Tyrosine, phenylalanine and tryptophan in gastro-esophageal malignancy – a systematic review

Running Title: Amino acids in gastro-esophageal cancer

Mr Tom Wiggins, MBChB MRCS,
Mr Sacheen Kumar, MBBS MRCS,
Mr Sheraz R. Markar, MBChB MRCS, MSc, MA
Mr Stefan Antonowicz, MBChB MRCS,
Professor George B. Hanna, PhD FRCS

Department of Surgery and Cancer
Imperial College London, St Mary’s Hospital, Praed Street, London, W2 1NY

Address for Correspondence: Professor George Hanna, Division of Surgery, Department of Surgery and Cancer, Imperial College London, 10th Floor, QEQM Building, St Mary’s Hospital, Praed Street, London, W2 1NY, UK.

E-mail: g.hanna@imperial.ac.uk
Telephone: +44 (0)20 3312 2124
Fax No: +44 (0)20 3312 6309

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Abstract

Gastro-esophageal cancer has a rapidly increasing incidence worldwide and reliable biomarkers are urgently required to facilitate earlier diagnosis and improve survival. The aromatic amino acids tyrosine, phenylalanine, and tryptophan represent potential biomarkers and their relation to gastro-esophageal cancer will be evaluated in this review.

An electronic literature search was performed to identify all published research relating to the measurement of tyrosine, phenylalanine or tryptophan in the biofluids or tissues of gastro-esophageal cancer patients.

Sixteen studies were included in this systematic review. Six studies investigated serum concentrations which all found decreased concentrations of these aromatic amino acids except one study which found increased phenylalanine. Five studies reported increased concentrations within gastric content of these patients and two reported increased urinary concentrations. Tissue concentrations of these aromatic amino acids were increased in three studies.

Tyrosine, phenylalanine and tryptophan represent potential biomarkers of gastro-esophageal cancer, and further research is necessary to definitively establish the mechanism responsible for altered concentrations of these compounds in gastro-esophageal cancer patients.

*Words: 163 words*
Introduction

The United Kingdom National Oesophagogastric Cancer Audit identified that there were 11,516 patients diagnosed with gastro-esophageal cancer between April 2011 and March 2012[1]. Globally there are an estimated 482,300 new esophageal cancer cases per annum. Gastric cancer affects nearly one million people worldwide per year and is responsible for around 10% of total cancer deaths[2]. There is significant geographical variation in the prevalence of gastric cancer with an incidence of up to 26.9 per 100 000 among males in Asia, compared with 3.4 per 100 000 among females in North America[3]. The prognosis of gastro-esophageal cancer is poor in Western countries as a result of the paucity of alarm symptoms in early stages of the disease resulting in late clinical presentation and delay in the institution of treatment[4]. Overall 5-year survival rates also vary geographically with better oncological outcomes in the Far East in part due to the existence of endoscopic or radiological screening programs that facilitate early detection of cancer[5]. The development of non-invasive, accurate biomarkers to determine gastro-esophageal cancer risk could help facilitate earlier diagnosis and improve patient survival.

Amino acids are compounds that have been muted as potential biomarkers of malignant disease. A recent review demonstrated that tryptophan was the metabolite with the most frequently altered concentrations across several cancer types[6]. Patients with esophageal cancer have reduced plasma levels of 14 separate amino acids including tyrosine, phenylalanine and tryptophan[7]. Cancer patients suffering with anorexia and malnutrition have the most distinctive changes in plasma amino acid profile[7]. Due to the obstructive nature of advanced esophageal cancer up to 79% of patients develop a degree of malnutrition prior to diagnosis[8][9]. Aromatic amino acid metabolism (specifically tyrosine, phenylalanine and tryptophan) may be dysfunctional in gastro-esophageal cancer[7]. Tryptophan and phenylalanine are both essential amino acids in the human diet. Tryptophan metabolism is linked to the production of serotonin. Phenylalanine is required for the production of the non-essential amino acid tyrosine. The production of tyrosine from phenylalanine was first discovered by Womack and Rose in 1934[10]. This conversion is catalyzed by the enzyme phenylalanine hydroxylase, which mainly functions in the liver, but
has been shown to be active in other body tissues including the kidney, pancreas and brain[11]. It has been shown that phenylalanine hydroxylase activity can be altered in inflammation or malignancy[12,13]. Tyrosine is broken down into several metabolites including L-DOPA, pyruvate, fumarate, and phenol. Tyrosine is converted to phenol via the action of the enzyme tyrosine phenol-lyase (β-tyrosinase). This reaction was first discovered in bacterial cultures by Kakihara & Ichihara[14]. Phenolic compounds are potential biomarkers of gastro-esophageal cancer with altered concentrations observed within exhaled breath[15], urine[16], and gastric content[17].

Investigation of aromatic amino acid profiles may yield suitable clinical assays to assess a patient's risk of gastro-esophageal cancer. This systematic review will investigate the current published literature to evaluate concentrations of tyrosine, phenylalanine and tryptophan within biofluids and tissues of gastro-esophageal cancer patients, and assess their potential as biomarkers of this disease.

**Materials and Methods**

A literature search (title and abstract) was performed using Ovid Medline® (1948-2012), Embase® (1974-2012), Web of Science® and PubMed® electronic databases up to and including 5th June 2014. This search was used to identify relevant studies that had measured tyrosine, phenylalanine or tryptophan within tissues or biofluids of patients with gastro-esophageal cancer and compared levels to an appropriate control group. The search was undertaken using the terms Tyrosine OR Phenylalanine OR Tryptophan AND (Cancer OR malignancy OR neoplasm) AND (Gastric OR Stomach OR Oesophageal OR Esophageal) as well as the medical subject headings (MeSH), Tyrosine OR Phenylalanine OR Tryptophan AND (stomach neoplasms OR esophageal neoplasms). The electronic search was supplemented by a hand-search of published abstracts from relevant conference proceedings (2010 – 2013). Two reviewers (T.W. and S.M.) independently screened titles and abstracts of studies identified via the electronic search. Full texts were retrieved for all potentially relevant articles. Searching of reference lists of appropriate texts identified further potentially relevant studies. Inclusion criteria were any study measuring tyrosine, phenylalanine or tryptophan in
gastro-esophageal cancer patients via any appropriate analytical method comparing biological samples from cancer patients and suitable controls. Exclusion criteria were any studies without an appropriate control group or animal studies. Studies that reported the same patient population in multiple publications were also excluded, except for the most recent or complete publication.

The two reviewers independently extracted data from the selected studies including: number and types of specimen; analytical platform; amino acid concentrations in cancer and control groups. The hypothesis under investigation was whether concentrations of these aromatic amino acids would be significantly different between cancer and control samples. The QUADAS-2 tool was used to assess the quality of the included studies[18]. This model consists of four domains including patient selection, index test, reference standard and flow of patients through the study. The reference standard is defined as the best available method to establish the presence or absence of the target condition[19], which in this review was considered to be histological confirmation of the diagnosis. Each domain is assessed in terms of bias and the first three are also assessed in terms of concerns regarding applicability.

Results

There were 16 studies that met our inclusion criteria for systematic review (see figure 1).

Summary Table

Please see Table 1.

Study Characteristics

Seven studies involved the investigation of patients with esophageal cancer, eight studies investigated gastric cancer patients and one study investigated both cancer types. In total there were 524 patients with gastric cancer and 296 esophageal cancer patients included.
from these 16 studies. Six studies investigated blood levels of these aromatic amino acids (four esophageal cancer, one gastric cancer and one for both cancers), five studies investigated gastric content (all gastric cancer patients), two investigated urinary concentrations in esophageal cancer patients, and three studies analyzed concentrations of these amino acids in cancer tissues (one esophageal and two gastric). Several analytical platforms were employed including liquid chromatography mass spectrometry (LC-MS) (n=7), gas chromatography mass spectrometry (GC-MS) (n=3), nuclear magnetic resonance (NMR) (n=5), capillary electrophoresis mass spectrometry (CE-MS) (n=1), automated amino acid analyzer (n=2), and chromatography (n=1). Some studies utilized more than one analytical platform.

Quality Assessment of Studies

Details of the QUADAS-2 study quality assessment are shown in ‘Supplementary Figure 1’ and ‘Supplementary Figure 2’. Unclear risk of bias in patient selection was recorded in all studies due to a lack of information regarding patient recruitment, as it was not specified whether consecutive patients were included or if any patients were excluded. The risk of bias for the index test was unclear due to lack of information regarding blinding of assessors. Several papers had a high risk of bias with regard to patient flow as not all of the controls received the reference standard investigation (histological examination). Twenty one percent of studies had an unclear or high risk of applicability concerns due to cancer and control groups not being appropriately matched.

Tyrosine

Tyrosine was measured in 13 of the studies included (five serum based, four gastric content, two urine based and two in tissue).

Miyagi et al. investigated concentrations of plasma free amino acids in various cancer types using high performance liquid chromatography electrospray ionization mass spectrometry[20]. This study included 199 gastric cancer cases and 985 healthy controls. Results from this
study showed a significantly decreased tyrosine concentration in gastric cancer cases compared to controls (<0.01). This difference was apparent in early (Stage I) as well as late (stage IV) disease stage. Zhang X et al. showed that serum tyrosine levels were decreased in esophageal cancer patients (n=25) compared to healthy controls (n=25) (p<0.002)[21], a finding also reported by Norton et al. (p<0.05)[22]. However, other studies by Zhang J et al.[23] and Ikeda et al.[24] showed no significant difference in serum tyrosine levels in esophageal and gastric cancer patients compared to controls.

Deng et al. have performed two studies to investigate levels of amino acids within gastric content[25,26]. The initial study compared concentrations within gastric content of gastric cancer patients (n=33) to a control group (n=68) using NMR and LC-MS. The median concentration of tyrosine in the cancer group was 54.9+/−11.0ug/mL, compared to 8.7+/−2.6ug/mL in the control group (p<0.05)[25]. Another study by the same group compared cases of early gastric cancer (n=49), advanced gastric cancer (n=66) and a control group of patients with non-neoplastic gastric disease (n=70). This study showed that median gastric content tyrosine concentrations in the early gastric cancer group was 19.4ug/mL (5.8-72.4), compared to 18.3ug/mL (6.4-52.3) in advanced gastric cancer patients and 3.8ug/mL (1.7-7.5) for the control group. A Receiver Operator Characteristic (ROC) curve analysis for the utilization of tyrosine concentrations to distinguish early gastric cancer from controls showed an area under the curve of 0.790 (95% CI, 0.703-0.877, p<0.001). This finding of increased tyrosine levels within gastric content is supported by an early study by Abasov et al. who used chromatography to detect the presence of aromatic amino acids in the gastric content of gastric cancer cases (n=100)[27]. This was compared to a control group containing healthy volunteers and benign gastric disease patients (n=140). They detected tyrosine in 65% of cancer cases compared to 46% of controls. Segawa et al. also showed that overall levels of amino acids (including tyrosine and phenylalanine) were higher in the gastric content of gastric cancer patients (n=16) compared to controls (n=40)[28].

Hasim et al. investigated levels of tyrosine in the urine of esophageal cancer patients (n=108) compared to controls (n=40) using NMR[29]. They showed that tyrosine levels were
increased within the urine of esophageal cancer patients (Correlation co-efficient -0.32). Davis et al. found increased tyrosine levels in the urine of esophageal cancer patients (n=44) compared to healthy controls (n=44) although this difference did not reach statistical significance[30]. This study also investigated urinary levels of tyrosine and tryptophan in Barrett's esophagus patients (n=31) against another healthy control group (n=31) but no significant differences were observed. Two studies investigated levels of tyrosine within gastric cancer tissue compared to normal controls. Wu et al. used paired tissue samples (cancer and healthy mucosa) from 20 esophageal cancer patients and utilized GC-MS to establish levels of tyrosine[31]. This showed significantly increased levels of tyrosine in cancer tissue compared to normal mucosa. Hirayama et al. used capillary electrophoresis mass spectrometry and showed levels of tyrosine were significantly increased in gastric cancer tissues compared to normal tissue (p<0.001)[32].

Phenylalanine

Phenylalanine levels were measured in 13 included studies (five blood based studies, four gastric content based studies, one urine based, and the three tissue studies).

Miyagi et al.[20] and Norton et al.[22] showed serum phenylalanine levels were decreased in gastric and esophageal cancer patients, respectively. However, Zhang X et al. demonstrated esophageal cancer patients to have raised serum phenylalanine levels compared to healthy controls (p=0.004)[21]. Ikeda et al. compared phenylalanine levels in gastric and esophageal cancer patients (n=15, and n=11 respectively) to healthy controls (n=12) but observed no significant difference[24]. Naini et al. also showed no significant change in plasma phenylalanine levels[33].

Deng et al. demonstrated that median concentrations of phenylalanine in gastric content were 54.3+/-11.ug/mL in the gastric cancer group, compared to 8.8+/-2.1ug/mL in the control group (p<0.05)[25]. In a second study by Deng et al., the area under the curve using gastric content phenylalanine concentrations to discriminate early gastric cancer cases from controls was 0.831 (95% CI, 0.750-0.911, p<0.001)[26]. The area under the curve for the advanced gastric
cancer cases compared to controls was 0.858 (95% CI, 0.794-0.922, p<0.001). Abasov et al. showed that phenylalanine was detected in 80% of cancer cases and 56% of controls[27]. Hasim et al. observed increased phenylalanine concentrations in the urine of esophageal cancer patients compared to controls (Correlation co-efficient -0.32)[29].

In the tissue-based studies, Hirayama et al. showed significantly higher levels of phenylalanine in gastric cancer tissues compared to controls (p<0.001)[32]. However, Wu et al. demonstrated no significant differences in phenylalanine concentrations between esophageal cancer tissue and normal mucosa[31]. Song et al. reported a trend towards increased phenylalanine concentrations in gastric cancer tissues but this observation was not statistically significant[34].

Tryptophan

Tryptophan levels were investigated in 10 studies (five blood-based, four in gastric content, and one in tissue).

Zhang J et al. measured fasting tryptophan levels in 67 esophageal cancer patients and a control group including three patients with Barrett’s esophagus, nine patients with high-grade dysplasia and 34 healthy volunteers[23]. This study showed that tryptophan levels were significantly reduced in esophageal cancer patients compared to controls (p=3.2E-0.5; fold change -1.2). This finding was supported by other groups including Zhang X et al.[21] and Naini et al.[33], who demonstrated decreased serum levels of tryptophan in these patients (Zhang X VIP 3.35, p=0.000; Naini p<0.05 for free tryptophan levels. Total tryptophan levels showed no significant change). Miyagi et al. also showed significantly decreased plasma tryptophan concentrations in gastric cancer cases compared to controls (p<0.01)[20]. However, Ikeda et al. found no significant difference in tryptophan between esophageal or gastric cancer patients when compared to healthy controls[24].
Lian et al. demonstrated tryptophan concentrations of greater than 6.0mg/L were found within the gastric content of 52.6% of gastric cancer patients (n=38) compared to 22.9% of controls with benign gastric disease (n=48) (p<0.05)[35]. This finding was further supported by Deng et al who showed that median concentrations of tryptophan in gastric content of gastric cancer patients was 19.4+/-4.7ug/mL, compared to 2.7+/-1.0ug/mL in controls (p<0.05)[25]. The second study by Deng et al. showed that the area under the curve for gastric content tryptophan concentrations was 0.819 (95% CI, 0.739-0.900, p<0.001) to discriminate gastric cancer from controls[26]. Abasov et al. showed tryptophan was found in 23% of cancer cases compared to 13% of controls[27]. Hirayama et al. found significantly increased tryptophan levels in gastric tissues compared to controls (p <0.05)[32].

**Discussion**

The results of this systematic review suggest the three aromatic amino acids under investigation (tyrosine, phenylalanine, and tryptophan) are increased within the gastric content and tissues of patients with gastro-esophageal cancer. Tyrosine and phenylalanine levels were also increased in the urine of these patients. The majority of studies investigating serum (or plasma) concentrations demonstrate that levels of these amino acids are decreased in the serum (or plasma) of gastro-esophageal cancer patients.

Malnourishment may also be a contributing factor to these reduced levels of plasma amino acids. Malnourished patients develop a hypermetabolic state with increased hepatic gluconeogenesis and protein catabolism. However, cachexia and malnutrition does not appear to be the primary factor contributing to decreased serum amino acid concentrations in gastro-esophageal cancer patients; this hypothesis is supported by Miyagi et al., who observed decreased plasma free amino acid levels in both early stage disease (stage I) as well as stage IV disease patients[20]. Kawamura et al. have also demonstrated that in tumor bearing rats, there is increased hepatic protein synthetic rates and increased muscle catabolism, particularly during later stages of disease[36]. They concluded that development of cachexia in cancer patients was not only related to reduced dietary intake but is linked to
increased whole-body protein turnover; this particularly occurs during the late stages of
disease where skeletal muscle protein is mobilized for increased tumor synthesis. This
increased protein demand in the tumor-bearing state reduces the availability of plasma amino
acids[7]. Increased demand and overutilization of amino acids by tumour tissue may account
for the reduced concentration of serum amino acids[29]. Certain amino acid transporters have
been reported to be up-regulated in cancer, in particular LAT1, which is involved in L-type
amino acid transport[37]. It has also been shown that blockade of amino acid transporters can
cause apoptosis in certain cancer cell lines[38].

The changes in blood levels of phenylalanine are of particular interest. Two studies in this
review showed reduced phenylalanine concentrations in gastro-esophageal cancer patients
and another identified increased concentrations. Previous work has shown that activity of
phenylalanine hydroxylase (which converts phenylalanine to tyrosine) is dysfunctional in
inflammatory or malignant disease states[12,13]. This raises the possibility that blood
phenylalanine levels could be altered in gastro-esophageal cancer patients due to reduced
action of this enzyme. This reduction in enzyme activity may also account for the decreased
tyrosine levels observed by Zhang X et al. [21]. However, the biological mechanism
responsible for these alterations in amino acid concentrations in cancer patients remains
unclear and further investigation is required to explore this relationship.

The increased levels of aromatic amino acids within the gastric content may be an especially
important finding. The two studies by Deng et al.[25,26] were both part of this review as only
six cancer cases (from 33 total in the initial study, and 115 total in the second study) were
included in both studies. In the second study, a logistic regression analysis was performed to
establish the predictive values of candidate biomarkers for the detection of early gastric
cancer. This identified four variables (age and elevated tyrosine, phenylalanine, and
tryptophan levels in gastric content) as being significant for detection of early gastric cancer
cases[26]. Various potential mechanisms have been proposed for the increased
concentrations of these aromatic amino acids within gastric content. One possibility involves
increased production of enzymes which degrade the basement membrane and extracellular
matrix by invasive cancer cells[39], and specifically certain matrix metalloproteinase enzymes have been shown to be upregulated by aggressive cancer cells[40]. This degradation may release aromatic amino acids into the gastric content causing increased concentrations of these compounds[25]. Other postulated mechanisms to explain this phenomenon include increased protein synthesis within rapidly growing malignant tissues thereby releasing these aromatic amino acids into the gastric content.

Aromatic amino acids within gastric content may be candidate biomarkers for gastro-esophageal cancer. However, the retrieval of gastric content for analysis requires an invasive procedure either via endoscopy or nasogastric tube insertion. Therefore the ‘gold-standard’ investigation for gastro-esophageal cancer remains endoscopy and histological examination. Gastric content and tissue may be sampled as part of diagnostic endoscopy to allow supplementary amino acid profiling in early stage gastro-esophageal cancer. Furthermore, evaluation of amino acid concentrations in gastric content and tissue of patients undergoing multiple endoscopic tests (e.g. endoscopic ultrasound or repeat endoscopy) may allow serial measurements to monitor therapeutic response during neoadjuvant therapy. The effect of neoadjuvant therapy upon amino acid profile as a marker of therapeutic response is an important area for future investigation.

These increased amino acid concentrations in gastric content are also important for the development of new biomarkers particularly given their link to phenol metabolism. Phenol production is linked to aromatic amino acid metabolism through the process of proteolytic fermentation. Specifically, tyrosine phenol-lyase is a bacterial enzyme involved in the conversion of tyrosine to phenol. It has been previously shown that phenols are a major product of tyrosine metabolism within the colon[41]. Once tyrosine has been released into the gastric content the action of tyrosine phenol-lyase from the microbiome could convert tyrosine to phenol causing increased concentrations within gastric content. This is in keeping with the findings of previous studies showing increased phenolic compound concentrations within gastric content of gastro-esophageal cancer patients[17]. Increased phenol concentrations have also been observed in the exhaled breath of gastro-esophageal cancer patients[15]. This raises
the potential for phenol measurement within exhaled breath to be used as a surrogate marker of gastro-esophageal cancer risk. Future studies may also help to definitively establish differences in aromatic amino acid profile within the urine of gastro-esophageal cancer patients compared to controls. Serial urinary sampling could represent another non-invasive method for assigning risk of gastro-esophageal cancer and monitoring therapeutic response. These methods could potentially be used for identifying at-risk individuals in need of urgent endoscopy and thereby help in diagnosing gastro-esophageal cancer at earlier disease stages. This could potentially increase the proportion of patients that are treated on an intention-to-cure basis if a sufficiently accurate clinical assay could be developed.

**Conclusion**

Tyrosine, phenylalanine and tryptophan concentrations are altered within the biofluids and tissue of gastro-esophageal cancer patients; these compounds represent promising biomarker targets in gastro-esophageal cancer and hence further studies are needed to investigate if the observed differences in tyrosine, phenylalanine and tryptophan are cancer-specific.

*Word count: 3,313*
References


41. Smith EA, Macfarlane GT. Enumeration of human colonic bacteria producing phenolic and indolic compounds: effects of pH, carbohydrate availability and retention time on

**Figure Legend**

*Figure 1*: Flow chart of included studies.
Table 1: Summary table of included studies.

<table>
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<th>Amino Acids Investigated</th>
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<th>Cancer</th>
<th>n</th>
<th>Control</th>
<th>Tyr (Cancer vs Control)</th>
<th>Phe (Cancer vs Control)</th>
<th>Trp (Cancer vs Control)</th>
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<td>Esophageal</td>
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<td>Eso n=15</td>
<td>Gas n=11</td>
<td>HC =12</td>
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<td>Esophageal</td>
<td>Amino acid analyzer</td>
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<td>Tyr, Phe, Trp</td>
<td>Gastric</td>
<td>LC-MS</td>
<td>33</td>
<td>68 (mix HC and BGD)</td>
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</table>
Key: ↑=raised levels in cancer patients; ↓=decreased levels in cancer patients; - =No difference shown; N/A = Not reported; * = p<0.05; ** p<0.001.

(Tyr = tyrosine; Phe=Phenylalanine; Trp = Tryptophan; LC-MS=Liquid chromatography mass spectrometry; GC-MS=Gas chromatography mass spectrometry; NMR=Nuclear magnetic resonance; CE-TOF-MS=Capillary electrophoresis time-of-flight mass spectrometry; EGC=Early gastric cancer; AGC=Advanced gastric cancer; HC=Healthy controls; BGD=Benign gastric disease; BE=Barrett’s esophagus;)

Abstracts assessed for eligibility
n=1442

Total number of abstracts screened
n=1442

Abstracts assessed for eligibility
n=25

Full-text articles assessed for eligibility
n=25

Full text articles included after N=16

Studies included in data analysis
n=16

Additional articles identified through hand searching of bibliography
n=0

Full-text articles excluded n=9
Reason:
5 = no comment upon compounds of interest
1 = not investigation into gastro-esophageal cancer.
3 = Insufficient methodological information or detail regarding control group.