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A Prospective Study of Pre-diagnostic Circulating Tryptophan and Kynurenine, and the Kynurenine/Tryptophan Ratio and Risk of Glioma

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Abstract

Background: Conversion of tryptophan to kynurenine may promote glioma growth and suppress antitumor immune response through activation of the aryl hydrocarbon receptor. Expression of the enzymes indoleamine 2,3-dioxygenase and tryptophan 2,3-dioxygenase-2 in the glioma microenvironment has been shown to mediate tryptophan catabolism, and the ratio between kynurenine and tryptophan is considered an indirect measure of this enzyme activity.

Methods: We explored whether tryptophan, kynurenine, and the ratio of kynurenine to tryptophan (KTR) in pre-diagnostic blood samples was related to risk of glioma in a nested case-control study of 84 cases and 168 matched controls from two cohort studies - the Nurses' Health Study, and the Health Professionals Follow-Up Study. Tryptophan and kynurenine were measured by liquid chromatography-tandem mass spectrometry. Conditional logistic regression models were used to estimate risk ratios (RRs) and 95% confidence intervals (95%CI) for the associations between tertiles of these analytes and glioma risk.

Results: We observed no significant associations for either analyte or the ratio for risk of glioma overall. The RR for the highest KTR tertile compared to the lowest for all gliomas was 0.74 (95% CI: 0.34–1.59). All results were essentially unchanged in lagged analyses excluding the first two or four years of follow up, though data were sparse.

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Declarations of interest: None

Conclusion: Our findings do not provide support for an association between pre-diagnostic circulating KTR and risk of glioma.

Malignant gliomas comprise nearly 80% of primary malignant brain tumors in adults (1) and arise from glial tissues in the brain. The etiology of glioma is poorly understood, and there are no well-established modifiable risk factors (2). Gliomas may be broadly classified into glioblastoma multiforme (GBM), the most aggressive grade IV tumors, and lower grade (grade II and III) gliomas (non-GBM). GBMs account for ~60% of gliomas, and have a poor prognosis regardless of treatment, with a median survival time of 15 months (3). High morbidity and mortality in glioma highlight need to identify factors influencing glioma development.

Catabolism of tryptophan (TRP), an essential amino acid, in the tumor microenvironment leads to generation of kynurenine (KYN), which can promote tumor progression and suppress antitumor immune response through varied mechanisms (4–6). Key enzymes involved in the catabolism of TRP include indoleamine 2,3-dioxygenase (IDO1) and tryptophan 2,3-dioxygenase-2 (TDO) (6). Expression of both IDO1 and TDO in the glioma microenvironment has been shown to mediate conversion of TRP to KYN (7). Binding of KYN to aryl hydrocarbon receptors (AhR) on effector T cells suppresses their activity, helping tumors to evade immune response (7–9). In addition, KYN generated by a tumor cell can bind to AhRs on the same cell, causing the receptor to move into the nucleus where it regulates transcription of genes related to tumor cell migration. Elevated levels of AhR in gliomas have been correlated with poor patient prognosis (9).

The ratio between KYN and TRP (KTR) is considered an indirect measure of IDO/TDO expression activity (10, 11) and a higher KTR was observed in serum or plasma from cases compared to noncases for lung (4, 12), bladder (13), and colorectal (14) cancers, whereas a lower KTR was observed in plasma from breast cancer cases compared to noncases (15). Among cervical cancer patients (16), the KTR was higher in tissue from cervical cancer tissue compared to normal cervix tissue. Two prospective studies have reported suggestive associations between plasma KTR and subsequent risk of colorectal (17) and lung (4) cancer.

Few studies have reported on the KTR in glioma. In one study, the mean KTR was significantly higher in plasma from 18 GBM cases compared to 18 healthy controls (6). In another, serum KTR was higher in 11 patients with grade III/IV gliomas when compared to 5 patients with grade I/II glioma and 18 non-glioma patients (7). However, the influence of prevalent tumor on KTR levels among the glioma patients in these studies is unclear. Other studies have found KTR to be a potential prognostic biomarker of improved overall survival in GBM patients treated with surgery followed by immunotherapy (18, 19). To further investigate the relationship between the KTR and glioma, we examined the associations of pre-diagnostic circulating TRP, KYN, and the KTR with glioma incidence in two prospective cohorts - the Nurses' Health Study (NHS), and the Health Professionals Follow-Up Study (HPFS).

Methods

The NHS was established in 1976 and included 121,701 female nurses aged 30 to 55 years. The HPFS began in 1986 and enrolled 51,529 male health professionals aged 40 to 75 years. All participants completed baseline questionnaires on demographics, lifestyle factors, and medical and other health-related information, and were followed biennially. Age, cigarette smoking habits, body weight, height, and physical activity, were assessed from questionnaires returned prior to blood collection. Energy expenditure of eight reported types of physical activity was first quantified by the metabolic equivalent task (MET) score, and then quantified as MET-hours per week by multiplying the corresponding MET score by the hours per week spent in each activity. Total physical activity was calculated by summing MET-hours per week over all eight activities.

Blood samples were returned by 32,826 NHS (20) participants from 1989–1990 and by 18,018 HPFS (21) participants from 1993–1995, through overnight mail. On arrival at a centralized laboratory, the samples were centrifuged to separate plasma from the buffy coat and red cells and were stored in well-monitored liquid nitrogen freezers until analyzed. Over 95 percent of samples arrived within 26 hours of phlebotomy. The study protocol was approved by the Institutional Review Boards of the Brigham and Women's Hospital and Harvard T.H. Chan School of Public Health, as well as participating registries, and all participants provide written informed consent.

Glioma cases were identified initially through self-report with additional cases confirmed by vital status or medical review post-death. Written consent for medical record review was collected from participants or their next of kin. Cases with confirmed ICD-9-CM diagnoses of 191.x were included, and information on tumor subtype (GBM, non-GBM) was extracted directly from medical records. This study includes first incident primary glioma diagnoses ascertained through 2006.

Among participants with available blood samples, two controls were randomly selected for each glioma case via incidence-density sampling among participants who were still alive and free of cancer at the date of the case diagnosis. Controls were individually matched to the cases on year of birth (± 1 year; 2 years was used for 10 matched sets), month of blood collection (± 1 month), race (white vs. non-white), fasting status (fasting vs. non-fasting), and, by study design, gender.

Measurement of TRP and KYN

Circulating levels of TRP and KYN were measured in plasma by liquid chromatography tandem mass spectrometry at Bevital (Bergen, Norway). Limits of detection were 400 nmol/L for TRP and 7 nmol/L for KYN. Coefficients of variation ranged from 2.5–6.0% for TRP and 3.1–8.7% for KYN (22). The laboratory staff were blinded to case-control status of the samples.

Statistical analysis

Conditional logistic regression models were used to estimate risk ratios (RRs) and 95% confidence intervals (95%CI) for the associations between tertiles (defined by the

distribution in the controls) of TRP, KYN, and KTR and glioma risk. In addition to conditioning on matching factors, we adjusted for body mass index (BMI, defined as weight in kg/height in meters squared) because KTR has been reported to increase with BMI (23–25). We also examined associations for men and women separately, and for GBM only. Data were too sparse to examine associations separately in non-GBMs. To examine potential reverse causation, we performed lagged analyses by excluding patients diagnosed within two and four years of blood draw. Analyses were conducted using SAS version 9.4 (SAS Institute, Cary, NC). Statistical tests were two-sided with p-values < 0.05 considered statistically significant.

Results

The final analysis included 84 glioma cases (54 GBM, 30 non-GBM) and 168 matched controls (Table 1). The distributions of TRP, KYN, and the KTR were similar between glioma cases and controls (Figure 1). We observed no significant associations between TRP, KYN, or KTR and overall glioma risk (Table 2). For all three biomarkers, inconsistent and nonsignificant associations were observed when comparing the highest tertile of the biomarker concentrations to the lowest tertile or when modeling biomarker concentrations continuously for risk of glioma overall and for men and women separately. Results were similarly nonsignificant in analyses restricted to GBM. However, data in all analyses were sparse and confidence limits were wide. Results did not materially change after excluding cases (and their matched controls) identified within two and four years of blood draw ($n = 4$ and 17 cases excluded, respectively; data not shown).

Discussion

We observed no statistically significant associations between prediagnostic TRP, KYN, or the KTR and risk of glioma in two nested case-controlled studies of health professionals including 84 glioma cases. All results were essentially unchanged in lagged analyses excluding cases diagnosed within two or four years of blood draw to rule out reverse causation, though data were sparse.

Strengths of this study include the prospective design and use of pre-diagnostic blood samples. TRP and KYN were measured in blood drawn on average 8.3 years prior to glioma diagnosis, reducing the possibility of protopathic bias. Results did not materially change after excluding cases diagnosed within two and four years of blood draw, leaving 80 and 67 glioma cases, respectively. An important limitation of this study is the small sample size and resulting limited power to detect associations between KTR and glioma overall or for GBM, and data were too limited to consider associations in lower grade gliomas (comprising 30 of the 84 cases). In addition, we could not examine other key proteins and enzymes in this pathway (including IDO and TDO), or factors that control TRP disposition by influencing plasma-free TP availability and its flux through the KYN pathway, in particular albumin and nonesterified fatty acids that are impacted by diet (26, 27). We relied instead on the KTR, which may not be directly correlated with IDO1/TDO activation (27). In summary, our results do not support an association between the KTR and subsequent glioma risk.

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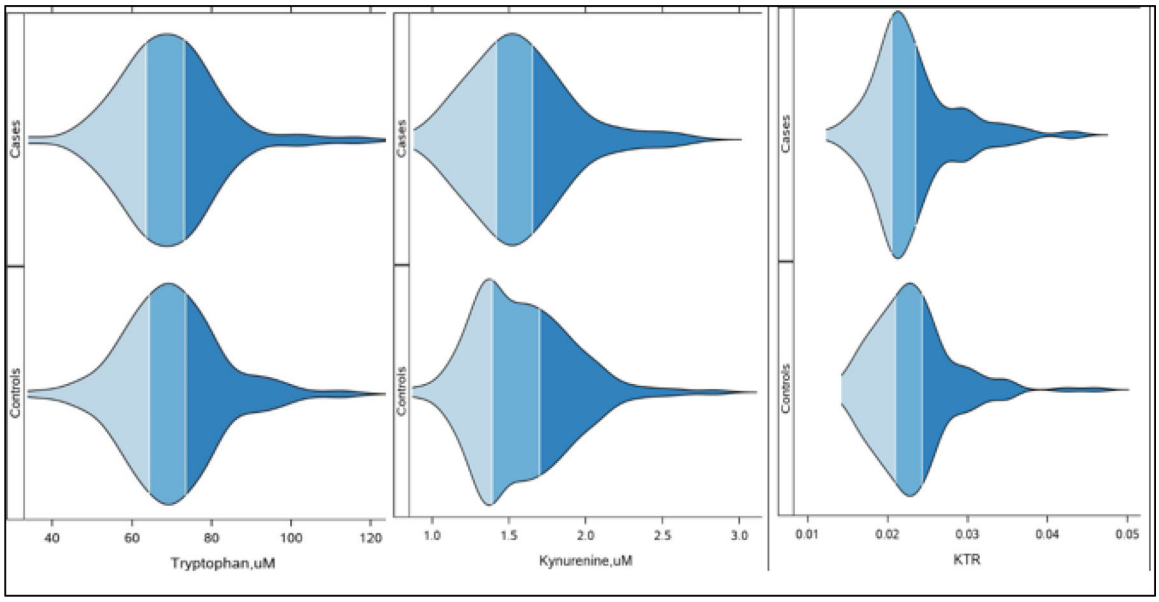

Figure 1.

Violin plots for tryptophan, kynurenine, and the kynurenine/tryptophan ratio (KTR) for 84 glioma cases and 168 controls. Shading corresponds to analyte distributions in tertiles (light blue=lowest tertile, dark blue=highest tertile).

Table 1.

Selected characteristics of glioma cases and controls from a nested case-control study of pre-diagnostic circulating tryptophan and kynurenine, and the kynurenine/tryptophan ratio in the Nurses' Health Study (NHS) and Health Professionals Follow-Up Study (HPFS).

	Women (NHS)		Men (HPFS)	
Characteristic	**Cases**	**Controls**	**Cases**	**Controls**
N	52	104	32	64
Age (years), mean (SD)	57.8 (6.1)	58.0 (6.1)	63.8 (9.2)	63.9 (9.1)
Body mass index (kg/m$^{2)}$, mean (SD)	24.5 (4.8)	26.6 (5.4)	25.7 (4.8)	25.3 (3.4)
Physical activity (MET-hours/week), mean (SD)	18.5 (24.1)	14.3 (17.1)	33.5 (31.4)	39.1 (43.8)
Current smokers, N (%)	7 (13.5)	11 (10.6)	1 (3.1)	1 (1.6)
Past smokers, N (%)	25 (48.1)	46 (44.2)	18 (56.3)	22 (34.4)
Tryptophan (μM), mean (SD)	67.6 (11.9)	70.4 (11.9)	71.9 (12.8)	69.5 (13.7)
Kynurenine (μM), mean (SD)	1.5 (0.3)	1.6 (0.3)	1.7 (0.4)	1.6 (0.4)
Kynurenine/Tryptophan ratio (KTR), mean (SD)	0.022 (0.005)	0.023 (0.005)	0.024 (0.005)	0.024 (0.005)

Abbreviations: NHS=Nurses' Health Study; HPFS=Health Professionals Follow-up Study; MET=metabolic equivalent task

Table 2.

Relationship between pre-diagnostic circulating tryptophan and kynurenine, and the kynurenine/tryptophan ratio (KTR), by tertiles and continuously, with risk of glioma overall, by gender, and for glioblastoma (GBM).[1]

	All glioma, overall		All glioma, women		All glioma, men		GBM, overall	
	# Gliomas	RR (95%CI)	# Gliomas	RR (95%CI)	# Gliomas	RR (95%CI)	# GBM	RR (95%CI)
Tryptophan, μM								
≤ 64.33	30	Ref	22	Ref	8	Ref	22	Ref
>64.33 to 73.53	28	0.86 (0.45–1.66)	14	0.49 (0.20–1.21)	14	1.85 (0.65–5.28)	20	0.71 (0.33–1.54)
> 73.53	26	0.80 (0.41–1.55)	16	0.50 (0.21–1.20)	10	1.21 (0.39–3.70)	12	0.52 (0.21–1.26)
p-value		0.50		0.13		0.70		0.14
Continuous	84	0.91 (0.69–1.19)	52	0.68 (0.45–1.03)	32	1.18 (0.79–1.75)	54	0.83 (0.58–1.20)
Kynurenine, μM								
≤ 1.39	24	Ref	20	Ref	4	Ref	14	Ref
>1.39–1.70	36	1.61 (0.83–3.11)	23	1.17 (0.50–2.74)	13	3.46 (1.03–11.69)	24	1.66 (0.75–3.65)
> 1.70	24	1.13 (0.57–2.26)	9	0.47 (0.18–1.21)	15	4.11 (1.12–15.07)	16	1.18 (0.52–2.71)
p-value		0.89		0.11		0.05		0.74
Continuous	84	0.98 (0.74-.28)	52	0.72 (0.48–1.08)	32	1.21 (0.82–1.77)	54	1.03 (0.75–1.40)
KTR								
≤ 0.0210	33	Ref	22	Ref	11	Ref	17	Ref
>0.0210 to 0.0243	29	0.94 (0.49–1.81)	21	1.18 (0.52–2.70)	8	0.58 (0.18–1.85)	23	1.99 (0.83–4.77)
> 0.0243	22	0.74 (0.34–1.59)	9	0.47 (0.15–1.51)	13	0.83 (0.26–2.62)	14	1.05 (0.39–2.79)
p-value		0.46		0.34		0.84		0.87
Continuous	84	1.07 (0.81–1.43)	52	1.07 (0.70–1.63)	32	1.05 (0.70–1.57)	54	1.21 (0.84–1.75)

Abbreviations: KTR= kynurenine/tryptophan ratio; GBM=glioblastoma; OR=Odds ratio; CI=confidence interval

[1]Controls were matched to cases on year of birth (± 1 year; 2 years was used for 10 matched sets), month of blood collection (± 1 month), race (white vs. non-white), and, by study design, gender. Conditional logistic regression models adjusted for body mass index (continuous, kg/m^2).