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Effect of SMOF Lipid Emulsion on Pro Inflammatory Cytokines and Oxidative Stress Markers in Sepsis Patient

KEYWORDS: SMOF, SIRS, OS, pro-inflammatory markers

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ABSTRACT *Sepsis is the systemic inflammatory response to infection and remains primary cause of death from infection. Cytokines and oxidative stress (OS) has main role in the pathophysiology of sepsis. Recently there has been increased interest in the lipid component of parenteral nutrition, which promises to modulate inflammatory responses and improve the outcome of patient with immune-mediated conditions.*

Aims and objectives: The present study is aimed to evaluate effect of parenteral lipid emulsion (SMOF) on oxidative stress and pro-inflammatory markers in sepsis - pre and post infusion and correlation between them.

Methodology: The study comprised of 40 sepsis patients, received SMOF lipid emulsion. Blood Samples were collected from septic patient; pre and post (after 48 and 96 hrs) on initiation of lipid emulsion. The pro-inflammatory markers IL-6, IL-8 and CRP and oxidative stress markers (MDA, NO•, SOD, CAT and Vitamin C) were estimated pre and post infusion.

Results: Levels of MDA, NO•, SOD, CAT and inflammatory markers IL-6, IL-8 and CRP were significantly decreased and vitamin C significantly increased on infusion of SMOF.

Conclusion: Present study suggests that SMOF infusion decreases the accumulation of toxic radicals i.e. lipid peroxidation products, pro-inflammatory response and increases the concentrations of antioxidants, which boost the immunity of the patient, which in turn decreases the severity of the sepsis.

Introduction:

Sepsis is a complex inflammatory syndrome with a wide spectrum of severity which is defined as a systemic inflammatory response syndrome (SIRS) in the presence of infection [1]. Severe sepsis and septic shock continue to be associated with high mortality rate worldwide, ranging between 30-60%, despite major advances in critical care medicine [2]. Severe sepsis is common in Indian Intensive Care Units [ICUs] and ICU mortality rate is higher compared with western countries [3].

Cytokines has main role in the pathophysiology of SIRS associated with sepsis. Pro-inflammatory cytokines especially tumor necrosis factor (TNF-α), interleukins IL-1, IL-6 and IL-8 are necessary for initiating an effective inflammatory process against infection. Phagocytic cells (neutrophils and macrophages) respond, to many of these mediators by releasing granular enzymes and producing reactive oxygen species (ROS) such as H_2O_2, a crucial product for the killing of bacteria. H_2O_2 is capable of causing tissue damage and its excess production leads multiple organ-system dysfunctions and mortality [4]. Immune status of sepsis patient can be evaluated by cytokines and OS markers. Therefore it is grey area for future investigation.

Nutritional support in ICUs represents a challenge but its delivery and monitoring can be followed closely. Parenteral nutrition (PN) represents an alternative or additional approach, when it is not possible or unsafe to use other routes. Total parenteral nutrition (TPN) provides all macronutrient and micronutrient [5]. Recently there has been increased interest in the lipid component of PN; it supplies energy, essential building blocks, bioactive and anti-inflammatory molecules. Traditionally used lipid emulsions are based solely upon soybean oil (SO), fish oil (FO) which are rich in the ω-6 fatty acid, long chain ω-3 fatty acids, or a 50:50 mixture of vegetable oil, rich in medium-chain saturated fatty acids and SO[6].

Considering the various limitations and risks of individual lipid emulsions as a PN, for example SO represents an imbalanced fatty acid (FA) supply, with an over abundance of ω-6 polyunsaturated fatty acids (PUFAs), medium chain triacylgrycerides (MCTs) increase risk of ketogenesis and acidosis[7]. Therefore it is suggested that the combination of lipid emulsion would be useful.

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Recently a new lipid emulsion with increased amounts of ω -3 PUFAs (EPA and DHA) and reduced content of ω-6 PUFA is developed. This emulsion is based on a physical mixture of soybean oil (30%), medium-chain triglycerides (30%), olive oil (25%), and fish oil (15%) and is supplemented with α-tocopherol (200 mg/L) to maintain adequate antioxidant status (SMOFlipid®) [8].

Recent study reported that a short infusion (6 hrs) of SMOF was well tolerated and at the end of infusion, lower serum TG concentrations were observed [9]. Therefore present study was designed to assess effects of SMOF lipid emulsion on pro inflammatory cytokines and OS markers in sepsis patients- pre and post infusion.

Material and Methods:

Study design

The present study is the prospective study carried out in Department of Biochemistry, MGM Medical College and MGM Group of Hospitals, Navi Mumbai. This study includes fourty male and female patients aged between 18 to 80 years and diagnosed with sepsis, admitted in ICUs of MGM Medical College, Kamothe and MGM Hospital, Vashi. Institutional Ethical clearance was obtained and written informed consent was taken from either patient or close relatives. Patients were recruited between June 2012 and Dec 2013.

Patient selection:

Sepsis patients were selected from ICU. This diagnosis was based on the presence the following criteria: Sepsis was defined as suspected or confirmed infection in addition to SIRS (i.e. presence of pyrexia, tachycardia, tachypnea and/or leukocytosis). Severe sepsis was defined as sepsis with organ dysfunction (hypotension, hypoxemia, oliguria, metabolic acidosis, and/or thrombocytopenia). Septic shock was defined as severe sepsis with hypotension despite adequate fluid resuscitation.

ICU sepsis patients, those needs PN (severe pancreatitis, excision surgery, multi -organ failure) were included in the study. These patients received SMOF lipid emulsion infusion at the rate of 1 to 2 gram/Kg/day.

Exclusion criteria:

- Patients <18 years age.
- Patients with known / suspected pregnancy.
- On treatment with corticosteroids within the previous 48 hours.
- Treatment with major immunosuppressive drugs.
- Infection with HIV, neutropenia not attributable to sepsis.
- Plasma TG >400 mg/dl (>4.6 mM).

Sample collection:

Blood samples were collected on admission, prior to starting SMOF infusion (i.e. Day 0), after initiating SMOF infusion 48 h (Day 2) and 96 h (Day 4). Blood was centrifuged at 3,000 rpm for 10 minutes and serum was stored at -70°C till analysis.

Oxidative stress markers and pro-inflammatory cytokines analysis: The following parameters were measured in serum by spectrophotometry: Malondialdehyde (MDA) by Satoh's K [10], nitric oxide ($NO^{\bullet}$) by Najawa KC and Nabil WW[11], superoxide dismutase (SOD) by Marklund and Marklund[12], catalase (CAT) by K Sinha[13] and vitamin C by DNPH method[14]. IL-6, IL-8 were estimated by ELISA (AviBion Human by Orgenium Laboratories, catalog number IL06001 and IL08001 respectively) and C- reactive protein by quantitative turbidimetric latex assay method[15].

Statistical analysis

Statistical analysis of the data was carried out with SPSS, version 16; Data was reported as mean ± SD. The comparison between two groups was tested by paired t-test. A 95% confidence interval was used. $P<0.05$ was considered statistically significant. Correlation between two continuous outcomes was evaluated using Pearson correlation coefficient.

Results:

Results were expressed as mean ± SD for each parameter. Statistically significant differences between pre and post infusion of SMOF are summarized in Table No.1 along with their significant values. Statistical correlation of pro-inflammatory cytokines and oxidative stress markers in pre infusion (zero day) of SMOF and post infusion (2^{nd} and 4^{th} day) are given in Table 2, 3, 4 respectively

Table 1: Comparison of serum pro-inflammatory cytokines and oxidative stress markers in sepsis patients – pre and post infusion of SMOF

Parameters	Sepsis patients (n=40) Mean SD		
	Pre infusion of SMOF(Day 0)	Post infusion of SMOF(Day 2)	Post infusion of SMOF (Day 4)
MDA (nmol/L)	5.95 ± 0.92	5.27 ± 0.99*	4.40 ± 0.73**,a
$NO^{\bullet}$ (µmol/L)	62.72 ± 9.44	53.03 ± 9.51**	43.93 ± 8.63**,a
SOD (U/L)	4.59 ± 0.64	4.39 ± 0.63	4.01 ± 0.59*
CAT (U/mg of protein/ml)	8.08 ± 0.84	7.59 ± 0.8 6	6.55 ± 0.85**, a
Vitamin C (mg/dl)	0.53 ± 0.16	0.59 ± 0.17	0.71 ± 0.16*
IL-6 (pg/ml)	231.32 ± 265.75	116.55 ± 171.76**	43.1 ± 59.79**,a
IL-8 (pg/ml)	291.30±128.45	144.23 ± 133.74**	87.53 ± 88.61**,a
CRP (mg/l)	6.13 ± 1.55	5.09 ± 1.80*	4.05 ± 1.98**,a

****p<0.0001 (highly statistically significant), * p<0.05 (statistically significant) VS. pre-infusion**

[a] p<0.05 (statistically significant) VS post-infusion (2^{nd} day)

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Table 2: Correlation of pro-inflammatory cytokines and oxidative stress markers in sepsis patients - pre infusion of SMOF

Parameters		MDA	NO•	CAT	SOD	Vit C	IL-6	IL-8
MDA	r- value	1	0.163	0.149	0.355*	-0.100	0.342*	0.213
NO•	r-value	0.163	1	0.244	0.226	-0.016	0.252	0.029
CAT	r-value	0.149	0.244	1	0.178	-0.146	0.143	0.175
SOD	r-value	0.355*	0.226	0.178	1	-0.0009	0.254	0.0067
Vit. C	r-value	-0.100	-0.016	-0.146	-0.0009	1	-0.053	-0.139
IL-6	r-value	0.342*	0. 252	0.143	0.254	-0.053	1	0.299*
Il-8	r-value	0.213	0.029	0.175	0.0067	-0.139	0.299*	1
CRP	r-value	0.432*	0.231	0.132	0.276*	-0.163	0.342*	0.423*

r = Pearson's correlation co-efficient. * p<0.05 (statistically significant)

Table 3: Correlation of pro-inflammatory cytokines and oxidative stress markers in sepsis patients - post infusion of SMOF (Day 2)

Parameters		MDA	NO•	CAT	SOD	Vit C	IL-6	IL-8
MDA	r- value	1	0.230	-0.165	0.454*	-0.0008	0.242	0.113
NO•	r-value	0.230	1	0.277*	0.309	-0.046	0.340*	0.029
CAT	r-value	0.165	0.277*	1	0.236	-0.143	0.143	0.075
SOD	r-value	0.454*	0.309*	0.236	1	-0.081	0.154	0.0067
Vit. C	r-value	-0.0008	-0.046	-0.143	-0.081	1	-0.153	-0.039
IL-6	r-value	0.242	0. 340*	0.143	0.154	-0.153	1	0.254
Il-8	r-value	0.113	0.029	0.075	0.0067	-0.039	0.254	1
CRP	r-value	0.332*	0.231	0.132	0.276*	-0.163	0.325*	0.412*

r = Pearson's correlation co-efficient. * p<0.05 (statistically significant)

Table 4: Correlation of pro-inflammatory cytokines and oxidative stress markers in sepsis patients - post infusion of SMOF (Day 4)

Parameters		MDA	NO•	CAT	SOD	Vit.C	IL-6	IL-8
MDA	r- value	1	0.024	-0.187	0.464*	-0.129	0.354*	0.131
NO•	r-value	0.024	1	0.349*	0.241	-0.022	0.349*	0.241
CAT	r-value	0.187	0.349*	1	0.184	-0.139	0.464*	0.131
SOD	r-value	0.464*	0.241	0.184	1	-0.006	0.364*	0.349*
Vit. C	r-value	-0.129	-0.022	-0.139	-0.006	1	-0.241	-0.147
IL-6	r-value	0.354*	0.349*	0.464*	0.364*	-0.241	1	0.351*
Il-8	r-value	0.131	0.241	0.131	0.349*	-0.147	0.351*	1
CRP	r-value	0.274*	0.022	0.184	0.241	-0.179	0.428*	0.278*

r = Pearson's correlation co-efficient. * p<0.05 (statistically significant)

Discussion:

The inflammatory response to critical illness, including sepsis, involves the activation of leukocytes and other inflammatory cells leading to an excessive production of inflammatory markers and ROS. ROS mediated oxidative stress has been implicated in apoptotic cell death and in turn can be harmful to the patient when the endogenous antioxidant defense mechanisms are overwhelmed [16]. It is now well documented that ROS is involved in the pathogenesis of multiple organ failure following sepsis which may lead to death [17].

Along with traditional therapies like antibiotics, nowadays clinicians are also considering some immunomodulators in septicemia [3]. Malnutrition is common with a prevalence of 20%–40% in ICU patients [18, 19]. It has an independent risk of increased hospital stay, complications, mortality and costs [20].

Recently, it has been suggested that mixture of lipid emulsion would be useful rather than single lipid emulsion, to reduce the complications associated with single lipid emulsion. Currently a novel emulsion has been developed (SMOF) which is a mix of SO, MCT, OO and FO resulting in a ratio of ω-6 to ω-3 PUFAs of 2.5:1. This ratio is within the optimal range to provide an anti-inflammatory effect [8].

We assessed effect of SMOF on pro inflammatory cytokines and OS markers in sepsis patients and correlated among each other at pre and post infusion of SMOF (i.e., on zero, 2nd and 4th the day).

Pro inflammatory and oxidative stress markers in post infusion SMOF

In our study on SMOF infusion, levels of IL-6, IL-8, CRP, MDA and NO• were significantly decreased on 2nd and 4th

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day as compared to pre-infusion, whereas SOD and CAT activities were non-significantly decreased on 2nd day of therapy but significantly decreased on 4th day of therapy as compared to pre-infusion. The vitamin C levels were non-significantly increased on 2nd day of therapy but significantly increased on 4th day of therapy as compared to pre-infusion, while this increase is non-significant on comparison with 2nd day ($p>0.05$).

IL-6, IL-8, CRP, MDA and NO levels were significantly decreased on 4th day as compared to 2nd day ($p<0.05$). SOD activity was non-significantly decreased on 4th day as compared to 2nd day of SMOF infusion ($p>0.05$) whereas CAT activity was significantly decreased on 4th day as compared to 2nd day ($p<0.05$).

Few studies have focused on clinical outcomes in response to the influence of parenteral SMOF on surgical patients, neonates and liver functions. Deshpande G et al. [21] reported SMOF lipid as a safe, well tolerated, and beneficial in terms of reduction of OS by reducing lipid peroxidation levels in high-risk preterm neonates. Antebi H et al.[22] studied effects of SMOF and SO on liver function and OS in metabolically stressed patients. SMOF lowers liver enzyme abnormalities and elevates antioxidants. Grim H et al. [23] carried out a double-blind, randomized study where they compared TPN based SMOF or SO in patients for 5 days after major abdominal surgery. They reported increased plasma ω-3 FA concentrations and decreased ω- 6 FA concentrations. SMOF also increased plasma EPA and DHA concentrations but had no effect on AA but the length of hospital stay decreased by 7 days.

A randomized trial by Mertees N et al. [24] on 200 patients after elective abdominal or thoracic surgery to receive TPN based on either SMOF or SO for 5 days postoperatively. Both emulsions were well tolerated and relevant laboratory variables were not different between groups, a trend toward a reduced length of hospital stay was observed with SMOF (16 compared with 18 day).

The anti-inflammatory effect of SMOF exerted by its various constituents plays a crucial role in relieving the oxidative stress in septic patients- post infusion of SMOF. SO of SMOF is rich in α-linolenic acid, an EFA. MCTs serve as an immediate source of energy. OO provides energy in the form of MUFAs. FO with its high content of EPA and DHA are precursors of eicosanoids and components of cell membrane respectively.

Anti-inflammatory properties of lipid emulsion may involves the cascade of mechanisms i.e. effects at membrane level, on signal transduction pathways lead to transcription factor activation and altered patterns of gene expression, and on the pattern of lipid mediator generation. EPA has been shown to decrease production of inflammatory eicosanoids and cytokines [7].

In our study on infusion of SMOF we observed significant reduction of pro inflammatory cytokines (IL-6, IL-8 and CRP), which lead to decrease production of ROS/RNS. SMOF lipid also contains α-tocopherol, a chain breaking antioxidant that reduces oxidative stress. Anti-inflammatory effect of SMOF reduces levels of ROS and RNS, which minimize the tissues injury and in turn lowers SOD and CAT activities. On infusion of SMOF α-tocopherol may neutralize harmful effects of ROS/RNS and restores the level of vitamin C.

Correlation between the oxidative stress and inflammatory marker support our results i.e. as inflammatory response increases, OS goes on increasing leading to tissue damage that is showed by positive correlation of SOD & CAT with MDA, $NO^{\cdot}$ and inflammatory markers in pre-infusion and post infusion of the SMOF.

Our study has certain limitations which include comparatively less number of sample sizes, not assessing mortality rate and hospital stay and not classifying sepsis patients into different subgroups. These are some of the limitations which will be addressed in our future studies.

Conclusion:

Our study shows, the SMOF lipid infusion is beneficial in septic patients and reduces pro-inflammatory and OS markers and mitigates the severity of sepsis by increasing and restoring antioxidants. Thus present study reveals the importance of estimation of pro-inflammatory cytokines (IL-6, IL-8 & CRP) and OS markers (MDA, NO, SOD, CAT and Vitamin C) in diagnosis and prognosis of septic patients.

To best of our knowledge this aspect is not better addressed in India. Therefore present study can be considered as a bench mark for future research. Such studies will be helpful to the clinicians to adopt an efficient treatment strategy for speedy recovery of sepsis patients i.e. shortens hospital stay, reduce mortality and economic burden.

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