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Investigation of Novel Urinary Biomarkers in Hepatocellular Carcinoma Risk in a Predominantly African American Population: A Case-Control Study

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Keywords
Biomarkers · Hepatocellular carcinoma · African American population · Thromboxane · Prostaglandin E2 · Prostacyclin

Abstract
Introduction: African Americans are at increased risk of hepatocellular carcinoma (HCC) compared to other racial and ethnic groups. We investigated the associations of four urinary biomarkers of prostaglandin E_2 (PGE-M), prostacyclin (PGI-M), and thromboxane ($11dTxB_2$) synthesis and the ratio of PGI-M to $11dTXB_2$ with HCC risk in a cohort of predominantly African American populations. ***Methods:*** We conducted a nested case-control study (50 cases; 43 with HCC, 151 controls) in the Southern Community Cohort Study (SCCS), a large prospective cohort study including over 80,000 study participants, of whom two-thirds are African Americans. Urine samples were collected at enrollment and subsequently analyzed to assess biomarker levels. Multivariable regression models adjusted for age, race, sex, BMI, smoking status, NSAID use, education level, income, and alcohol consumption were used to assess the relationship between the biomarker and HCC risk. ***Results:*** Only $11dTxB_2$ (OR = 11.50; 95% CI [2.34–56.47] for highest tertile vs. lowest tertile, $p = 0.004$) and the PGI-M/$11dTXB_2$ ratio of the second quartile (0.25–0.49) (OR = 5.16; 95% CI [1.44–18.47]; $p = 0.01$) were significantly associated with increased risk of liver cancer. ***Conclusion:*** $11dTXB_2$ and PGI-M/$11dTXB_2$ ratio may be urinary markers of HCC risk, particularly among African Americans, and future prospective studies are needed to evaluate this finding further and to develop accessible methods.

Introduction

Hepatocellular carcinoma (HCC) is the third leading cause of cancer deaths in the world and the sixth most diagnosed cancer based on 2020 statistics [1]. The

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majority of HCC occurs in patients with underlying chronic liver disease, which usually produces liver inflammation [2]. Racial disparities in death from HCC in the USA have previously been linked to the risk of HCC-related death in Asian Americans, American Indian/Alaskan natives, and African Americans in comparison to non-Hispanic whites [3]. African Americans have also been more frequently diagnosed with advanced liver disease and have lower survival than whites [4]. The molecular etiology of these differences has not been elucidated.

Activation of inflammatory signaling pathways produces an environment that is rich in various cytokines, prostaglandins, and reactive oxygen species (ROS). The cyclooxygenases (COX-1 and COX-2) are key enzymes responsible for conversion of arachidonic acid to prostaglandins, such as prostaglandin E_2 (PGE_2), prostaglandin D (PGD_2), thromboxane A_2 (TXA_2), and prostaglandin I_2 (PGI_2), also known as prostacyclin. Nonsteroidal anti-inflammatory medications (NSAIDs) inhibit the COXs and decrease the production of their downstream products. NSAID use has previously been associated with reduced risks of colorectal and other digestive tract cancers, with several in vivo studies now reporting decreased incidence and recurrence of HCC associated with use as well [5, 6]. Thus, COX inhibition may play a role in inhibiting carcinogenesis (Figure 1).

Of the prostaglandins, PGE_2 is also likely to be the mediator of the effects of COX-2 in liver carcinogenesis. Overexpression of COX-2 or treatment with PGE_2 has been shown to increase HCC invasion and growth [7]. Furthermore, PGE_2 was described as increasing human HCC cell proliferation. Importantly, PGE_2 levels are increased in HCC tissue and adjacent non-malignant tissues when compared to normal hepatic parenchyma [8]. The half-life of PGE_2 in humans is very short; thus, quantification of the major urinary metabolite of PGE_2 (9,15-dioxo-11α-hydroxy-13,14-dihydro-2,3,4,5-tetranor-prostan-1,20-dioic acid, PGE-M) is an accurate and precise method to assess endogenous PGE_2 production in humans [9].

PGD_2, on the other hand, seems to decrease tumor progression, and higher concentrations of PGD_2 tend to slow down tumor growth and metastasis in animal models [10]. Elevated levels of PGD_2 can decrease the number of ROS and increase apoptosis in non-small cell lung cancer, slow down the growth of gastric adenocarcinoma, and be associated with decreased number of metastasis in melanoma [11–13]. The data on association between urinary PDG_2 metabolite (PGD-M) and cancers is limited [14, 15].

PGI_2 is another [16] product of arachidonic acid metabolism by COX-1 and COX-2. It counteracts the effects of ROS in the liver [17] and mediates normal hepatic perfusion. Prostacyclin analogs have been investigated as therapeutic targets for delaying hepatic fibrosis formation [18]. It also has antimetastatic activity on liver metastasis of human pancreatic cancer in a mouse model [19]. The best way to estimate systemic biosynthesis of PGI_2 is to measure its urinary metabolite, 2,3-dinor-6-keto-$PGF_{1\alpha}$ aka PGI-M.

Thromboxane A_2 (TxA_2) is a mediator of platelet activation. TxA_2 increases cancer proliferation, invasion, and angiogenesis in colorectal and bladder cancers [20, 21]. Furthermore, TxA_2 inhibitors suppress formation of metastasis in pulmonary and hepatic tissue in experimental mouse models [22, 23]. TxA_2 is usually produced in response to physiological stress and has been implicated in mediating hepatic inflammation and injury [24]. 11-dehydro-TxB_2 is a major urinary metabolite of TxA_2 and is readily measured in urine. 11dTxB_2 has previously been shown to be increased in patients with cirrhosis [25, 26]. However, the role of TxA_2 or 11dTxB_2 in HCC has not been investigated. In addition, previous reports identified alterations in the ratio of prostacyclin to thromboxane in the development of adverse cardiovascular events in patients on antiplatelet therapy or with breast cancer or preeclampsia [27–29].

The Southern Community Cohort Study (SCCS) is a large prospective cohort study including over 80,000 study participants and was designed to evaluate health disparities in cancer. About two-thirds of participants are African Americans and about two-thirds live below poverty. SCCS is also one of the few cohort studies in which baseline urine samples have been banked. In this study, we aimed to prospectively evaluate baseline urinary PGE-M, PGI-M, 11dTxB_2, and TN-E in the SCCA cohort and correlate them with risk of incident HCC.

Methods

Study Population

Detailed methods for the Southern Community Cohort Study (SCCS) have been previously published [30]. Briefly, the SCCS was designed to study cancer disparities including racial (black/white), socioeconomic, and rural/urban disparities within 12 southeastern US states [30]. SCCS participants were recruited in-person from community health centers (86%) or from a random sample of adults who responded to a mailed survey (14%). A total of 86,000 participants enrolled in the study between 2002 and 2009. All study participants were aged 40–79 years at the time of enrollment. Study participants completed an extensive questionnaire during enrollment which solicited information on the participants' behaviors, health, and environment.

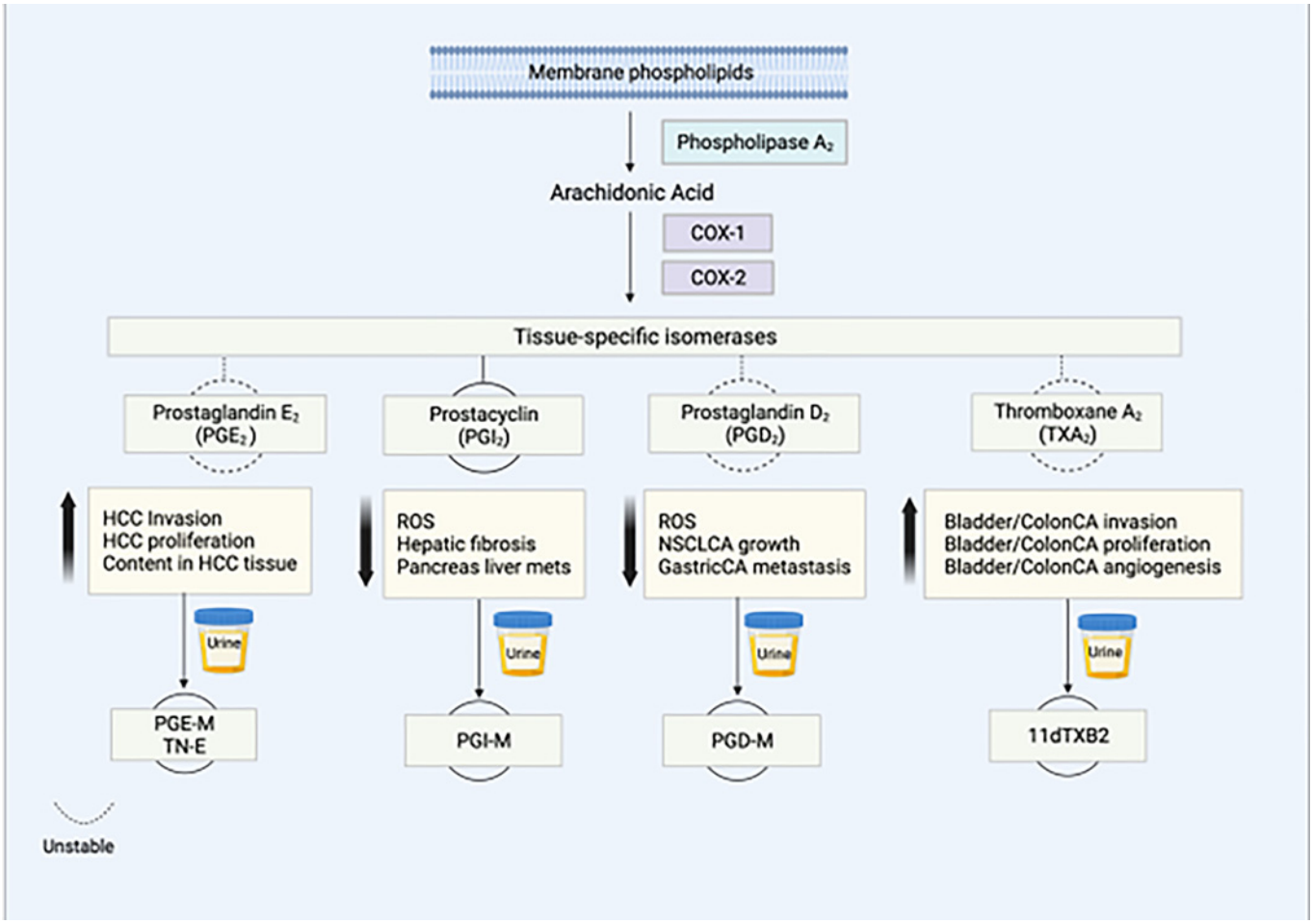

Fig. 1. Schematic representation of potential biomarkers of HCC. HCC, hepatocellular carcinoma; ROS, reactive oxygen species; NSCLCa, non-small cell lung carcinoma; GastricCa, gastric carcinoma; ColonCa, colorectal carcinoma.

Urine Collection

During 2004–2009, a subset of participants (n = 23,400) provided an approximately 30 mL spot urine sample at baseline. Ascorbic acid was added to the samples, and they were shipped cold daily to the study laboratory. Urine samples were spun at 2,000 × g for 10 min using a refrigerated centrifuge (at 4°C). The urine was then removed and pipetted into 7 sterile 3.5 mL cryovials. The pellet (including exfoliated cells) was suspended in 1.5 mL TE buffer and transferred to a 3.5 mL cryovial. All samples are stored in −80°C freezers until use.

Case and Control Selection

In this case-control study, 50 incident liver cancer cases diagnosed after enrollment and who had a urine sample were identified. Out of 50 cases, 48 were identified from state cancer registries, with 43 identified as HCC and 5 as "neoplasm-malignant." An additional 2 cases were identified from the National Death Index and their morphology is unknown. These 50 cases were matched to 50 controls who did not develop liver cancer and were cancer-free at the time of the diagnosis of the case. Matching was based on sex (male/female), racial group (black, white, other/unknown), NSAID use (defined as ever/never use of two or more times/week for 1 month of low-dose aspirin, aspirin, Advil, Motrin, Celebrex, Vioxx, Bextra), age at enrollment (+/−5 years), and date of urine collection (+/−12 months). An additional 101 cancer-free controls were included in this analysis from a separate nested case-control study of colorectal cancer. Cancer diagnosis was obtained through annual linkage with the 12 state cancer registries and the National Death Index (NDI). Participants were followed for a median of 10 years for cancer incidence. No participants had a cancer diagnosis prior to enrollment.

Measurement of Urinary PGE-M, 11dTxB2, PGI-M, and TN-E Levels

Urine samples from all cases and controls were analyzed at the same time. Concentrations of 9,15-dioxo-11α-hydroxy-13,14-dihydro-2,3,4,5-tetranor-prostan-1,20-dioic acid (PGE-M), tetranor-PGE_1 (TN-E), 9α-hydroxy-11,15-dioxo-13,14-dihydro-2,3,4,5-tetranor-prostan-1,20-dioic acid (tetranor PGD-M), 11-dehydro-thromboxane B_2 ($11dTxB_2$), and 2,3-dinor-6-keto-$PGF_1\alpha$

(PGI-M) in urine were measured in the Eicosanoid Core Laboratory at Vanderbilt University Medical Center. Urine (1 mL) was acidified to pH 3 with HCl. [$^2H_4$]-2,3-dinor-6-keto-PGF1a, [$^2H_4$]-11-dehydro-TxB_2, and [$^2H_{11}$]-tetranor-PGE_1 were added, and the sample was treated with methyloxime HCl to convert analytes to the *O*-methyloxime derivative. The derivatized analytes were extracted using a C-18 Sep-Pak (Waters Corp., Milford, MA, USA) and eluted with ethyl acetate as previously described [31, 32]. A [$^2H_6$]-*O*-methyloxime PGE-M deuterated internal standard was then added for PGE-M and PGD-M quantification. The sample was dried under a stream of dry nitrogen at 37 °C and then reconstituted in 75 μL mobile phase A (see below) for LC/MS analysis.

LC was performed on a 2.0 × 50 mm, 1.7 mm particle Acquity BEH C18 column (Waters Corporation, Milford, MA, USA) using a Waters Acquity I-Class UPLC. Mobile phase A was 95/5/0.01 (v/v/v) water/acetonitrile/acetic acid, and mobile phase B was 10/90/0.01 (v/v/v) water/acetonitrile/acetic acid. Samples were separated by a gradient of 85–5% of mobile phase A over 12 min at a flow rate of 0.375 mL/min prior to delivery to a Waters Xevo TQ-XS triple quadrupole mass spectrometer.

Urinary creatinine levels were measured using a test kit from Enzo Life Sciences. The urinary metabolite levels in each sample are normalized using the urinary creatinine level of the sample and expressed in ng/mg creatinine. The ratio of PGI-M to 11dTXB$_2$ was calculated to reflect the balance of vasodilator to vasoconstrictor in the environment.

The mean CVs for intra-and inter-assay variability of PGE-M were found to be 4.1% and 6.7%, respectively. The mean CVs for intra-and inter-assay variability of TN-E were 8.1% and 6.1%, respectively.

Data Analysis

Differences in potential confounders by case/control status were derived from χ^2 (categorical) or *t* tests (continuous). The biomarkers were ranked into tertiles according to the control distribution. The PGI-M to 11dTXB$_2$ ratio was calculated. In order to avoid division by zero, we imputed the lower limit of detection for PGI-M (0.01) and 11dTXB$_2$ (0.005) and divided it first by the creatinine value and then by the square root of 2. The ratio was then grouped into four categories (0.001–0.24, 0.25–0.49, 0.5–0.99 and ≥1) to approximate quartiles in controls. Any covariates which significantly differed among the two groups were considered potential confounding factors. The potential confounding factors were age (years), sex (male/female), race (Black, white), body mass index (BMI, <25, 25–29, 30–34, 35+ kg/m^2), household income (less than $15 K, $15–49 K, more than $50 K per year), education (less than high school, high school or equivalent, college or more), smoking status (current, former, and never smokers), alcohol use [none, moderate (>0 and ≤1 drink per day for females or >0 and ≤2 drinks per day for males), and heavy drinkers (>1 drink per day for females, and >2 drinks per day for males)], and regular NSAID use (yes, no). BMI was missing for 2 participants, education level was missing for 1 participant, and income was missing for 1 participant. The median or mode was assigned to these participants. Odds ratios (ORs) and 95% confidence intervals (CIs) were derived from multivariate logistic regression models. Tests for linear trend were performed by entering the categorical tertile variables as continuous variables in the model. A sensitivity analysis was performed by using conditional logistic regression for the liver cancer cases and their matched controls. Effect estimates were similar to the full pooled study, although the confidence intervals were wide due to the small sample size. All *p* values were 2-sided with a significance level of <0.05. All statistical analyses were performed using SAS 9.4.

Results

Among the 50 liver cancer cases, 10 were diagnosed in less than 24 months after enrollment and 7 were diagnosed at or over 10 years after, median time to diagnosis of 6 years (range 0–11; results not shown in table). In comparison to controls, patients with liver cancer were more likely to be male (74 vs. 49%, $p = 0.002$), be a current smoker (72 vs. 45%, $p = 0.001$), use more NSAIDs (48 vs. 28%, $p = 0.01$), and have higher rates of moderate and heavy alcohol consumption (46 vs. 38% and 34 vs. 21%, respectively, $p = 0.02$) (Table 1).

Overall, in an unadjusted analysis, the levels of PGE-M were higher in cases versus controls (20.2 vs. 14.7 ng/mg Cr, $p = 0.04$) as were TN-E levels (7.9 vs. 5.3 ng/mg Cr, $p = 0.03$), PGI-M levels (0.49 vs. 0.22, $p = 0.03$), and 11dTxB$_2$ levels (1.24 vs. 0.40, $p = 0.004$). A similar pattern was seen using geometric means (see online suppl. Table 1; for all online suppl. material, see https://doi.org/10.1159/000538131). However, after adjustment for potential confounders (Table 2), only 11dTxB$_2$ was significantly associated with incident cancer (OR = 11.50; 95% CI [2.34–56.47], $p = 0.004$). This association was present among those diagnosed either less than 5 years after baseline or 5 years or more since baseline (OR = 14.21; 95% CI [1.08–187.36]; $p = 0.0005$ and OR = 12.02; 95% CI [1.56–92.53]; $p = 0.009$, respectively).

We then proceeded to analyze PGI-M to 11dTXB$_2$ ratio. In order to avoid division by zero, for undetectable 11dTXB$_2$ values, we used the creatinine standardized logarithm of the odds values divided by square root of 2. The ratio was then grouped into four categories (0.001–0.24; 0.25–0.49; 0.5–0.99; and ≥1). PGI-M to 11dTXB$_2$ ratio of 0.25–0.49 was associated with a fivefold increased risk of liver cancer in comparison to a ratio greater than 1 (Table 3). This association was strengthened after adjustment for the other biomarkers (OR = 5.16; 95% CI [1.44–18.47]; $p = 0.001$).

Discussion

In this study, we investigated 5 potential urinary biomarkers of liver cancer, predominantly HCC risk, within the Southern Community Cohort Study, a

Table 1. Characteristics of liver cancer cases and controls, Southern Community Cohort Study

Characteristic	Liver cancer status		*p* value
	cases (*N* = 50)	controls (*N* = 151)	
Age, years, mean (std)	53.0 (6.0)	53.4 (8.4)	0.77
Age <65 years, *n* (%)	47 (94)	133 (88)	0.24
Age ≥65 years, *n* (%)	3 (6)	18 (12)	
Sex, *n* (%)			0.002
Female	13 (26)	77 (51)	
Male	37 (74)	74 (49)	
Race, *n* (%)			0.62
Black	39 (78)	107 (71)	
White	10 (20)	40 (26)	
All others	1 (2)	4 (3)	
Income, *n* (%)			0.14
<15 K	38 (76)	91 (61)	
15 K–49 K	10 (20)	51 (34)	
50 K+	2 (4)	8 (5)	
Education, *n* (%)			0.09
<HS	21 (42)	46 (30)	
HS or equivalent/some college	27 (54)	84 (56)	
College+	2 (4)	21 (14)	
Smoking status, *n* (%)			0.001
Current	36 (72)	68 (45)	
Former	9 (18)	30 (20)	
Never	5 (10)	53 (35)	
NSAID use, *n* (%)			0.01
Yes	24 (48)	43 (28)	
No	26 (52)	108 (72)	
BMI, *n* (%)	28.4 (6.3)	29.0 (6.2)	0.55
<25	19 (38)	40 (27)	0.50
25–29	14 (28)	52 (35)	
30–34	9 (18)	32 (21)	
35+	8 (16)	25 (17)	
Alcohol consumption status, *n* (%)			0.02
None	10 (20)	61 (40)	
Moderate	23 (46)	58 (38)	
Heavy	17 (34)	32 (21)	
Cr, mg/mL, mean (std)	1.2 (0.6)	1.2 (0.7)	0.79
PGE-M, ng/mg Cr, mean (std)	20.2 (18.2)	14.7 (14.9)	0.04
PGD-M, ng/mg Cr, mean (std)	3.5 (6.3)	3.9 (5.2)	0.68
PGI-M, ng/mg Cr, mean (std)	0.49 (0.84)	0.22 (0.21)	0.03
11dTxB_2, ng/mg Cr, mean (std)	1.24 (1.94)	0.40 (0.63)	0.004
TN-E, ng/mg Cr, mean (std)	7.9 (8.5)	5.3 (7.2)	0.03

p value was derived from *t* test for continuous variables and χ^2 test for categorical variables.

population of largely black or low-income participants. We found statistically significant associations of 11dTxB_2 and PGI-M/11dTxB_2 with subsequent risk of liver cancer, while PGD-M, PGI-M, PGE-M, and TN-E failed to show a statistically significant association.

Table 2. Associations between urinary biomarkers and risk of liver cancer, Southern community cohort study

Biomarker	Overall		Cases diagnosed <5 years from baseline		Cases diagnosed ≥5 years from baseline	
	cases/controls	OR (95% CI)[a]	cases/controls	OR (95% CI)[a]	cases/controls	OR (95% CI)[a]
PGE-M						
T1 (low)	16/49	1.00 (ref)	7/49	1.00 (ref)	9/49	1.00 (ref)
T2	12/52	0.34 (0.09–1.33)	2/52	0.16 (0.02–1.69)	10/52	0.30 (0.05–1.70)
T3	21/50	0.39 (0.10–1.45)	8/50	0.55 (0.08–4.00)	13/50	0.21 (0.04–1.26)
p for trend		0.44		0.47		0.18
PGD-M						
T1 (low)	16/50	1.00 (ref)	5/50	1.00 (ref)	11/50	1.00 (ref)
T2	22/52	1.63 (0.57–4.64)	8/52	1.41 (0.25–7.96)	14/52	1.32 (0.37–4.64)
T3	12/49	0.39 (0.11–1.44)	5/49	0.59 (0.07–4.67)	7/49	0.34 (0.07–1.67)
p for trend		0.99		0.06		0.06
PGI-M						
T1 (low)	10/50	1.00 (ref)	4/50	1.00 (ref)	6/50	1.00 (ref)
T2	10/51	0.56 (0.14–2.26)	2/51	0.42 (0.03–5.69)	8/51	0.70 (0.13–3.68)
T3	30/50	1.55 (0.40–6.12)	12/50	1.17 (0.11–12.42)	18/50	1.75 (0.32–9.68)
p for trend		0.12		0.28		0.06
$11dTxB_2$						
T1 (low)	5/50	1.00 (ref)	2/50	1.00 (ref)	3/50	1.00 (ref)
T2	10/49	4.08 (0.86–19.50)	2/49	2.94 (0.20–42.78)	8/49	6.58 (0.91–47.49)
T3	35/50	**11.50 (2.34–56.47)**	14/50	**14.21 (1.08–187.36)**	21/50	**12.02 (1.56–92.53)**
p for trend		0.004		0.0005		0.009
TN-E						
T1 (low)	10/50	1.00 (ref)	5/50	1.00 (ref)	5/50	1.00 (ref)
T2	21/51	1.70 (0.49–5.85)	8/51	0.65 (0.10–4.32)	13/51	6.16 (1.00–38.07)
T3	19/50	1.30 (0.31–5.47)	5/50	0.26 (0.03–2.78)	14/50	5.69 (0.73–44.03)
p for trend		0.82		0.18		0.37

p for trend was calculated by including the biomarkers as continuous variables in the model. [a]Multivariate logistic regression model adjusted for all of the five biomarker levels in tertiles, age, sex, race, BMI, smoking status, NSAID use, household income, education, and alcohol drinking status.

Table 3. Association between risk for liver cancer and the ratio of PGI-M to 11dTXB2

PGI-M: $11dTXB_2$ ratio	Cases/controls	OR (95% CI)	
		model 1[a]	model 2[b]
0.001–0.24	12/37	2.98 (0.86–10.38)	2.34 (0.62–8.80)
0.25–0.49	17/24	**5.16 (1.44–18.47)**	**5.68 (1.46–22.15)**
0.5–0.99	15/48	2.55 (0.78–8.40)	2.54 (0.72–8.95)
≥1	6/42	1.00 (Ref)	1.00 (Ref)

[a]Adjusted for age, race, sex, BMI, smoking status, NSAID use, household income, education, and alcohol drinking status. [b]Additionally adjusted for PGE-M, PGD-M, and TN-E level in tertiles.

This is the first study to report on association of $11dTxB_2$ or PGI-M/$11dTxB_2$ ratio with liver cancer and HCC risk. The association persisted for both cases diagnosed within and longer than 5 years since urine collection. Thromboxane A2 synthase inhibitors have been previously demonstrated to decrease hepatitis C

infectivity in ex vivo models. Since viral hepatitis data is not available in our population and only one control subject self-reported hepatitis without further specification, this may represent a surrogate marker of viral hepatitis infection [33]. TxA_2 has prothrombotic properties (platelet aggregation) and vasoconstrictor properties activated as a part of inflammatory response. TxA_2 production increases in the setting of tissue injury and subsequent inflammation, platelet aggregation, and vasoconstriction, thus decreasing the blood supply to the injured site. Platelets have been shown to promote cancer development through angiogenesis and protection of metastatic cells which allows evasion of the immune system in circulation [22, 34]. The observed elevation of $11dTxB_2$ in our cohort who went on to develop liver cancer is in line with previous reports of $11dTxB_2$ correlation with breast cancer growth and progression [35] as well as with diagnosis of colorectal cancer [36].

As a vasoconstrictor, TxA_2 exists in equilibrium with its physiological antagonist prostacyclin which is a potent vasodilator. We showed that decreased PGI-M/$11dTxB_2$ ratio, which reflects relative abundance of TxA_2 as compared to prostacyclin, predicts future liver cancer development in the SCCS. Similar findings were reported in breast cancer [37], endometrial cancer [38], and thyroid cancer [39]. This finding likely reflects inflammatory milieu which is associated with tissue injury and possibly carcinogenesis.

PGE-M has been shown to be elevated in individuals with gastric cancer, colorectal cancer, as well as in smokers and overweight/obese patients [40–42]. 2,3,4,5-tetranor-PGE_1 (TN-E) is a secondary metabolite of PGE_2 excreted in urine [43]. Formation of this metabolite is not dependent upon the action of 15-prostaglandin dehydrogenase (15-PGDH). 15-PGDH has been shown to be downregulated in human hepatoma cells with high expression of COX-2. Quantification of both PGE-M and TN-E gives a more comprehensive representation of endogenous PGE_2 production. However, our analysis of two primary urinary metabolites of PGE_2 was not associated with subsequent liver cancer risk. These findings are in concordance with the only previously published study of PGE-M levels and HCC risk in an Asian population, which also failed to show a significant association with HCC [44]. Possible confounding of this lack of correlation may lie in our inability to assess for concurrent liver disease at the time of urine collection. Underlying liver disease may interfere with PGE_2 metabolism, thus resulting in lower systemic levels of PGE_2 and its metabolite, TN-E. Our study also failed to demonstrate an association between PGD-M levels and the risk of liver cancer. There are no previous studies for comparison.

Our study has some limitations. Although it is the first study to explore several of these biomarkers with liver cancer risk, only a small number of cases were included which may have affected our ability to detect an association. Unfortunately, the incidence of cirrhosis and viral hepatitis was not available in our dataset. We acknowledge that this represents a major limitation in methodology of this paper, considering that the presence of cirrhosis and hepatitis B are major factors for liver cancer. Another limitation is that observed relationship could be a result of reverse causation bias, whereas the unrecognized presence of HCC many leads to elevated levels, which may make these biomarkers a useful attribute in diagnosis of HCC. Likewise, the urine sample obtained provides a single, baseline measurement of urinary metabolites and may not reflect the most etiologically relevant time period. However, the relationships were consistent for diagnoses that occurred within 5 years of biospecimen collection and diagnoses that occurred more than 5 years after collection as well as analyses that excluded cases diagnosed within the first 2 years (results not shown). Future studies should evaluate longer time periods. The prospective analysis and well-characterized population are additional strengths of this study which has newly evaluated several of these biomarkers with liver cancer risk.

Despite these limitations, we believe that this study provides grounds for future prospective studies of urinary TxA_2 markers in HCC risk. $11dTxB_2$ and PGI-M/$11dTxB_2$ ratio may also be urinary markers of HCC risk that warrant future study as well as the development of easily accessible methods.

Acknowledgments

This work has been presented at Digestive Disease Week 2022 as a poster presentation entitled Tu1278: Investigation of Novel Urinary Biomarkers in Hepatocellular Carcinoma Development in Predominantly African American Patient Population, available at https://www.sciencedirect.com/science/article/abs/pii/S0016508522636990?via%3Dihub.

Statement of Ethics

All participants provided written informed consent through the Vanderbilt University Medical Center Institutional Review Board. The IRB number is 010345.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

All authors contributed equally to development of the project, data analysis, and manuscript write-up and review; approved the final version of the manuscript; and agree to be accountable for all aspects of the work. Alexandra Shingina, Xijing Han, Lei Fan, Harvey Murff, Robert Coffey, Ginger L. Milne, Qi Dai, and Martha Shrubsole contributed to design of the project and data acquisition, analysis, and interpretation. Alexandra Shingina and Martha Shrubsole drafted the manuscript, and Xijing Han, Lei Fan, Harvey Murff, Robert Coffey, Ginger L. Milne, and Qi Dai critically reviewed it.

Data Availability Statement

Data are available upon request through the Southern Community Cohort Study Data and Biospecimen Use Committee when consistent with the informed consent of study participants. Further inquiries can be directed to the corresponding author. Instructions are available at https://www.southerncommunitystudy.org/.

References

1 Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209–49. doi: 10.3322/caac.21660.
2 El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology. 2007;132(7):2557–76. doi: 10.1053/j.gastro.2007.04.061.
3 Ashktorab H, Kupfer SS, Brim H, Carethers JM. Racial disparity in gastrointestinal cancer risk. Gastroenterology. 2017;153(4):910–23. doi: 10.1053/j.gastro.2017.08.018.
4 Momin BR, Pinheiro PS, Carreira H, Li C, Weir HK. Liver cancer survival in the United States by race and stage (2001–2009): findings from the CONCORD-2 study. Cancer. 2017;123(Suppl 24):5059–78. doi: 10.1002/cncr.30820.
5 Yeh CC, Lin JT, Jeng LB, Ho HJ, Yang HR, Wu MS, et al. Nonsteroidal anti-inflammatory drugs are associated with reduced risk of early hepatocellular carcinoma recurrence after curative liver resection: a nationwide cohort study. Ann Surg. 2015;261(3):521–6. doi: 10.1097/SLA.0000000000000746.
6 Sahasrabuddhe VV, Gunja MZ, Graubard BI, Trabert B, Schwartz LM, Park Y, et al. Nonsteroidal anti-inflammatory drug use, chronic liver disease, and hepatocellular carcinoma. J Natl Cancer Inst. 2012;104(23):1808–14. doi: 10.1093/jnci/djs452.
7 Han C, Michalopoulos GK, Wu T. Prostaglandin E2 receptor EP1 transactivates EGFR/MET receptor tyrosine kinases and enhances invasiveness in human hepatocellular carcinoma cells. J Cell Physiol. 2015;230(6):1401. doi: 10.1002/jcp.24869.
8 Zang S, Ma X, Wu Y, Liu W, Cheng H, Li J, et al. PGE2 synthesis and signaling in malignant transformation and progression of human hepatocellular carcinoma. Hum Pathol. 2017;63:120–7. doi: 10.1016/j.humpath.2017.02.018.
9 Murphey LJ, Williams MK, Sanchez SC, Byrne LM, Csiki I, Oates JA, et al. Quantification of the major urinary metabolite of PGE2 by a liquid chromatographic/mass spectrometric assay: determination of cyclooxygenase-specific PGE2 synthesis in healthy humans and those with lung cancer. Anal Biochem. 2004;334(2):266–75. doi: 10.1016/j.ab.2004.08.019.
10 Jara-Gutiérrez Á, Baladrón V. The role of prostaglandins in different types of cancer. Cells. 2021;10(6):1487. doi: 10.3390/cells10061487.
11 Gomes RN, Felipe da Costa S, Colquhoun A. Eicosanoids and cancer. Clinics. 2018;73(Suppl 1):e530s. doi: 10.6061/clinics/2018/e530s.
12 Dai DF, Swanson PE, Krieger EV, Liou IW, Carithers RL, Yeh MM. Congestive hepatic fibrosis score: a novel histologic assessment of clinical severity. Mod Pathol. 2014;27(12):1552–8. doi: 10.1038/modpathol.2014.79.
13 Lai YH, Liu H, Chiang WF, Chen TW, Chu LJ, Yu JS, et al. MiR-31-5p-ACOX1 Axis enhances tumorigenic fitness in oral squamous cell carcinoma via the promigratory prostaglandin E2. Theranostics. 2018;8(2):486–504. doi: 10.7150/thno.22059.
14 Cui Y, Shu XO, Li HL, Yang G, Wen W, Gao YT, et al. Prospective study of urinary prostaglandin E2 metabolite and pancreatic cancer risk. Int J Cancer. 2017;141(12):2423–9. doi: 10.1002/ijc.31007.
15 Johnson J, Schmidt C, Shrubsole M, Billheimer D, Joshi P, Morrow J, et al. Urine PGE-M: a metabolite of prostaglandin E2 as a potential biomarker of advanced colorectal neoplasia. Clin Gastroenterol Hepatol. 2006;4(11):1358–65. doi: 10.1016/j.cgh.2006.07.015.
16 Castro-Sanchez L, Agra N, Llorente Izquierdo C, Motino O, Casado M, Bosca L, et al. Regulation of 15-hydroxyprostaglandin dehydrogenase expression in hepatocellular carcinoma. Int J Biochem Cell Biol. 2013;45(11):2501–11. doi: 10.1016/j.biocel.2013.08.005.
17 Tanaka J, Malchesky PS, Omokawa S, Goldcamp JB, Harasaki H, Vogt DP, et al. Effects of prostaglandin I2, superoxide dismutase, and catalase on ischemia-reperfusion injury in liver transplantation. ASAIO Trans. 1990;36(3):M600–3.
18 Zardi EM, Vespasiani Gentilucci U, Picardi A, Ambrosino G, Fazio VM, Dobrina A, et al. Iloprost: an adjunctive approach to chronic viral hepatitis treatment. Med Hypotheses. 2005;64(1):46–52. doi: 10.1016/j.mehy.2004.05.017.
19 Schwalke MA, Tzanakakis GN, Vezeridis MP. Effects of prostacyclin on hepatic metastases from human pancreatic cancer in the nude mouse. J Surg Res. 1990;49(2):164–7. doi: 10.1016/0022-4804(90)90256-2.

20 Pradono P, Tazawa R, Maemondo M, Tanaka M, Usui K, Saijo Y, et al. Gene transfer of thromboxane A(2) synthase and prostaglandin I(2) synthase antithetically altered tumor angiogenesis and tumor growth. Cancer Res. 2002;62(1):63–6.
21 Moussa O, Yordy JS, Abol-Enein H, Sinha D, Bissada NK, Halushka PV, et al. Prognostic and functional significance of thromboxane synthase gene overexpression in invasive bladder cancer. Cancer Res. 2005;65(24):11581–7. doi: 10.1158/0008-5472.CAN-05-1622.
22 Nie D, Lamberti M, Zacharek A, Li L, Szekeres K, Tang K, et al. Thromboxane A(2) regulation of endothelial cell migration, angiogenesis, and tumor metastasis. Biochem Biophys Res Commun. 2000;267(1):245–51. doi: 10.1006/bbrc.1999.1840.
23 Yokoyama I, Hayashi S, Kobayashi T, Negita M, Yasutomi M, Uchida K, et al. Prevention of experimental hepatic metastasis with thromboxane synthase inhibitor. Res Exp Med. 1995;195(4):209–15. doi: 10.1007/BF02576790.
24 Yokoyama Y, Nimura Y, Nagino M, Bland KI, Chaudry IH. Role of thromboxane in producing hepatic injury during hepatic stress. Arch Surg. 2005;140(8):801–7. doi: 10.1001/archsurg.140.8.801.
25 Laffi G, Cinotti S, Filimberti E, Ciabattoni G, Caporale R, Marra F, et al. Defective aggregation in cirrhosis is independent of in vivo platelet activation. J Hepatol. 1996;24(4):436–43. doi: 10.1016/s0168-8278(96)80164-4.
26 Ferro D, Basili S, Iuliano L, Camastra C, Giammarresi C, Santarone S, et al. Increased thromboxane metabolites excretion in liver cirrhosis. Thromb Haemost. 1998;79(4):747–51. doi: 10.1055/s-0037-1615058.
27 Chavarría ME, Lara-González L, González-Gleason A, García-Paleta Y, Vital-Reyes VS, Reyes A. Prostacyclin/thromboxane early changes in pregnancies that are complicated by preeclampsia. Am J Obstet Gynecol. 2003;188(4):986–92. doi: 10.1067/mob.2003.203.
28 Montine TJ, Sonnen JA, Milne G, Baker LD, Breitner JC. Elevated ratio of urinary metabolites of thromboxane and prostacyclin is associated with adverse cardiovascular events in ADAPT. PLoS One. 2010;5(2):e9340. doi: 10.1371/journal.pone.0009340.
29 Vergote IB, van Dam PA, Laekeman GM, Keersmaeckers GH, Uyttenbroeck FL, Herman AG. Prostacyclin/thromboxane ratio in human breast cancer. Tumour Biol. 1991;12(5):261–6. doi: 10.1159/000217713.
30 Signorello LB, Hargreaves MK, Blot WJ. The southern community cohort study: investigating health disparities. J Health Care Poor Underserved. 2010;21(1 Suppl):26–37. doi: 10.1353/hpu.0.0245.
31 Mohebati A, Milne GL, Zhou XK, Duffield-Lillico AJ, Boyle JO, Knutson A, et al. Effect of zileuton and celecoxib on urinary LTE4 and PGE-M levels in smokers. Cancer Prev Res. 2013;6(7):646–55. doi: 10.1158/1940-6207.CAPR-13-0083.
32 Drew DA, Schuck MM, Magicheva-Gupta MV, Stewart KO, Gilpin KK, Miller P, et al. Effect of low-dose and standard-dose aspirin on PGE2 biosynthesis among individuals with colorectal adenomas: a randomized clinical trial. Cancer Prev Res. 2020;13(10):877–88. doi: 10.1158/1940-6207.CAPR-20-0216.
33 Abe Y, Aly HH, Hiraga N, Imamura M, Wakita T, Shimotohno K, et al. Thromboxane A2 synthase inhibitors prevent production of infectious hepatitis C virus in mice with humanized livers. Gastroenterology. 2013;145(3):658–67.e11. doi: 10.1053/j.gastro.2013.05.014.
34 Im JH, Fu W, Wang H, Bhatia SK, Hammer DA, Kowalska MA, et al. Coagulation facilitates tumor cell spreading in the pulmonary vasculature during early metastatic colony formation. Cancer Res. 2004;64(23):8613–9. doi: 10.1158/0008-5472.CAN-04-2078.
35 Ferroni P, Santilli F, Cavaliere F, Simeone P, Costarelli L, Liani R, et al. Oxidant stress as a major determinant of platelet activation in invasive breast cancer. Int J Cancer. 2017;140(3):696–704. doi: 10.1002/ijc.30488.
36 Sciulli MG, Filabozzi P, Tacconelli S, Padovano R, Ricciotti E, Capone ML, et al. Platelet activation in patients with colorectal cancer. Prostaglandins Leukot Essent Fatty Acids. 2005;72(2):79–83. doi: 10.1016/j.plefa.2004.10.006.
37 Kij A, Kus K, Smeda M, Zakrzewska A, Proniewski B, Matyjaszczyk K, et al. Differential effects of nitric oxide deficiency on primary tumour growth, pulmonary metastasis and prostacyclin/thromboxane A(2) balance in orthotopic and intravenous murine models of 4T1 breast cancer. J Physiol Pharmacol. 2018;69(6). doi: 10.26402/jpp.2018.6.05.
38 Aitokallio-Tallberg A. Prostacyclin and thromboxane synthesis by endometrial cancer and leiomyomas. Prostaglandins. 1990;39(3):259–66. doi: 10.1016/0090-6980(90)90045-w.
39 Kajita S, Ruebel KH, Casey MB, Nakamura N, Lloyd RV. Role of COX-2, thromboxane A2 synthase, and prostaglandin I2 synthase in papillary thyroid carcinoma growth. Mod Pathol. 2005;18(2):221–7. doi: 10.1038/modpathol.3800285.
40 Altobelli E, Angeletti PM, Latella G. Role of urinary biomarkers in the diagnosis of adenoma and colorectal cancer: a systematic Review and meta-analysis. J Cancer. 2016;7(14):1984–2004. doi: 10.7150/jca.16244.
41 Kim S, Taylor JA, Milne GL, Sandler DP. Association between urinary prostaglandin E2 metabolite and breast cancer risk: a prospective, case-cohort study of postmenopausal women. Cancer Prev Res. 2013;6(6):511–8.
42 Gross ND, Boyle JO, Morrow JD, Williams MK, Moskowitz CS, Subbaramaiah K, et al. Levels of prostaglandin E metabolite, the major urinary metabolite of prostaglandin E2, are increased in smokers. Clin Cancer Res. 2005;11(16):6087–93. doi: 10.1158/1078-0432.CCR-05-0733.
43 Green K. Metabolism of prostaglandin E2 in the rat. Biochemistry. 1971;10(6):1072–86. doi: 10.1021/bi00782a022.
44 Yuan JM, Grouls M, Carmella SG, Wang R, Heskin A, Jiang Y, et al. Prediagnostic levels of urinary 8-epi-prostaglandin F2α and prostaglandin E2 metabolite, biomarkers of oxidative damage and inflammation, and risk of hepatocellular carcinoma. Carcinogenesis. 2019;40(8):989–97. doi: 10.1093/carcin/bgy180.