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Abstract:

Objectives: This research is aimed to evaluate plasma free amino acid in gastric cancer patients without metastasis (early gastric cancer post gastrectomy) and with metastasis (advanced gastric cancer). Amino acids level of postoperative gastric cancer (M0) patients are compared with metastatic gastric cancer (M1) patients in search of biomarker which can predict the metastasis of gastric cancer. We have made clinical correlation of patients’ vital signs, respiratory rate, pulse rate, blood pressure, body temperature, disease stages, chief complaints, complications and survival curve within light of metastatic and nonmetastatic domain.

Background: Majority of cancer patients are diagnosed after seeding of metastatic cells to adjacent organs and distant sites. At this point, treatment is palliative and supportive. The cellular propagation of cancer cells and tumor micro-environment plays vital role in genesis of gastric cancer. Genetic alteration leading to faulty nucleotides to amino acids, then to protein, and finally formation of tumor is the natural sequence of pathogenesis of gastric cancer. Prediction of metastasis by use of plasma free amino acid profile may be of great significance because it will help to tailor the patient specific cancer treatment.

Plasma Amino acids are ideal for being developed as tool for prediction of metastasis as they are affordable, less expensive and convenient.

Method: This study includes total 54 patients, among which 27 had metastasis of Gastric cancer and rest 27 had undergone gastric surgery at early stage with no recurrence at the time of the study. Twenty-three amino acids were studied. Student’s t test was performed to find out statistically significant values of amino acids. The p value of ≤ 0.05 was considered statistically significant. Amino acids with significant p values were investigated with multivariate logistic regression. Partial Least Squares Discriminant Analysis (PLS DA) was done using Microsoft SPSS 23 version software®. Variable Importance of Projection (VIP) was estimated, values ≥ 1 was considered statistically significant.

Result: Performance Score (PS) (p= 0.004) and Body Mass Index (BMI) (p= 0.035) were statistically significant between M0 and M1 groups. Staging (I, II vs. III, IV) (p< 0.001) was significant. Seven amino acids, Asp, Cys, Hey, His, Leu, Orn and Ser were significant between M0 and M1 in first month evaluation. Eight amino acids, Cys, Hey, His, Leu, Met, Thr, Trp and Tyr were significant between M0 and M1 in sixth month evaluation. PLS DA regression analysis, VIP test showed Cys, Ser, Hey, Thr, His, Met, Tyr, Trp to be more important amino acids of significance. Kaplan Meier Overall Survival (OS) = 34.979 months. Mean survival time in M0 was 43.53± 1.741 months. Mean survival in M1 was 26.29± 2.635 months.

Conclusion: We found BMI and PS as most important variables in defining and determining the disease status of gastric cancer patients. Nutrition and physical activity is very much characteristic of disease outcome from a physician’s perspective. This study propounds amino acids can be valuable biomarkers of predictive and prognostic importance in metastasis in gastric cancer patients.
INTRODUCTION:
Cancer of stomach can spread through local extension to regional lymph nodes or develop lymphatic, peritoneal, hematogenous spread to distant sites. Gastric cancer is second most common cause of cancer-related deaths in the world, the epidemiology of which has changed within last decades [1]. Approximately 50% gastric cancer are localized and surgically resectable, during initial diagnosis. Effective cure by surgery alone isn’t adequate in many patients. Cancer relapse in patients who undergo complete surgery for gastric cancer, often occurs commonly in tumor bed, nodal regions and systemically [2]. In early upper GI cancers absence of particular presenting symptoms often enables these lesions to progress in an obscure way till they reach advanced stage and metastasize. Thus, discovery of biomarkers is exigent to help in the early diagnosis and treatment [3,4]. Overall prognosis of metastatic gastric cancer remains poor. Currently endoscopy, biopsy and histopathology examination are implemented for diagnosis of early gastric cancer or tumor recurrence [5,6]. This is an invasive procedure and can lead to complications. Diagnosis depends on quality of instrument and experience of operator [7].

In early stages of gastric cancer mostly patients don’t have specific symptoms. There are insufficient screening techniques now, thus advanced stage accounts for approximately 90% diagnosed cases. Five-year survival rate is 20% or less. This shows us the importance of research in mechanism of gastric cancer occurrence, which involves up- and down-regulation of numerous metabolomes. It can lead to identification of specific protein and amino acids markers for diagnosis and treatment of the disease [8]. The tumor microenvironment, including the immune cells and related cytokines, is crucial during all the steps of gastric carcinogenesis. Metabolomic products are seen in various physiological and pathological processes, which can be used as prognostic marker [9]. Gastric cancer is most frequently diagnosed malignancy in adults. China has substantial health burden of GC. Patients with metastatic GC prognosis is unfavorable, with median survival does usually not exceeding 1 year. It is very important to explore new diagnostic biomarkers and therapeutic targets for GC [10].

MATERIALS AND METHOD:
Initially 74 patients, age from 35 to 75 with gastric cancer were selected, 20 patients were excluded on the scientific basis of exclusion criteria. First group with early stage gastric cancer, who underwent gastric surgery and had no metastasis to distant organs (M0). Second group, patients who presented with advanced metastatic gastric cancer (M1) and came for palliative care. Fasting blood samples from 54 patients were obtained and stored by standard techniques and protocols including 30–60 min coagulation at room temperature, centrifuged for 10 min. ekspert™ ultraLC Systems – SCIEX 100 Liquid Chromatography machine and ABSCIEX 4000 QTRAP LCMS/MS Spectrometer was used.

STATISTICAL ANALYSIS:
This is a multivariate statistical analysis coupled with metabolomic mass spectrometric values. Statistical Analysis was done using SPSS® 23 along with Python 2.7 and PLS plugin. Pearson’s Chi Square test ($\chi^2$) was used to analyze demographic, clinical and pathological characteristics. Student’s t test was done to estimate the differences in means of amino acids values between metastatic gastric cancer and nonmetastatic
gastric cancer patients. Data were marked as mean ± SD. The differentially expressed amino acid variables with p value < 0.05 was considered statistically significant. Several diagnostic models were constructed. Regression model via PLS DA (Partial Least Squares Discriminant Analysis) was performed followed by VIP (Variable Importance of Projection) by was using SPSS. Finally, OS (Overall survival) of patients was analyzed with Kaplan Meier method.

**RESULT:**

**CLINICO-PATHLOGICAL FEATURES OF PATIENTS ADMITTED DUE TO GASTRIC CANCER:**
The overall mean age of all M1 patients was 61.00 (SD ± 9.41) years and M0 patients was 61.85(SD± 8.06) years (Fig 4.0). Patients with age ≥ 60 years constituted 64.82%. Patients with age < 60 years were 35.18% in this study respectively (Fig 5.0).

In M1 group, 23 males and 4 females participated. In M0 group, 21 males and 6 females participated.

The data set displayed gender wise distribution of patients in both metastatic and non-metastatic groups. Pearson’s Chi Square test ($\chi^2$) is statistically insignificant (p=0.484) for male and female genders.

Comparison of means of ages between metastatic and non-metastatic groups is statistically insignificant (p=0.723). Pearson Chi Square test ($\chi^2$) for age group<60 and ≥60 years is statistically insignificant (p=0.393). Chi Square test ($\chi^2$) is statistically significant (p=0.004) for ECOG (Eastern Cooperative Oncology Group) Performance Score (PS). Independent t test of BMI (Kg/m²) is statistically significant (p=0.035). Independent t test was statistically insignificant for patient’s temperature (p=0.674), respiratory rate (p=0.302), pulse rate (p=0.536), systolic (p=0.076) and diastolic (p=0.924) blood pressure. (Table 1)

**Table 1.0 Demographic and clinical characteristics of gastric cancer patients**

| Serial No. | Features                        | Metastatic mean±SD | Non-Metastatic Mean±SD | Statistical test | p value |
|------------|---------------------------------|--------------------|------------------------|------------------|---------|
| 1.         | Age                             | 61.00 (±9.41)      | 61.85 (± 8.06)         | t = -0.357       | 0.723   |
| 2.         | Age group                       |                    |                        | $\chi^2$ =0.731  | 0.393   |
|            | < 60 years                      | 8                  | 11                     |                  |         |
|            | ≥ 60 years                      | 19                 | 16                     |                  |         |
| 3.         | Gender                          |                    |                        | $\chi^2$ =0.491  | 0.484   |
|            | Male                            | 23                 | 21                     |                  |         |
|            | Female                          | 4                  | 6                      |                  |         |
| 4.         | Performance Score (ECOG-PS)     | 0,1,2,3            | 0,1,2,3                | $\chi^2$ = 13.54 | 0.004 * |
| 5.         | Body Mass Index (BMI)           | 21.21(±2.89)       | 22.92(± 2.65)          | t = -2.17       | 0.035 * |
| 6.         | Temperature                     | 36.78(±0.86)       | 36.75(±0.71)           | t = 0.423       | 0.674   |
| 7.         | Respiratory rate                | 19.74(±1.52)       | 19.33(±1.35)           | t = 1.043       | 0.302   |
| 8.         | Pulse rate                      | 82.25(±14.00)      | 85.88(±27.08)          | t = -0.623      | 0.536   |
| 9.         | Blood Pressure                  |                    |                        |                  |         |
|            | Systolic                        | 119.59(±17.95)     | 129.74(±24.37)         | t = -1.808      | 0.076   |
|            | Diastolic                       | 76.44(±9.31)       | 76.70(±11.09)          | t = -0.969      | 0.924   |

*p Value < 0.05 considered statistically significant.

The vital body functions were as following in metastatic gastric cancer patients.

Respiratory rate: 18-22/min (19.92, SD ± 0.4), pulse rate 58-110/min (80.44, SD ± 14), temperature 36.3-36.7°C (36.45, SD ± 0.16), systolic blood pressure 73-148 mmHg (119.8, SD ± 17.95), diastolic blood pressure 54-94 mmHg (76.16, SD ± 9.31) and BMI 16.60-27.34 kg/m² (21.36, SD ± 2.89) respectively.

The vital body functions were as following in non-metastatic patients.

Respiratory rate: 18-21/min (19.76, SD ± 0.83), pulse rate 58-110/min (86.92, SD ± 27.08), temperature 36.3-37.2°C (36.56, SD ± 0.16), systolic blood pressure 74-168 mmHg (129.88, SD ± 24.37), diastolic blood pressure 53-94 mmHg (76.64, SD ± 11) and BMI 16.94-27.34 kg/m² (22.53, SD ± 2.65) respectively.

The performance status in metastatic group were, 37% patients PS0, 48% patients PS1, 4% patients PS2 and 11% patients PS3. The performance status in non-metastatic group were, 76% patients PS0, 20% patients PS1, 4% patients PS2 and no patients with PS3 in this study.

The patients’ chief complain was identified as a cause of visit to hospital for seeking care. Patients presented with symptoms of abdominal pain, melena, abdominal bloating, vomiting, hematemesis, abdominal mass, nausea and dyspnea respectively. The factors like well/poorly differentiated (p= 0.143), degree of local invasion(p=0.761) and local lymph node metastasis (p=0.735) didn’t show statistically significant differences between metastatic and non-metastatic groups.

The major histological type of gastric carcinoma in both groups were gastric adenocarcinoma,81.48% in non-metastatic group and 66.67% in metastatic group. The major site of metastasis of
gastric cancer was peritoneal/ retroperitoneal space and organs. The second most common site of metastasis was liver. Pancreas, bone, ovaries etc. were also observed. The amino acid levels were obtained in the first month after diagnosis of gastric cancer and again after sixth months. Independent t test was performed to compare means of amino acids of first month between metastatic and non-metastatic patients which showed statistically significant values of seven amino acids. Asp (p= 0.042), Cys (p= 0.005), Hcy (p= 0.007), His (p= 0.022), Leu (p= 0.029), Orn (p= 0.011) and Ser (p= 0.013). (Table 2.0)

Independent t test was performed to compare means of amino acids of sixth month between metastatic and non-metastatic patients which showed statistically significant values of eight amino acids. Cys (p= 0.02), Hcy (p= 0.004), His (p= 0.04), Leu (p= 0.031), Met (p= 0.008), Thr (p= 0.007) Trp (p= 0.003) and Tyr (p= 0.035). (Table 3.0).

Table 2.0 Concurrent comparison of means of amino acids between metastatic and non-metastatic gastric cancer patients in 1st month with Independent Student’s T test.

| No. | Amino Acids | Metastatic cancer (μ mol/L) | Non-Metastatic Cancer (μ mol/L) | Student’s t Test | Sig. p value |
|-----|-------------|----------------------------|--------------------------------|-----------------|-------------|
| 1   | Ala         | 148.13 ± 53.51             | 165.84 ± 61.79                  | -1.126          | 0.265       |
| 2   | Arg         | 8.35 ± 5.01                | 8.35 ± 5.91                     | 0.002           | 0.999       |
| 3   | Asn         | 64.34 ± 16.47              | 64.80 ± 18.86                   | -0.95           | 0.925       |
| 4   | Asp         | 29.94 ±11.95               | 37.47 ± 14.47                   | -2.084          | 0.042 *     |
| 5   | Cit         | 22.37 ± 12.38              | 23.96 ± 15.55                   | -0.416          | 0.679       |
| 6   | Cys         | 1.43 ± 0.67                | 0.93 ± 0.57                     | 2.945           | 0.005 *     |
| 7   | Gln         | 9.46 ± 3.55                | 8.63 ± 3.52                     | 0.869           | 0.389       |
| 8   | Glu         | 165.16 ±71.29              | 149.82 ±49.21                   | 0.920           | 0.362       |
| 9   | Gly         | 203.18 ±76.67              | 217.59 ±76.19                   | -0.693          | 0.492       |
| 10  | Hcy         | 8.67 ± 0.75                | 7.99 ± 1.02                     | 2.783           | 0.007 *     |
| 11  | His         | 90.51 ±48.26               | 134.00 ±83.00                   | -2.354          | 0.022 *     |
| 12  | Leu         | 96.10 ± 25.02              | 118.83 ± 46.21                  | -2.248          | 0.029 *     |
| 13  | Lys         | 120.50 ± 42.16             | 134.24 ± 58.01                  | -0.996          | 0.324       |
| 14  | Met         | 18.9563 ± 15.04            | 18.16 ± 6.45                    | 0.251           | 0.803       |
| 15  | Orn         | 25.07 ± 17.20              | 40.59 ± 25.38                   | -2.630          | 0.011 *     |
| 16  | Phe         | 37.62 ± 10.28              | 37.11 ± 10.99                   | 0.175           | 0.862       |
| 17  | Pip         | 241.97 ± 88.01             | 228.59 ± 127.43                 | 0.449           | 0.655       |
| 18  | Pro         | 449.31 ±150.16             | 404.68 ± 158.21                 | 1.063           | 0.293       |
| 19  | Ser         | 65.00 ± 22.40              | 87.69 ± 39.76                   | -2.584          | 0.013 *     |
| 20  | Thr         | 29.25 ± 18.11              | 27.96 ± 13.17                   | 0.300           | 0.765       |
| 21  | Trp         | 46.89 ± 12.91              | 49.80 ± 14.52                   | -0.778          | 0.440       |
| 22  | Tyr         | 50.20 ± 15.13              | 47.23 ± 10.10                   | 0.849           | 0.400       |
| 23  | Val         | 130.39 ± 34.82             | 118.82 ± 40.44                  | 1.126           | 0.265       |

* p value <0.05 considered statistically significant.

Table 3.0 Concurrent comparison of means of amino acids between metastatic and non-metastatic gastric cancer patients in 6th month with Independent Student’s T test.

| No. | Amino Acids | Metastatic cancer (μ mol/L) | Non-Metastatic Cancer (μ mol/L) | Student’s t Test | Sig. p value |
|-----|-------------|----------------------------|--------------------------------|-----------------|-------------|
| 1   | Ala         | 168.23 ± 68.59             | 161.49 ± 54.73                  | 0.399           | 0.691       |
| 2   | Arg         | 7.25 ± 4.96                | 8.40 ± 5.39                     | -0.816          | 0.418       |
| 3   | Asn         | 62.90 ± 23.20              | 61.33 ± 20.24                   | 0.266           | 0.791       |
| 4   | Asp         | 30.69 ± 12.45              | 31.18 ± 12.66                   | -0.144          | 0.886       |
| 5   | Cit         | 20.74 ± 10.77              | 22.72 ± 16.67                   | -0.518          | 0.607       |
| 6   | Cys         | 0.91 ± 0.61                | 1.47 ± 1.06                     | -2.393          | 0.020 *     |
A paired sample t-test was performed to compare the means of amino acids of metastatic groups of patients in the first and sixth months, which showed statistically significant values for three amino acids: Cys (p=0.001), Glu (p=0.002), and Trp (p=0.006).

A paired sample t-test was also performed to compare the means of amino acids of non-metastatic groups of patients in the first and sixth months, which showed statistically significant values for six amino acids: Asp (p=0.044), Cys (p=0.017), Hcy (p=0.006), His (p=0.001), Met (p=0.030), and Orn (p=0.017). During all statistical analysis with a t-test, a p-value of <0.05 was considered statistically significant.

Partial Least Squares Regression Discriminant Analysis was performed with the selected amino acids obtained from the t-test. A Variable Importance of Projection (VIP) with latent factors was constructed using SPSS. All these values were used to build a PLS DA model. Regression model with PLS was established. A value of ≥1.00 was considered statistically significant for VIP.

PLS Regression Model in the First Month between metastatic and non-metastatic groups of patients: Three amino acids with a VIP value more than 1 were statistically significant in this test: Cys, Ser, and Hcy.

PLS Regression Model in the Sixth Month between metastatic and non-metastatic groups of patients: Four amino acids with a VIP value more than 1 were statistically significant in this test: Hcy, Met, Thr, and Tyr. Table (4.0)

Table 4.0 Variable Importance of Projection (VIP) in First and Sixth Months

| No. | Amino Acids | Metastatic cancer (μ mol/L) | Non-Metastatic Cancer (μ mol/L) | Student’s t Test | Sig. p value |
|-----|-------------|-----------------------------|---------------------------------|-----------------|-------------|
| 7   | Gln         | 7.93 ± 4.89                 | 8.12 ± 3.84                    | -0.166          | 0.869       |
| 8   | Glu         | 199.60 ± 72.90              | 157.16 ± 82.48                 | 2.003           | 0.050       |
| 9   | Gly         | 221.21 ± 65.70              | 208.46 ± 68.55                 | 0.697           | 0.489       |
| 10  | Hcy         | 7.60 ± 2.54                 | 10.77 ± 4.85                   | -3.012          | 0.004*      |
| 11  | His         | 109.64 ± 84.88              | 70.41 ± 46.11                  | 2.110           | 0.040*      |
| 12  | Leu         | 96.43 ± 38.99               | 122.65 ± 47.38                 | -2.221          | 0.031*      |
| 13  | Lys         | 124.39 ± 60.10              | 119.43 ± 68.24                 | 0.284           | 0.778       |
| 14  | Met         | 16.86 ± 7.71                | 23.83 ± 10.60                  | -2.759          | 0.008*      |
| 15  | Orn         | 27.98 ± 18.24               | 27.84 ± 13.81                  | 0.030           | 0.976       |
| 16  | Phe         | 41.04 ± 12.52               | 44.69 ± 35.71                  | -0.501          | 0.618       |
| 17  | Pip         | 251.46 ± 109.7              | 279.41 ± 137.44                | -0.826          | 0.413       |
| 18  | Pro         | 460.88 ± 134.22             | 448.69 ± 166.58                | 0.296           | 0.768       |
| 19  | Ser         | 70.38 ± 25.43               | 69.31 ± 28.30                  | 0.145           | 0.885       |
| 20  | Thr         | 32.66 ± 12.86               | 22.86 ± 12.69                  | 2.819           | 0.007*      |
| 21  | Trp         | 60.58 ± 21.45               | 49.34 ± 16.47                  | 2.159           | 0.035*      |
| 22  | Tyr         | 59.08 ± 24.12               | 41.56 ± 15.78                  | 3.157           | 0.003*      |
| 23  | Val         | 120.49 ± 46.55              | 118.72 ± 37.42                 | 0.154           | 0.878       |

*p value <0.05 considered statistically significant.

Paired sample t-test was performed to compare the means of amino acids of metastatic groups of patients in the first and sixth months, which showed statistically significant values for three amino acids: Cys (p=0.001), Glu (p=0.002), and Trp (p=0.006).

Paired sample t-test was also performed to compare the means of amino acids of non-metastatic groups of patients in the first and sixth months, which showed statistically significant values for six amino acids: Asp (p=0.044), Cys (p=0.017), Hcy (p=0.006), His (p=0.001), Met (p=0.030), and Orn (p=0.017). During all statistical analysis with a t-test, a p-value of <0.05 was considered statistically significant.

Partial Least Squares Regression Discriminant Analysis was performed with the selected amino acids obtained from the t-test. A Variable Importance of Projection (VIP) with latent factors was constructed using SPSS. All these values were used to build a PLS DA model. Regression model with PLS was established. A value of ≥1.00 was considered statistically significant for VIP.

PLS Regression Model in the First Month between metastatic and non-metastatic groups of patients: Three amino acids with VIP more than 1 were statistically significant in this test: Cys, Ser, and Hcy.

PLS Regression Model in the Sixth Month between metastatic and non-metastatic groups of patients: Four amino acids with VIP more than 1 were statistically significant in this test: Hcy, Met, Thr, and Tyr. Table (4.0)

Table 4.0 Variable Importance of Projection (VIP) in first and sixth months

| Variables | Latent Factors |
|-----------|----------------|
|           | 1   | 2   | 3   | 4   | 5   |
| Orn       | 0.982 | 0.900 | 0.897 | 0.899 | 0.899 |
| Cys*      | 1.060 | 1.142 | 1.137 | 1.136 | 1.136 |
| Asp       | 0.896 | 0.828 | 0.833 | 0.832 | 0.832 |
| Leu       | 0.880 | 0.808 | 0.812 | 0.811 | 0.812 |
| Ser*      | 1.066 | 0.989 | 0.992 | 0.995 | 0.995 |
| His       | 0.833 | 0.797 | 0.809 | 0.808 | 0.808 |
| Hcy*      | 1.227 | 1.431 | 1.424 | 1.423 | 1.423 |
| Sixth     | 1.163 | 1.189 | 1.189 | 1.190 | 1.190 |

*p value <0.05 considered statistically significant.
| Variables | Latent Factors |
|-----------|---------------|
| Met*      | 1.078         |
| Leu       | 0.888         |
| Tyr*      | 1.210         |
| Trp       | 0.866         |
| Thr*      | 1.099         |

*VIP ≥ 1.0 considered statistically significant.

PLS Regression Model in metastatic group in first month and sixth month: Two amino acids with VIP more were statistically significant in this test. Cys and Trp.

PLS Regression Model in non-metastatic group in first month and sixth month: Two amino acids with VIP more were statistically significant in this test Hcy and His.

Figure 1. PLS Regression matrix plot in sixth month between M1 and M0.

Figure 2. Kaplan Meier Overall Survival (OS) curve of gastric cancer patients
It is seen in our study that amino acid Cysteine is profoundly altered in process of metastasis. In our study the level of Cysteine in metastatic gastric cancer in first month 1.43 (±0.067) μmol/L decreased to 0.91 (± 0.61) μmol/L in sixth months. Threonine is higher in metastatic gastric cancer patients than non-metastatic gastric cancer. Our findings are consistent with the results obtained by Poschke et al., 2013.[21].

Serine is rapidly absorbed by cancer cells and largely used as intermediate metabolite. Our result showed that Serine is in low values in metastatic gastric cancer 65 (±22.40) μmol/L than in non-metastatic gastric cancer 87.69 (±39.76) μmol/L. This phenomenon can be explained with excessive consumption of Serine for proliferation of metastasis by cancer cells. Serine biosynthesis and consumption has been reported in some other cancer like melanoma, breast cancer, non-small cell lung cancer and ovarian cancer etc. [22]

Serine level is found to decrease in several other cancer types also, like Pancreatic cancer, Cervical cancer, Colorectal cancer and Gastric cancer. Patients of gastric cancer showed decrease in levels of Serine and Cysteine [23]. We found Histidine is high 134.00 (±83.00) μmol/L in first month which decreased to 70.41(±46.11) μmol/L in sixth month in case of non-metastatic cancer [24]. Decrease in histidine in gastric cancer patients was similarly reported in study done in 2011. But same study reported decrease in Trp in gastric cancer which is quite opposite in our study. Trp values in metastatic gastric cancer patients in first months, 46.89 (± 12.91) μmol/L has increased to 60.58 (± 21.45) μmol/L in six months [25].

In our study the plasma level of Serine was down regulated in metastatic group 65±22.40 μmol/L compared to non -metastatic group 87.69±39.76 μmol/L.

We found statistical significance in Trp between first month and sixth month in metastatic gastric cancer patients. The Trp level was 46.89 ±12.91 μmol/L in first month which increased to 60.58±21.45 μmol/L in sixth months which supports the important role of Trp during course of disease itself. Increased levels of tryptophan in various cancers like lung, breast, blood and colon have been discovered in previous studies. Tryptophan, Phenylalanine and Tyrosine increased signals in malignant tumors of lung and bladder in comparison to normal organs have been observed. Despite this, plasma amino acids may decrease severely in cancer patients suffering from significant malnutrition at the terminal stage [26, 27] Thus, Tryptophan is a key amino acid related to development of cancer. Tryptophan is utilized in biosynthesis of various proteins which form tumor parenchyma [28].

Retroperitoneal and peritoneal metastasis were highest in our study measuring up to 37.84%, followed by hepatic site 27.52%. It is supported by previous studies, done by Kishi K et al. and Ushimaru et al., found that seeding of free cancer cells and dissemination into peritoneum was most common type of metastasis in advanced gastric cancer. Despite doing extensive surgery for peritoneal metastasis the invasion of serosa by metastatic cells renders high mortality [29, 30, 31]. Prognosis is poor for these conditions. Peritoneal and hepatic are common distant metastasis sites as it was also seen in study done by Adachi Y et al., in 2000 [32, 33.34]. The pattern of metastasis...
has not changed in two decades. There was no statistically significant (p value = 0.735) relationship between metastatic and non-metastatic gastric cancer due involvement of lymph node. We found presence of metastasis to regional lymph node does not rule out metastasis to distant sites or organ. This finding is also supported by previous researchers Sobin et al., 2009 and Ichinose M et al., 2015, demonstrated that there is no significance of metastatic lymph nodes in gastric cancer [35, 36]. 

Construction of regression model PLS DA (Partial Least Squares Discriminant Analysis) was done followed by VIP (Variable Importance of Projection). Metastasis of cancer can be considered as an essential factor in prognosis and can be described by using amino acid levels in above model, Hu JD et al., 2011. The PLS DA score plots revealed clustering of gastric cancers sample values locate in different regions. Hence, we can conclude that several amino acid metabolites play crucial role as marker in metastasis of cancer cells, Lario S et al., 2017[37, 38]. 

Overall survival: Kaplan Meier analysis showed survival curve of metastatic group 26.29 ± 2.63 (95%CI) months which was significantly lower than the nonmetastatic group 43.53 ± 1.79(95%CI) months (p-value 0.001). Overall Survival was 34.979 ~ i.e. 35 months. The mean survival time of patients with metastatic gastric cancer was significantly lower than those with nonmetastatic gastric cancer. Thus, it can be inferred that expression of specific type of amino acids can point out occurrence of metastasis. 

CONCLUSION: 
Plasma free amino acids are substantially altered during the progress of disease and metastasis. Gastric cancer related amino acid markers have the potential to be developed as biomarker. Variations in plasma amino acid levels can be developed in the form of predictive biomarkers, which can then tell us about metastasis occurrence. It can be useful tool for assessment of gastric cancer patients. It can be used to monitor recurrence of tumor in patients after tumor resection or therapy. We are not very far from developing comprehensive set of biomarkers which can be very handy tool to be used in outpatient clinics and oncology wards in hospitals. 

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