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RESEARCH ARTICLE

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Analysis of *TP53* aflatoxin signature mutation in hepatocellular carcinomas from Guatemala: A cross-sectional study (2016-2017)

Christian S. Alvarez[1] | Jeremy Ortiz[2] | Giovanna Bendfeldt-Avila[3] | Yi Xie[1] | Mingyi Wang[4] | Dongjing Wu[4] | Herbert Higson[4] | Elisa Lee[4] | Kedest Teshome[4] | Joaquin Barnoya[5] | David E. Kleiner[6] | John D. Groopman[7,8] | Roberto Orozco[9] | Katherine A. McGlynn[1] | Eduardo Gharzouzi[5] | Michael Dean[1]

[1]Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland

[2]Instituto de Cancerología/INCAN, Guatemala City, Guatemala

[3]Hospital Centro Médico Militar, Guatemala City, Guatemala

[4]Cancer Genetics Research Laboratory, Division of Cancer Epidemiology and Genetics, Leidos Biomedical Research Inc, Frederick National Laboratory for Cancer Research, Gaithersburg, Maryland

[5]Integra Cancer Institute, Guatemala City, Guatemala

[6]Laboratory of Pathology, Center for Cancer Research, NCI, NIH, Bethesda, Maryland

[7]Department of Environmental Health and Engineering, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland

[8]Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins, University, Baltimore, Maryland

[9]Department of Pathology, Hospital General San Juan de Dios, Guatemala City, Guatemala

Correspondence
Christian S. Alvarez, PhD, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Shady Grove. 9609 Medical Center Drive, Rockville, MD 20850.
Email: christian.alvarez@nih.gov

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Abstract

Background and aims: Guatemala has the highest incidence of hepatocellular carcinoma (HCC) in the Western hemisphere. The major risk factors in Guatemala are not well characterized, but the prevalence of hepatitis B virus (HBV) and hepatitis C virus (HCV) appears to be low, while the prevalence of aflatoxin (AFB_1) exposure appears to be high. To examine whether AFB_1 may contribute to the elevated incidence of HCC in Guatemala, this study examined the frequency of the AFB_1-signature mutation in the *TP53* gene (R249S) as well as other somatic mutations. In addition, we assessed whether the frequency of the *TP53* mutation differed by sex.

Methods: Formalin-fixed, paraffin-embedded (FFPE) HCC tissues were obtained from three hospitals in Guatemala City between 2016 and 2017. In addition, tumor tissues preserved in RNAlater were also obtained. Sociodemographic and clinical information including HBV and HCV status were collected. Targeted sequencing of *TP53* was performed in the FFPE samples, and a panel of 253 cancer-related genes was sequenced in the RNAlater samples.

Results: Ninety-one FFPE tissues were examined, from 52 men and 39 women. Median (IQR) age at diagnosis was 62 (51-70). Among those with known HBV and HCV status, two were HBV+ and three were HCV+. Overall, 47% of the HCCs had a *TP53* mutation. The AFB_1-signature R249S mutation was present in 24%. No overlap between the R249S mutation and HBV+ was observed in this cohort. Among 18 RNAlater samples examined, 44% had any *TP53* mutation and 33% had the R249S mutation. Other somatic mutations were identified in known HCC driver genes.

Conclusions: The presence of the *TP53* R249S mutation in the samples studied suggests that AFB_1 may contribute to the high incidence of HCC in Guatemala. The

Abbreviations: AFB_1, aflatoxin B_1; FFPE, formalin-fixed paraffin-embedded; HBV, Hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; INCAN, Instituto de Cancerología; IQR, interquartile range; NAFLD, non-alcoholic fatty liver disease; NCI, National Cancer Institute; R249S, codon 249 mutation of *TP53* gene.

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proportion of HBV+ tumors was low, suggesting that AFB_1 may be associated with HCC in the absence of concomitant HBV infection. Further investigation of AFB_1 and other risk factors for HCC in Guatemala is warranted.

KEYWORDS

aflatoxin, Guatemala, hepatocellular carcinoma, R249S mutation, *TP53* mutation

1 | INTRODUCTION

Liver cancer is the seventh most common cancer and the second leading cause of cancer mortality globally.[1] The most common histological subtype is hepatocellular carcinoma (HCC), which accounts for 80% of all liver cancers.[2] Sex and geographic variation in HCC incidence has been reported across regions worldwide, as has the variability in the prevalence of known risk factors.[2-4] Major risk factors for HCC, such as hepatitis B virus (HBV), hepatitis C virus (HCV), excessive alcohol consumption, and aflatoxin B_1 (AFB_1) exposure vary between high-rate and low-rate areas. In many high-rate areas (eg, regions in Asia and Africa), HBV and AFB_1 exposure are dominant factors, while in low-rate areas, HCV and alcohol consumption are more common.[3-5] In addition, non-alcoholic fatty liver disease (NAFLD) is becoming recognized as an important risk factor for HCC in both high and low incidence regions.[6]

In Guatemala, the estimated incidence and mortality rates of liver cancer are the highest in the Western hemisphere (age-standardized rates [ASRs]: 14.9 cases per 100 000 person-years and 14.5 deaths per 100 000 person-years), with 1787 new cases of liver cancer estimated to have occurred in 2018.[7] Unlike most other countries, where men have a two- to three-fold higher rate of liver cancer than women, Guatemala has a unique 1:1 ratio of liver cancer rate between the sexes.[8] A recent cross-sectional study that assessed risk factors for liver cancer in Guatemala found a very low prevalence of both HBV and HCV infection (<1%).[8] In contrast, the study found high serum levels of AFB_1-albumin adducts among the participants, with significantly higher geometric mean levels among men (10.93 pg/mg albumin) than women (7.92 pg/mg albumin).[8] The results were consistent with previous evidence of high AFB_1 levels in maize samples across the country.[9] In addition, the study demonstrated that NAFLD (60.1%), obesity (30.9%), and metabolic syndrome (64.2%) are highly prevalent in Guatemala.[10]

AFB_1 forms DNA adducts at the N^7 position of guanine, inducing primarily G -> T transversions. One particular G -> T mutation in codon 249 (AGG to ATT, arginine to serine, R249S) of *TP53* is a molecular signature of AFB_1 exposure in HCC.[11] Studies in AFB_1 endemic regions in Asia and Africa have reported a wide range in the prevalence of the R249S mutation in HCC, from 4.8% to 67%.[12-19] In the Americas, the R249S mutation prevalence reportedly ranges between 1.3% and 28%.[20-23] In the U.S., a recent study reported that 7% of the HCCs among Hispanic patients in southern Texas had the R249S mutation.[24] No studies have previously been conducted in northern Central America, a region characterized by high AFB_1 exposure but low prevalence of chronic HBV infection.[25]

To evaluate the possible role of AFB_1 in the elevated incidence of HCC in Guatemala, tumor samples were retrieved from three major hospitals in Guatemala City, and targeted sequencing analysis of *TP53* was performed. Among a smaller set of RNA later-preserved samples, 253 cancer-related genes were sequenced. In addition, the frequency of *TP53* mutations by sex was assessed.

2 | METHODS

2.1 | Study population

Formalin-fixed, paraffin-embedded (FFPE) HCC tissue slides and blocks were obtained from the pathology departments of hospitals in Guatemala City between 2016 and 2017. The three hospitals that provided tissue were: Hospital General San Juan de Dios, a large public hospital; Hospital Militar, a hospital serving military personnel and their families; and the Instituto de Cancerología (INCAN), an adult cancer hospital. The HCC diagnoses were histologically confirmed by Dr. David Kleiner, at the U.S. National Cancer Institute (NCI). In addition, HCC tissues stored in RNAlater were obtained from INCAN.

Socio-demographic information, such as age, sex, residence (Guatemala and contiguous departments vs other departments), as well as HBV and HCV status were abstracted, when available, from medical records.

2.2 | DNA extraction

One half of the tissue from each pathology slide was scraped from the slide and extracted by a phenol-chloroform procedure.[26] FFPE blocks were sectioned in a microtome, and curls (10 um sections) were collected for DNA extraction. Tissue stored in RNAlater (ThermoFisher Scientific, Catalog # AM7020) was stored at −20°C until DNA was extracted using the AllPrep DNA/RNA Micro kit (Qiagen, Catalog # 80284). DNA was quantified using the PicoGreen dsDNA assay method (ThermoFisher Scientific, Catalog #P7581).

2.3 | DNA sequencing

Targeted sequencing of *TP53* was performed on the HCC FFPE samples using a primer panel provided by Ion Torrent (Ampliseq) using the manufacturer's protocol. Positive PCR products were quantified using Kapa's

Library Quantification Kit (Catalog # KK4824), normalized, pooled, amplified via emulsion PCR using the One Touch v2, enriched on the ES2, and sequenced on either the PGM or S5 sequencers according to the manufacturer's instructions. An average of 300-1000 reads per amplicon was obtained for each sample. Sequences were aligned to the hg19 reference human genome, and mutations called through a custom analysis workflow utilizing the aligned reads and a dual variant calling process, Torrent Suite Variant Caller (TSVC), and a modified GATK (Genome Analysis Toolkit) variant caller optimized for Ion Torrent data.[27] Variants from the reference sequence were annotated, and mutations confirmed by manual review in the Integrated Genome Viewer (IGV).

To obtain a more complete picture of somatic mutations in HCC samples from Guatemala, DNA from the tumor tissues in RNAlater (ThermoFisher Scientific, Catalog # AM7020) were examined in a Nimblegen custom targeted capture of all exons of 253 known cancer-related genes (Roche NimbleGen, Inc., Madison, WI, USA) (see below). Following library preparation with the Kapa HyperPlus kit (Catalog # KK8510), libraries were quantified using PicoGreen dsDNA Reagent (ThermoFisher Scientific, catalog #P7581), normalized, and pooled. The pooled samples were captured with the custom Nimblegen Roche SeqCap EZ Choice custom panel (Catalog # 06740251001), and 2x150bp sequencing was performed on either an Ilumina HiSeq4000 or NovaSeq. Sequence reads were trimmed and aligned to the hg19 reference genome using the NovoAlign software (v3.00.05). High-quality alignments for each individual were created, local realignments refined, and BAM file-level recalibrations performed using the Genome Analysis Toolkit (GATK v3.1). Mutations were identified using the UnifiedGenotyper and HaplotypeCaller from the Genome Analysis Toolkit (GATK v3.1) and the FreeBayes variant caller (v9.9.2). An ensemble variant calling pipeline (v0.2.2) was implemented to integrate analysis results from the three callers in *TP53* and other known cancer driver genes. In order to compare the proportion of tumors carrying mutations in the 253 known cancer-related genes, the results of the current study were contrasted with those of other studies indexed in PubMed that were published in the 5 years prior to the collection of the data of the current study. Eight studies with at least 50 HCCs that performed genome sequence analyses of more than 10 genes, between 2012 and 2018, were included in the comparison.[28-35]

An analysis of the mutation spectrum of *TP53* across all tumors, as well as the somatic variants and targeted sequence of the 253 genes in the 18 samples with fresh tumor DNA is presented in the Supplemental materials. In addition, the list of the 253 cancer-related genes is presented in Table S2.

2.4 | Statistical analysis

To examine whether the prevalence of *TP53* mutations varied by sex, the frequencies were evaluated for statistical significance by Fisher's exact test. A p-value <0.05 was considered statistically significant. SAS software v 9.4 (SAS Institute, Cary, NC) was used for the analysis.

2.5 | Ethical considerations

The study was approved by the ethical review board at INCAN, the Hospital General San Juan de Dios, and the Hospital Militar. Cases provided written informed consent at INCAN, and a waiver of consent was approved by the other two hospitals. In addition, there was an exemption by the NIH Office of Human Subjects Research (OHSR) to receive the samples without personal identifiers at NCI.

3 | RESULTS

In total, 91 FFPE HCC tissues and 18 additional tumor tissues in RNAlater were collected. Of the 91 HCC samples examined, 52 were from men and 39 were from women. The median age at diagnosis among men was 62 years (Interquartile range [IQR]: 48-73) and among women, 61 years (IQR: 52-68). Among the persons for whom information on residence was recorded (34.0%, 31/91), more than 70% lived in the department of Guatemala or in contiguous departments. Among the persons for whom HBV (27.5%, 25/91) and HCV status (28.6%, 26/91) was recorded, only two were HBV positive (defined as being positive for HBsAg) and only three were HCV positive (defined as being positive for anti-HCV) (Table 1).

3.1 | Prevalence of TP53 mutations in the FFPE samples

Of the 91 FFPE samples, two yielded insufficient DNA; thus, a total of 89 samples were successfully sequenced. Overall, 47% of the FFPE HCCs (42/89) had any *TP53* mutation (Table 2). The mutation prevalence was somewhat higher in the tumors from women (58%) than in the tumors from men (39%), but the difference was not statistically significant (P = .09, Fisher's exact test). The prevalence of the R249S mutation was 24%, with no major difference observed in the prevalence of the mutation in the tumors from men (22%) and women (26%) (P = .62, Fisher's exact test). The prevalence of any G -> T transversion, including the R249S mutation, was 27%, and the prevalence of any C -> T transition at CpG was 32%; we observed no statistically significant difference in the prevalence by sex (P = .47 and P = .17, respectively).

3.2 | Cancer gene sequence analysis of the tumor samples preserved in RNAlater

Table 3 shows the results of the gene sequence analysis for the 18 tumor tissues in RNAlater that were analyzed for targeted capture of the exons of 253 known cancer-related genes, compared to the genome sequence analysis results of HCCs in other populations. Among the 18 cases, 6 tumors (33%) had the *TP53* R249S mutation. The proportion with any *TP53* mutation was 44%, while the prevalence in other studies ranged between 21%

TABLE 1 Demographic characteristics of the study sample by sex, Guatemala 2016–2017

Characteristics	Total (N = 91)	Men n = 52 (57.1%)	Women n = 39 (42.9%)
Age, y, median (IQR)	62 (51, 70)	62 (48, 73)	61 (52, 68)
Hospital, n (%)			
INCAN	52 (57.1)	29 (55.8)	23 (59.0)
Hospital General San Juan de Dios	26 (28.6)	13 (25.0)	13 (33.3)
Hospital Militar	13 (14.3)	10 (19.2)	3 (7.7)
Residence[a], n (%)			
Guatemala and contiguous departments	22 (71.0)	13 (61.9)	9 (90.0)
Other departments	9 (29.0)	8 (38.1)	1 (10.0)
HBV status[a,b], n (%)			
Positive	2 (8.0)	2 (11.8)	0 (0.0)
Negative	23 (92.0)	15 (88.2)	8(100.0)
HCV status[a,c], n (%)			
Positive	3 (12.0)	3 (16.7)	0 (0.0)
Negative	23 (88.0)	15 (83.3)	8 (100.0)

[a]Missing for residence: 60; Missing for HBV status: 66; Missing for HCV status: 65.
[b]Defined as being positive for HBsAg.
[c]Defined as being positive for anti-HCV.

TABLE 2 Prevalence of TP53 mutations in HCCs by sex, Guatemala 2016-2017

Mutation	Total, n (%)	Men, n (%)	Women, n (%)	*P* value[a]
Any *TP53* mutation				
Yes	42 (47.2)	20 (39.2)	22 (57.9)	.09
No	47 (52.8)	31 (60.8)	16 (42.1)	
Total	89 (100)	51 (100)	38 (100)	
***TP53* R249S mutation**				
Yes	21 (23.6)	11 (21.6)	10 (26.3)	.62
No	68 (76.4)	40 (78.4)	28 (73.7)	
Total	89 (100)	51 (100)	38 (100)	
G -> T transversion				
Yes[b]	24 (27.0)	12 (23.5)	12 (31.6)	.47
No	65 (73.0)	39 (76.5)	26 (68.4)	
Total	89 (100)	51 (100)	38 (100)	
C -> T transition at CpG				
Yes	28 (31.5)	13 (25.5)	15 (39.5)	.17
No	61 (68.5)	38 (74.5)	23 (60.5)	
Total	89 (100)	51 (100)	38 (100)	

[a]*P* value from Fisher's exact test.
[b]G -> T transversions: codon 249 (n = 21); codon 248 (n = 1); codon 157 (n = 2).

and 82%.[28-35] Mutations were also observed in known HCC driver genes such as AT-rich interaction domain 2 (*ARID2*) (28%), AT-rich interaction domain 1 (*ARID1*) (17%), adenomatous polyposis coli (*APC*) (17%), and catenin beta-1 (*CTNNB1*) genes (17%). In addition, mutations were observed in axis inhibition protein 1 (*AXIN1*), SWI/SNF related, matrix associated, actin-dependent regulators of chromatin, subfamily a, member 4, (*SMARCA4*), guanine nucleotide-binding protein (*GNAS*), retinoblastoma (*RB1*), Fms-like tyrosine kinase 3 (*FLT3*), DNA (cytosine-5)-methyltransferase 3A (*DNMT3A*), cyclin-dependent kinase inhibitor 2A (*CDKN2A*), albumin (*ALB*), ribosomal protein S6 kinase, 90 kDa, polypeptide 3 (*RPS6KA3*), ataxia-telangiectasia mutated (*ATM*), and fibroblast growth factor receptor 3 (*FGFR3*).

Table S1 shows the mutation spectrum of the *TP53* and the 253 cancer-related genes grouped into the six possible classes of base substitutions. The analysis found an excess of C>A, G>T mutations in the *TP53* genes (57%) compared to 22% in all 253 genes. A deficit in the T>A, T>C and T>G classes is also seen in the *TP53* mutations. The mutation profile of all 253 cancer-related genes grouped into 16 possible 3 base pairs (bp) sequence context is displayed in Figure S1.

4 | DISCUSSION

This is the first report of *TP53* mutations in HCCs from northern Central America. Although the region is one of the three high-rate HCC regions in the world,[1] it is characterized by a low prevalence of chronic HBV infection.[8] The study found an overall *TP53* mutation rate of nearly 50% in both FFPE and RNAlater samples. In addition, among all the samples analyzed, the proportion with the AFB_1-signature mutation (R249S) was 25%.

Prior studies conducted in high-rate HCC areas have reported a *TP53* R249S mutation prevalence as high as 67%.[14,15,20,36] In Western Africa, a study in the Gambia found the R249S mutation in 36% (19/53) of HCCs, while a study in Senegal reported a prevalence of 67% among 15 HCCs.[14,18] In China, studies from high-rate areas have reported the R249S mutation in 36% (18/50) of HCCs from Guangxi, and 54% (97/181) of HCCs from Qidong in the early 2000s.[15,16] More recently, a study in Thailand found the R249S mutation in 34% of HCCs.[37] The majority of HCCs in sub-Saharan Africa and eastern Asia, however, are HBV positive (>50%), and it has been suggested that HBV sensitizes hepatocytes to the effects of AFB_1.[15]

The proportion of tumors with the R249S mutation found in the current study is similar to that reported in other regions in the Americas. For example, a study in Brazil found the mutation in 28% (21/74) of HCCs, with 16% (13/74) being HBV positive.[20] In Colombia, the R249S mutation was found in only four of the 38 HCC cases (11%), with 25 of the cases being positive for HBV infection.[22] A study in Peru found the aflatoxin signature mutation in only one tumor out of 80 HCCs.[23] In Mexico, 18% of HCC samples (3/16) had the AFB_1-signature mutation, with only three cases being positive for HBV.[21] As maize is commonly consumed in a number of Latin American countries, exposure to AFB_1 is likely. The high levels of AFB_1

TABLE 3 Prevalence of genetic mutations in HCCs reported by different studies

	Zhang et al[29]	Ahn et al[31]	Fujimoto et al[32]	Guichard et al[33]	Li et al[34]	Huang et al[28]	Schulze et al[30]	TCGA cBioPortal[35]	Current study 2020
Population	**China**	**Korea**	**Japan**	**Not provided**	**Not provided**	**China**	**Europe**	**US**	**Guatemala**
N	49	231	300	125	139	110	243	366	18
TP53	82%	32%	28%	21%	28%	59%	25%	33%	44%
TP53 R249S									33%
AXIN1	20%	7%	5%	15%	20%		12%	2%	6%
CTNNB1	20%	23%	26%	33%			38%		17%
KIT	12%								
SMARCA4	8%								6%
JAK3	8%								
PBRM1	8%								
GNAS	8%								6%
MED12	8%								
RB1	8%	8%	6%				7%	11%	6%
RET	8%								
ARID1A	6%		12%	17%		33%	12%	10%	17%
ARID2	6%		9%	6%	6%	4%	9%		28%
DNMT1	6%								
DNMT3A	6%								6%
FLT3	6%								6%
ABL1	6%								
FGFR2	6%								
MAP3K1	6%								
SETD2	6%								
ARID1B	6%								
CDKN2A	4%	6%	6%	7%			10%	8%	6%
ALB		4%	14%				12%	14%	6%
RPS6KA3		4%	6%	10%			9%		6%
CDKN1B		4%							
MYC		4%							
APC		2%		2%					17%
ATM		2%	44%				8%		11%
NFE2L2				6%			9%	7%	
IRF2				5%					
IL6ST				2%				4%	
PIK3CA				2%					
DMXL1					4%				
KRAS				2%				3%	
PTEN			4%					7%	
CDKN1A								4%	
FGFR3									11%
CASP8									0%

reported in Guatemala[8,9] are comparable to the levels found in some high-rate parts of China before the transition there from a maize-based to a rice-based diet.[38]

In the current study, we observed no overlap between the R249S mutation and HBV infection among those individuals with known HBV status. As AFB_1 exposure appears to be high in Guatemala, these

results suggest that the R249S mutation in HCC may be less common in regions where HBV is not endemic than in HBV-endemic regions. However, results from a previous meta-analysis reported only a 6% mean difference (95%CI: −1, 13%, P = .11) in the proportion of the R249S mutation between HBV positive and HBV negative cases with similar AFB_1 exposures.[16]

In total, there were 24 mutations with a G -> T transversion. The majority of the G -> T transversions occurred in codon 249, and the rest were in codon 157 (n = 2) and codon 248 (n = 1).These somatic mutations have been reported in HCCs previously.[39] Furthermore, one third of the HCCs in the current study has a C -> T transition at a CpG, suggesting that DNA methylation changes could play a role in these tumors. For example, a study that characterized genome-wide DNA methylation patterns in HCC identified a large subset of CpG sites associated with HCV infection, liver cirrhosis, or HCC.[40]

The p53 protein plays an important role in growth regulation as well as in tumor suppression and DNA repair.[41] Somatic mutations in *TP53* are common alterations in the majority of human cancers, including HCC.[41] The most common site for *TP53* mutations is in the DNA-binding domains, which decreases the binding affinity to responsive elements, leading to reduced activity of the p53 protein.[42,43] Etiological factors, in addition to AFB_1, such as HBV, HCV, and alcohol have been implicated in generating *TP53* mutations in HCC.[42,44] Inactivation of p53 by core proteins produced by HBV (eg, HBx and CtHBx) and HCV (eg, NS3 and NS5A) may lead to the development and progression of HCCs.[44] Furthermore, in animal studies, p53 has been implicated in the progression of steatosis to non-alcoholic steatohepatitis (NASH), involving mechanisms such as upregulation of *TP53* activity with increased mRNA levels of p21 and p66ShC, which are associated with fibrosis severity.[44]

The current study also identified somatic mutations in other known HCC driver genes, including *CTNNB1*, *ARID1A*, *ARID2*, *AXIN1*, among others.[45] A recent review of 20 studies with HCC genome sequence data reported recurrent mutations in 12 genes, including *TP53*, *CTNNB1*, *AXIN1*, *ALB*, *ARID2*, *ARID1A*, *RPS6KA3*, *APOB*, *RB1*, *CDKN2A*, *LRP1B*, and *PTEN*.[28] Mutations in *CTNNB1* have commonly been reported in HCCs,[46] with prevalences ranging from 20% to 38%.[29,30] The results of the current study are in line with prior findings, as *CTNNB1* mutations were identified in 17% of the HCCs.[29-33] In addition, mutations in *ARID1A* and *ARID2*, which are involved in WNT (cell-differentiation) pathway activation, have also been reported.[46] Deregulation of ARID1/2 signaling appears to affect 6%-18% of HCC tumors,[46] similar to the mutation prevalence found in the current study (17% *ARID1A* and 28% in *ARID2*). Furthermore, inactivating mutations in *ARID2* have been found in HCCs of various etiologies. A European study that performed exome sequencing analysis of 243 HCCs reported associations between some known risk factors and mutational patterns.[30] The study reported that alcohol-related HCCs were more likely to have mutations in *CTNNB1*, *TERT*, *CDKN2A*, *SMARCA2*, and *HGF*, while HBV-related HCCs were more likely to have *TP53* mutations. In contrast, no mutations were identified in either HCV- or NAFLD-related HCCs.[30] Overall, nearly 100 somatic mutations have been determined as HCC driver mutations, and approximately five to six driver mutations are considered necessary to cause cancer within a particular patient.[47]

The current study provides evidence that the elevated incidence and mortality of HCC in Guatemala could at least partially be explained by AFB_1. However, other factors, such as other mycotoxin exposure, metabolic disorders (eg, obesity, diabetes, and NAFLD), among others, may also be contributors. For example, our previous work reported high prevalences of NAFLD (60.1%), diabetes (21.6%), and obesity (30.9%) among Guatemalan adults (≥40 years old).[10] In addition, a study found high levels of fumonisin B_1 (FB_1) in maize samples across Guatemala.[9] No studies have examined the role of these risk factors for liver cancer in either Guatemala or other Central American countries where there is also an unusual 1:1 ratio of liver cancer incidence between men and women.[8]

To our knowledge, this study is the first to examine mutations in HCC from Guatemala. Other strengths include the sizable number of HCCs included and the histologic confirmation of all diagnoses by a liver cancer pathologist. Limitations of the study include that the tumors were not collected as part of a systematic protocol, so they may not be representative of all HCCs seen at the study hospitals, or in the country. In Guatemala, it is estimated that approximately 40% of HCCs are biopsied. Another limitation is that there was incomplete information available on risk factors, so it was not possible to determine the extent to which the *R249S* mutation corresponded to AFB_1 exposure. It was also not possible to determine HBV or HCV status of all cases. In addition, there was incomplete clinical information available on the tumors, so the number of somatic mutations could not be correlated with extent of disease. Furthermore, mutations in the *TERT* gene, the most commonly mutated gene in HCC,[45,48] could not be examined because it was not included in the panel of 253 cancer-related genes that were sequenced in the RNAlater samples.

In conclusion, the presence of the *TP53* AFB_1-signature mutation suggests that AFB_1 may play a role in the high incidence of HCC in Guatemala. As the prevalence of HBV was low among those with known HBV status, the current results suggest that AFB_1 is associated with HCC in the absence of concomitant HBV infection. These results suggest that further investigation of AFB_1 and other risk factors for HCC in Guatemala is warranted.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Conceptualization: Michael Dean, Katherine McGlynn, Eduardo Gharzouzi.

Resources: Jeremy Ortiz, Giovanna Bendfeldt-Avila, Roberto Orozco, Eduardo Gharzouzi, Joaquin Barnoya.

Investigation: Michael Dean, Yi Xie, Mingyi Wang, Dongjing Wu, Herbert Higson, Elisa Lee, Kedest Teshome, David Kleiner.

Methodology: Michael Dean, Yi Xie, Mingyi Wang, Dongjing Wu, Herbert Higson, Elisa Lee, Kedest Teshome.

Validation: David Kleiner.

Formal analysis: Christian S. Alvarez, Michael Dean.

Visualization: Christian S. Alvarez, Katherine McGlynn, Michael Dean.
Writing - original Draft Preparation: Christian S. Alvarez.
Writing - review & editing: Christian S. Alvarez, Katherine McGlynn, Michael Dean, Eduardo Gharzouzi, Roberto Orozco, Joaquin Barnoya, John Groopman.

All authors have read and approved the final version of the manuscript.

Christian S. Alvarez, the corresponding author, had full access to all the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis.

TRANSPARENCY STATEMENT

The lead author, Christian S. Alvarez, affirms that this manuscript is honest, accurate, and transparent account of the study being reported; no aspects of the study have been omitted; any discrepancies from the study as planned have been explained.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Christian S. Alvarez https://orcid.org/0000-0002-5338-0444

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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