DEPRESSION IN PATIENTS WITH HEAD AND NECK CANCER AND A FUNCTIONAL GENETIC POLYMORPHISM OF THE SEROTONIN TRANSPORTER GENE

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Abstract: Background. Polymorphism of the serotonin transporter gene, 5-HTTLPR (short allele) has been associated with depression. The purpose of this study was to show the prevalence of depression in patients with head and neck cancer and a possible association with the 5-HTTLPR.

Methods. The Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders (SCID for DSM-IV) was administered to 94 patients with head and neck cancer, of which 33 patients were genotyped for 5-HTTLPR. We also evaluated the prevalence of 5-HTTLPR polymorphism in 121 patients with head and neck cancer and 97 controls.

Results. Forty-three percent of the patients met the criteria for a depressive diagnosis, 19% of which was new onset. In depressed patients, 85.7% (n = 12 of 14) had at least 1 short allele versus 68.4% (n = 13 of 19) of the patients without depressive diagnosis (p < .04). No difference was noted in the prevalence of the short allele in head and neck cancer cases versus controls (odds ratio = 0.8; p = .490).

Conclusion. Despite the high rate of depressive diagnosis, patients with head and neck cancer did not demonstrate a higher prevalence of this short allele of the 5-HTTLPR compared with a control population.

Keywords: serotonin; head and neck cancer; depression; 5-HTTLPR; polymorphism

Depression represents a significant problem for patients with head and neck cancer. Depressive symptoms negatively affect quality of life, decision-making, treatment adherence, and survival. Approximately 40% to 50% of patients with head and neck cancer report depressive symptoms at some point during treatment or recovery, underscoring the need to better understand this behavioral morbidity.¹⁻⁴ The prevalence of depression in patients with head and neck cancer significantly exceeds that in the general U.S. population.⁵⁻⁷ Head and neck cancer demonstrates among the highest rates of depression within the cancer population, ranking in the top 5 types of cancers with the highest prevalence.⁸⁻⁹ Depressive symptoms may also persist long after treatment is completed. As the survival of patients with head and neck cancer has significantly improved with aggressive combined-modality therapy, it is important to recognize and study this common problem to improve the quality of survivorship.

An important challenge in understanding depression in patients with cancer is identifying both mediators and moderators of depression risk in the complex setting of cancer. A mediator is considered a causal factor linking an independent variable (here, head and neck cancer) and a dependent variable (depression).¹⁰ A moderator in this context is an independent third variable that modifies the likelihood of an outcome (depression) in the presence of a causal factor (head and neck cancer).¹⁰ Although investigators have noted several factors that are associated with risk for depression in patients with head and neck cancer, the biology of this phenomenon has not been well described.

The neurotransmitter serotonin plays a significant role in mood and behavior. Altered homeostasis of the serotonergic system may contribute to depression, including depression in the context of head and neck cancer. In fact, inhibition of serotonin reuptake remains 1 of the most successful mechanisms of pharmacologic antidepressant therapy. A common functional promoter polymorphism of the serotonin transporter gene (serotonin transporter-linked
polymorphic region (5-HTTLPR), a tandem repeat variant present in long (l) and short variants, has been identified as a potential contributing factor. The short variant is associated with reduced expression of the transporter, and has been associated with increased risk of depression in some, but not all, studies. The association links 5-HTTLPR and depression in the context of stressful life events. However, the contribution of 5-HTTLPR to depression risk associated with cancer has not been investigated.

The purpose of the current study was intended to determine the prevalence of depression of outpatients in posttreatment head and neck cancer using an established and validated structured interview, the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders (SCID for DSM IV). It also sought to test an association between 5-HTTLPR and depression in the context of patients with head and neck cancer.

**METHODS**

**Setting and Participants.** This study was approved by the Vanderbilt University Institutional Review Board and the Scientific Review Committee of the Vanderbilt–Ingram Cancer Center, and written informed consent was provided after participants received a complete description of the study. Patients were recruited from the Henry Joyce Cancer Clinic at the Vanderbilt University Medical Center. All participants had a history of head and neck cancer and had completed treatment. Patients were not required to be disease free at the time of enrollment and study. Patients were at least 18 years of age. At the time of enrollment, patients underwent a psychiatric interview administered by trained study personnel using the SCID DSM-IV. Patients also completed a Functional Assessment of Cancer Therapy Head and Neck Version self-assessment questionnaire, described below. However, only the SCID was used to determine a depressive diagnosis. The SCID was only administered once and this occurred at study enrollment. Patients who reported (or were found to have) clinically significant levels of distress were referred for treatment.

Blood was obtained for DNA extraction from 121 patients with head and neck cancer and 97 controls. Genotyping was performed on all samples for 5-HTTLPR using methods previously described. All data were completely de-identified before banking and analysis.

**Measures**

**Medical Information.** Data were obtained from the patients’ medical records and included the site of the primary tumor, stage of the tumor, date of diagnosis, treatment regimen, and date of completion of treatment regimen. The time from the end of the treatment to the time of study enrollment was also noted.

**Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders.** The SCID for DSM-IV is a standardized structured psychiatric interview yielding DSM-IV Axis I diagnoses, including major depression, substance use disorders, and anxiety disorders. Diagnoses are made both for current and lifetime periods, allowing for the assessment of precancer and postcancer diagnoses. The SCID is reliable and valid in psychiatric populations with coefficients of agreement between 0.70 and 1.0 for depressive, anxiety, and substance use disorders, and is significantly superior to standard clinical interview. The SCID was performed at the time of trial enrollment. The test was administered by a psychologist trained in administering this test.

**Genetic Polymorphisms.** Patient samples were banked using a separate Institutional Review Board–approved biospecimen collection protocol for the Head and Neck Biospecimen Repository at Vanderbilt. Before studying samples from patients and from healthy volunteers (collected during an oral cancer–screening day), an additional protocol for evaluation of the serotonin transporter gene was approved by the Vanderbilt Medical Center Institutional Review Board and the Scientific Review Committee of the Vanderbilt–Ingram Cancer Center.

DNA was isolated from whole blood using the PureGene kit according to the manufacturer’s instructions (Genta Systems, Minneapolis, MN). The 5-HTTLPR genotypes were determined using size discrimination of polymerase chain reaction (PCR) products on 3% NuSeive agarose gels. The short allele corresponded to a product of 484 bp whereas the longer allele was 528 bp. Amplifying PCR primers have been previously described. PCR was used to amplify the region containing the polymorphism and the samples were screened via temperature gradient capillary electrophoresis to determine the genotypes of the serotonin transporter (L/L, L/S, and S/S).

**Statistical Analysis.** Statistical summaries and analysis of the data were completed using SPSS (version 17). Frequency distributions were used to summarize the categorical and ordinal data; means and SDs summarized continuous data. Bivariate cross-tabulations and chi-square tests of association were used to describe and assess the associations of demographic and medical characteristics with a diagnosis of depression. Bivariate cross-tabulations were used to summarize the distributions of the short allele in both the subsetted sample consented to the biomarker study and the larger case/control sample. Logistic regression methods were used to calculate the odds.
ratio for the association of the short allele with a diagnosis of depression and with head and neck cancer, as well as to test the statistical significance of those associations.

**RESULTS**

**Patient Characteristics.** Ninety-four eligible patients consented to participate and completed the SCID. Thirty-three of those patients simultaneously enrolled on the biospecimen collection protocol and had blood samples available for genotyping of the 5-HTTLPR. Patient characteristics of the entire sample and the genotyped subset are summarized in Table 1. The majority of the patients were men, white, married or living with a partner, and had a little over a year of college. The mean age of the patients was 55.4 years old (SD = 9.7; minimum = 24; maximum = 84). Most had locally advanced disease (stage III or IV), and no patients were diagnosed with recurrent disease. Forty patients (42.6%) had received surgical intervention with concurrent chemotherapy and radiation, whereas the remainder had received radiation with concurrent chemotherapy and radiation. Within the subset (n = 33) participating in the genotyped study, a slightly higher percentage had received surgical intervention (54.5% vs 45.5% concurrent chemoradiation [CCR] alone). As shown in Table 1, approximately 11% of the entire sample was currently in treatment at the time of the study. Of the participants who had completed treatment, the average time from completion of concurrent radiotherapy and study enrollment was 11.4 months for the entire sample (minimum = 0.5, maximum = 60.0) and 7.1 months for the genotyped sample (minimum = 0.5, maximum = 29.0).

Approximately 50% (Table 1) of all of the patients were taking some type of antidepressant, antianxiety, or sleep medication at the time of evaluation. A slightly higher percentage (58%) was taking such medication in the genotyped subset. Approximately 26% in the entire sample and genotyped subset reported having lifetime problems with alcohol to the point where they met criteria for alcohol abuse or dependence. Only 2 patients in the entire sample (1 in the genotyped subset) acknowledged currently having active symptoms of alcohol use disorders.

**Rate of Depressive Diagnosis.** Forty of the 94 patients (43%) met the criteria for a depressive diagnosis. Of those, 18 patients (19% of the total) met the criteria for a new onset depressive disorder after the cancer diagnosis without a medical history of depression. Within the subset of patients who also consented to biospecimen repository, 14 of those 33 patients (approximately 42%) met the criteria for a depressive diagnosis.

There was no statistically significant relationship between depressive symptoms and the time from completion of CCR. Sixteen of 44 depressed patients demonstrated a depressive diagnosis between 0 and 4 months after completion of CCR; between 4 and 12 months, 11 of 24 patients (46%) experienced a depressive diagnosis; and at >12 months after completion, 13 of 26 patients experienced a depressive diagnosis (50%; p = .500). At least 9 patients (approximately 10%) in the entire sample had a precancer history of depression, but had not experienced a recurrence because of their cancer diagnosis (n = 1 of 33 or 3% in the genotyped subset).

Seventy-five percent of the patients (n = 30 of 40) with depressive symptoms were taking medications compared to 33% of those patients (n = 18 of 54)
without depressive symptoms ($p < .001$). There were no statistically significant differences between the groups with and without depressive symptoms on the other demographic and medical characteristics shown in Table 1.

Prevalence of Genetic Polymorphism of the Serotonin Transporter in Patients with Head and Neck Cancer with Depressive Symptoms. Thirty-three of the 94 patients who participated in the depression prevalence study also consented to the tissue biospecimen repository. Thus, samples for evaluation of 5-HTTLPR polymorphism were available from 33 of the 94 patients. Prevalence of the 5-HTTLPR short allele in the subset of patients who consented to be genotyped is summarized in Table 2. In this sample of patients with head and neck cancer with depressive symptoms, 85.7% ($n = 12$ of 14) had either LS or SS genotypes, as opposed to 68.4% ($n = 13$ of 19) of the patients not manifesting those symptoms (chi-square = 1.31; df = 1; $p = .252$). A patient with at least 1 copy of the short allele had an approximately 2.8 times greater likelihood of expressing depressive symptoms than a patient without the short allele (odds ratio [OR], = 2.77; $p = .263$).

Prevalence of Genetic Polymorphism of the Serotonin Transporter in Patients with Head and Neck Cancer and Controls. We then evaluated the prevalence of the short allele (LS, SS) of the 5-HTTLPR in patients with head and neck cancer and in a control samples, taken from the Vanderbilt Ingram Cancer Center Head and Neck Tissue Repository. Control samples were identified based on sex and ethnicity that matched with a case sample. A total of 121 patients with head and neck cancer and 97 controls were available for this evaluation. Only 33 patients in this evaluated population of patients with head and neck cancer had participated in the depression prevalence study. Most of the patient samples were taken from the wider population of patients with head and neck cancer seen at the Vanderbilt Ingram Cancer Center. The prevalence of the genotypes is summarized in Table 3. No statistically significant difference was noted in the prevalence of the short allele in the head and neck cancer cases versus the controls (OR = 0.8; $p = .490$).

DISCUSSION

Multiple investigators have described the high prevalence of depression in the setting of head and neck cancer, with prevalence estimates ranging from 22% to 57%. The reason behind the high rate of depression is not entirely clear. While investigators have noted several factors that predict for depressive symptomatology in patients with head and neck cancer, the biology of this phenomenon has not been well described.2,4,18–23

Historically, 1 obstacle to accurately defining depression prevalence has been the methodology used to identify depression in patients with cancer. Whereas some studies have used self-reported measures, others have used tools that only evaluate major depression, not the spectrum of depressive disorders. Thus, the SCID provides a validated tool to describe the prevalence of depressive disorders in this population.

The neurotransmitter serotonin plays a significant role in mood and behavior.24,25 Altered homeostasis of the serotonergic system can contribute to depression. In fact, inhibition of serotonin reuptake remains 1 of the most successful mechanisms of pharmacologic antidepressant therapy. In the general population, a functional genetic polymorphism of the 5-HTTLPR has been identified as a potential contributing factor.12 To our knowledge, this association has not been studied previously in a cancer population.

In this study, posttreatment head and neck cancer survivors experienced significant psychopathology, both historically and during survivorship. We were able to demonstrate a spectrum of depressive disorders in this population. Interestingly, although most patients with a psychiatric disorder at evaluation had a history of that disorder, a significant number of patients developed psychiatric diagnoses only after being diagnosed with cancer; in the majority of instances, this was a DSM-IV depression diagnosis. The rate of current depressive disorders in this study was in the mid range of the previous diagnostic studies. However, this rate is higher than the 16.2% prevalence rates for the general U.S. population. The well-known associations of tobacco and alcohol with

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Control</th>
<th>Head and neck cancer</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>17 (17.5)</td>
<td>15 (12.4)</td>
<td>32 (14.7)</td>
</tr>
<tr>
<td>LS</td>
<td>56 (57.7)</td>
<td>71 (58.7)</td>
<td>127 (58.3)</td>
</tr>
<tr>
<td>LL</td>
<td>24 (24.7)</td>
<td>35 (28.9)</td>
<td>59 (27.1)</td>
</tr>
<tr>
<td>Total</td>
<td>97</td>
<td>121</td>
<td></td>
</tr>
</tbody>
</table>

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Table 2. Depression and genetic polymorphism of the serotonin transporter.

<table>
<thead>
<tr>
<th>Depression</th>
<th>No. of patients (%) by genotype</th>
<th>SS</th>
<th>LS</th>
<th>LL</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>6 (54.5)</td>
<td>7 (50.0)</td>
<td>6 (75.0)</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>5 (45.5)</td>
<td>7 (50.0)</td>
<td>2 (25.0)</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>14</td>
<td>8</td>
<td>33</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Head and neck cancer and genetic polymorphism of the serotonin transporter in a population of patients with head and neck cancer and controls in the Vanderbilt Ingram Cancer Center Head and Neck Tissue Repository.
head and neck cancer may select for a population with maladaptive coping strategies and a higher likelihood of depression.

Given the relatively high rate of new-onset depression in the setting of CCR, the question remains whether the biology of the treatment itself may influence the development of this psychological morbidity. The rate of new-onset depression after CCR, suggests a strong interactive effect of treatment on depression. Both head and neck cancer tumors and CCR produce proinflammatory cytokines, including the ones associated with depression in the general population: IL-6, IL-1β, and TNF-α. Although the mechanism by which inflammation contributes to depression is not known, inflammatory cytokines can alter the metabolism and release of central serotonin in the central nervous system. An additional mechanistic interaction between inflammation and serotonin homeostasis has recently been described. In vitro, IL-1β and TNF-α exert a concentration and time-dependent stimulation of serotonin transporter activity. Additionally, evidence supports the p38 mitogen-activated protein kinase pathway in mediating these effects and suggests that other peripheral cytokines that stimulate p38 mitogen-activated protein kinase may have similar effects on the serotonin transporter. Therefore, activation of inflammatory mediators as seen in CCR done in the context of head and neck cancer may interact with a vulnerable serotonin system associated with functional variants of the serotonin transporter to produce depression in vulnerable individuals. Further research is needed to elucidate a possible role of the proinflammatory state of CCR in patients with head and neck cancer in generating depression.

One potential contributing factor may be a higher rate of the 5-HTTLPR short allele in the head and neck cancer population. The impact of the short allele on depression risk in head and neck cancer is substantially higher than the impact seen for the short allele with depression. The short allele has been associated with tobacco and substance abuse in earlier research. However, that was not demonstrated in the present study. We did not find a significant difference between patients with head and neck cancer and healthy control volunteers without an evidence of cancer.

This study has several limitations. Although provocative, the relatively small sample size of specimens available for both the depression evaluation and genetic polymorphism analysis limits the ability to make definitive conclusions. Additionally, the 33 genotyped samples of patients who participated in the depression prevalence study were retrieved from the Vanderbilt Head and Neck Tissue repository and were not collected prospectively as part of the original depression prevalence study. Therefore, we evaluated patients with a known depression status (from the depression prevalence study) who had also agreed to undergo tissue banking under a separate Institutional Review Board–approved protocol. Thus, the findings reported in this manuscript do not have sufficient “power” for definitive association as this genotypic evaluation was performed retrospectively. This association will require a well-powered case-control design for further clarification of the relationship between the 5-HTTLPR and depression in patients with head and neck cancer.

Another study limitation is the inability to correct for multiple confounding factors that may influence the prevalence of depressive diagnosis in this patient population. Whereas multiple potential confounders were described in Table 1, future investigation should include measurements of thyroid function, and social support, among others. Moreover, a symptom cluster of depression, pain, and fatigue has been described in patients with cancer. The cause and effect of these 3 factors is complex and multidirectional and should be evaluated simultaneously in future investigations. Additionally, other biological mechanisms may play a role in depression in this population. For example, sustained activation of the hypothalamic-pituitary-adrenal axis has a putative role in depression and hypothalamic-pituitary-adrenal deregulation is well-described in patients with cancer. Moreover, cognitive dysfunction may affect mood and depression. Formal cognitive testing was not performed on the study population.

In conclusion, depressive disorders are common in patients with head and neck cancer. The 19% incidence of new-onset depression in the setting of CCR suggests an interactive treatment effect. Although our data remain provocative with an association of the 5-HTTLPR and depression, the findings did not reach statistical significance and will need to be prospectively evaluated in a larger study population of patients with head and neck cancer. Investigation of the biology of this morbidity is essential to developing effective therapies, prevention strategies, and to maximize the quality of survivorship.

REFERENCES