

**ROLE OF GLUTAMINE SUPPLEMENTATION ON IMPROVING THE NUTRITIONAL, FUNCTIONAL AND IMMUNOLOGICAL STATUS OF MALIGNANT OBSTRUCTIVE JAUNDICE PATIENTS AT A TERTIARY CARE CENTRE.**

**Induja Awasthi<sup>1\*</sup>**, Abhijit Chandra<sup>1</sup>, Lav Kumar Kacker<sup>1</sup>, Anup Kumar Wahal<sup>1</sup>, S M Natu<sup>1</sup> and Pankaj Kumar<sup>1</sup>

<sup>1</sup>Department of Surgical Gastroenterology, CSMMU.

**Corresponding author\*:** [induja.awasthi@gmail.com](mailto:induja.awasthi@gmail.com)

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**SUMMARY**

**Introduction:** Incidence of carcinoma gall bladder in India ranges from 1.01/100000 for males to 10.1/100000 for females. Glutamine decreases intestinal permeability, maintain intestinal barrier and attenuate systemic inflammatory response.

**Objectives:** Assessment of Nutritional status, functional status and mortality in Non-supplemented (Gp.A) / Glutamine supplemented (Gp.B) Malignant Obstructive Jaundice (MOJ) patients in which endoscopic drainage /surgery was impossible.

**Methods:** Fifty patients Gp.A-25 and Gp.B-25, were included in the study. Supplementation was 50 ml (20g)/day intravenously to the patients for 3 days and 30g/day orally for 4 days. BMI, Karnofsky Score and mortality were assessed on 42<sup>nd</sup> day.

**Results:** Gp. A mean BMI was 16.32±2.64 on admission and 16.37±3.42 on 42<sup>nd</sup> day. In Gp.B mean BMI on admission was 18.15±1.27 which improved to 21.35±2.53 on 42<sup>nd</sup> day. In Gp.A mean Karnofsky score was 55.60±20.02 on admission and 21.20±25.71 on 42<sup>nd</sup> day. In Gp.B mean Karnofsky score on admission was 60.00±19.58 and 24.00±33.54 on 42<sup>nd</sup> day. The mean IL-2 Level in Gp.A was 13.93±26.80 pg/ml on admission and 81.30±79.46 pg/ml on 42<sup>nd</sup> day. The mean IL-2 Level in Gp.B was 9.07±26.93 pg/ml on admission which reduced to 8.27±15.21 pg/ml on 42<sup>nd</sup> day. IL-6 Level in Gp.A was 79.43±96.83pg/ml on admission and 173.10±217.94pg/ml on 42<sup>nd</sup> day. IL-6 Level in Gp.B was 390.06±345.22 pg/ml on admission which reduced to 230.71±201.65pg/ml on 42<sup>nd</sup> day. TNF-α Level in Gp.A was 59.53±56.63pg/ml on admission and 202.30±224.84pg/ml on 42<sup>nd</sup> day. TNF-α Level in Gp.B was 95.33± 72.21 pg/ml on admission which reduced to 67.80±52.35pg/ml on 42<sup>nd</sup> day.

**Conclusion:** Glutamine supplementation helps in improving the Nutritional status and reducing mortality of MOJ patients.

**KEY WORDS:** IL-2, IL-6, TNF-α

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**INTRODUCTION**

Gallbladder cancer is the most common malignancy of the biliary tract. Women are affected three times more commonly

than men, and the vast majority patients stricken with this disease are older than 40 years of age. Among the highest incidences in the world are in women from Chile (27/100,000), Poland

(14/100,000), India (10/100,000), Japan (7/100,000), and Israel (5/100,000). Other high-risk areas include parts of Pakistan, Korea, Slovakia, Spain, Ecuador, and Colombia.<sup>5</sup> Patients with unresectable or metastatic GBC have a poor prognosis.<sup>[1]</sup> Due to its non specific clinical presentation, it is seldom diagnosed preoperatively except in advanced cases.<sup>9</sup> Cancer epidemiology articles often point out that cancer rates tend to be higher among males than females yet rarely is this theme the subject of investigation.<sup>3</sup> The aetiology of carcinoma gall bladder is poorly understood. Chronic cholecystitis and gallstones, choledochal cysts, female gender, age and exposure to carcinogens are some of the factors implicated in the causation of gall bladder cancer but a definite cause - effect relationship has yet to be established for any of these factors.<sup>9</sup> Glutamine, the most abundant amino acid in the body, is thought to become conditionally essential in critical illness. It plays a central role in nitrogen transport within the body, is a fuel for rapidly dividing cells, such as in the gut and immune system and has many other metabolic functions. Under normal physiological conditions, Glutamine is synthesised in large amounts by the human body and is therefore considered non-essential. It has been hypothesized that glutamine may become a conditionally essential amino acid in patients with catabolic disease.<sup>11</sup>

## Materials and Methods

### *PATIENTS ELIGIBILITY CRITERIA*

Fifty malignant obstructive jaundice patients in which endoscopic drainage was not possible (the obstruction was not negotiable by guide wire) were included in the study. Glutamine Non-

supplemented (Gp.A-25) /supplemented (Gp.B-25). The sample size was determined statistically. The patients were taken from the OPD of Department of Surgical Gastroenterology, CSMMU, Lucknow, Uttar Pradesh (Letter no. 5029/R.Cell-10, Dated:11/01/10, Ref.code-XLEM/A-P4). Patients were between 20-80 years of age. They complained of icterus, pruritus, pain in abdomen, yellow sclera and skin, pale urine, clay coloured stools, nausea, vomiting etc. Patients were eligible for inclusion in the study when they presented with obstructive jaundice with bilirubin concentrations  $\geq 5\text{mg}\%$  and if introduction of an endoprosthesis by endoscopic retrograde cholangiopancreatography (ERCP) was not possible. Malignancy was confirmed by FNAC/Biopsy/Brush cytology. Glutamine supplementation was 50ml (20g) / day intravenously (Aminoven manufactured and marketed by Fresenius kabi India Pvt. Ltd.) the patient for 3 days and 30g/day of Glutamine Orally (Kabimmune manufactured by Drytech process India Pvt. Ltd. and marketed by Fresenius kabi India Pvt. Ltd.) for 4 days. Prior consent was obtained by the patients.

### *PATIENTS EXCLUSION CRITERIA:*

Patients were excluded from the study when they presented with obstructive jaundice with bilirubin concentrations  $< 5\text{mg}\%$  and if Patients were unfit for ERCP/ surgery.

### *STUDY DESIGN*

The study was designed so that all patients were attempted an ERCP and stent placement. The patients in which ERCP was not possible were taken in the study. A prospective, randomized,

controlled trial was performed. The study was an intervention study.

### *SAMPLING*

5ml blood sample, four times (procedure day, 7<sup>th</sup> day, 21<sup>st</sup> day and 42<sup>nd</sup> day) was obtained from the patients. Serum was centrifuged from it then stored at -20°C until testing. A questionnaire was used to obtain the general information and biochemical test results. Biochemical tests were performed in the laboratory of Pathology Department, CSMMU.

### *ASSAYS*

Serum concentrations of Cytokines were assessed by the highly sensitive, AviBion Human IL-2, IL-6 and TNF- $\alpha$  ELISA kits according to the manufacturer's instructions (Orgenium Laboratories Business Unit, Vantaa Finland). Nutritional status was determined by BMI, Functional status was determined by Karnofsky Score, mortality was obtained by assessing the period of survival of the patients for all fifty patients.

*BMI:* Was determined by using formula: mass (kg) / (height (m))<sup>2</sup>.

### *ETHICAL APPROVAL*

The study was approved by Ethics Committee of CSMMU (Letter no. 5029/R.Cell-10, Dated: 11/01/10, Ref.code-XLEM/A-P4).

### **Statistical Methods**

Results are expressed as mean  $\pm$  SD and proportion, as appropriate. Statistical differences between haematological, chemical, and cytokine data were

analysed using the Wilcoxon rank sum test or two sample t-test adjusting for unequal variance, if required. Stata 11.1 was used to analyze the data. A p value <0.05 was considered to be significant.

### **Results**

**Age group:** The mean age of the patients in Gp.A was 48.92 $\pm$ 8.3 years and in Gp. B it was 46.56 $\pm$ 13.3 years (Table-1). Maximum patients were between 41-50 years of age.

**Sex:** In Gp.A- 10 i.e. 55.56% patients were females and in Gp.B-8 i.e. 44.44% patients were female (Table-1).

**Duration of Jaundice in patients:** In Gp.A, mean duration of jaundice was since last 89.52 $\pm$ 54.9 days. Whereas, in Gp.B mean duration of illness was 97.56 $\pm$ 43.8 days. (Table-1).

**Cholelithiasis association with MOJ:** It was found that in Gp.A, 10 patients i.e. 32.26% had Cholelithiasis and in Gp. B 21 patients i.e. 67.74 % had cholelithiasis (Table-1).

**Nutritional status:** In Gp.A the mean BMI was 16.32 $\pm$ 2.64 on admission which was improved to 16.37 $\pm$ 3.42 on 42<sup>nd</sup> day. In Gp.B the mean BMI on admission was 18.15 $\pm$  1.27 which was improved to 21.35 $\pm$ 2.53 on 42<sup>nd</sup> day (Table-2).

**Diet:** The dietary intake of patients was very poor due to persistent nausea and vomiting. Most of the patients at the time of admission were malnourished.

**Functional status:** The mean Karnofsky score in Gp. A was 55.60 $\pm$  20.02 on admission which was reduced to 21.20 $\pm$  25.71 on 42<sup>nd</sup> day. In Gp.B the mean

Karnofsky score on admission was  $60.00 \pm 19.58$  which was reduced to  $24.00 \pm 33.54$  on 42<sup>nd</sup> day (Table-2).

**LFT:** The S.Bilirubin levels (total) in Gp. A were  $13.30 \pm 4.40$  mmol/L on admission, which was increased to  $17.47 \pm 4.31$  mmol/L on 42<sup>nd</sup> day. In Gp. B the levels were  $14.65 \pm 5.54$  mmol/L on admission which was increased to  $16.14 \pm 4.83$  mmol/L on 42<sup>nd</sup> day (Table-2). The S.Bilirubin levels (direct) in Gp. A were  $9.94 \pm 3.77$  mmol/L on admission which was increased to  $15.60 \pm 3.31$  mmol/L on 42<sup>nd</sup> day. In Gp. B the levels were  $9.42 \pm 2.69$  mmol/L on admission, which was reduced to  $7.93 \pm 2.76$  mmol/L on 42<sup>nd</sup> day (Table-2). The SGPT levels in Gp.A were  $127.43 \pm 74.41$  IU/L on admission, which was increased to  $154.39 \pm 66.45$  IU/L on 42<sup>nd</sup> day. In Gp. B the levels were  $66.45 \pm 35.29$  IU/L on admission, which was reduced to  $56.27 \pm 31.46$  IU/L on 42<sup>nd</sup> day (Table-2). The SGOT levels in Gp. A were  $146.14 \pm 75.69$  IU/L on admission, which was increased to  $169.82 \pm 76.96$  IU/L on 42<sup>nd</sup> day. In Gp. B the levels were  $55.01 \pm 38.33$  IU/L on admission, which was reduced to  $53.11 \pm 38.57$  IU/L on 42<sup>nd</sup> day (Table-2). The S.Alkaline Phosphatase levels in Gp. A were  $2183.75 \pm 1299.48$  IU/L on admission, which were increased to  $2414.63 \pm 1335.30$  IU/L on 42<sup>nd</sup> day. In Gp. B the levels were  $1736.09 \pm 1478.42$  IU/L on admission, which was reduced to  $1114.27 \pm 877.05$  IU/L on 42<sup>nd</sup> day (Table-2).

**Interleukin-2:** The mean IL-2 Level in Gp.A was  $13.93 \pm 26.80$  pg/ml on admission, which was increased to  $81.30 \pm 79.46$  pg/ml on 42<sup>nd</sup> day. The mean IL-2 Level in Gp.B was  $9.07 \pm 26.93$  pg/ml on

admission, which was reduced to  $8.27 \pm 15.21$  pg/ml on 42<sup>nd</sup> day (Table-3).

**Interleukin-6:** The mean IL-6 Level in Gp.A was  $79.43 \pm 96.83$  pg/ml on admission, which was increased to  $173.10 \pm 217.94$  pg/ml on 42<sup>nd</sup> day. The mean IL-6 Level in Gp.B was  $390.06 \pm 345.22$  pg/ml on admission, which was reduced to  $230.71 \pm 201.65$  pg/ml on 42<sup>nd</sup> day (Table-3).

**Tumor Necrosis Factor - $\alpha$ :** The mean TNF- $\alpha$  Level in Gp.A was  $59.53 \pm 56.63$  pg/ml on admission, which was increased to  $202.30 \pm 224.84$  pg/ml on 42<sup>nd</sup> day. The mean TNF- $\alpha$  Level in Gp.B was  $95.33 \pm 72.21$  pg/ml on admission, which was reduced to  $67.80 \pm 52.35$  pg/ml on 42<sup>nd</sup> day (Table-3).

**Duration of survival of patients:** In Gp. A, the mean survival of patients was  $60.56 \pm 94.0$  days. Whereas, in Gp. B the mean survival of patients was  $40.92 \pm 57.7$  days (Table-1).

**Overall mortality:** In Gp. A, 17 patients (54.84%) died within 42 days, the male:female ratio being 13:4. Whereas, in Gp. B only 14 patients (45.16%) died within 42 days in which the male:female ratio was 8:6. (Table-1).

**Viral markers:** None of the patients out of enrolled 50 patients were positive for HIV, HbSAg or HCV.

**Special Observation:** The special observation was that Glutamine supplementation helped in reducing the levels of all the liver function tests significantly.

**Total bilirubin after completion of study:** Significant improvement was seen

in the levels of total bilirubin after completion of study. The levels in Gp. A were  $13.30 \pm 4.40$  mmol/L on admission, which was increased to  $17.47 \pm 4.31$  mmol/L on 42<sup>nd</sup> day. In Gp. B the levels were  $14.65 \pm 5.54$  mmol/L on admission which was increased to  $16.14 \pm 4.83$  mmol/L on 42<sup>nd</sup> day. There was an increase in bilirubin levels of Gp.A by  $4.17 \pm 0.90$  mmol/L, whereas in Gp.B it was by only  $1.49 \pm 1.72$  mmol/L.

## Discussion

This study analyzed the effect of Glutamine supplementation on nutritional status, functional status and immunological status of patients with obstruction of the biliary tract. The average age of the patients in Gp.A was similar to that of patients in Gp. B i.e. patients were between 45-60 years of age. In a study, it was observed that majority of patients with obstructive jaundices were in the range of 60 to 69 years old in male and 50 to 59 years in female.<sup>4</sup> In Gp.A, 55.56% patients were females and in Gp.B, 44.44% patients were female which shows that GB carcinoma has a female preponderance. Another study stated that the female-to-male ratio is 3:1.<sup>2</sup> In a study it was found that 44.36% were male and a female to male ratio was 1.25.<sup>4</sup> The average duration of jaundice was since last 125-140 days. A study stated the average duration of jaundice was 1.15 months i.e. 45-46 days.<sup>10</sup> In Gp.A, 32.26% patients had Cholelithiasis and in Gp.B, 67.74% patients had cholelithiasis. Earlier studies have reported Cholelithiasis to be one of the causal factors of MOJ.<sup>9</sup> In a study, it was found that Cholecystolithiasis, is present in 70-90% of patients.<sup>2</sup> The risk of carcinoma gall bladder in patients with gall stones

may be increased 4 to 7 times.<sup>13</sup> In another study, association of gall stones with carcinoma gall bladder has been found.<sup>8</sup> Significant difference was found in the improvement in BMI of the supplemented group. A showed that GBC influences the nutritional status of the patients. Forty-three percent of GBC patients were malnourished with low body mass index (BMI).<sup>7</sup> An elevated body mass index and multiparity are also correlated with an increased risk of developing gallbladder cancer. A Norwegian cohort study including over two million people and 1715 cases of gallbladder cancer showed a relative risk developing gallbladder cancer of 2.53 for women aged 20-44 years patients with a body mass index greater than 30.<sup>5</sup> No significant improvement was seen in the functional status of both the group. This might be because of carcinoma being in very advanced stage. The levels S.Bilirubin levels (total), S.Bilirubin levels (direct), SGPT, SGOT and S.Alkaline Phosphatase levels increased on 42<sup>nd</sup> day in Gp.A but the levels decreased significantly in the Glutamine supplemented group i.e. Gp.B. In a study, mean serum bilirubin level in patients with obstructive jaundice was  $230 \pm 110$  mmol/L, mean SGPT levels were  $172 \pm 175$  IU/L, mean SGOT levels were  $205 \pm 188$  IU/L and mean S.Alkaline Phosphatase levels were  $817 \pm 868$  IU/L.<sup>12</sup> IL-2 is a cytokine which is normally produced by the body during an immune response. The levels of IL-2 were increased in Gp.B but were significantly decreased in Gp.A after supplementation. IL-6 is a pro-inflammatory cytokine secreted by T cells and macrophages to stimulate immune response to trauma, especially burns or other tissue damage leading to inflammation. The IL-6 levels were also decreased significantly in the supplemented group than in the non-supplemented group. TNF  $\alpha$  is a

cytokine involved in systemic inflammation and is a member of a group of cytokines that all stimulate the acute phase reaction. Dysregulation and, in particular, overproduction of TNF have been implicated in a variety of human diseases, as well as cancer. The TNF- $\alpha$  levels were also decreased significantly in the supplemented group than in the non-supplemented group. The mean TNF- $\alpha$  and CRP concentrations were significantly lower in the supplemental group than in the control group ( $P < 0.05$ ), and the increase in TNF- $\alpha$  levels postoperative was significantly lower in the supplemental group than in the control group ( $41.02 \pm 27.56$  vs  $160.09 \pm 35.17$ ,  $P < 0.05$ ).<sup>6</sup> The mean survival of patients was higher in the Glutamine supplemented group. Patients with GB carcinoma have an overall mean survival rate of 6 months, and the 5-year survival rate is 5%.<sup>2</sup> No significant difference was found in death of patients within 42 days. None of the patients out of enrolled 50 patients were positive for HIV, HbSAg or HCV. This shows that these viral markers have no association with malignant obstructive jaundice. The above study suggests that Glutamine supplementation may help the patients of malignant obstructive jaundice to fight against malnutrition and reducing the levels of proinflammatory cytokines.

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**Table 1: Baseline patients' characteristics**

Variables	Gp.A (n=25) Mean±SD	Gp.B (n=25) Mean±SD	p-value
Age (yrs)	48.92±8.3	46.56±13.3	0.1539
Duration of jaundice(days)	89.52±54.9	97.56±43.8	0.5383
Survival (days)	60.56±94.0	40.92±57.7	0.6202
Female sex	10 (55.56)	8 (44.44)	0.769
Presence of Cholelithiasis	10 (32.26)	21 (67.74)	0.001*
Death within 42 days	17 (54.84)	14 (45.16)	0.382

*Figures in parenthesis are %*

Table 2: Effect of glutamine on various parameters studied

Variables	Group A				Group B				p-value of change in groups
	n	Baseline Mean±SD	42 <sup>nd</sup> day Mean±SD	Change Mean±SD	n	Baseline Mean±SD	42 <sup>nd</sup> day Mean±SD	Change Mean±SD	
BMI	08	16.32± 2.64	16.37± 3.42	0.05± 2.27	9*	18.15± 1.27	21.35±2.53	3.20±3.72	0.0318*
Karnofsky score	25	55.60± 20.02	21.20± 25.71	-34.40± 30.29	25	60.00± 19.58	24.00± 33.54	-36.00± 34.76	0.8600
S. Bil (total)	08	13.30± 4.40	17.47± 4.31	4.17± 0.90	11	14.65±5.54	16.14±4.83	1.49±1.72	0.0007*
S.Bil (direct)	08	9.94± 3.77	15.60± 3.31	5.66± 1.79	11	9.42± 2.69	7.93± 2.76	-1.49± 0.46	0.0003*
SGPT	08	127.43±74.41	154.39±66.45	26.97± 17.15	11	66.45±35.29	56.27± 31.46	-10.18± 7.69	<b>0.0004*</b>
SGOT	08	146.14±75.69	169.82±76.96	23.68± 37.67	11	55.01±38.33	53.11± 38.57	-1.91± 9.20	0.0026*
Alkaline phosphatase	08	2183.75± 1299.48	2414.63± 1335.30	230.88± 216.14	11	1736.09± 1478.42	1114.27± 877.05	-621.82± 773.07	0.0003*

\* 2 patients had ascites.

**Table 3: Effect of glutamine on cytokines**

Variables	Group A				Group B				p-value of change in groups
	n	Baseline Mean±SD	42 <sup>nd</sup> day Mean±SD	Change Mean±SD	n	Baseline Mean±SD	42 <sup>nd</sup> day Mean±SD	Change Mean±SD	
IL-2	08	13.93± 26.80	81.30± 79.46	67.37± 56.58	11	9.07± 26.93	8.27± 15.21	-0.80± 18.73	0.0011*
IL-6	08	79.43± 96.83	173.10± 217.94	93.66± 122.92	11	390.06± 345.22	230.71± 201.65	-159.35± 144.80	0.0002*
TNF- $\alpha$	08	59.53± 56.63	202.30± 224.84	142.77± 171.71	11	95.33± 72.21	67.80± 52.35	-27.53± 27.61	0.0004*

**Abbreviations:**

1. Gln. = Glutamine
2. IL-2 = Interleukin-2
3. IL-6 = Interleukin-6
4. TNF- $\alpha$  = Tumor Necrosis Factor Alpha
5. BMI = Body Mass Index
6. SGOT = Serum glutamic oxaloacetic transaminase
7. SGPT = Serum glutamic pyruvic transaminase
8. S.Alp. = Serum Alkaline phosphatase
9. S.Bil. = Serum Bilirubin
10. ELISA = Enzyme-linked immunosorbent assay
11. MOJ = Malignant Obstructive Jaundice