ARTICLE

Kitchen-based diet versus commercial polymeric formulation in acute pancreatitis: a pilot randomized comparative study

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INTRODUCTION: Nutrition plays an important role in management of acute pancreatitis (AP) and decreases its severity and infectious complications. Various formulations of enteral nutrition (EN) are available and are costly. For developing countries, cost and availability is a major issue and kitchen-based diet should be explored in patients with AP.
AIM: Comparison of kitchen-based diet with a commercially available polymeric formulation in terms of various outcomes in patients with AP within 14 days after the onset of pain.
METHODS: Sixty patients (39 male, mean age 36.1 ± 12.7 years) of moderately severe and severe AP of any etiology were randomized (30 in each group) to either kitchen-based diet or commercial polymeric formulation group. Outcome measures were refeeding pain, tolerability, infectious complications, mortality, total hospital/intensive care unit stay; and change in serum C-reactive protein (CRP), transferrin and pre albumin.
RESULTS: There was no significant difference in baseline demographic and biochemical parameters in both groups. No difference was observed in refeeding pain (7.1% vs 8%, $p = 0.99$), tolerability (28.6% vs 12%, $p = 0.17$), infectious complications (57.14% vs 36%, $p = 0.12$), mortality (31.7% vs 20%, $p = 0.69$), hospital stay (19.5 vs 23.5 days, $p = 0.86$), CRP (74.4 vs 59 mg/L, $p = 0.97$), transferrin levels (23.6 vs 25.6 mg/dL, $p = 0.75$) and pre albumin (9.45 vs 13.09 mg/dL, $p = 0.68$) in both groups.
CONCLUSION: Kitchen-based diet is comparable to commercial polymeric formulation for the early initiation of enteral nutrition in patients with severe or moderately severe acute pancreatitis.
CLINICAL TRIAL REGISTRATION: Trial registered with the Clinical Trials registry-India (CTRI/2018/01/011188).

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INTRODUCTION

Acute pancreatitis (AP) is a leading cause of hospitalization among gastrointestinal disorders with significant morbidity and mortality [1, 2]. Acute pancreatitis is an acute inflammatory and severe catabolic state leading to prolonged hospitalization and delayed recovery [3]. The management of AP is mainly supportive including fluid management, analgesics for pain, nutrition, organ support for organ failure and treatment of complications like intra-abdominal collections etc. In the past, patients were kept nil per oral to give rest to the pancreas and the preferred route for nutrition was parenteral which was associated with higher rate of complications [4]. Now, enteral nutrition is considered an important cornerstone in the management of AP [5]. Enteral nutrition (EN) is now the recommended mode of nutritional support and meta-analyses have shown that EN decreases the systemic infections, multiorgan failure, hospital stay, mortality, and need for surgical interventions [4, 6, 7]. There is good evidence on various components of EN like timing (early enteral nutrition) and route of EN (either nasogastric or nasojejunal) [8, 9].

Various formulations of EN like semi-elemental diet, immuno-nutrition, probiotics etc. have been used in patients with AP [10–12]. No specific type of enteral nutrition formulation has shown any benefit over another for early enteral nutrition [13, 14]. Semi-elemental formulations are commercial feeds with predigested enteral formulations, whereas polymeric formulations have intact macronutrient components. Most of the earlier studies showing benefit with early EN when compared to parental nutrition or no nutrition were done with semi-elemental diet while some of the recent studies done with polymeric formulations also showed benefit [14–16]. A single pilot RCT comparing the semi-elemental formula with polymeric formula showed that both formulations were similarly tolerated while the hospital stay, and weight loss were lower with semi-elemental diet [17]. A meta-analysis comparing semi-elemental and polymeric formulations indirectly using parenteral nutrition as a reference found that feeding tolerance, complications and mortality were similar in both groups [14]. Hence, the guidelines recommend a standard polymeric formulation for enteral nutrition in acute pancreatitis including critically ill patients [5, 18].

The polymeric diet can be obtained from two sources: commercial formulation and kitchen-based diet. Commercial formulas have recently become more desirable as they are easy to prepare, less prone to microbial contamination and provide desired amount of nutrients. However commercial formulations are more expensive, and are less palatable compared to the kitchen-based diet. The kitchen-based diet is easily available, cost

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effective in healthcare settings with limited resources, more palatable, and more acceptable to a patient although this diet has its own concerns like time from preparation to consumption, increased risk of microbiological contamination and uncertainty on their nutritional value especially with non-standardized recipes [19, 20]. All the studies on polymeric formulations in enteral nutrition in AP have used commercial formulations with none considering kitchen-based diet [15–17, 21–24]. Therefore we conducted a pilot randomized comparative trial to assess whether economical and more palatable kitchen-based diet was comparable to commercial polymeric formulation in patients with moderately severe and severe AP.

MATERIALS AND METHODS

This study was a single center pilot, open label randomized-comparative trial, conducted at a tertiary care center in North India. Institutional ethical approval was obtained. The study was conducted in compliance with the Declaration of Helsinki and Good Clinical Practice guidelines. Informed written consent was obtained from all the patients before enrollment in the study. The trial was registered with the Clinical Trials Registry-India (CTRI/2018/01/011188) in January 2018 and study was initiated in July 2018.

All the consecutive admitted patients (between 18 and 65 years of age) of moderately severe (MSAP) and severe AP (SAP) of any etiology and within 14 days after the onset of pain were included. The patients who were already started on oral diet at the time of admission, those with mild AP, pregnant and lactating women, co-morbid diseases such as chronic liver disease, chronic renal failure or malignancy, and refusal to consent were excluded from the study. The diagnosis and severity of AP was based on the revised Atlanta classification [25]. All patients underwent blood investigations which included complete hemogram, liver and kidney function test, serum calcium level, lipid profile and in select group of patient's serum parathormone level. 5 mL serum was stored for further analysis for pre-albumin, C-reactive (CRP) and transferrin. The patients who required radiological imaging preferably underwent either ultrasonography (USG) or contrast enhanced computed tomography (CECT) of abdomen, based on treating clinician decision.

Initially nutrition screening was done in a subset of patients using Nutrition Risk Screening, which revealed every patient to be in 'at risk' category. Assuming all the patients under 'at risk' category, nutrition screening was not done for rest of these patients. Along with nutritional intervention, standard medical treatment for AP and support for organ failure was given to the patients as described in detail elsewhere [3].

Randomization and allocation concealment

After assessing the eligibility of the patients, they were randomly allocated in two groups: kitchen-based diet (liquid, semisolid or solid diet as per tolerance) (group 1) and exclusive commercial polymeric formulation (group 2). Randomization was done by using computer generated sequence by pseudo random code. Allocation of patients to receive the intervention was done by sequentially numbered opaque sealed envelope (SNOSE) method. Generation of random numbers and preparation of the envelopes was done by a statistician not associated with the conduct of the study. Enrollment of participants, and assignment of interventions to the participants was done by investigator.

Interventions and type of feed formulations

The weight-based equation was used to determine energy (25–30 kcal/kg/d) and protein requirements (1.2–2 g/kg/d). Diet was started within 24 h after inclusion. An attempt was made to increase their caloric intake from 250 kcal/d to nutritional goal (25–30 kcal/kg/d of ideal body weight) in 3–4 days duration. If patient developed pain or intolerance, this was recorded, and diet was stopped; diet was gradually introduced and increased again when the patient was able to tolerate. The feed in both groups was aimed to be isocaloric (1 kcal/ml). Patients were monitored daily for nutrient supply, tolerance, gastrointestinal symptoms, and recurrence of pain. Oral route was preferred for the patients. If oral diet was not tolerated then nasogastric or nasojejunal route was tried to meet the calorie requirements. The duration of intervention was for 14 days.

Kitchen-based diet: Group 1

Kitchen-based diet contained all the constituents from each food group (cereals, pulses, vegetables, milk and milk products, eggs etc). In this group, patients were given kitchen or home-made diet (solid/semisolid/liquid diet as tolerated). Liquid diet denotes curd or milk-based feed enriched with oil, sugar, and starch (approximate macronutrient composition- carbohydrate: 50%, fat: 32% and protein: 18%). The ingredients, composition and method of preparation is detailed in supplementary material. In case of intolerance to regular food, the kitchen-based liquid feed was additionally given to increase their nutrients intake either through oral route or tube feeding.

Commercial polymeric formulation: Group 2

Commercially available polymeric enteral formulation (Essential BN/DM, Azzura Pharmaconutrition Pvt. Ltd., Delhi-NCR, India) was used in this group. In case of uncontrolled blood sugar levels, diabetic formula was used. This contained 50% to 55% complex carbohydrates, 15% to 20% intact proteins and 30% fats, mainly long chain triglycerides.

Study outcomes and their definitions

The outcome measures for our study were: proportion of patients with refeeding pain, tolerability of feed, infectious complications, length of hospital stay and mortality in each diet group.

Refeeding pain was defined as occurrence of pain requiring stopping of feeds [26]. The feed/diet was reintroduced slowly once the pain subsided. Abdominal discomfort was not considered to be refeeding pain if feeding was continued and tolerated by patients. Feed tolerability was defined as ability to take the prescribed diet without gastrointestinal symptoms such as bloating, diarrhea, constipation, nausea, vomiting and aspiration. Other outcomes measured were infectious complications, duration of hospital and ICU stay, change in serum pre-albumin and serum transferrin. Biochemical parameters (CRP, pre albumin and transferrin) were done at day 1 (i.e., initiation of diet) and repeated at day 14 (i.e., completion of intervention). The patients were further followed till discharge or death during hospitalization (Fig. 1). Follow-up investigations could not be obtained for some patients due to various reasons e.g.,, death (during 14 days of intervention), ileus and protocol violation (Fig. 1), and for these patients only baseline data was reported.

Serum pre-albumin was measured with Human Prealbumin ELISA kit (Immunology Consultants Laboratory, USA); whereas serum transferrin was measured by Human Transferrin ELISA kit (Thermo Scientific, USA) and CRP was measured by CRP ultra-EIA kit (XEMA Co, Ltd ELISA kit, Russia) as per manufacturer's instructions.

Sample size

This was a pilot study, as there is lack of previous studies comparing kitchen-based diet to commercial formulations. Approximately 90–100 patients with moderately severe and severe acute pancreatitis get admitted at our institute every year. Considering >50% patients to be excluded due to various exclusion criteria we planned to recruit a total of 60 patients in two years.

Changes in protocol after trial commencement

Initially we planned to recruit patients with moderately severe or severe AP presenting within 7 days of onset of pain and planned to recruit 50 patients in each arm. We observed that majority of these patients presented after 7 days of pain onset as our center is a tertiary and referral center. So, we expanded our inclusion criteria to moderately severe or severe AP presenting within 14 days of onset of pain. In initial 8 months of study, we could recruit only 18 patients out of 90 screened patients with acute pancreatitis. Assuming a similar trend, we planned to recruit a total of 60 patients in next one and half year in this pilot study.

Statistical analysis

Data were analyzed using statistical software STATA 14.0. Categorical data were expressed as frequency and percentage. Quantitative data were expressed as mean ± standard deviation or median for variables after normal or skewed distribution, respectively. Chi-square or fisher exact test was used to compare proportion of categorical variable between two groups. Those variables followed normal distribution was compared by independent t test between the groups. Those variables did not follow

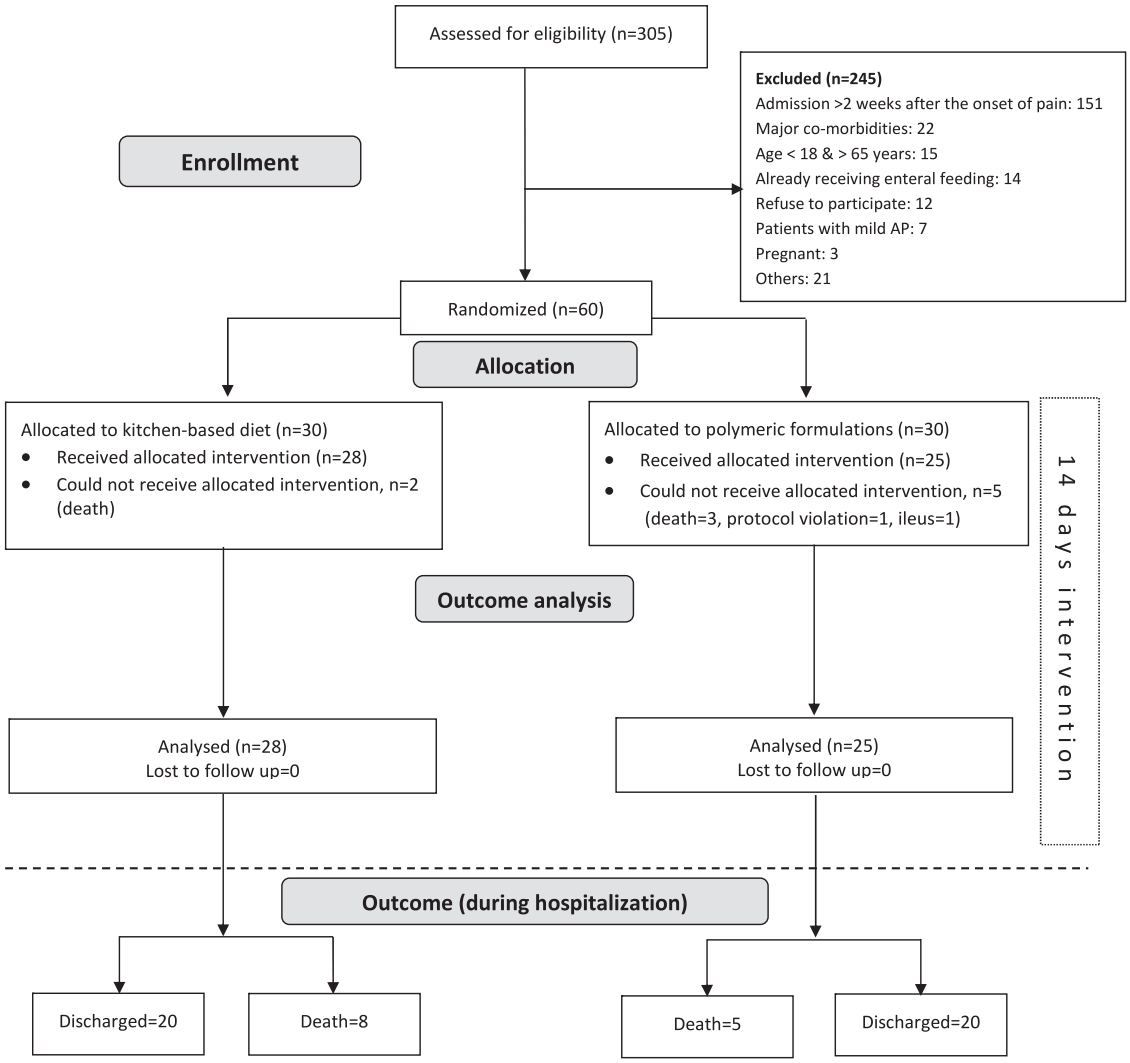

Fig. 1 Consort flow diagram: Patient recruitment, randomization and study design.

normal distribution were compared by Rank sum test. P value ≤ 0.05 was considered significant.

RESULTS

During the study period from July 2018 to July 2021, a total of 305 patients with AP were admitted. Of these, 245 patients were excluded and the major reason for exclusion was admission after 14 days of disease onset to our hospital (Fig. 1) and 60 patients were enrolled in this study, 30 in kitchen-based diet (group 1) and 30 in commercial polymeric formulation (group 2). In group 1 and group 2, the mean age was similar (36.26 ± 14.3 and 36.03 ± 11.4 years, respectively; $p = 0.94$). Sixty percent in group 1 and 70% in group 2 were males ($p = 0.41$). Additional demographic variables, etiology, and severity of acute pancreatitis were also comparable in both groups at baseline. The most common etiology of AP was gallstone (45%) followed by alcohol (33.3%). Overall, 31.7% of patients had moderately severe and 68.3% had severe AP Table 1.

Outcome measures

Of all included patients, 20 patients (66.66%) in group 1 and 22 (73.33%) in group 2 were able to take prescribed feed orally ($p = 0.52$). Ten patients (33.33%) in group 1 and eight patients (26.66%) in group 2 were on tube feeding as they were not able to tolerate diet orally ($p = 0.52$). Parenteral nutrition was required in those patients who could not tolerate feeding immediately after randomization and was required in 2 and 3 patients in group 1 and group 2, respectively ($p = 0.57$).

Two patients in each group had refeeding pain ($p = 0.99$), and one patient in group 1 and three patients in group 2 required feed discontinuation due to other GI symptoms ($p = 0.99$). Only 8 (28.6%) patients in kitchen-based diet and 3 (12%) in polymeric formulation group were able to tolerate feed/diet without any complications like pain, nausea, vomiting and bloating with no significant difference between the groups ($p = 0.17$). There was no significant difference in tolerability measures like abdominal pain, bloating, nausea, and vomiting (71.4% in kitchen diet group vs 88% in polymeric formulation group, $p = 0.25$) except the incidence of diarrhea which is significantly less in the group 1 taking kitchen-based diet (14.29% vs 48%, $p = 0.02$). No significant difference was observed in incidence of infections (57.14% vs 36%, $p = 0.12$), new onset organ failure (10.7% vs 12%, $p = 0.99$) and mortality (31.7% vs 20%, $p = 0.69$) (Table 2). Most of the patients with infected necrosis were treated conservatively with antibiotics during the trial while two patients in group 1 and one patient in group 2 required surgery. Most of the deaths occurred after two weeks which were due to septic shock and multi organ failure.

There was no significant difference between the nutritional parameters i.e. serum pre-albumin, serum transferrin and serum CRP level at baseline or at day 14 after the dietary intervention in

Table 1. Baseline demographic and biochemical characteristics of the patients in the study.

Parameters	Kitchen-based diet (Group 1) ($n = 30$)	Polymeric formulation (Group 2) ($n = 30$)	*P* value
Age (years)	36.26 ± 14.06	36.03 ± 11.43	0.94
Sex (M/F)	18/12	21/9	0.41
BMI (kg/m^2)	24.22 ± 3.66	24.08 ± 2.95	0.87
Etiology *n* (%)			
Gallstones	13 (43.33)	14 (46.67)	0.67
Alcohol	10 (33.33)	9 (30.00)	
Hyperparathyroidism	1 (3.33)	0 (0.00)	
Idiopathic	6 (20.00)	7 (23.33)	
Hemoglobin (g/dL)	9.30 ± 1.85	9.95 ± 1.98	0.19
TLC (μL)	18,010 (5250–45,650)	18,060 (7130–81,000)	0.59
Platelets (×10^9/L)	265.5 (91–752)	208 (82–968)	0.11
Urea (mg/dL)	28.5 (6–331)	22 (8–299)	0.93
Creatinine (mg/dL)	0.7 (0.3–13.2)	0.6 (0.1–15.0)	0.89
Total Bilirubin (mg/dL)	0.75 (0.4–2.9)	1.0 (0.4–12.8)	0.09
ALT (IU/L)	40 (8–735)	31.5 (11–570)	0.74
AST (IU/L)	37 (5–293)	26.5 (7–730)	0.30
ALP (IU/L)	243.5 (70–633)	203 (24–610)	0.62
Sodium (mEq/L)	138.7 ± 8.04	141 ± 6.79	0.23
Potassium(mEq/L)	4.17 ± 0.67	3.88 ± 0.58	0.09
Calcium (mg/dL)	7.74 ± 0.59	7.59 ± 1.02	0.51
Phosphorous (mg/dL)	3.45 (1.4–9.4)	2.6 (1.2–16.6)	0.08
Total protein (g/dL)	5.84 ± 0.71	5.52 ± 0.42	0.04
Albumin (g/dL)	2.70 ± 0.38	2.77 ± 0.55	0.55
Amylase (IU/L)	900 (28–7850)	1161 (107–5520)	0.10
APACHE-II score	11(3–32)	9(4–28)	0.27
Days between onset of pain and initiation of feed	11.5 (6–14)	9.5 (5–14)	0.10
Organ failure [n(%)]			
No organ failure	3 (10.00)	6 (20.00)	0.55
Single organ failure	17 (56.67)	15 (50.00)	
Multiple organ failure	10 (33.33)	9 (30.00)	
Severity of pancreatitis			
MSAP	9 (30.00)	10 (33.33)	0.99
SAP	21 (70.00)	20 (66.66)	
Route of feeding			
Oral	20 (66.66)	22 (73.33)	0.52
Tube feeding	5 (16.66)	6 (20.00)	
Mixed(oral+tube feeding)	5 (16.66)	2 (6.66)	

Expressed in mean ± SD and median(min-max).
ALP alkaline phosphatase, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *APACHE-II* acute physiology and chronic health evaluation II, *BMI* body mass index, *INR* international normalized ratio, *MSAP* moderately severe acute pancreatitis, *SAP* severe acute pancreatitis, *TLC* total leukocyte count.

both groups (Table 3). Analysis restricted to subgroups of MSAP and SAP also did not show any significant differences in any primary or secondary outcomes.

DISCUSSION

The present study was a pilot randomized control trial where we compared two types of enteral feeding formulations in patients with MSAP and SAP i.e., kitchen-based diet vs commercial polymeric formulation feed. In patients with severe or moderately severe AP, our results indicate that both kitchen-based diet and commercial polymeric formulation were similarly tolerated for early initiation of feeding with no significant difference in refeeding pain, infectious complications, nutritional and inflammatory markers. The kitchen-based diet was additionally associated with significantly lower frequency of diarrhea.

The incidence of refeeding pain in acute pancreatitis was equal in both groups and required temporary interruption of diet with gradual reintroduction. The incidence of pain exacerbation with both the diets was similar to previous studies and a recent meta-analysis analyzing the nasogastric and nasojejunal feeds in severe AP [9, 26]. Parenteral nutrition was required in 8% of patients when the calories required were inadequate or patients were not tolerating enteral feed. Other outcomes, including infectious

Table 2. Comparison of the study parameters in kitchen-based diet vs polymeric formulation in patient with acute pancreatitis.

Parameters	Kitchen-based diet (Group 1), $n = 28$	Polymeric formulation (Group 2), $n = 25$	*P* value
Patient requiring feed discontinuation (total)			–
A. Due to Refeeding pain (*n*)	2	2	0.99
B. Due to other symptoms (*n*)	1	3	–
	(ileus – 1)	(ileus-1,diarrhoea-1,gastrointestinal bleed- 1)	–
Tolerability			
Patients who tolerated feeds without any symptoms	8	3	0.17
Complications:			
A. GI Symptoms, *n* (%):			
Abdominal pain	13 (46.42)	11 (44.00)	0.86
Abdominal bloating	15 (53.57)	16 (64.00)	0.44
Nausea	13 (46.43)	12 (48.00)	0.91
Vomiting	4 (14.28)	7 (28.00)	0.22
Diarrhea	4 (14.29)	12 (48.00)	0.02
B. Infectious complications, *n*			
1. New onset fever	5	8	0.23
2. Culture positive infections	6	6	0.82
Blood	8	8	0.79
Urine	9	5	0.32
Pus	6	4	0.73
Endotracheal	4	1	0.36
Ascitic fluid	3	1	0.61
Pleural fluid	2	1	0.99
Others (Bile, Central line tip, Tissue)	3	3	0.99
C. New onset organ failure, *n*			
Partial parenteral nutrition	2 (7.14)	3 (12.00)	0.57
Cumulative total energy prescribed (kcal) in 14 days	22,215 ± 2894	21,856 ± 2933	0.66
Cumulative total energy delivered (kcal) in 14 days	12,864 ± 4732	15,490 ± 4913	0.05
Mortality	8 (28.6)	5 (20)	0.69
Hospital stay* (days)	19.5 (7–82), $n = 20$	23.5 (9–54), $n = 20$	0.86
ICU stay (days)	10.5 (1–27), $n = 10$	6 (1–16), $n = 11$	0.41

Expressed in *n* (%), mean ± SD and median (min-max).
*Hospital stay among the patients who improved and were discharged ($n = 40$).

complications, length of hospital stay, and outcome (death or discharge) were similar in both groups.

We did not find any significant difference in tolerance with feed initiation between the two groups except for lower incidence of diarrhea with kitchen-based diet. Kitchen-based diet is good in soluble fibers and could be better in maintaining a healthy gut microbiome. This might be the probable reason for less incidence of diarrhea in group taking kitchen-based diet. Another important point to note was that the total cumulative energy received by patients was less than the prescribed energy intake in both groups. In critically ill patients, permissive underfeeding (40 to 60% of estimated caloric requirements) is believed to be acceptable and has similar outcomes to the standard calorie intake [27]. We however still need to devise strategies to deliver more calorie intake to these patients as all of them have increased metabolic demand but can't tolerate feeds in initial period of the disease. There was no significant difference in nutritional (serum prealbumin and transferrin) and inflammatory markers (serum CRP) in both groups. Improvement in pre-albumin and decline in CRP and TLC levels at the end of 2 weeks after intervention in both groups shows equivalence of both type of feeds. Cost analysis of both the feeds showed that kitchen-based feed was approximately 8 folds cheaper than commercially available polymeric formulation.

Our present study reveals a completely different aspect of feeding that kitchen-based diet can be used in patients with AP. We believe that kitchen-based feeds are economical, gut friendly, easily available, and more palatable than commercial formulations. The strength of the present study is that easily available kitchen-based diet was compared to commercial polymeric feed with reasonable sample size for early enteral nutrition in AP. A major limitation of our study is that large number of exclusions occurred due to patients presenting late at our center probably due to referral bias. Ours was also a pilot study, and in all likelihood it was underpowered to detect differences in primary outcome. We also did not evaluate patient satisfaction or subjective palatability with the prescribed diet. We believe that our results could be generalizable to the patients who fulfill the

Table 3. Comparison of biochemical (nutritional and inflammatory markers) parameters inkitchen-based diet vs polymeric formulation in patient with acute pancreatitis.

	Day 1 parameters			Day 14 parameters			Within group (Day 1 vs Day14) comparison	
Parameter	**Kitchen-based diet ($n = 30$)**	**Polymeric formulation ($n = 30$)**	***P* value**	**Kitchen-based diet ($n = 28$)**	**Polymeric formulation ($n = 25$)**	***P* value**	***P* value (Kitchen based diet)**	***P* value Polymeric (formulation)**
Hemoglobin (g/dL)	9.3 ± 1.8	9.9 ± 1.9	0.19	9.5 ± 2.24	9.1 ± 2.06	0.64	0.39	0.04
Serum albumin (g/dL)	2.7 ± 0.38	2.8 ± 0.55	0.55	2.93 ± 0.63	2.91 ± 0.47	0.89	0.06	0.34
Serum pre albumin (mg/dL)	4.05 (0.90–15.67)	4.59 (0.83–19.65)	0.69	9.45 (1.42–75.91)	13.09 (0.91–88.85)	0.68	0.01	0.01
Serum transferrin (mg/dL)	13.88 (0.12–86.23)	20.05 (1.02–50.85)	0.17	23.58 (5.45–80.68)	25.56 (2.71–62.82)	0.75	0.02	0.12
CRP (mg/L)	459.8 (7.1–3052)	415.3 (16.9–2334)	0.73	74.37 (8.59–94.55)	59 (6.26–778.4)	0.97	0.00	0.00
TLC/μL	18010 (6800–45650)	18060 (7130–81000)	0.59	10,040 (3360–31,540)	10,775 (4650–27,330)	0.53	0.01	0.01

Expressed in mean ± SD and median (min-max).
CRP C-reactive protein, *TLC* total leukocyte count.

study inclusion and exclusion criteria, however there remains a need for a larger, multicenter study including a broader group of patients to assess these outcomes fully and validate our findings.

To conclude, in patients with moderately severe or severe AP, the tolerability and clinical outcomes with the kitchen-based diet are comparable to commercial polymeric formulation for early enteral nutrition. Kitchen-based diet was associated with lower incidence of diarrhea. Kitchen-based diet is economical, easily available, palatable and can be used in the patients with acute pancreatitis. We suggest that commercial formulations which are expensive and non-palatable can be replaced by kitchen-based diet in patients with acute pancreatitis in a resource constrained setting, with no significant impact on patient tolerability or clinical outcomes.

DATA AVAILABILITY

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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AUTHOR CONTRIBUTIONS

IG - conceptualization of the study, acquisition of data, analysis of data, drafting of manuscript. DG - patient care, drafting of manuscript, critical revision of the manuscript. NS - patient care, analysis of data, drafting of manuscript. SG - patient care, drafting of manuscript. HS- statistical analysis. VS - laboratory work. AS - conceptualization of the study, patient care, analysis of data, drafting of manuscript, critical revision of the manuscript

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COMPETING INTERESTS

The authors declare no competing interests.

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