestinal flora and intestinal inflammation begin to develop.[3] Eliminating the cause of ischemia within the first 6 h of the onset is important in improving the prognosis. As the duration of ischemia increases, bacterial and endotoxin absorption, which cause an inflammatory response, increase. With necrosis that develops after ischemia, sepsis, acidosis, septic shock, and death occur.[2]

It is not always easy to diagnose AMI, because it is rarely observed and progresses with non-specific symptoms. With the early diagnosis and early initiation of treatment, mortality rates may decrease.[4]

For the diagnosis of AMI, blood markers with high sensitivity and specificity have not been identified in studies performed so far.[5–8] In cases where the diagnosis is suspected, besides the clinical examination, routine blood tests (such as complete blood count, D-Dimer, and blood gas) and imaging methods assist the diagnosis. Although computed tomography angiography has a very high specificity in diagnosing AMI, it has disadvantages such as high-level radiation and contrast-induced nephropathy.[9]

In new biomarker studies for the diagnosis of AMI, concentrations of D-lactate, intestinal fatty acid-binding protein, ischemia modified albumin, α-glutathione S-transferase, and

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citrulline were associated with intestinal barrier dysfunction. It is stated that it can often be abnormal; therefore, it can be used to diagnose acute intestinal diseases.[10]

Our study aims to evaluate the usability of pro-inflammatory and anti-inflammatory cytokines and chemokines in the early diagnosis of AMI and the changes of these markers according to the ischemia duration.

MATERIALS AND METHODS

This study was performed after the approval of the Laboratory Animals Local Ethics Committee. Twenty-one male Sprague–Dawley rats (mean weight was 436 g and mean age was 11 months) were divided into three equal groups.

The superior mesenteric artery (SMA) and superior mesenteric vein were isolated after laparotomy and tied tightly with silk sutures to cut the blood flow. The abdomen was closed in Group 1 and Group 2. The animals in groups 1 and 2 were exposed to ischemia for 2 and 6 h, respectively. Laparotomy was performed after 2 h for Group 1 and 6 h of ischemia for Group 2, and the occurrence of ischemia was confirmed (Fig. 1a and b, respectively). There was no intervention for Group 3, and it was selected as a control group. Intracardiac blood samples were collected from the rats for cytokine and chemokine measurement; the rats were sacrificed by cervical dislocation.

The interleukin (IL)-1α, 1β, 6, 10, 12p70, 17A, 18, 33, CXCL1/KC, CCL2/MCP-1, GM-CSF, interferon-gamma (IFN-γ), and tumor necrosis factor-alpha (TNF-α) were measured using flow cytometry (Cube 8™, Sysmex, Japan, cat no: CY-S-3068R_V3) and a cytokine measurement kit (LEGENDplex™ Rat Inflammation Panel, Biolegend, USA, cat no: 740251).

Statistical Analysis

The statistical data analysis was performed with the SPSS version 22.0 program. The Kolmogorov–Smirnov test was used for the distribution of variables. The Kruskal–Wallis test was used for independent, non-normally distributed data. Dunn's post hoc test was used if there were any significant differences between groups. The Spearman correlation coefficient was used for non-normally distributed data. The correlation analysis was interpreted according to: 0.00–0.19, very weak, 0.20–0.39, weak; 0.40–0.59, moderate; 0.60–0.79, strong; and 0.80–1.0, very strong. P-value was accepted as statistically significant if it was below 0.05.

RESULTS

Comparisons of blood cytokine and chemokine values of the groups are given in Table 1. Significant differences were observed between the groups in IFN-Gamma, CXCL1, MCP1, TNF-alpha, and IL-6 parameters. In the correlation analyses performed according to the mesenteric ischemia time, a very strong correlation is observed in CXCL1, with a strong level of MCP1, TNF alpha, and IL-6. A moderate level of correlation was found in IFN-Gamma, IL-10, and IL-18.

The differences between groups in the subgroup analyzes are shown in Table 2. Accordingly, significant differences were observed in CXCL1, MCP1, TNF-alpha, and IL-6 in the control and 2-h AMI groups. Furthermore, significant differences in IFN-Gamma, CXCL1, MCP1, TNF-alpha, and IL-6 in the control and 6-h AMI groups.

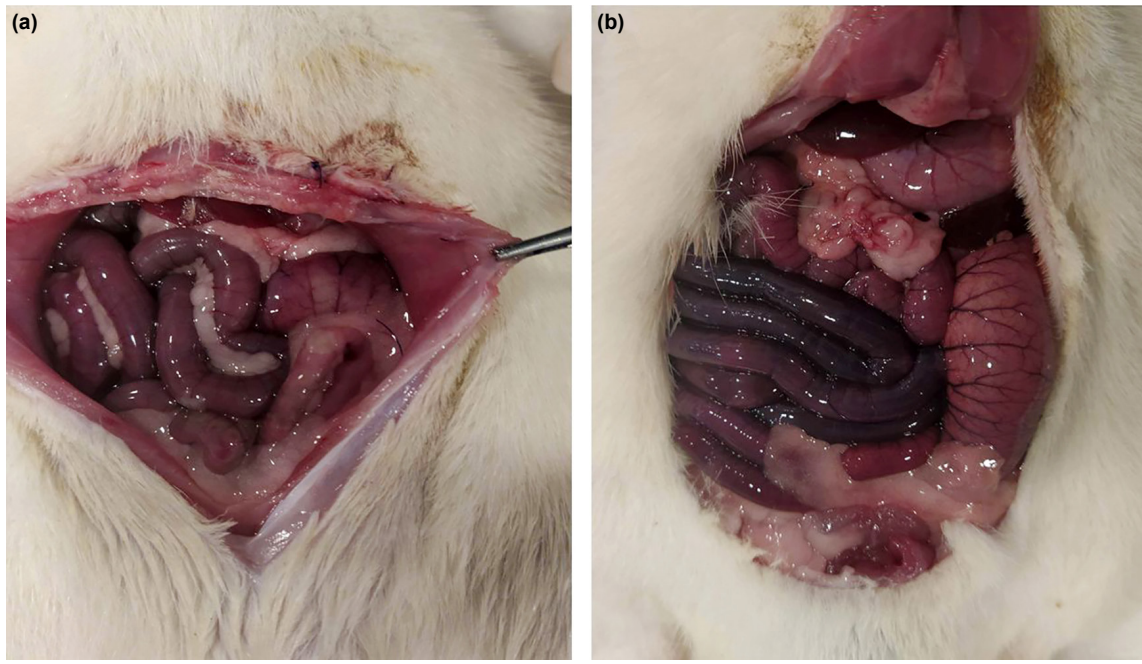

Figure 1. Intra-abdominal view of the acute mesenteric ischemia groups **(a)** 2 h Ischemia group and **(b)** 6 h ischemia group.

Table 1. The changes and corelations of cytokines and chemokines between groups

	2 hr ischemia (n=7)	6 hr ischemia (n=7)	Control (n=7)	p-value	Correlation
IL-10 (pg/ml) (median, min-max)	0.57 (0.57–2.38)	1.44 (0.57–3.32)	0.57 (0.57–0.57)	0.062	r=-0.523 p=0.015
IFN-Gamma (pg/ml) (median, min-max)	1.95 (1.95–2.56)	2.56 (1.95–7.44)	1.95 (1.95–1.95)	0.050	r=-0.544 p=0.011
CXCL1 (pg/ml) (median, min-max)	5246.70 (1566.64–6456.80)	7898.08 (3580.00–19471.90)	5.02 (4.52–12.41)	0.001	r=-0.800 p=0.000
MCP-1 (pg/ml) (median, min-max)	6980.61 (3759.90–11654.60)	9371.01 (4104.84–28399.45)	1584.32 (781.16–3585.17)	0.001	r=-0.799 p=0.000
TNF alpha (pg/ml) (median, min-max)	4.78 (1.95–13.78)	8.16 (1.95–173.32)	1.95 (1.95–2.29)	0.014	r=-0.637 p=0.000
GM-CSF (pg/ml) (median, min-max)	17.38 (17.38–17.38)	17.38 (17.38–17.38)	17.38 (17.38–17.38)	1.000	r=NA p=NA
IL-18 (pg/ml) (median, min-max)	10.60 (10.06–56.46)	17.45 (10.06–134.01)	10.60 (10.06–10.06)	0.058	r=-0.531 p=0.013
IL12p70 (pg/ml) (median, min-max)	1.75 (1.75–1.75)	1.75 (1.75–1.75)	1.75 (1.75–1.75)	1.000	r=NA p=NA
IL-1 beta (pg/ml) (median, min-max)	6.48 (6.48–6.68)	6.48 (6.48–6.68)	6.48 (6.48–6.68)	1.000	r=NA p=NA
IL-17A (pg/ml) (median, min-max)	1.56 (1.56–1.56)	1.56 (1.56–1.56)	1.56 (1.56–1.56)	1.000	r=NA p=NA
IL33 (pg/ml) (median, min-max)	6.55 (6.55–6.55)	6.55 (6.55–6.55)	6.55 (6.55–6.55)	1.000	r=NA p=NA
IL-1 alpha (pg/ml) (median, min-max)	1.60 (1.60–9.54)	3.93 (1.60–27.73)	1.60 (1.60–2.94)	0.253	r=-0.353 p=0.117
IL-6 (pg/ml) (median, min-max)	4890.94 (3865.65–8463.87)	4857.32 (1364.01–21442.71)	4.36 (2.27–3.48)	0.001	r=-0.723 p=0.000

IL-10: Interleukin-10; IFN: Interferon; CXCL1: C-X-C Motif Chemokine Ligand 1; MCP-1: Monocyte chemoattractant protein-1; TNF: Tumor necrosis factor; GM-CSF: Granulocyte-macrophage colony-stimulating factor.

Table 2. Pairwise comparisons of groups that had a significant difference in the Kruskal-Wallis Test

	IFN-Gamma	CXCL1	MCP1	TNF alpha	IL-6
Control vs mesenteric ischemia 2 hours	0.330	0.006	0.006	0.046	0.002
Control vs mesenteric ischemia 6 hours	0.015	0.000	0.000	0.004	0.001
Mesenteric ischemia 2 hours vs mesenteric ischemia 6 hours	0.144	0.413	0.413	0.339	0.897

IFN: Interferon-gamma; CXCL1: C-X-C Motif Chemokine Ligand 1; MCP-1: Monocyte chemoattractant protein-1; TNF: Tumor necrosis factor; IL-6: Interleukin-6.

DISCUSSION

Due to its high mortality, it is important to diagnose AMI early. It is thought that systemic inflammatory response syndrome and septic complications are generally responsible for its high mortality.[11] In recent studies, no specific biomarker with high diagnostic potential that could increase the opportunity for survival has been identified.[9,12,13]

In the case of suspected AMI, the sensitivity and specificity of the blood leukocytes, CRP, procalcitonin, and lactate levels (that are increased in many diseases) are evaluated as low. Although mesenteric angiography and computed tomography angiography are very helpful in diagnosis, they have disadvantages, such as being invasive or causing contrast nephropathy.[9]

In a study performed on rats by creating SMA occlusion, sig-

nificant increases were found in TNF-α, IL-6, and IL-1. Their levels increased from the 2nd h in the ischemia group compared to the control group.[14] In another study, serum IL-6 levels were higher in patients with AMI than in the healthy control group.[15] Procalcitonin, a pro-inflammatory cytokine-like mediator, is elevated in severe infections, sepsis, multiple organ deficiency syndromes, and mesenteric ischemia. Procalcitonin and its expression are regulated by pro-inflammatory cytokines, such as TNF-α and IL-6.[16] In our study, IL-6 and TNF-α levels were significantly higher in mesenteric ischemia. A study evaluating ischemia-reperfusion injury after AMI observed that MCP-1, CXCL1, IL-6, and IL-10 levels increased as the ischemia time increased in rats, reperfused after 30, 45, and 75 min of mesenteric ischemia.[17] In the literature, there are inconsistent results regarding MCP-1 levels in the presence of ischemia and subsequent reperfusion. In one study, no change was reported in MCP-1 levels in ischemia-reperfusion conditions, while in another study, an increase in MCP-1 levels was found in lung and intestinal ischemia-reperfusion conditions.[18,19] Although acute inflammatory mediators such as IL-2, IL-6, and TNF are not specific for intestinal damage, a cohort study suggested that IL-6 is both sensitive and specific for AMI.[20] In our study, IL-6, CXCL1, and MCP-1 values highly correlate with increased ischemia time.

It has been reported that IFN-γ, a regulatory cytokine, may be associated with ischemic events, and there are very few publications on this subject.[21,22] Another study reported that IFN-γ ratios were significantly higher in the portal vein at 24 h than in the control group in macaques who underwent reperfusion after 1 h of SMA occlusion. No significant difference was observed in peripheral blood.[22] In our study, IFN-γ levels were higher in rats with only long-term ischemia compared to the control group.

Most studies evaluating the relationship between IL-10, one of the anti-inflammatory cytokines, and intestinal ischemia focus on ischemia-reperfusion injury.[23,24] Deficiency of the gene encoding IL-10 or administration of IL-10 neutralizing antibodies has been shown in animal studies to increase susceptibility to endotoxemia.[25,26] Experimentally, pharmacological doses of IL-10 have been reported to have a protective effect on acute endotoxemia, cecal ligation-puncture, and intestinal ischemia.[27,28] In our study, IL-10 levels also increase with ischemia, and the possible reason for this is the anti-inflammatory response created to suppress the immune response against ischemia.

It has been reported that reperfusion with mesenteric angiography, one of the mesenteric ischemia treatment options, is more successful, especially in the early stages of ischemia (before peritonitis develops).[29] However, objective criteria are not used in the pre-operative period for ischemia duration. Considering the CXCL1, MCP-1, TNF-α, and IL-6 parameters, which increase in our study, especially as the ischemia time increases, we can predict that very high levels of these cytokines and chemokines lead the clinician toward a longer ischemia time and apply the surgical option.

Conclusion

Evaluating the increased CXCL1, MCP-1, TNF-α, and IL-6 levels, which especially had a high correlation with the duration of ischemia, in patients with suspected intestinal ischemia may help clinicians with diagnoses and treatment decision-making.

Ethics Committee Approval: This study was approved by the Bezmialem Vakıf University Animal Experiment Ethics Committee (Date: 22.02.2021, Decision No: 2021/21).

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Conflict of Interest: None declared.

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DENEYSEL ÇALIŞMA - *ÖZ*

Mezenterik iskeminin erken teşhisinde sitokinler ve kemokinler önemli midir?

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AMAÇ: Akut mezenterik iskemi (AMİ) çok yüksek mortalite ve morbiditeye sahiptir ve AMİ'nin prognozunda en önemli faktör iskeminin süresidir. Bu çalışmanın amacı, AMİ'nin erken tanısında sitokin ve kemokinlerin olası kullanımı ve iskemi süresine göre bu belirteçlerin değişimini değerlendirmektir.

GEREÇ VE YÖNTEM: Yirmi bir erkek Sprague Dawley sıçanı üç eşit gruba ayrıldı. İlk iki grupta superior mezenterik arter ve superior mezenterik ven sıkıca bağlanarak grup 1'de iki, grup 2'de altı saat iskemiye maruz bırakıldı. Grup 3'e ise herhangi bir işlem uygulanmayarak kontrol grubu olarak belirlendi. Grup 1'de iskemiden iki saat sonra ve grup 2 iskemiden altı saat sonar ve grup 3'te ise laparotomiden altı saat sonar intrakardiyak kan örnekleri alınarak IL-1α, 1β, 6, 10, 12p70, 17A, 18, 33, CXCL1/KC, CCL2/MCP-1, GM-CSF, IFN-γ ve TNF-α düzeyleri flow sitometri kullanılarak ölçüldü.

BULGULAR: IFN-γ, CXCL1, MCP-1, TNF-α ve IL-6 parametrelerinde gruplar arasında önemli farklılıklar gözlendi. Mezenterik iskemi süresine göre yapılan korelasyon analizlerinde CXCL1'de çok yüksek, MCP-1, TNF-α ve IL-6'da yüksek düzeyde korelasyon gözlenmektedir. Ayrıca IFN-γ, IL-10 ve IL-18'de orta düzeyde bir korelasyon mevcuttu.

TARTIŞMA: Mezenterik iskemi olan hastalarda iskemi süresi ile yüksek korelasyon gösteren CXCL1, MCP-1, TNF-α ve IL-6 düzeylerinin artması, klinisyenin tanı koymasına ve doğru tedaviyi seçmesine yardımcı olabilir.

Anahtar sözcükler: Akut mezenterik iskemi; kemokin; sitokin.

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