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MINIREVIEWS

Role of T-box transcription factor 3 in gastric cancers

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Abstract

The expression of T-box transcription factor 3 (TBX3) has been identified in various cancers, including gastric cancers. Its role in breast cancers and melanomas has been intensively studied, and its contribution to the progression of cancers through suppressing senescence and promoting epithelial-mesenchymal transition has been reported. Recent reports on the role of TBX3 in gastric cancers have implied its involvement in gastric carcinogenesis. Considering its pivotal role in the initiation and progression of cancers, TBX3 could be a promising therapeutic target for gastric cancers.

Key Words: Aging; Wnt; β-catenin; Transforming growth factor-β; Stomach; Carcinogenesis

Core Tip: Expression of T-box transcription factor 3 (TBX3) has been reported in a variety of cancers. Preceding reports have shown that TBX3 contributes to the progression of cancers by suppressing cellular senescence and promoting epithelial-mesenchymal transition. Recent reports on the role of TBX3 in gastric cancers have implied its involvement in aging-related gastric carcinogenesis.

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INTRODUCTION

The T-box gene family is involved in embryonic development[1] and is conserved among species[2]. Currently, seventeen transcription factors have been identified as members of the T-box gene family in mammals. The T-box gene family consists of five subfamilies, namely, T, Tbx1, Tbx2, Tbx6, and Tbr1 (Table 1).

T-box transcription factor 3 (TBX3), which belongs to the Tbx2 subfamily, was initially reported as the gene responsible for ulnar-mammary syndrome, an autosomal dominant human development disorder that affects limb, apocrine gland, tooth, hair, and genital development[3]. Studies with genetically engineered mice revealed that Tbx3 homozygous mutant mice were embryonic lethal and exhibited yolk sac defects, lack of mammary glands, and limb defects[4]. Subsequent studies have revealed the involvement of TBX3 in the development of numerous organs, including the heart[5], retina[6], ureter [7], and inner ear[8].

The TBX3 protein consists of 723 amino acids and is encoded by 2169 bp nucleotides in 7 exons. Differential splicing of the second intron leads to the addition of the 2a exon, resulting in the production of the TBX3+2a isoform. Both TBX3 and TBX3+2a are widely expressed in humans and mice, and alternative splicing of *TBX3* was shown to be tissue- and species-specific[9]. TBX3 contains a DNA-binding T-domain[10], two repression domains, and an activation domain[11]. The protein is recruited to the T-box binding sites in the promoter regions of its downstream genes and acts both as a repressor and an activator. The functional similarity between TBX3 and its isoform is still controversial. Fan *et al* [9] reported that the TBX3+2a isoform lacked the ability to bind to the T-box binding site, and that while TBX3 immortalized mouse embryonic fibroblasts, the TBX3+2a isoform accelerated the senescence in those cells. On the other hand, Hoogaars *et al*[12] reported that both TBX3 and TBX3+2a were able to bind to the T-box binding site and inhibit cardiac chamber formation in mouse embryonic hearts. Another report from Zhao *et al*[13] showed that overexpression of either Tbx3 or Tbx3+2a induced the differentiation of mouse embryonic stem cells, but only Tbx3+2a was able to interact directly with Nanog. This discrepancy could be due to the difference in tissues and cells, and future studies are needed to elucidate this issue.

THE FUNCTION AND REGULATION OF TBX3

As expected from its broad expression, TBX3 has important functions. As mentioned earlier, it plays a crucial role in development. TBX3 binds to DNA through its T-domain, and functions as a repressor or an activator owing to its repression domains and an activation domain. Regarding cell cycle-related molecules, Tbx3 has been reported to repress $p19^{ARF}$ ($p14^{ARF}$ in humans) and inhibit cellular senescence [14,15]. This repression was either regulated through direct binding of TBX3 to the $p14^{ARF}$ promoter[15] or through interactions of TBX3 with histone deacetylases (HDAC) 1, 2, 3, and 5[16]. Tbx3 has also been shown to suppress p53[17], while another preceding report demonstrated that it repressed $p21^{CIP1/WAF}$ in a p53-independent manner[18]. In addition, Burgucu *et al*[19] reported that TBX3 suppressed phosphatase and tensin homolog by repressing its promoter activity, which led to augmented phosphatidylinositol-3-kinase activity. Collectively, these findings indicate that TBX3 possesses the ability to enhance cellular proliferation by regulating these molecules.

Several studies have reported that TBX3 suppresses apoptosis in addition to cellular senescence. Huang *et al*[20] showed that knocking down TBX3 in hypopharyngeal cancer cells increased annexin V-positive cells and the level of cleaved caspase 3. Ito *et al*[21] demonstrated that transfection of anti-sense Tbx3 into a rat bladder cancer cell line increased annexin V-positive cells, and the floating cells in the transfected culture exhibited DNA ladders on gel electrophoresis. These two previous studies reported that suppressing TBX3 led to increased apoptosis. On the other hand, Wensing and Campos[22] showed that overexpressing TBX3 and TBX3+2a reduced apoptosis in mesangial cells as assessed by caspase 3 activity. Carlson *et al*[17] also reported the anti-apoptotic function of TBX3 in overexpression experiments showing that transfection of TBX3 rescued primary mouse embryonic fibroblasts from Myc-induced apoptosis. Taken together, these preceding studies demonstrated that TBX3 possesses an anti-apoptotic function.

Another reported function of TBX3 is the repression of E-cadherin, which contributes to the promotion of epithelial-mesenchymal transition (EMT). Rodriguez *et al*[23] showed that TBX3 bound to the T-box binding site in the promoter of the *E-cadherin* gene and repressed E-cadherin expression, which resulted in enhanced invasiveness of melanomas. Dong *et al*[24] also reported that TBX3 repressed E-cadherin expression in hepatocellular carcinomas (HCC), but the repression occurred through the interaction of TBX3 with HDAC5. Peres *et al*[25] demonstrated that phosphorylation of TBX3 by AKT serine/threonine kinase 3 (AKT3) stabilized and promoted the nuclear translocation of TBX3, which was essential for E-cadherin repression. Although the effects were exerted through different mechanisms, these reports demonstrated that TBX3 negatively regulates E-cadherin expression, which can promote tumor invasion and metastasis.

Table 1 T-box gene family

T subfamily	Tbx1 subfamily	Tbx2 subfamily	Tbx6 subfamily	Tbr1 subfamily
T	Tbx1	Tbx2	Tbx6	Tbr1
Tbx19 (Tpit)	Tbx10	Tbx3	Mga	Tbr2 (Eomes)
	Tbx15	Tbx4		Tbx21 (Tbet)
	Tbx18	Tbx5		
	Tbx20			
	Tbx22			

For the regulation of TBX3, TBX3 has been regarded as one of the target genes of the Wnt/β-catenin signaling pathway[7,26-28], and a preceding report showed that β-catenin directly bound to the Tcf binding site in the promoter region of *Tbx3* and induced Tbx3 expression[29]. However, the Wnt/β-catenin signaling pathway is not the only signaling pathway that regulates Tbx3 expression. Transforming growth factor-β (TGF-β) is another signaling molecule that has been shown to induce Tbx3. Li *et al*[30] showed that Smad3 and Smad4, downstream signaling molecules of TGF-β, bound to the Smad-binding element in the *TBX3* promoter together with JunB and enhanced *TBX3* promoter activity. Lee *et al*[31] also reported that TBX3 was upregulated by TGF-β, although they demonstrated that this induction was dependent on the MAPKK-like protein kinase TOPK. Notch signaling has been shown to facilitate the nuclear translocation of Smad3 and activate TGF-β signaling[32,33], and considering that Notch signaling activates Tbx5[34], another member of the Tbx2 subfamily, it is possible that Notch signaling also regulates Tbx3 expression, but further studies are needed to clarify whether Notch signaling regulates TBX3 expression (Figure 1).

TBX3 IN BREAST CANCERS AND MELANOMAS

Overexpression of TBX3 has been reported in various cancers[35]. Among them, breast cancers and melanomas are the cancers in which the role of TBX3 has been intensively studied.

Sequencing of 100 primary breast cancers identified driver mutations in several genes, including *TBX3*[36], and another comprehensive study of 817 breast tumors identified that mutations in *TBX3* were enriched in invasive breast cancers[37]. In addition, genomic sequencing of 1918 breast cancers also indicated that alterations in *TBX3* were enriched in breast cancers[38]. These studies demonstrated that TBX3 is one of the key players in breast cancers. Recently, Kostecka *et al*[39] reported that sequencing of cancer-associated genes, including *TBX3*, in normal mammary glands of 52 patients with reportedly sporadic breast cancer revealed that subclonal somatic pathogenic variants of these genes were identified at considerable allelic frequencies. This suggests that TBX3 plays an important role in the initiation of breast cancers.

Functionally, TBX3 has been shown to promote the progression of breast cancers by suppressing cellular senescence and enhancing EMT, as described earlier in this review. However, although overexpression of TBX3 alone accelerated mammary epithelial cell proliferation and led to mammary gland hyperplasia, it did not lead to tumor development[40], which implies that overexpression of TBX3 alone is inadequate to initiate breast cancers.

Preceding studies have also reported the overexpression of TBX3 in melanomas[23,25,41], and interestingly, the constitutively active *B-RAF* mutation observed in melanomas was reported to induce TBX3[42]. Recently, a comprehensive study of 189 cohorts and 178 individual patients identified TBX3 as a marker of poorly differentiated melanomas[43]. Mechanistically, TBX3 was determined to promote tumor progression through inhibition of cellular senescence and promotion of EMT, similar to its role in breast cancers. However, in contrast to its role in breast cancers, the overexpression of TBX3 alone was sufficient to promote the formation and invasion of melanomas[44].

Taken together, the preceding studies suggested that TBX3 promotes tumor progression and invasion by suppressing senescence and enhancing EMT, but whether TBX3 can initiate cancers seems to depend on the type of cancer.

TBX3 IN COLORECTAL CANCERS, PANCREATIC CANCERS, AND LIVER CANCERS

The involvement of TBX3 has also been reported in colorectal cancers. A genome-wide meta-analysis revealed the association of polymorphisms in *TBX3* with increased colorectal tumor risk[45]. Shan *et al*[46] reported that aberrant TBX3 expression was associated with a large tumor size, poor differentiation,

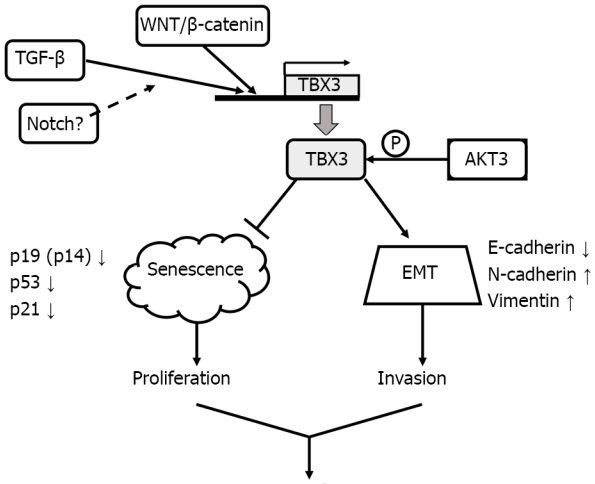

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Figure 1 The role of T-box transcription factor 3 in cancers. A schema describing the regulation and function of T-box transcription factor 3 in cancers. P: Phosphorylation; EMT: Epithelial-mesenchymal transition; TGF-β: Transforming growth factor-β; TBX3: T-box transcription factor 3.

invasion, lymph node metastasis, and advanced TNM stage in colorectal cancers, resulting in poor prognosis. They also showed through multivariate analysis that TBX3 can independently predict the outcome of colorectal cancer patients.

Similar to the findings in colorectal cancers, augmented TBX3 expression was associated with poor prognosis in pancreatic cancer patients and was reported to be an independent prognostic factor for overall survival[47]. Regarding the mechanism of TBX3, Perkhofer *et al*[48] demonstrated that TBX3 enhanced migration, invasion, and angiogenesis in pancreatic cancers through *in vitro* and *in vivo* studies.

TBX3 has also been reported to be associated with histological grade, tumor size, metastasis, and Ki-67 expression in HCC[49] and the expression of TBX3 in HCC was found to be induced by Wnt/β-catenin signaling[26,29,50]. Interestingly, Tbx3 in the hepatic microenvironment has been reported to play a crucial role in determining the fate of transformed hepatic cells and whether they develop HCC or intrahepatic cholangiocarcinoma[51].

Collectively, these studies demonstrated that TBX3 plays a major role in these cancers.

TBX3 IN GASTRIC CANCERS

Concerning gastric cancers, Miao *et al*[52] reported that TBX3 was overexpressed in 46 of 98 primary gastric cancer tissues, and its overexpression correlated with advanced TNM stage and with a higher relapse incidence. In vitro studies demonstrated that overexpression of TBX3 augmented cellular proliferation, whereas knockdown of TBX3 suppressed proliferation in gastric cancer cell lines. Regarding the mechanism involved in TBX3-induced accelerated proliferation, they showed that TBX3 overexpression led to a reduction in the percentage of cells in G1 phase and an increase in the percentage of cells in S and G2 phases in addition to augmented c-Myc and cyclin D1 expression, suggesting that TBX3 facilitated cell cycle progression. The *in vitro* studies also indicated that TBX3 downregulated E-cadherin and induced N-cadherin and vimentin expression, which suggested the enhancement of EMT. This enhancement of proliferation and EMT could be the reason why the expression of TBX3 is associated with advanced tumor stage in gastric cancers, similar to its correlation with poor prognosis in colorectal cancer patients[46].

Takeuchi *et al*[53] recently reported the essential role of Tbx3 in aging-related gastric carcinogenesis. Analysis of gastric organoids established from young and aged mice revealed that cellular proliferation was enhanced in aged gastric organoids due to Tbx3-induced repression of cellular senescence. Aged gastric organoids exhibited suppressed expression of Dickkopf3 (Dkk3), a Wnt antagonist, due to methylation of the *Dkk3* gene, and consequently, the enhanced Wnt/β-catenin signaling induced Tbx3 expression. Epigenetic alterations, such as the methylation of the *Dkk3* gene, are considered as one of the hallmarks of aging[54]. The stochastic process that involves alterations of the methylation state over time is referred to as epigenetic drift and is considered to track biological tissue aging[55]. Indeed, Takeuchi *et al*[53] showed that DKK3 expression in human gastric tissues decreased as the patient aged, whereas TBX3 expression in human gastric tissues exhibited a positive correlation with patient age. Furthermore, they showed that gastric cancer tissues exhibited lower DKK3 expression and higher TBX3 expression than normal oxyntic glands, suggesting the central role of TBX3 in aging-related gastric

carcinogenesis.

Another study of gastric precancerous lesions in 449 patients identified *TBX3*, along with *CDX2* and *MYC*, as one of the top 7 core genes that contributed to the progression from low-grade intraepithelial neoplasia to high-grade intraepithelial neoplasia[56], a finding that emphasizes the involvement of TBX3 in the early stage of gastric carcinogenesis.

Taken together, these studies imply that TBX3 plays a pivotal role in aging-related carcinogenesis and the progression of gastric cancers. Further studies are awaited to confirm the role of TBX3 in aging-related gastric carcinogenesis.

TBX3 AS A THERAPEUTIC TARGET

Since TBX3 is expressed in various cancers and possesses the ability to promote the progression of these tumors, it has been considered a therapeutic target in these cancers[57]. As TBX3 has been shown to promote cancer progression, its suppression will be required for therapies. Several microRNAs (miR) have been reported to inhibit TBX3. In adipocytes, miR-93 has been shown to inhibit TBX3 and negatively control adipogenesis[58]. On the other hand, miR-137 was reported to inhibit TBX3 in breast cancers[59] and melanomas[60]. In pancreatic cancers, members of the miR-17-92 cluster have been shown to inhibit TBX3 together with p21 and p57[61]. Furthermore, miR-183 was found to suppress TBX3 and enhanced sensitivity to chemotherapy in laryngeal cancers[62]. These miRNAs could be considered candidates for the treatment of TBX3-expressing cancers.

Concerning chemical reagents, an integrated computational approach indicated that two alkaloids, Jervine and Diflomotecan, can form stable complexes with TBX3 and suggested them as new effective drugs against breast cancers[63]. In another study, an aqueous extract of Fructus ligustri lucidi, a common Chinese herbal medicine, was reported to suppress TBX3 and enhance sensitivity to doxorubicin in colon cancer cells[64].

Recently, Willmer *et al*[65] reported that the multifunctional phosphoprotein nucleolin is required for TBX3 to function and that the nucleolin-targeting aptamer AS1411 exhibited an anticancer effect against sarcomas. These reagents could contribute to anticancer therapy against TBX3-overexpressing cancers.

In addition to its role as a therapeutic target, TBX3 can also contribute to treatment by aiding in the selection of medication for chemotherapy. Freeman *et al*[43] proposed using *TBX3* to predict the outcomes of immune checkpoint inhibitors against melanomas. They showed that patient stratification into risk groups regarding *TBX3* and *MAP4K1* expression was associated with overall survival; hence, evaluating the expression of these genes could enable individualized treatment for each patient. Similar findings may be found in other TBX3-expressing cancers, and further studies are warranted.

CONCLUSION

In this review, we discussed the role of TBX3 in cancers. TBX3 is expressed in various cancers and contributes to their progression mainly through the repression of senescence and the promotion of EMT. Given its crucial role in tumor progression, TBX3 could be a promising therapeutic target in malignant tumors, including gastric cancers.

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ORIGINAL ARTICLE

Case Control Study

Polymorphism of genes encoding drug-metabolizing and inflammation-related enzymes for susceptibility to cholangiocarcinoma in Thailand

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Abstract

BACKGROUND

Cholangiocarcinoma (CCA) is an intractable cancer, and its incidence in north-

eastern Thailand is the highest worldwide. Infection with the liver fluke *Opisthorchis viverrini* (OV) has been associated with CCA risk. However, animal experiments have suggested that OV alone does not induce CCA, but its combination with a chemical carcinogen like nitrosamine can cause experimentally induced CCA in hamsters. Therefore, in humans, other environmental and genetic factors may also be involved.

AIM

To examine relations between risk for CCA and genetic polymorphisms in carcinogen-metabolizing and inflammation-related genes.

METHODS

This hospital-based case-control study enrolled 95 case-control pairs matched by age (± 5 years) and sex. We examined relations between risk for CCA and genetic polymorphisms in carcinogen-metabolizing and inflammation-related genes, serum anti-OV, alcohol consumption, and smoking. Polymorphisms of *CYP2E1*, *IL-6* (-174 and -634), *IL-10* (-819), and *NF-κB* (-94) and their co-occurrence with polymorphisms in the drug-metabolizing enzyme gene *GSTT1* or *GSTM1* were also analyzed.

RESULTS

Although CCA risk was not significantly associated with any single polymorphism, persons with the *GSTT1* wild-type and *CYP2E1* c1/c2 + c2/c2 genotype had an increased risk (OR = 3.33, 95%CI: 1.23-9.00) as compared with persons having the *GSTT1* wild-type and *CYP2E1* c1/c1 wild genotype. The presence of anti-OV in serum was associated with a 7- to 11-fold increased risk, and smoking level was related to an OR of 1.5-1.8 in multivariable analyses adjusted for each of the seven genetic polymorphisms.

CONCLUSION

In addition to infection with OV, gene-gene interactions may be considered as one of the risk factors for CCA development.

Key Words: *Opisthorchis*; Glutathione transferase; Cytochrome P-450 *CYP2E1*; Case-control study

Core Tip: Cholangiocarcinoma (CCA) is an intractable cancer, and its prevalence in northeastern Thailand is the highest worldwide. An inflammatory condition produced by infection with the liver fluke *Opisthorchis viverrini* (OV) has been associated with CCA risk, but the susceptibility of individuals has not been fully examined. Our study revealed that persons with the *GSTT1* wild-type and *CYP2E1* c1/c2 + c2/c2 genotype had an increased risk for developing CCA (OR = 3.33, 95%CI: 1.23-9.00). Therefore, both gene-gene interactions and OV infection should be considered as risk factors for cholangiocarcinogenesis.

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INTRODUCTION

Cholangiocarcinoma (CCA) is a cancer of the hepatobiliary tract, and its incidence is extremely high in northeastern Thailand[1]. This is related to the lifestyle of the people of this area who often consume raw fish, which carries the risk of ingesting fish-borne parasites[2]. Infection with the liver fluke, *Opisthorchis viverrini* (OV), is a known CCA risk factor for inhabitants of northeastern Thailand[3-5], where approximately one-third of the population has been infected with OV[6,7]. Although infection with OV has been listed as a carcinogen to humans by The International Agency for Research on Cancer (IARC)[8], infection with OV alone is not sufficient for CCA development. Indeed, experiments with hamsters have suggested that parasitic infection alone is not sufficient to develop CCA. In fact, co-administration of a chemical carcinogen such as *N*-nitrosodimethylamine is necessary to induce CCA in hamsters[9]. In addition, genetic background related to the activation or detoxification of chemical

carcinogens is reported to be involved in CCA risk[10]. Also, elevated plasma IL-6 was associated with increased risk of CCA in patients infected with OV[11]. Thus, maintenance of chronic infection, exposure to a chemical carcinogen(s), and genetic background may explain CCA risk in northeastern Thailand. We previously reported that infection with OV and genetic polymorphism of a drug-metabolizing enzyme gene, namely *GSTM1*, is related to CCA risk[5] and that the combined effect of polymorphisms of the genes *8-oxoguanine glycosylase 1* and *GSTM1* is also relevant CCA risk[12] in northeastern Thailand. Here, we report our analysis of the association between polymorphisms of inflammation-related genes *(IL-6*, *IL-10* and *NF-kB*), and *CYP2E1*, *GSTT1* and *GSTM1* with risk of developing CCA. We also analyzed potential interactions among genetic polymorphisms of these genes.

MATERIALS AND METHODS

This work was conducted after receiving the approval from the ethics committees of the Nagahama Institute of Bio-Science and Technology, Shiga, Japan, and the National Cancer Institute, Bangkok, Thailand.

Study subjects

All cases with CCA were identified between 1999 and 2005 upon a visit to the Ubon Ratchathani Cancer Centre in the northeastern province of Thailand, one of the cancer centers administered by the National Cancer Institute of Thailand. Diagnosis was based on abdominal ultrasonography by a single radiologist at Ubon Ratchathani Cancer Centre with serological supportive evidence including an elevated carbohydrate antigen 19-9 level (≥ 40 μ/mL). Each case was matched by sex and age (within 5 years) with each control subject who lived in the same Ubon Ratchathani area and visited Ubon Ratchathani Cancer Centre for health check-up. All control subjects were without any clinical, ultrasonographical, or serological abnormalities. Finally, the 95 case-control pairs having data for an antibody against infection with OV were employed although the number of subjects for each of the analyses involving genetic polymorphisms was not equal because the amount of blood samples was limited. Still, there was a narrow range (91-95) for the number of case-control pairs for each of the genetic polymorphisms examined (Table 1).

OV infection

Infection with OV was determined with an enzyme-linked immunosorbent assay using an antibody ("anti-OV") raised against an OV antigen[13].

DNA extraction

Blood samples were frozen and stored at -80°C. DNA was extracted from 2 mL blood with the QIAGEN DNA Blood Midi kit[14].

Analysis of GSTM1 and GSTT1 polymorphisms

Polymorphisms in each of *GSTM1* and *GSTT1* were determined with polymerase chain reaction (PCR) [5] using the following primers: *GSTM1*, 5′-GAA CTC CCT GAA AAG CTA AAG C-3′ and 5′-GTT GGG CTC AAA TAT ACG GTG G-3′; *GSTT1*, 5′-TTC CTT ACT GGT CCT CAC ATC TC-3′ and 5′-TCA CCG GAT CAT GGC CAG CA-3′; *CYP1A1* (internal control), 5′-GAA CTG CCA CTT CAG CTG TCT-3′ and 5′-CAG CTG CAT TTG GAA GTG CTC-3′. Each 20-μl reaction contained 0.2 μM of each primer, 200 μM deoxyribonucleoside triphosphates (dNTPs), 50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.5), 1.5 mmol/L $MgCl_2$, approximately 6 ng DNA, and *Taq* DNA polymerase (AmpliTaqGold; Cetus PerkinElmer, Norwalk, CT). The PCR cycling parameters were 3 min at 95°C (initial denaturation) followed by 45 cycles of 1 min at 95°C, 30 sec at 59°C, and 1 min at 72°C, with a final 10-min extension step. The PCR products (299 bp for *GSTM1*, 507 bp for *GSTT1*) were separated on a 2% agarose gel.

Analysis of CYP2E1 polymorphisms

The 5′-flanking *Rsa*I site polymorphism of *CYP2E1* was detected by PCR combined with restriction-fragment-length polymorphism analysis (PCR-RFLP). The forward and reverse primers were 5′-CCA GTC GAG TCT ACA TTG TCA-3′ and 5′-TTC ATT CTG TCT TCT AAC TGG-3′, respectively. Each 20-μl reaction contained 0.2 μM of each primer, 200 μM dNTPs, 50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.5), 1.5 mmol/L $MgCl_2$, approximately 6 ng DNA, and *Taq* DNA polymerase. The PCR cycling parameters were 12 min at 95°C (initial denaturation) followed by 40 cycles of 1 min at 95°C, 30 sec at 60°C, and 1 min at 72°C, with a final 10-min extension step at 72°C. A 10-μl aliquot of the PCR products was digested with 0.2 U of the restriction enzyme *Rsa*I (GTaU) (TOYOBO Co., Ltd., Japan) for 16 h at 37°C. The resulting fragments were separated by 3% agarose gel electrophoresis and stained with ethidium bromide. The expected PCR product was 410 bp. Upon digestion with *Rsa*I, the c1/c1 wild-type homozygotes had fragments of 360 and 50 bp, the c1/c2 heterozygotes had fragments of 410, 360 and 50 bp, and the c2/c2 variant homozygotes had a fragment of 410 bp[15].

Table 1 Number of subjects with non-missing and with missing values for serum anti-*Opisthorchis viverrini*, alcohol consumption, and smoking dependent on each matched-pair analysis

Principle independent variable	Number of matched pairs	Anti-OV		Alcohol consumption		Smoking	
		Missing	Non-missing	Missing	Non-missing	Missing	Non-missing
Anti-OV	95	Not applicable		3	187	2	188
GSTM1	95	10	180	2	188	2	188
GSTT1	95	10	180	2	188	2	188
CYP2E1	93	9	177	2	184	2	184
IL6, -634 G/C	91	10	172	2	180	2	180
IL6, -175 G/C	92	10	174	2	182	2	182
IL10, -819 T/C	91	10	172	2	180	2	180
NF-kB, -94 ins/del ATTG	92	10	174	2	182	2	182

OV: *Opisthorchis viverrini*.

Analysis of IL-6 (rs1800795) polymorphisms

Polymorphism -174 (G/C) (rs1800795) of *IL-6* was detected by PCR-RFLP with forward primer 5′-ATG CCA AGT GCT GAG TCA CTA-3′ and reverse primer 5′-TCG AGG GCA GAA TGA GCC TC-3′. Each 20-μl reaction contained 0.2 μM of each primer, 200 μM dNTPs, 50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.3), 1.5 mmol/L $MgCl_2$, approximately 6 ng DNA, and *Taq* DNA polymerase. The PCR cycling parameters were 10 min at 95°C (initial denaturation) followed by 40 cycles of 30 sec at 95°C, 30 sec at 54°C, and 40 sec at 72°C, with a final 10-min extension step at 72°C. A 10-μl aliquot of the PCR products was digested with 0.2 U of the restriction enzyme *Nla*III (CATG↓) (New England Biolabs, Japan) at 37°C for 16 h and separated by PAGE (10% polyacrylamide gel). The expected PCR product was 308 bp. Upon digestion with *Nla*III, the G/G wild-type homozygotes had fragments of 233 and 75 bp, the G/C heterozygotes had fragments of 233, 121, 112 and 75 bp, and the C/C variant homozygotes had fragments of 121, 112 and 75 bp[16].

Analysis of IL-6 (rs1800796) polymorphisms

Polymorphism -634 G/C (rs1800796) of *IL-6* was analyzed by PCR with confronting two-pair primers [17]. The four primers used were *IL-6*-634 F1 5′-CCT CTA AGT TGG GCT GAA GCA GG-3' and *IL-6*-634 R1 5′-GTT CTG GCT CTC CCT GTG AGG-3′ for amplifying the variant type, and *IL-6*-634 F2 5′-CCA GGC AGT TCT ACA ACA GCC G-3′ and *IL-6*-634 R2 5′-TGA GTT TCC TCT GAC TCC ATC GC-3′ for amplifying wild type. Each 25-μl reaction contained 0.2 μM of each primer, 200 μM dNTPs, 50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.3), 2 mmol/L $MgCl_2$, approximately 6 ng DNA, and *Taq* DNA polymerase. The PCR cycling parameters were 10 min at 95°C (initial denaturation) followed by 36 cycles of 1 min at 95°C, 1 min at 65°C, and 1 min at 72°C, with a final 5-min extension step at 72°C. The PCR products were separated by PAGE. The G/G wild-type homozygotes had fragments of 157 and 240 bp, G/C heterozygotes had fragments of 125, 157, 240 and 75 bp, and the C/C variant homozygotes had fragments of 125 and 240 bp.

Analysis of IL-10 (rs1800871) polymorphisms

Polymorphism -819 T/C (rs1800871) of *IL-10* was analyzed by PCR-RFLP. The forward and reverse primers were *IL-10*-819 F 5′-TCA TTC TAT GTG CTG GAG ATG G-3′ and *IL-10*-819 R 5′-TGG GGG AAG TGG GTA AGA GT-3′, respectively. Each 20-μl reaction contained 0.2 μM of each primer, 200 μM dNTPs, 50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.3), 2 mmol/L $MgCl_2$, approximately 6 ng DNA, and *Taq* DNA polymerase. The PCR cycling parameters were 5 min at 95°C (initial denaturation) followed by 40 cycles of 30 sec at 95°C, 45 sec at 59°C, and 1 min at 72°C, with a final 10-min extension step at 72°C. A 10-μl aliquot of the PCR products was digested with 0.03 U of the restriction enzyme *Mae* III (↓GTNAC) (Roche Diagnostics GmbH Germany) for 16 h at 55°C and separated by PAGE. The expected PCR product was 209 bp. Following digestion with *Mae*III, the T/T wild-type homozygotes had a fragment of 209 bp, the T/C heterozygotes had fragments of 209, 125 and 84 bp, and the C/C variant homozygotes had fragments of 209 and 84 bp[18].

Analysis of NF-κB polymorphisms

Polymorphism -94 ins/del ATTG of the *NF-κB* promoter was determined by PCR-RFLP. The forward and reverse primers were 5′-TTT AAT CTG TGA AGA GAT GTG AAT-3′ and 5′-CTA GCA GGG CGC TCC CGA AT-3′, respectively. Each 20-μl reaction contained 0.2 μM of each primer, 200 μM dNTPs, 50

Table 2 Relationship between serum anti-*Opisthorchis viverrini* and cholangiocarcinoma risk, Ubon Ratchathani, Thailand

Number of case-control pairs according to serum anti-OV status: positive (+) *vs* negative (-)				Crude OR	95%CI		*P* value	Adjusted[1] OR	95%CI		*P* value	Adjusted[2] OR	95%CI		*P* value
Case (+) / Control (+)	Case (+) / Control (-)	Case (-) / Control (+)	Case (-) / Control (-)		LL	UL			LL	UL			LL	UL	
67	23	2	3	11.50	2.71	48.78	< 0.001	8.96	2.06	38.99	0.001	9.40	2.16	40.85	0.003

[1]Adjusted for alcohol consumption and smoking.
[2]Adjusted for alcohol consumption and smoking. Missing values for these variables were imputed.
OV: *Opisthorchis viverrini;* CCA: Cholangiocarcinoma; LL: Lower limit; UL: Upper limit.

mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.3), 1.5 mmol/L $MgCl_2$, approximately 6 ng DNA, and *Taq* DNA polymerase. The PCR cycling parameters were 10 min at 95°C (initial denaturation) followed by 40 cycles of 30 sec at 95°C, 1 min at 56°C, and 1 min at 72°C, with a final 10-min extension at 72°C. A 10-µl aliquot of the PCR products was digested with 2 U of the restriction enzyme *PflMI* (New England Biolabs Inc., Japan) at 37°C for 16 h and separated by PAGE. The expected PCR product was 254 bp. Upon digestion with *PflMI*, the del/del ATTG wild-type homozygotes had a fragment of 254 bp, the ins/del heterozygotes had fragments of 254 and 206 bp, and the ins/ins variant homozygotes had a fragment of 206 bp[19].

Data collection for lifestyle-related factors

Smoking and alcohol consumption status were ascertained alongside dietary habits at the hospital by local health personnel using a structured questionnaire used in previous studies[5,12]. Smoking status was classified into four categories: Never, occasional, former, and current. A current smoker was defined as smoking at least 1 cigarette *per* day, whereas the former smoker was defined as having stopped smoking regularly at least 1 year prior to our study. Alcohol consumption status was classified the same as for smoking status into four categories: Never, occasional, former, and current. "Current" drinker was defined as drinking more than once a week, whereas "former" drinker was defined as having stopped regular drinking at least 1 year prior to the study.

Statistical analysis

CCA risk attributable to infection with OV was defined as positivity for anti-OV, and the potential contributions of genetic polymorphisms of genes encoding inflammation-related enzymes were examined by calculating an OR. Each OR was calculated using a conditional logistic regression model, keeping matched case-control pairs. Interactions between each of the genetic polymorphisms of the inflammation-related genes and each of the metabolic enzyme genes were first examined using a log-likelihood ratio test. The tests compared the main effect with no interaction terms with a full model that included the interaction term for the variables concerned. When the p value for the log-likelihood ratio statistic was < 0.1, we calculated the OR owing to the co-occurrence of two genetic polymorphisms. Three subjects lacked data for alcohol consumption, and two lacked data for smoking status (Table 1). Most of the multivariable statistical analyses were performed with imputation for the missing data for these five subjects and produced comparable results (Tables 2-4). All statistical analyses were performed

Table 3 Relationship between serum anti-*Opisthorchis viverrini*, alcohol consumption, and smoking and cholangiocarcinoma risk, Ubon Ratchathani, Thailand, shown by analysis for each of the genetic polymorphisms

Independent variable	Gene adjusted for	Adjusted[1] OR	95%CI		*P* value	Adjusted[2] OR	95%CI		*P* value
			LL	UL			LL	UL	
Positive serum anti-OV	*GSTM1*	9.16	2.00	41.90	0.004	7.12	1.76	28.81	0.006
	GSTT1	8.16	1.85	35.96	0.006	7.33	1.74	30.93	0.007
	CYP2E1	7.75	1.73	34.69	0.007	4.90	1.41	17.05	0.013
	IL-6, -174 G/C	8.07	1.80	36.07	0.006	5.39	1.51	19.29	0.010
	IL-6, -634 G/C	7.21	1.57	32.98	0.011	6.13	1.54	24.32	0.010
	IL-10, -819 T/C	9.04	1.97	41.39	0.005	6.03	1.65	22.04	0.007
	NF-kB	11.81	2.31	60.44	0.003	8.14	1.90	34.90	0.005
Alcohol drinking	*GSTM1*	1.06	0.70	1.60	0.779	1.12	0.76	1.67	0.560
	GSTT1	1.05	0.70	1.58	0.817	1.10	0.74	1.63	0.643
	CYP2E1	1.05	0.70	1.58	0.801	1.10	0.75	1.63	0.614
	IL-6, -174 G/C	1.03	0.68	1.56	0.907	1.09	0.73	1.62	0.674
	IL-6, -634 G/C	1.00	0.65	1.52	0.989	1.06	0.70	1.59	0.784
	IL-10, -819 T/C	0.98	0.63	1.53	0.933	1.06	0.70	1.61	0.785
	NF-kB	0.99	0.63	1.54	0.956	1.05	0.68	1.60	0.836
Smoking	*GSTM1*	1.56	1.01	2.40	0.044	1.49	0.99	2.23	0.053
	GSTT1	1.56	1.01	2.42	0.044	1.51	1.00	2.27	0.049
	CYP2E1	1.55	1.00	2.41	0.052	1.46	0.98	2.18	0.060
	IL-6, -174 G/C	1.73	1.05	2.85	0.030	1.62	1.04	2.54	0.035
	IL-6, -634 G/C	1.79	1.10	2.91	0.018	1.73	1.08	2.76	0.022
	IL-10, -819 T/C	1.80	1.09	2.98	0.022	1.65	1.05	2.59	0.030
	NF-kB	1.78	1.08	2.94	0.023	1.80	1.11	2.91	0.016

[1]Adjusted for alcohol consumption and smoking.
[2]Adjusted for alcohol consumption and smoking. Missing values for these variables were imputed.
OV: *Opisthorchis viverrini;* CCA: Cholangiocarcinoma; LL: Lower limit; UL: Upper limit.

with the statistical package STATA 16.1 (College Station, TX, USA), and statistical significance was defined as $P < 0.05$ unless indicated otherwise.

Although the CCA-related risk of infection with OV among the current study subjects was reported in the multivariable analyses that included the interaction terms between the *GSTM1* and *hOGG1* polymorphisms in our previous study[12], a crude or adjusted OR for each polymorphism has not been reported. Data for CCA risk owing to polymorphism of *GSTM1* or *GSTT1* alone also has not been reported[12]. Findings for those factors are, thus, reported in the present study.

RESULTS

Concerning environmental factors, we confirmed the strong association between anti-OV-positive individuals and CCA risk adjusted for smoking status and alcohol consumption (Table 2). The OR values (95%CI) for a one-category change in smoking category (never, occasional, former, current) were approximately 1.5 regardless of the particular genetic polymorphism(s) examined or whether imputed data were included (Table 3). Alcohol consumption was not materially related to CCA risk (Table 3).

There was no significant association between polymorphisms for any of the inflammation-related genes or drug-metabolizing genes and CCA risk. When an inflammation-related gene (*IL-6*, *IL-10*, *NF-kB*) wild-type genotype was used as the reference, CCA risk was not significantly associated with any homozygous or heterozygous genotype (Table 4). Likewise, when the *GSTT1* wild-type genotype was used as the reference, CCA risk was not significantly associated with the null genotype. Similarly, when

Table 4 Relationship between polymorphisms of drug-metabolizing and inflammation-related enzyme genes and cholangiocarcinoma risk, Ubon Ratchathani, Thailand

Gene	Number of pairs according to gene polymorphism of case and control					Polymorphism	Crude OR	95%CI		*P* value	Adjusted[1] OR	95%CI		*P* value	Adjusted[2] OR	95%CI		*P* value
								LL	UL			LL	UL			LL	UL	
GSTM1																		
			Case															
			Wild	Null														
	Control	Wild	8	18		Wild	1.00	Reference			1.00	Reference			1.00	Reference		
		Null	18	51		Null	1.00	0.52	1.92	1.000	0.67	0.29	1.56	0.357	0.81	0.37	1.75	0.594
GSTT1																		
			Case															
			Wild	Null														
	Control	Wild	44	23		Wild	1.00	Reference			1.00	Reference			1.00	Reference		
		Null	21	7		Null	1.10	0.61	1.98	0.763	0.94	0.44	1.98	0.865	1.02	0.52	2.02	0.957
CYP2E1																		
			Case															
			c1/c1	c1/c2	c2/c2													
	Control	c1/c1	56	14	4	c1/c1	1.00	Reference			1.00	Reference			1.00	Reference		
		c1/c2	12	4	2	c1/c2	1.11	0.52	2.38	0.782	1.02	0.39	2.70	0.966	1.14	0.47	2.77	0.773
		c2/c2	1	0	0	c2/c2	6.19	0.74	52.08	0.093	2.02	0.20	20.67	0.552	4.17	0.45	38.24	0.207
						c1/c2 or c2/c2	1.38	0.68	2.83	0.371	1.14	0.47	2.77	0.767	1.35	0.58	3.11	0.484
IL-6																		
			Case			-174												
			G/G	G/C	C/C													
	Control	G/G	90	0	0	G/G	1.00	Reference			1.00	Reference			1.00	Reference		
		G/C	1	0	0	G/C	0.00	-	-	-	0.003	0.00	-	0.995	0.0037	0.00	-	0.999
		C/C	0	0	0	C/C	-	-	-	-	-	-	-	-	-	-	-	-
IL-6						-634												

			Case													
			G/G	G/C	C/C											
	Control	G/G	0	4	5	G/G	1.00	Reference		1.00	Reference		1.00	Reference		
		G/C	2	13	12	G/C	1.93	0.63	5.93	0.252	1.90	0.49	7.30	0.351	1.69	0.46	6.25	0.432
		C/C	4	18	34	C/C	1.28	0.44	3.73	0.654	1.38	0.40	4.71	0.607	1.45	0.43	4.87	0.551
						G/C or C/C	1.50	0.53	4.21	0.442	1.53	0.46	5.05	0.488	1.52	0.46	4.98	0.489
IL-10						-819 T/C												
			Case													
			T/T	T/C	C/C											
	Control	T/T	23	15	6	T/T	1.00	Reference		1.00	Reference		1.00	Reference		
		T/C	15	17	4	T/C	0.81	0.41	1.60	0.537	0.66	0.27	1.63	0.371	0.75	0.34	1.70	0.497
		C/C	8	0	3	C/C	1.19	0.46	3.06	0.715	1.59	0.43	5.93	0.487	1.63	0.51	5.17	0.405
						T/C or C/C	0.91	0.51	1.65	0.763	0.85	0.39	1.87	0.686	0.95	0.47	1.93	0.891
NF-kB						-94 ins/del ATTG												
			Case													
			del/del	ins/del	ins/ins											
	Control	del/del	3	5	3	del/del	1.00	Reference		1.00	Reference		1.00	Reference		
		ins/del	4	16	13	ins/del	0.83	0.32	2.17	0.711	1.11	0.27	4.61	0.884	0.79	0.24	2.67	0.708
		ins/ins	9	20	19	ins/ins	0.48	0.19	1.23	0.128	0.32	0.08	1.20	0.090	0.33	0.10	1.03	0.057
						ins/del or ins/ins	0.91	0.51	1.65	0.763	0.49	0.14	1.69	0.258	0.47	0.16	1.38	0.167

[1]Adjusted for alcohol consumption and smoking.
[2]Adjusted for alcohol consumption and smoking. Missing values for these variables were imputed.
CCA: Cholangiocarcinoma; LL: Lower limit; UL: Upper limit.

the *GSTM1* wild-type genotype was used as the reference, CCA risk was not significantly associated with the null genotype. When the *CYP2E1* c1/c1 wild-type genotype was used as the reference, CCA risk was not significantly associated with c1/c2, c2/c2, or the combination of the c1/c2 + c2/c2 genotypes (Table 4).

Gene-gene interactions between GSTT1 and CYP2E1

Interaction of the *CYP2E1* polymorphism and the *GSTT1* polymorphisms was suggested by a p value of

Table 5 Risk of cholangiocarcinoma due to the combination of polymorphisms of *GSTT1* and *CYP2E1*: Matched case-control study, Ubon Ratchathani, Thailand

CYP2E1	*GSTT1* wild		*GSTT1* null		*CYP2E1*	*GSTT1* wild				*GSTT1* null				
	Number		Number			Odds ratio[1]	95%CI		*P* value	Odds ratio[1]	95%CI		*P* value	*P* for interaction
	Case	Control	Case	Control			LL	UL			LL	UL		
c1/c1	49	59	25	18	c1/c1	1.00	Reference			2.04	0.95	4.39	0.07	< 0.01
c1/c2 + c2/c2	24	9	5	11	c1/c2 + c2/c2	3.33	1.23	9.00	0.02	0.54	0.14	2.13	0.38	

[1]Based on the conditional logistic regression model.

CCA: Cholangiocarcinoma; CI: Confidence interval; LL: Lower limit; UL: Upper limit.

0.003, whereas other interactions between drug-metabolizing enzyme genes (*GSTM1* or *GSTT1*) and other genetic polymorphisms were not substantiated ($P > 0.10$). Persons with the *GSTT1* wild-type plus *CYP2E1* c1/c2 + c2/c2 genotype had an increased risk for CCA (OR = 3.33, 95%CI: 1.23-9.00) as compared with persons having the *GSTT1* wild-type plus *CYP2E1* c1/c1 wild-type genotype that was used as the reference (Table 5).

DISCUSSION

Early diagnosis of CCA is difficult, and most patients die within a year after diagnosis[20]. Identification of risk factors and means of preventing cholangiocarcinogenesis is thus highly desirable. We confirmed that positivity for anti-OV constitutes a significant risk factor for CCA, as reported previously[3,5,21]. Smoking was not a significant risk factor for CCA, whereas alcohol consumption was in fact related to increased risk as we reported previously for subjects (and matched controls) recruited from another part of northern Thailand[5] In the present study, control subjects were persons seeking a health check-up and thus possibly may have led a relatively healthier lifestyle compared with our experimental subjects. However, the discordance between the findings on smoking and alcohol drinking of the two studies are not yet explained. Although we did not find any significant association between CCA risk and polymorphisms in inflammation-related genes (*IL-6*, *IL-10*, *NF-kB*) or in drug metabolism-related genes (*CYP2E1, GSTT1* and *GSTM1*), persons with the *GSTT1* wild-type plus *CYP2E1* c1/c2 + c2/c2 genotype had a 3-fold greater risk than persons having the *GSTT1* wild-type plus *CYP2E1* c1/c1 wild-type genotype that was used as the reference. Hayashi *et al*[22] reported that the c2/c2 homozygous sequence placed upstream of the SV40 promoter and *chloramphenicol acetyltransferase* gene enhanced the expression of that gene, and the enhancement of expression by the c2/c2 sequence was about 10-fold that by the c1/c1 sequence. Thus, it is possible that CYP2E1 is expressed at a higher level in the presence of the c2/c2 sequence than in the presence of the c1/c1 sequence, which is consistent with the known function of CYP2E1 as a carcinogen-activating enzyme[23,24]. Because the numbers of cases and controls were not large, the conclusions from this work should be confirmed in a future study with

more cases and controls. In addition, genes encoding other drug-metabolizing enzymes should also be tested with respect to gene-gene interactions.

CONCLUSION

The presence of anti-OV in serum was associated with a 7- to 11-fold increased risk for CCA independently of the genetic polymorphisms of carcinogen-metabolizing and inflammation-related genes. While any single polymorphism was not significantly associated with CCA risk, persons with the *GSTT1* wild-type and *CYP2E1* c1/c2 + c2/c2 genotype had a 3-fold increased risk as compared with persons having the *GSTT1* wild-type and *CYP2E1* c1/c1 wild genotype. In addition to infection with OV, gene-gene interactions may be considered as one of the risk factors for CCA development.

ARTICLE HIGHLIGHTS

Research background

Cholangiocarcinoma (CCA) is a cancer of the hepatobiliary tract, and its incidence is extremely high in northeastern Thailand. This is related to the lifestyle of the inhabitants of this area consuming often raw fish, which carries the risk of ingesting fish-borne parasites, *Opisthorchis viverrini* (OV), a known CCA risk factor. While infection with OV has been listed as a carcinogen to humans by The International Agency for Research on Cancer, the parasitic infection alone is not sufficient to develop CCA; in fact, co-administration of a chemical carcinogen such as *N*-nitrosodimethylamine is necessary to induce CCA in animal model. In addition, genetic background related to the activation or detoxification of chemical carcinogens is reported to be involved in CCA risk. Also, elevated plasma IL-6 was associated with increased risk of CCA in patients infected with OV.

Research motivation

We already reported that infection with OV and genetic polymorphism of a drug-metabolizing enzyme gene, namely *GSTM1*, is related to CCA risk and that the combined effect of polymorphisms of the genes *8-oxoguanine glycosylase 1* and *GSTM1* is also relevant CCA risk in northeastern Thailand. In the present study, we further investigated possible associations of maintenance of chronic infection, exposure to a chemical carcinogen(s) and genetic background with CCA risk in northeastern Thailand.

Research objectives

To examine relations between risk for CCA and genetic polymorphisms in carcinogen-metabolizing (*CYP2E1*, *GSTT1* and *GSTM1*) and inflammation-related genes (*IL-6, IL-10* and *NF-kB*), and potential interactions among genetic polymorphisms of these genes on the CCA risk.

Research methods

All cases with CCA were identified between 1999 and 2005 upon a visit to the Ubon Ratchathani Cancer Centre in the northeastern province of Thailand. This hospital-based case-control study enrolled 95 case-control pairs matched by age (± 5 years) and sex. We examined relations between risk for CCA and genetic polymorphisms in carcinogen-metabolizing and inflammation-related genes, serum anti-OV, alcohol consumption, and smoking. Smoking and alcohol consumption status were ascertained using a structured questionnaire used in previous studies. Conditional logistic regression was employed to estimate CCA risk as OR due to each of genetic polymorphisms and possible interactions of those polymorphisms.

Research results

Although any single polymorphism was not significantly associated with CCA risk, persons with the *GSTT1* wild-type and *CYP2E1* c1/c2 + c2/c2 genotype had a 3-fold increased risk as compared with persons having the *GSTT1* wild-type and *CYP2E1* c1/c1 wild genotype. The presence of anti-OV in serum was associated with a 7- to 11-fold increased risk for CCA independently of the genetic polymorphisms of carcinogen-metabolizing and inflammation-related genes.

Research conclusions

An inflammatory condition produced by infection with OV indicated as raised anti-OV in serum has been associated with CCA risk. Our study added the finding that persons with the *GSTT1* wild-type and *CYP2E1* c1/c2 + c2/c2 genotype had a 3-fold increased risk for developing CCA. Therefore, both gene-gene interactions and OV infection should be considered as risk factors for cholangiocarcinogenesis in northeastern Thailand.

Research perspectives

CCA is still an intractable cancer. While our study revealed the interaction of polymorphisms of *GSTT1* and *CYP2E1* possibly contributes to development of CCA, the numbers of cases and controls were not large. The conclusions from this work should be confirmed in a future study with more cases and controls. In addition, genes encoding other drug-metabolizing enzymes should also be tested with respect to gene-gene interactions.

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FOOTNOTES

Author contributions: Miwa M secured funds and started the collaborative study in 1998 with the late Srivatanakul P who had been Thai side's active organizer based on the National Cancer Institute, Bangkok, until her death in 2020; Viwatthanasittiphong C, Muangphot M, Chenvidhya D, Jedpiyawongse A, Sripa B, Honjo S, and Miwa M designed and conducted the epidemiological study; Sripa B measured anti-OV and providing microbiological advice; You G, Zeng L, Tanaka H, Ohta E, Fujii T, Ohshima K, Tanaka M, Hamajima N performed analyses concerning genetic polymorphisms; You G, Zeng L, Miwa M and Honjo S conducted statistical analyses and prepared the manuscript; All authors have read and approved the final manuscript.

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ORIGINAL ARTICLE

Observational Study

Novel CABIN score outperforms other prognostic models in predicting in-hospital mortality after salvage transjugular intrahepatic portosystemic shunting

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Abstract

BACKGROUND

Transjugular intrahepatic portosystemic shunt (TIPS) is now established as the salvage procedure of choice in patients who have uncontrolled or severe recurrent variceal bleeding despite optimal medical and endoscopic treatment.

AIM

To analysis compared the performance of eight risk scores to predict in-hospital mortality after salvage TIPS (sTIPS) placement in patients with uncontrolled variceal bleeding after failed medical treatment and endoscopic intervention.

METHODS

Baseline risk scores for the Acute Physiology and Chronic Health Evaluation (APACHE) II, Bonn TIPS early mortality (BOTEM), Child-Pugh, Emory, FIPS, model for end-stage liver disease (MELD), MELD-Na, and a novel 5 category CABIN score incorporating Creatinine, Albumin, Bilirubin, INR and Na, were calculated before sTIPS. Concordance (C) statistics for predictive accuracy of in-hospital mortality of the eight scores were compared using area under the receiver operating characteristic curve (AUROC) analysis.

RESULTS

Thirty-four patients (29 men, 5 women), median age 52 years (range 31-80) received sTIPS for uncontrolled (11) or refractory (23) bleeding between August 1991 and November 2020. Salvage TIPS controlled bleeding in 32 (94%) patients with recurrence in one. Ten (29%) patients died in hospital. All scoring systems had a significant association with in-hospital mortality ($P < 0.05$) on multivariate analysis. Based on in-hospital survival AUROC, the CABIN (0.967), APACHE II (0.948) and Emory (0.942) scores had the best capability predicting mortality compared to FIPS (0.892), BOTEM (0.877), MELD Na (0.865), Child-Pugh (0.802) and MELD (0.792).

CONCLUSION

The novel CABIN score had the best prediction capability with statistical superiority over seven other risk scores. Despite sTIPS, hospital mortality remains high and can be predicted by CABIN category B or C or CABIN scores > 10. Survival was 100% in CABIN A patients while mortality was 75% for CABIN B, 87.5% for CABIN C, and 83% for CABIN scores > 10.

Key Words: Transjugular intrahepatic portosystemic shunt; Risk score; Portal hypertension; Variceal bleeding; Mortality

Core Tip: This study compared the performance of a new CABIN score with seven existing risk scores to predict in-hospital mortality after salvage transjugular intrahepatic portosystemic shunt (TIPS) placement in 34 patients with uncontrolled variceal bleeding after failed medical treatment and endoscopic intervention. Using concordance statistics for predictive accuracy of in-hospital mortality the novel 5 category CABIN score incorporating Creatinine, Albumin, Bilirubin, INR and Na outperformed the APACHE II, BOTEM, Child-Pugh, Emory, FIPS, MELD and MELD-Na scores when compared by area under the receiver operating characteristic curve (AUROC) analysis. Survival was 100% in CABIN A patients while mortality was 75% for CABIN B, 87.5% for CABIN C, and 83% for CABIN scores > 10.

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INTRODUCTION

Transjugular intrahepatic portosystemic shunt (TIPS) is now established as the salvage procedure of choice in patients who have uncontrolled or severe recurrent variceal bleeding despite optimal medical and endoscopic treatment[1]. Key clinical distinctions exist in the spectrum of patients undergoing TIPS, ranging from high-risk cirrhotic patients with liver decompensation and uncontrolled variceal bleeding necessitating an emergent salvage TIPS (sTIPS) to those with well-preserved liver function undergoing an elective TIPS for refractory bleeding. Current risk stratification of patients who have refractory variceal bleeding and require sTIPS is however imperfect. Although TIPS is a minimally invasive procedure, appropriate patient selection is crucial to identify patients who would benefit from the procedure, considering the substantial risks of hepatic encephalopathy, liver failure and increased overall morbidity and mortality in high-risk individuals[2,3].

Several prognostic and risk scores have been developed to identify patients at risk for a poor clinical outcome after sTIPS. These include the Acute Physiology and Chronic Health Evaluation (APACHE) II [4], Bonn TIPS early mortality (BOTEM)[5], Child-Pugh (C-P)[6], Emory[7], Freiburg index of post-TIPS survival (FIPS)[8], model for end-stage liver disease (MELD)[9], and Model for End-Stage Liver Disease sodium (MELD-Na)[10] scores. In this study the accuracy of a novel CABIN score, which was developed to overcome limitations of existing scoring systems, was compared to established risk scores for the prediction of in-hospital mortality following sTIPS.

MATERIALS AND METHODS

In this retrospective observational analysis, eight risk scores were evaluated in a cohort which included all adult patients who underwent sTIPS for uncontrollable or life-threatening refractory variceal

bleeding in the Surgical Gastroenterology Unit at Groote Schuur Hospital and the University of Cape Town Private Academic Hospital between August 1991 and November 2020. The study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for reporting observational studies[11]. Baseline demographic, clinical and endoscopic data and biochemical variables were collected on admission. The anonymized and de-identified information were retrieved from a prospectively maintained ethics approved registry for patients treated for esophageal varices (Table 1).

Details of the acute bleeding management protocol and the endoscopic interventional techniques used in our unit have been published previously[12-15]. In patients who had endoscopically uncontrolled bleeding a Minnesota balloon tube or a Danis esophageal stent (Ella-CS, Hradec Kralove, Czech Republic) was inserted to tamponade variceal bleeding and endotracheal intubation was used for airway protection when indicated[16]. In this high-risk group with uncontrolled variceal bleeding and those with refractory life-threatening bleeding despite endoscopic intervention and somatostatin infusion, sTIPS was performed as an emergency procedure under general anaesthesia with placement of an expandable uncovered 10 mm Wallstent (Boston Scientific, Marlborough, MA, United States)[15].

The study protocol followed the Baveno recommendations and defined uncontrolled or persistent variceal bleeding as the need for a transfusion of 4 units of blood or more within 6 h and the inability to achieve an increase in systolic blood pressure to 70 mmHg or more or a pulse reduction to less than 100/min. Contraindications to sTIPS in our unit were severe pulmonary hypertension, severe tricuspid regurgitation, congestive heart failure, fibropolycystic liver disease, uncontrolled systemic sepsis and unrelieved biliary obstruction. Relative contraindications were congenital hepatic fibrosis, portal vein thrombosis, obstruction of all hepatic veins and severe coagulopathy (INR > 5).

Details of the newly developed five component CABIN score are given in Supplementary Table 1. Each CABIN variable is scored from one to five and the cumulative total is calculated by adding the individual values of the five biochemical components (Creatinine, Albumin, Bilirubin, INR (international normalized ratio) and Na (sodium). The best total CABIN score computes at 5 points and the worst at 25 points. Four CABIN categories (A-D) were established (A: 5-10 points, B: 11-15, C: 16-20, D: 21-25).

The CABIN score and seven previously described scoring systems, APACHE II, BOTEM, Child-Pugh, Emory, FIPS, MELD, and MELD-Na scores were calculated based on clinical evaluation and laboratory values obtained before the sTIPS procedure. The primary study outcome measure was prediction of in-hospital mortality after sTIPS and compared the relative performances of the seven established scoring models and the new CABIN score.

Statistical analysis

All clinical data and variables were collected and managed using the REDCap electronic data capturing software licensed to the University of Cape Town[17]. Statistical computations were made using IBM SPSS statistics (version 26.0, IBM, United States). Statistical significance was set at $P < 0.05$. Continuous data were reported as mean ± SD or medians and range and discrete data as percentages. To evaluate the performance of the various scoring systems to predict in-hospital mortality the concordance C-statistic [area under the curve (AUC) of the receiver operating characteristic (ROC) curves] was used.

Ethical considerations

The study protocol was approved by the Human Research Ethics Committee (HREC Ref No. 120/2019) of the University of Cape Town and the research was conducted in accordance with the Declaration of Helsinki.

RESULTS

A total of 564 patients with variceal bleeding were treated during the study period. In 530 patients (94%), bleeding was controlled by endoscopic intervention and medication. In 34 patients (6%) who constitute the study population and underwent sTIPS, bleeding was either uncontrollable *ab initio* (n = 11) or life-threatening refractory (n = 23) despite optimal endoscopic and pharmacological management.

The demographic and clinical data of the patients are summarized in Table 1. No patients had a concomitant HCC or portal vein thrombosis at the time of TIPS insertion. Before sTIPS 19 patients had a median of three (1-9) injection sclerotherapy treatment (IST) sessions and 20 had a median of two (1-6) endoscopic variceal ligation (EVL) sessions with a median of 10 bands placed per session. Five patients had both IST and EVL. Median units of blood transfused before sTIPS was six (3-12), and 14 patients required either Minnesota balloon tamponade (n = 12) or placement of a Danis stent (n = 2) for temporarily control of bleeding before the sTIPS procedure. Eleven patients required endotracheal intubation and mechanical ventilation and nine required inotropic support.

Technical success for sTIPS was 100% and therapeutic success (control of bleeding) was achieved in 31 of 34 (91%) patients. Bleeding persisted in two patients (6%) despite a patent sTIPS on repeat US-doppler examination and one patient developed recurrent bleeding in hospital during the index

Table 1 Demographic, clinical characteristics and risk prediction scores of 34 patients undergoing salvage transjugular intrahepatic portosystemic shunt, *n* %

Variable	Total cohort (*n* = 34)	Survived (*n* = 24)	In-hospital death (*n* = 10)	*P* value
Demographics				
Age (mean ± SD)	52 ± 11.6	50 ± 10.5	57 ± 12.9	0.107
Sex				
Male	29 (85)	22 (92)	7 (70)	0.104
Female	5 (15)	2 (8)	3 (30)	
Cause of cirrhosis				
Alcohol related	22 (65)	15 (63)	7 (70)	0.938
Non-alcohol related	12 (35)	9 (37)	3 (30)	
Child-Pugh grade				
A	3 (9)	3 (12)	0	0.022
B	19 (56)	16 (67)	3 (30)	
C	12 (35)	5 (20)	7 (70)	
Risk prediction scores				
APACHE II (mean ± SD)	13.4 ± 4.7	11.4 ± 3.3	18.3 ± 3.8	0.196
BOTEM (mean ± SD)	5.4 ± 1.1	5.0 ± 0.9	6.3 ± 0.7	0.964
CABIN (mean ± SD)	10.9 ± 5.0	8.3 ± 1.8	17.0 ± 3.8	0.133
CHILD-PUGH (mean ± SD)	8.9 ± 1.8	8.2 ± 1.8	10.6 ± 2.0	0.001
EMORY (mean ± SD)	3.2 ± 0.9	2.8 ± 0.7	4.3 ± 0.5	0.497
FIPS (mean ± SD)	-0.3 ± 0.9	-0.6 ± 0.9	0.5 ± 0.5	0.205
MELD (mean ± SD)	15.0 ± 6.2	13 ± 4.8	19.8 ± 6.7	0.007
MELD-Na (mean ± SD)	16.9 ± 7.4	14 ± 5.3	23.9 ± 7.1	< 0.001

SD: Standard deviation; APACHE II: Acute physiology and chronic health evaluation II; BOTEM: Bonn TIPS early mortality; CABIN: Creatinine, Albumin, Bilirubin, INR, Sodium score; FIPS: Freiburg index of post-TIPS survival; MELD: Model for end-stage liver disease; MELD-Na: Model for end-stage liver disease sodium.

admission after initial control of bleeding by sTIPS.

Ten patients (29.4%) died in hospital at a median of 5 d following the procedure (range 1-10 d) of progressive liver failure (*n* = 4), MOF (2), alcoholic cardiomyopathy (*n* = 2) or uncontrolled variceal bleeding (*n* = 2). Mortality in C-P grade A patients was 0%, in C-P grade B patients 16% and C-P grade C patients 58%. In patients who died the median C-P score was 11, (range 7-13), median MELD score was 18 (range 11-29) and median MELD Na score was 25 (range 11-33). Nine of the 12 (75%) patients who required pre-sTIPS balloon tamponade died, while all nine (100%) patients who were hypotensive (systolic blood pressure < 70 mmHg) and with the combination of > 8 unit blood transfusion, inotropic support, balloon tamponade and mechanical ventilation died.

The two patients with persistent bleeding after TIPS underwent repeat endoscopy and ultrasound-guided Histoacryl and coil injection of residual gastric varices with resolution. The patient with recurrent bleeding in hospital underwent a gastric devascularization for control of gastric varices.

Figure 1 shows the graphic representation of the comparative performances of the eight risk scores in predicting in-hospital death following sTIPS. The CABIN score (AUROC 0.967) had the highest discriminative ability in predicting in-hospital death compared to the APACHE II (AUROC 0.948), BOTEM (AUROC 0.877), C-P (AUROC 0.802), EMORY (AUROC 0.942), FIPS (AUROC 0.892), MELD (AUROC 0.792), and MELD-Na (AUROC 0.865) scores as detailed in Table 2. The median CABIN score in the 24 in-hospital TIPS survivors was 8 (range 5-18) compared to a median of 17 (range 11-22) in the 10 deaths. CABIN A patients had a 100% survival, compared to 25% and 12.5% survival in CABIN B and CABIN C category patients respectively. CABIN points of 11 or more provided a clear survival cut-off. No patients with CABIN scores < 10 died while 83% of patients with CABIN scores of > 11 died.

Table 2 Performance of various risk prediction scores in predicting in-hospital death following salvage transjugular intrahepatic portosystemic shunt

	In-hospital deaths			
	AUC	STD error	*P* value	95% confidence interval
APACHE II	0.948	0.035	0	0.879–1.000
BOTEM	0.877	0.059	0.001	0.762–0.992
CABIN	0.967	0.028	0	0.912–1.000
CHILD-PUGH	0.802	0.084	0.006	0.638–0.967
EMORY	0.942	0.038	0	0.868–1.000
FIPS	0.892	0.055	0	0.783–1.000
MELD	0.792	0.082	0.008	0.631–0.952
MELD Na	0.865	0.077	0.001	0.713–1.000

AUC: Area under the curve; SD: standard deviation; APACHE II: Acute physiology and chronic health evaluation II; BOTEM: Bonn TIPS early mortality; CABIN: Creatinine albumin bilirubin INR sodium score; FIPS: Freiburg index of post-TIPS survival; MELD: Model for end-stage liver disease; MELD-Na: Model for end-stage liver disease sodium.

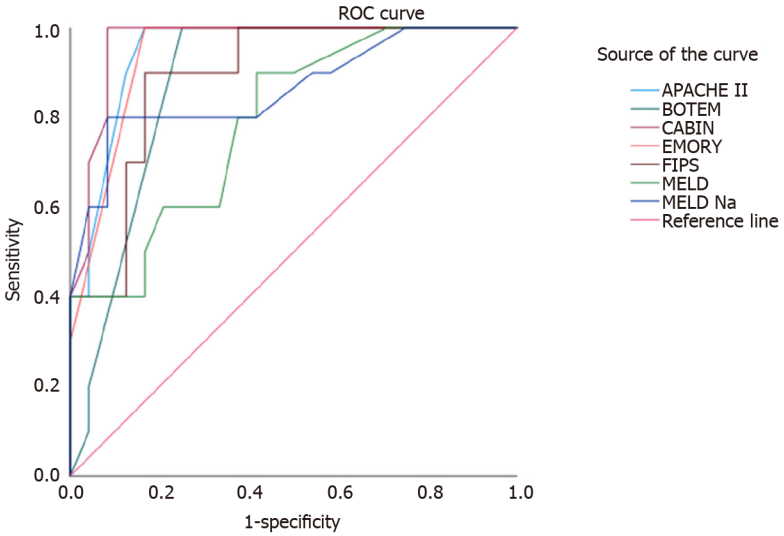

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Figure 1 Performance of various risk prediction scores in predicting in-hospital death following salvage transjugular intrahepatic portosystemic shunt. APACHE II: Acute physiology and chronic health evaluation II; BOTEM: Bonn TIPS early mortality; CABIN: Creatinine, Albumin, Bilirubin, INR, Sodium score; FIPS: Freiburg index of post-TIPS survival; MELD: Model for end-stage liver disease; MELD-Na: Model for end-stage liver disease sodium; TIPS: Transjugular intrahepatic portosystemic shunt; ROC: Receiver operating characteristic.

DISCUSSION

The unique safety profile and minimally invasive characteristics conferred by TIPS provide an effective reduction in portal pressure and make the procedure the ideal rescue intervention for variceal bleeding not controlled by endoscopic intervention and pharmacological therapy[18]. In this study we compared the relative performances of eight scoring models, including the novel CABIN score, in predicting in-hospital mortality in a high-risk cohort of patients who underwent sTIPS placement. Although sTIPS controlled variceal bleeding in 94% of patients, over-all in-hospital mortality was 29.4% and increased exponentially in those who required > 8 unit blood transfusion, inotropic support, esophageal balloon tamponade and mechanical ventilation. Log-rank comparisons of survival curves showed that of the eight scores evaluated, the CABIN, APACHE II and Emory scores had the highest AUROC values and the best discriminatory ability with C-statistic values all exceeding 0.9. Of these three top contenders, the CABIN score (0.967) had the best discriminatory and predictive capability. As a collorary, this study also demonstrates the predictive ability of the CABIN score with 100% survival observed in patients in the CABIN A category (< 10 points) after sTIPS.

The reported mortality rate after TIPS placement varies widely due to differing inclusion criteria, timing of TIPS placement, the spectrum and severity of the underlying liver disease and inclusion in some reports of patients with active bleeding during urgent TIPS as well as stable patients undergoing elective TIPS[19-21]. In the 22 studies exclusively reporting salvage or rescue TIPS in patients with uncontrolled life-threatening or endoscopically unmanageable variceal bleeding, as in this study, in-hospital mortality rates range from 17% to 56% which are significantly higher than for elective TIPS[22-43] (Table 3). Accurate prediction of outcome following sTIPS is thus a crucial element of management and the optimal prognostic score should ideally be able to distinguish two groups, patients with a better prognosis and likely to survive and those with a high or prohibitive risk of death.

Most of the current prognostic scores used in sTIPS patients have intrinsic limitations due to the selection and weighting of the constituent components. The MELD score, which was initially created to predict survival following elective placement of TIPS, is currently the most widely used liver-related prognostic score both in clinical practice and research and especially as a tool for organ allocation[9]. Although the MELD score was a prospectively developed and validated indicator of the severity of end-stage liver disease that utilizes quantitative and objective measures, including bilirubin, creatinine and INR values, the score has potential limitations. A further caveat is the maximum assigned value of serum creatinine which is capped at four even when the measured serum level is higher. Modifications to overcome MELD shortcomings have included reweighting the model's coefficients, altering the laboratory components and the addition of new variables including serum sodium ('MELD-Na'), albumin [termed '5-variable MELD' (5vMELD)][44] and female gender (MELD 3.0)[45]. These modifications are more discriminative than either MELD or MELD-Na in transplant assessment and use similar elements as the CABIN score.

The inclusion of subjective clinical components in other proposed prognostic models may also limit precision and reproducibility of score assignments. The C-P, Emory and BOTEM scores all have at least one component that may be perceived as subjective while the APACHE II and BOTEM scores lack specificity for liver disease which limits their capacity to predict outcomes after liver interventions such a sTIPS. In addition the C-P, Emory, and BOTEM scores are limited by a ceiling effect in which laboratory values above a particular cut-off level are not distinguished from one another in terms of higher scoring[4-7,9,10]. The FIPS overcomes some of these limitations by using four objective components, age, bilirubin, albumin, and creatinine levels[8].

In a meta-analysis, which included 11 studies and 2037 patients Zhou *et al*[46] found that MELD was superior to the C-P score in predicting 3-mo survival after TIPS but not 1-mo, 6-mo or 12-mo survival. Zhang *et al*[47] found that C-P grade C and MELD > 10 but not the Emory, BOTEM or SB/PLT scores were predictors of survival in Chinese cirrhotic patients treated with TIPS. Gaba *et al*[48] reported that MELD and MELD-Na scores had the best capability to predict early mortality in an American population compared with bilirubin and the C-P, Emory, PI, APACHE II, and BOTEM scores. In a comparison of the MELD, C-P and Emory scores Schepke *et al*[49] found that all three models predicted 3-mo survival with similar accuracy, but the MELD score was marginally superior to the C-P score for both 12- and 36-mo survival. In patients with refractory variceal bleeding Rubin *et al*[39] found that survival was inversely proportional to C-P class and APACHE II scores. The single determinant most closely associated with decreased survival in the first month following TIPS was the APACHE II score, with a score of 18 stratifying patients into low and high mortality risk groups (Table 3). Only one of 13 patients with C-P class C cirrhosis and an APACHE II score exceeding 18 survived > 30 d[39]. In the Hermie study early mortality was associated with a MELD score of at least 19 and hemodynamic instability at the time of admission[32] (Table 3). If hemodynamic instability was combined with a high MELD score, the 6-week mortality peaked at 77.8%[32]. In a multicentre French study Walter *et al*[50] reported that sTIPS mortality was > 90% in patients who had lactate levels ≥ 12 mmol/L and/or a MELD score ≥ 30.

In view of these differing outcomes, the development of a prognostic model to accurately stratify the risk profile of patients undergoing sTIPS may be invaluable in guiding treatment. The novel CABIN score used in this study was developed as a point-based tool to improve prognostic prediction specifically for patients undergoing emergent sTIPS and circumvents the complex computations of the MELD and other scores. This new score avoids subjective elements and can be calculated at the bedside providing a refined, granular grading system from a minimal laboratory dataset with scores ranging from 5 to 25. The CABIN score achieved significant prognostic discrimination reflected by in-hospital survival of 100% in patients in the CABIN A category (5-10 points), while patients in the CABIN B category (11-15) score had a 25% and those in the CABIN C category (16-20) a 12.5% survival. Our model predicted in-hospital mortality with high accuracy and showed statistical superiority over the other seven contenders, including MELD and C-P scores. Moreover, of all the examined models, only the CABIN, APACHE II and Emory scores exceeded a C-statistic value of 0.9.

There are inevitable and specific limitations to our study. Firstly, this investigation is limited by its small sample size, retrospective design, and lack of a control group. Secondly, the study has a clear selection bias which restricts universal applicability as these patients were treated in a single, well-resourced tertiary care referral center with round the clock skilled endoscopic and TIPS access. Thirdly, because patients were accrued over three decades, technical differences in TIPS placement and improvements in medical care during the study period would have contributed to differences in clinical

Table 3 Published series of salvage transjugular intrahepatic portosystemic shunt for uncontrolled variceal bleeding

Ref.	Country	No. of patients	C-P grade A/B/C	Initial control of bleeding %	30-d mortality %	Persistent/Recurrent rebleeding	Survival %	Prognostic factors
Azoulay *et al* [22], 2001	France	58	3/8/47	90	29	17	51.7 (12 mo)	Sepsis, vasoactive drugs, balloon tamponade
Bañares *et al* [23], 1998	Spain	56	11/22/23	95	28	22 (1 mo)	72 (30 d)	Ascites, HE, albumin
Barange *et al* [24], 1999	France	32	3/14/15	90	25	14	75 (30 d)	ND
Bizollon *et al* [25], 2001	France	28	0/11/17	96	25 (40 d)	18	52 (2 yr)	↑Creatinine, ↑bilirubin
Casadaban *et al* [26], 2015	United States	101	2/46/52	89	31	21	44 (12 mo)	↑Bilirubin, ↑creatinine, ↑INR, non-alcoholic liver disease
Chau *et al*[27], 1998	England	84	4/17/63	98	34	30 (30 d)	66 (30 d)	ND
Encarnacion *et al*[28], 1995	United States	64	2/32/31	98	19	29 (6 mo)	56 (12 mo)	Haemodynamic instability
Gazzera *et al* [29], 2012	Italy	82	ND	94	25.6	13.4	74.4 (30d)	Child-Pugh C, ↑ creatinine, ↑PT
Gerbes *et al* [30], 1998	Germany	11		91	27	27	73 (12 mo)	ND
Helton *et al* [31], 1993	United States	23	0/15/18	74	56 (in hospital)	39	ND	Emergency TIPS, active bleeding
Hermie *et al* [32], 2018	Belgium	32	ND/ND/14	97	31	0	69	MELD > 19, Haemodynamic instability
Jabbour *et al* [33], 1996	United States	25	ND/ND/8	96	44	ND	56 (30 d)	Child-Pugh C, urgent TIPS
Jalan *et al*[34], 1995	Scotland	19	3/3/13	100	42	15.6	58 (30 d)	Liver failure, sepsis
Maimone *et al* [36], 2019	England	144	11/55/78	ND	36 (6 wk)	29	64 (6 wk)	↑MELD, ↑Child-Pugh score
Le Moine *et al* [35], 1994	Belgium	24	3/13/9	96	17	25	29 (5 mo)	ND
McCormick *et al*[37], 1994	England	20	1/7/12	100	60 (40 d)	40	30	ND
Patch *et al*[38], 1998	England	54	5/20/29	91	48 (6 wk)	11	53 (6 mo)	Ventilation, ↑WBC, platelets, ↑creatinine
Rubin *et al*[39], 1995	United States	49	3/23/23	84	40%	16	ND	C-P grade C, APACHE II > 18
Sanyal *et al* [40], 1996	United States	30	1/7/22	100	37	7	60 (6 wk)	> 70 yr, bilirubin >6 mg/dL, creatinine > 3 mg/dL, HE, ARDS
Tyburski *et al* [41], 1997	United States	33	0/5/28	ND	27	15	58 (12 mo)	Albumin < 2.5 g/dL, bilirubin > 3 mg/dL, PT > 15 s
Tzeng *et al* [42], 2009	Taiwan	107	ND	ND	28	ND	50 (12 mo)	C-P score > 11, MELD > 20
Zhu *et al*[43], 2019	China	58	5/36/7	91.2	12.3 (6 wk)	10.5 (6 wk)	81.8 (12 mo)	Ventilation, ICU

APACHE: Acute Physiology and Chronic Health Evaluation; ARDS: Adult Respiratory Distress Syndrome; C-P: Child-Pugh; INR: International normalized ratio; TIPS: Transjugular intrahepatic portosystemic shunt; MELD: Model for end-stage liver disease; HE: Hepatic encephalopathy; ND: No data; ICU: Intensive care unit; WBC: White blood cells.

outcomes over time. Fourthly, this new score has been developed using a derivation dataset and requires confirmation and external validation in a similar sTIPS patient group. The robustness of this study is enhanced by the prospective data collection, supervision by the same investigators during the study period, restriction of subjects to a well-defined cohort of cirrhotic patients with uncontrolled exsanguinating bleeding and complete follow-up. The use of all-cause mortality as the primary outcome provided a consistent and objective end point.

CONCLUSION

In conclusion, the novel CABIN prognostic score, which is objective, quantitative, and reproducible, combines five easily obtained laboratory test results and provides improved statistical power predicting in-hospital mortality in patients with uncontrolled variceal bleeding undergoing sTIPS. The CABIN score identified high-risk patients and outperformed other scoring systems in predicting in-hospital mortality. Despite the fact that mortality was 75% for CABIN B, 87.5% for CABIN C, and 83% for CABIN scores > 10 in this study, this high-risk category should not be denied consideration for an emergency TIPS and should be assessed on a case-by-case basis especially in units where there is prompt access to liver transplantation after sTIPS. This study was based on a small defined cohort of predominantly alcoholic decompensated cirrhotic patients undergoing emergent TIPS and this newly developed derivative CABIN score will need further prospective external validation before being considered for general clinical application.

ARTICLE HIGHLIGHTS

Research background

Transjugular intrahepatic portosystemic shunt (TIPS) is now established as the salvage procedure of choice in patients who have uncontrolled or severe recurrent variceal bleeding despite optimal medical and endoscopic treatment.

Research motivation

Although TIPS is a minimally invasive procedure, appropriate patient selection is crucial to identify patients who would benefit from the procedure, considering the substantial risks of hepatic encephalopathy, liver failure and increased overall morbidity and mortality in high-risk individuals.

Research objectives

In this study the accuracy of a novel CABIN score, which was developed to overcome limitations of existing scoring systems, was compared to established risk scores for the prediction of in-hospital mortality following sTIPS.

Research methods

Eight risk scores were evaluated in a cohort which included all adult patients who underwent sTIPS for uncontrollable or life-threatening refractory variceal bleeding. A new five component CABIN score was devised in which each CABIN variable was scored from one to five and the cumulative total is calculated by adding the individual values of the five biochemical components (Creatinine, Albumin, Bilirubin, INR (international normalized ratio) and Na (sodium). The best total CABIN score computes at 5 points and the worst at 25 points. Four CABIN categories (A-D) were established (A: 5-10 points, B: 11-15, C: 16-20, D: 21-25). The CABIN score and seven previously described scoring systems, Acute Physiology and Chronic Health Evaluation (APACHE) II, Bonn TIPS early mortality (BOTEM), Child-Pugh, Emory, FIPS, model for end-stage liver disease (MELD), and MELD-Na scores were calculated based on clinical evaluation and laboratory values obtained before the sTIPS procedure. The primary study outcome measure was prediction of in-hospital mortality after sTIPS and compared the relative performances of the seven established scoring models and the new CABIN score.

Research results

In 34 patients (6%) who underwent sTIPS, bleeding was either uncontrollable *ab initio* (n = 11) or life-threatening refractory (n = 23) despite optimal endoscopic and pharmacological management. Ten patients (29.4%) died in hospital at a median of 5 d following the procedure (range 1-10 d). Nine of the 12 (75%) patients who required pre-sTIPS balloon tamponade died, while all nine (100%) patients who were hypotensive (systolic blood pressure < 70 mmHg) and with the combination of > 8 unit blood transfusion, inotropic support, balloon tamponade and mechanical ventilation died. The CABIN score [area under the receiver operating characteristic curve (AUROC) 0.967] had the highest discriminative ability in predicting in-hospital death compared to the APACHE II (AUROC 0.948), BOTEM (AUROC

0.877), C-P (AUROC 0.802), EMORY (AUROC 0.942), FIPS (AUROC 0.892), MELD (AUROC 0.792), and MELD-Na (AUROC 0.865) scores. The median CABIN score in the 24 in-hospital TIPS survivors was 8 (range 5-18) compared to a median of 17 (range 11-22) in the 10 deaths. CABIN A patients had a 100% survival, compared to 25% and 12.5% survival in CABIN B and CABIN C category patients respectively. CABIN points of 11 or more provided a clear survival cut-off. No patients with CABIN scores < 10 died while 83% of patients with CABIN scores of > 11 died.

Research conclusions

The novel CABIN prognostic score, which is objective, quantitative, and reproducible, combines five easily obtained laboratory test results and provides improved statistical power predicting in-hospital mortality in patients with uncontrolled variceal bleeding undergoing sTIPS. The CABIN score identified high-risk patients and outperformed other scoring systems in predicting in-hospital mortality. Despite the fact that mortality was 75% for CABIN B, 87.5% for CABIN C, and 83% for CABIN scores > 10 in this study, this high-risk category should not be denied consideration for an emergency TIPS and be assessed on a case by case basis especially in units where there is prompt access to liver transplantation after sTIPS.

Research perspectives

This study was based on a small defined cohort of predominantly alcoholic decompensated cirrhotic patients undergoing emergent TIPS and this newly developed derivative CABIN score will need further prospective external validation before being considered for general clinical application.

FOOTNOTES

Author contributions: Krige J, Jonas E and Robinson C designed the research study; Krige J, Jonas E, Robinson C and Kotze U collected the data and performed the research; Krige J, Jonas E, Robinson C, Kotze U, Beningfield S, Bernon M, Burmeister S, and Kloppers C analyzed the data and wrote the manuscript; All authors have read and approve the final manuscript.

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Informed consent statement: Since this was a retrospective observational study using existing anonymized data, the requirement for informed consent from the study participants was waived by the Institutional Review Board.

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