Volume : 5 | Issue : 6 | June 2016 • ISSN No 2277 - 8179 | IF : 3.508 | IC Value : 69.48

Research Paper

# Assessment of Immuno- Modulatory Effects of Ω-3 Pufa in Patients of Predicted Severe Acute Pancreatitis Using The Positive Predictive Value of Il-6

Medical science

**KEYWORDS :** Severe acute pancreatitis; Interleukin- 6; Omega-3 fatty acids; Parenteral nutrition; Immunomodulators.

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## ABSTRACT

*Fish oil derived omega-3 polyunsaturated fatty acids (ω-3 PUFA) have been reported to have a beneficial effect on the immune response, hence improving outcomes in acute pancreatitis (AP). This trial was planned to assess the effect of early ω- 3 PUFA supplementation on clinical outcomes of predicted severe acute pancreatitis (PSAP) by using the positive predictive value (PPV) of IL- 6 as a marker for clinical outcomes. The PPV of IL-6 at a value of >17pg/mL was 96.5% in the control group. The number of patients who actually progressed to severe acute pancreatitis (SAP) in the ω-3 PUFA group were significantly lesser than the number predicted to progress by the cut- off value of IL-6 (p= 0.02938), thus demonstrating the effectiveness of ω-3 PUFA in improving early clinical outcomes in acute pancreatitis.*

**Introduction**

The early peak morbidity and mortality (upto 50%) [1] associated with acute pancreatitis (AP) is the result of pro-inflammatory cytokines which have shown to escalate the localized pancreatic inflammation into a generalized systemic inflammatory response syndrome (SIRS) with significantly higher incidence of pulmonary, renal, and cardiovascular complications , longer hospital stays and overall higher mortality rates unless early targeted therapy is instituted [2-6]. A variety of newer inflammatory markers are now used to assess the severity of the SIRS, such as IL-6, IL-8, IL-10 and CRP. Of these, IL- 6, with a positive predictive value (PPV) of upto 91% has been shown to be the best early predictive marker for clinical outcomes of acute pancreatitis [7-15].

Fish oil derived omega-3 polyunsaturated fatty acids (ω-3 PUFA) when administered along with adequate nutritional support has been reported to have a beneficial effect on the immune response; enhancing immunity, reducing inflammatory response, altering cytokine production and hence improving outcomes in AP [16, 17]. This trial was planned to assess the effect of early ω- 3 PUFA supplementation on clinical outcomes of predicted severe acute pancreatitis (PSAP) by using PPV of IL- 6 as a marker for clinical outcomes.

**Materials and Methods**

The study was conducted over a period of 17 months in a publicly funded tertiary care centre in Mumbai as per the International Conference on Harmonization (ICH) Good Clinical Practice (GCP) standards. Approval was obtained from the Institutional Ethics Committee.

***Study Design:*** The study was a single center, open-label, randomized, controlled, comparative phase IV study to evaluate the immunomodulatory effects of intravenous fish oil derived ω-3 PUFA (Omegaven®-Fresenius Kabi, Bad Homburg, Germany) and the related clinical outcomes in 80 consecutive patients with predicted severe acute pancreatitis, by using the positive predictive value of Interleukin-6 (IL-6) as a marker for clinical outcomes.

**Inclusion Criteria:**

1. 18-60 years old patients of either sex, diagnosed with acute pancreatitis. Diagnosis of acute pancreatitis was established by the Atlanta guidelines criteria [19] namely, any two of the following three criteria to be fulfilled:
   a. Clinical features suggestive of acute pancreatitis.
   b. Serum amylase or lipase levels elevated to more than three times the upper limit of normal.
   c. USG or CT showing features of acute pancreatitis.
2. Onset of pain within 24 hours of admission to the hos pital.
3. Patients "predicted" to have Severe Acute Pancreatitis (PSAP) by fulfilling the following criteria on admission:

   a. Patients showing the signs of a Systemic Inflammatory response syndrome (SIRS), defined by the presence of two or more of the following criteria1:

   i Rectal temperature >380C (100.4F) or <360C (96.8F)
   ii Heart rate >90 beats/min
   iii Respiratory rate >20/min or PaCO2<32mmHg.
   iv WBC count >12000/mm3, <4000/mm3 or >10% bands.

**Exclusion Criteria**

1. Patients with known immunodeficient status
2. Primary hypertriglyceridemia
3. On long term cyclo-oxygenase inhibitors (more than 3 months)
4. Severe cardiac disease
5. Known hepatic disorders (total bilirubin >1.5 times the upper limit of normal)
6. Psychiatric disorders
7. Known renal compromise (serum creatinine >2.0mg/dl).
8. Received parenteral nutrition within 2 weeks of the study.

**Study procedure**

At the initial screening visit, the diagnosis of acute pancreatitis and presence of SIRS was confirmed on clinical, biochemical (Sr. Lipase, Renal function tests, Liver function tests, Sr. Electrolytes, Complete Hemogram, Arterial Blood Gas analyses) and radiological investigations (USG, CECT abdomen). After verifying the absence of any exclusion criteria, such patients, with a predicted severe acute pancreatitis were inducted into the trial.

Body weight measurements were taken of all the patients, and daily caloric requirement was calculated. All patients in the trial received 25kcal/kg/day plus 1.5g of proteins/kg/

Volume : 5 | Issue : 6 | June 2016 • ISSN No 2277 - 8179 | IF : 3.508 | IC Value : 69.48

day of nutrition. Patients were initially started on Total Parenteral Nutrition (TPN). TPN formulation used was Kabiven™ Peripheral (1400kcal, 1920mL emulsion for infusion). The parenteral nutrition was transitioned to enteral nutrition as per dietician's charts as early as possible, keeping the daily nutritional intake constant. Sr. IL-6 levels were sent of all the patients on Day 0. All patients received antibiotic and supportive therapy as per standard protocols for the management of acute pancreatitis.

Patients were then randomly allocated into one of the two treatment arms in a ratio of 1:1. Computer based randomization list was generated and the allocation was done by one of the investigators using sealed envelopes. Both groups were matched for age, sex distribution and levels of inflammatory markers at onset.

The treatment arm (Group I) received ω-3 PUFA (Omegaven®, Fresenius Kabi, Bad Homburg, Germany) at the dosage of 2ml/kg/day i.e., 0.2g/kg/day of 10% ω-3 PUFA at the rate of 0.05g/kg/hr i.e., 8 drops per minute for a duration of 5 days whereas the control arm (Group II) received a placebo (2ml/kg/day of normal saline) for 5 days.

The patients were assessed at specified intervals for the following parameters:

Marshall score [20] done on Day 0 and Day 3 to assess for organ failure. Patients having a Marshall score of 2 or more on day 0 as well as day 3 were diagnosed to have persistent organ failure for > 48hrs, thereby confirming the progression to Severe Acute Pancreatitis. Marshall scores were assessed by the following parameters:

1. PaO2/FiO2
2. Sr. Creatinine (mg/dL)
3. Systolic blood pressure
4. Arterial pH

Liver function tests, Sr. proteins and Blood Glucose levels were serially recorded to assess for TPN and IVFO related toxicities.

**Study end points and Statistical analyses**

Five cardinal statistics were calculated for the study population:

- Cut- off value of IL-6 with the highest PPV for progression to SAP in the control group at Day 0. ($V_c$)
  The PPV for IL-6 levels on day 0 for prediction of progression from PSAP to SAP were calculated and the cut- off level with highest PPV was determined.
- Number of patients in the ω-3 PUFA group above Vc. (Nω)
- Number of patients predicted to progress to SAP among Nω by applying the calculated PPV of IL-6. (Np)
  The PPV for the cut-off value calculated from the control group was applied to the ω-3 PUFA group and the number of patients who were predicted to progress to SAP in that group was calculated.
- Number of patients actually observed to progress to SAP in Nω. (No)
  The actual number of patients progressing from PSAP to SAP above the calculated IL-6 cut- off level in the ω-3 PUFA group was calculated.
- Comparison of Np v/s No.
  For the determined cut- off of IL-6, the actual number of patients of PSAP progressing to SAP (No) was compared to the number of patients predicted to progress to SAP in this group (Np).

Qualitative data was analysed using standard two by two tables. A p-value of ≤0.05 was considered as significant. All the statistical analysis were done using SPSS version 17.0 (SPSS Inc. released 2007. SPSS for Windows, Version 17.0. Chicago, SPSS Inc.) and Graphpad prism Instat version 3.0.

**Results**

**Demographic characteristics**

Both the groups comprised of 40 patients of clinically predicted severe acute pancreatitis (PSAP), with onset within 24 hours of admission to the hospital.

Mean age of patients in the control group was 40.1 (13.28) years and that in the ω-3 PUFA group was 40.05 (13.28) years. Both groups were comparable with respect to age (p= 0.9866).

The control group comprised of 35 men and 5 women, and the ω-3 PUFA group comprised of 33 men and 7 women. Both groups were comparable with respect to sex distribution within the groups.

Both groups were comparable with respect to median levels of IL-6 [44.35pg/mL (3.4 – 1178) v/s 30.3pg/mL (2- 452); p= 0.0965] on day 0.

***- Cut- off value of IL-6 with the highest PPV for progression to SAP in the control group.*** IL-6 levels ranged from 2- 452pg/mL with median levels of 30.3pg/mL n the control group.

29 out of 40 patients had an IL-6 level of >17pg/mL (Vc). Of these, 28 patients progressed to SAP. Of the remaining 11 patients who had IL-6 levels <17pg/mL, 7 patients progressed to SAP.

At a cut- off value of >17pg/mL, PPV of IL-6 for progression to SAP was found to be 96.5%.

- Number of patients in the ω-3 PUFA group above Vc. Nω = 33; 33 out of 40 patients in the ω-3 PUFA group had IL-6 levels higher than Vc (17pg/mL)Np - Number of patients predicted to progress to SAP among Nω by applying the calculated PPV of IL-6. (Vc ) Np=

32; 32 (96.5%) patients from among Nω (33) were predicted to progress to SAP in the ω-3 PUFA group.

Np= 32 out of 33 patients were predicted to progress to SAP in the ω-3 PUFA group.No= 26 out of 33 patients actually progressed to SAP in the ω-3 PUFA group.

P value as calculated by a standard two by two table was 0.02938, which is statistically significant.

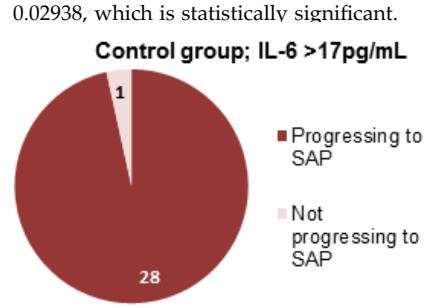


**Fig. 1: 29 patients in the control group had IL-6 level of >17pg/mL on day 0. Of these, 28 (96.55%) patients progressed to SAP.**

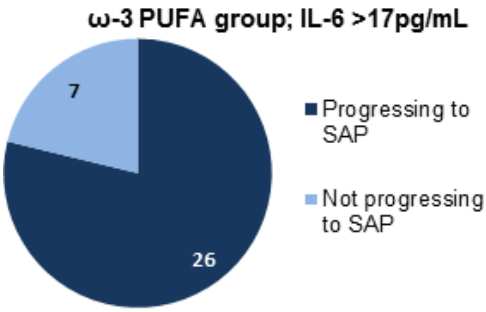


**Fig. 2: 33 patients in the ω-3 PUFA group had an IL-6 level of >17pg/mL on day 0. Of these, 26 (78.78%) progressed to SAP whereas 7 (21.21%) did not.**

**Discussion**

The early peak morbidity and mortality (upto 50%) [1] associated with acute pancreatitis (AP) is the result of pro-inflammatory cytokines which have shown to escalate the localized pancreatic inflammation into a generalized systemic inflammatory response syndrome (SIRS) with significantly higher incidence of pulmonary, renal, and cardiovascular complications , longer hospital stays and overall higher mortality rates unless early targeted therapy is instituted [2-6]. The degree of cytokine increase correlates with the severity of the inflammatory response leading to the use of IL-6, IL-8, IL-10, TNF-$\alpha$ and CRP to assess the severity of AP, which has been validated by several studies [7-15].

Interleukin 6 (IL-6) is a pro- inflammatory cytokine produced by monocytes/ macrophages, endothelial cells, fibroblasts, and smooth muscle cells10. Twenty-four hours after admission, raised plasma IL-6 concentrations can reliably predict the severity of pancreatitis and organ failure, early in the course of the disease. [3, 8- 12, 14, 15, 22- 27] and correlate with the mortality rate [7, 8, 21] of patients with AP.

Several studies show IL-6 to be the most useful inflammatory cytokine in the prediction of severity of acute pancreatitis [7- 15]. Heath et al. (1993) showed in a study of 24 patients, the sensitivity and specificity of IL-6 in distinguishing severe from mild pancreatitis at peak levels of >130u/mL to be 100% and 71% respectively [3]. Pezzilli et al. (1995), in a study of 38 patients of all pancreatitis demonstrated a sensitivity, specificity and positive predictive value of IL-6 at a cut off value of 2.7pg/mL on day 1 in identifying patients of SAP to be 100%, 86%, and 91% respectively [15]. Aoun et al. (2010) performed a meta- analysis of relevant articles published regarding inflammatory markers as predictive factors for SAP. For IL-6, the pooled sensitivity ranged from 81.0 and 83.6%, specificity ranged between 75.6 and 85.3% with positive likelihood ratio of 3.43 on day 1 and 4.90 on day 2 [28].

The treatment of acute pancreatitis at present is largely supportive. However, a therapeutic window for intervention with modulators of inflammation exists between the onset of clinical symptoms and peak pro- inflammatory cytokine expression. Polyunsaturated fatty acids (omega-6 and omega-3 fatty acids) are the precursors of the lipid mediators and play an important role in regulation of inflammation. Omega-6 fatty acids (e.g. arachidonic acid) promote inflammation whereas omega-3 fatty acids (e.g. eicosapentanoic acid and docosahexanoic acid) have anti-inflammatory properties, dampening inflammation by inhibiting the formation of omega-6 fatty acids-derived pro-inflammatory eicosanoids (e.g. PGE2 and LTB4), and suppressing the activity of nuclear transcription factors, such as NFκB [16, 17].

Evidence exists that suggests that the production of inflammatory cytokines takes place systemically and not just locally within the pancreas. If these mediators are activated within end organs, such as the lungs and kidneys, the ability to attenuate their production may have similar beneficial effects within these organs-as demonstrated within the pancreas [2, 29, 30]. Omega- 3 PUFAs have been shown to reduce levels of pro- inflammatory cytokines like IL-6, in several human trials [16, 17]

The patients in the control group in our study were managed according to standard protocols for severe acute pancreatitis. We calculated the PPVs for various levels of IL-6 in this group, and found a cut off level of 17pg/ml of IL-6 to have the highest PPV of 96.5% with a good sensitivity of 80% for patients progressing to SAP from PSAP

This value was applied to the ω-3 PUFA treated group to see if the actual number of patients with PSAP progressing to SAP was different than the predicted number. Of the patients in the ω-3 PUFA group with IL-6 levels above the cut- off value of 17pg/mL, the number of patients observed to progress to SAP in the first 3 days were significantly lesser than the number predicted to progress [26 (78.78%) v/s 32 (96.5%); p= 0.02938], thus demonstrating the efficacy of ω-3 PUFA in improving the early clinical outcomes of the patients of moderate to severe AP and due consideration should be given to making IV Omega- 3 PUFA supplementation part of the standard management protocols for moderate to severe AP.

**Ethical approval and consent**

All procedures performed in this study were in accordance with the ethical standards of the institutional research ethics committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

**Disclosure**

The authors report no conflict of interest.

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