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Review

The Threat of Multiple Liver Carcinogens in the Population of Laos: A Review

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Abstract: Laos is a landlocked country in South East Asia, ranking fifth for primary liver cancer incidence worldwide. Risk factors that might explain this worrying situation are poorly known. We conducted a review of the literature concerning the etiologies of terminal liver diseases in Laos. A double infectious burden with hepatitis B and C viruses and the liver fluke *Opisthorchis viverrini* seems to be the main cause of the high liver cancer incidence. Moreover, it was also suggested that mutagenic substances frequently found in tobacco, alcoholic beverages, fermented fish, and mold-contaminated cereals or nuts, which are all substances heavily consumed by Lao people, lead to the accumulation of DNA mutations in the liver cell genome causing tumor processes. However, the respective proportions of liver cancer cases attributable to each category of infections and substances consumed, as well as the histological nature of the neoplasia are still not precisely documented in Laos. The international medical and scientific communities as well as public health stakeholders should urgently consider the alarming situation of liver health in Laos to stimulate both research and subsequent implementation of prevention policies.

Keywords: primary liver cancer; hepatocellular carcinoma; cholangiocarcinoma; hepatitis virus; *Opisthorchis viverrini*; mutagens

1. Introduction

Laos is the least densely populated country with 30 people/km² and with the lowest life expectancy of 66 years in mainland Southeast Asia/Indochinese peninsula. According to Coker et al., the endemic presence of severe infectious diseases is a factor both preventing population expansion and increase of life expectancy in the region [1]. Furthermore, Laos is affected by a worrying pattern of non-communicable diseases that includes a high age-adjusted death rate in the 15–59-years population, high levels of tobacco use in men of 64%, and a significant proportion of the population is overweight (>35%) [2].

It was estimated that Laos has an incidence of primary liver cancer (PLC) of 22.4 cases/100,000 people, which is currently the second highest rate in Southeast Asia, behind Vietnam (see Figure 1). It was estimated that around 1100 new cases occur yearly in the country, equally affecting men and women, an unusual situation as worldwide women are usually relatively less affected by this

disease. However, these figures are mere estimates from the World Health Organization (WHO), taking neighboring nations as references. There have been significant variations in estimates of the incidence of PLC in Laos, illustrated by the last two GLOBOCAN reports. These variations are not supported by any obvious changes in the epidemiology of risk factors. There is currently no comprehensive cancer registry in Laos and very few pathologists. For reasons that will be discussed below, it is thus highly plausible that PLC incidence is largely underestimated in Laos. The underestimation of PLC cases is common worldwide, even in high-income countries [3,4].

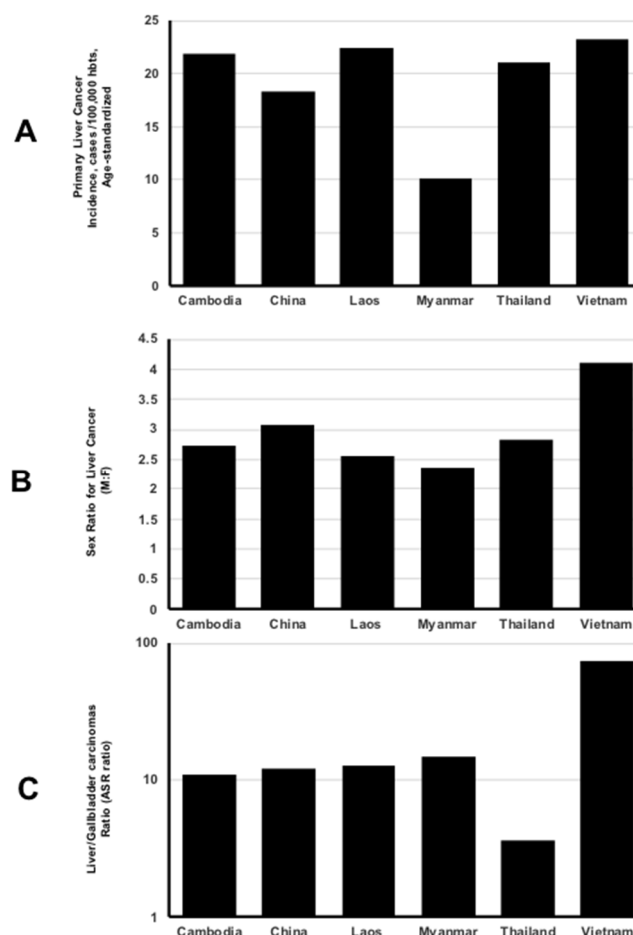


Figure 1. (A) Incidence of primary liver cancer (PLC) in Laos and neighboring countries. Myanmar is clearly less affected than all other countries of the region. (B) Male to female M:F sex ratios of PLC in the same countries. An increased sex ratio is observed in Vietnam suggesting a strong dominance of hepatitis B virus in PLC etiology. (C) Ratios of liver cancer/gallbladder cancer in the region. The low ratio observed in Thailand indicates the epidemiological importance of gallbladder and bile ducts carcinomas cases supposedly triggered by *O. viverrini*. In contrast, PLC is hyper-dominant over gallbladder in Vietnam, suggesting that the role played by flukes is less important in this country. All values have been calculated from Globocan 2018 <https://gco.iarc.fr/today/home>, accessed on 15 October 2020.

To explain the high incidence of PLC in Laos, we should first state that various infectious burdens, including chronic hepatitis B and C and frequent liver infestation with the fluke *Opisthorchis viverrini* (OV) afflict Lao people. The respective contributions of the different viruses and parasites to the local burden of PLC are, however, still unknown and therefore national incidences of cholangiocarcinoma (CCA), due to OV or hepatocellular carcinoma (HCC), due to hepatitis viruses are hypothetical. Furthermore, the impact of their interactions on liver carcinogenesis in case of co-presence has not yet

been properly characterized [5]. More remarkably, no description of PLC in the Laotian population is currently available in the medical literature.

2. Methods

Relevant literature included in this short review was collected in keeping with the guidelines proposed by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines (PRISMA) [6]. Records were retrieved from relevant online databases' (Medline, PubMed, Google Scholar) collection of publications ending in October 2020. Our search was targeted on terms corresponding to liver diseases and carcinogenic factors in the region (hepatitis viruses B and C, *O. viverrini*, aflatoxin B1, nitrosamines, alcoholic beverage, fatty liver, liver cirrhosis, liver cancer, cholangiocarcinoma, hepatocellular carcinoma) and country names. We primarily focused our search on the literature treating Laos (or Lao People's Democratic Republic, Lao PDR, also frequently used in the medical literature), but when no record was available, we explored publications from immediately neighboring countries (Cambodia, Myanmar, Thailand, Vietnam). Relevant medical literature from China was only marginally approached and mostly focused on the Yunnan province, the only Chinese province to share a border with Laos. Articles were first selected after reviewing the title and abstract, and subsequently by reading the full text of papers initially retained.

3. Results

3.1. Viral Risk Factors

3.1.1. Hepatitis B Virus

In Laos, the situation of persistent infection with hepatitis B virus (HBV) corresponds to that of a highly endemic country. More than 8% of the population are chronically infected. A decade ago, 8.7% of blood donors were seropositive for HBV surface antigen (HBsAg) and this rate remained stable, but steadily increasing (at 9.6%), in a more recent study [7,8]. Additional evidence in adolescents from Vientiane and Luang Prabang confirms these preliminary estimates, detailing rates of 10.2% [9]. Furthermore, it was shown that more than 10% of HBsAg-blood donors present circulating HBV DNA, i.e., an occult hepatitis B infection (OBI). This observation implies that in reality, around 20% of the Laotian population might be chronically infected with HBV. Moreover, in a study conducted in Vientiane on 3857 HBV-infected patients, it was shown that 50% of patients were under 37 and almost 60% had a viral load higher than 10,000 IU/mL, the threshold value for high risk of developing liver cancer. Among the patients, 10.6% had both a high level of HBV DNA over 10,000 IU/mL and elevated aminotransferases. However, only 2.4% of them received treatment for chronic hepatitis [10,11].

Finally, several surveys conducted on mothers and immunized newborns indicated that hepatitis B vaccination is remarkably inefficient in Laos, and results in protection only in a minority of newborns [12–14]. In addition, despite mass vaccination campaigns, only a minority of newborns actually receive the birth dose of anti-hepatitis B vaccine [15]. Taken together, the data show that HBV will still be a significant public health problem in Laos in the next decades. Finally, it was demonstrated that the HBV strains circulating in Lao blood donors predominantly belong to subtypes C1 and B4. However, mixed infections are frequent and more importantly, recombinant viruses between B and C genotypes, including the rare genotype I, which is dominant in around 5% of cases [16]. This is surprising, as HBV is believed to be transmitted pseudo-vertically at birth from mother to newborn in Laos. The circumstances that explain multiple strain infections are poorly known, as are the consequences of this situation for disease progression.

3.1.2. Hepatitis C Virus

In comparison, hepatitis C virus (HCV) is a lesser problem, as seroprevalence for anti-HCV virus reaches "only" 1.1% in Lao people. In Laos, seropositivity for HCV affects older subsets of

the population, suggesting the virus circulated more widely in the past than now. In this regard, a case-control survey on acute hepatitis conducted two decades ago measured anti-HCV prevalence around 60% in patients over 50 years [17]. More recently, a retrospective study conducted on 1765 HCV-infected patients showed that, in Vientiane, 28% had high viral loads ($>6\log_{10}$). About 23% of patients had levels of aminotransferases indicative of liver damage >40 IU/mL, but less than 20% of them received antiviral drugs [10,11]. In Laos, circulating strains overwhelmingly belong to genotype 6 $>95\%$, the Southeast Asian version of HCV, with frequent viral isolates specific to the local population [18–20]. Overall, Laos exhibits the highest HCV genotype 6 diversity in Asia, suggesting that the country might be the place where this virus originated. Another specificity of Laos is that no rapid expansion of HCV subtype was observed in Laos during the 20th century, a situation that contrasts with neighboring countries. As for other HCV genotypes, the origin of genotype 6 entry in human populations is remote, dating back 1100–1350 years ago [18]. Its original host is unknown. During recent decades, a large number of novel hepacivirus species have been identified in various mammals including bats [21]. In this context, the well-known capacity of hepaciviruses to cross species barriers, illustrated by the recent transmission of equine hepacivirus to dogs, appears to be especially relevant [22]. In this regard, although animal hepaciviruses circulating in Laos are still unknown, there are a large diversity of chiropters [23]. These animals, well-known for their capacity to transmit viruses to humans, are occasionally consumed as bushmeat by the Laotian population, and also found in highly frequented Buddhist cave temples such the Pak Ou caves. These circumstances may possibly represent an appropriate configuration for the efficient crossing of species barriers by hepaciviruses [24,25].

Subsequent to its entry in the human population of Laos, HCV circulation could have been maintained for centuries through the practice of *Sak Yant* tattooing, Buddhist monk tattoos, or by variolation practiced by traditional healers for smallpox protection since its possible import in the early 19th century by the French [26,27].

Altogether, for both HBV and HCV, the medical literature describing disease presentation in Laos is scarce, while references describing the roles of the viruses in terminal diseases such as liver cirrhosis or PLC are non-existent [10,11].

3.2. Parasitic Risk Factors

3.2.1. Opisthorchiasis

The epidemiology of opisthorchiasis, responsible for bile duct inflammation cholangitis, cholecystitis, periportal fibrosis, and intra-hepatic cholangiocarcinoma, is well described in Laos. The prevalence of OV infection is high in Laos; an estimated 2 million people are infected. Southern provinces of the country, with a high proportion of populations living on the banks of the Mekong river are more heavily affected, with a 50% prevalence, while central areas like Vientiane are around 20%. A survey conducted in Saravane province showed that the prevalence increases with age, reaching 85% in older population subsets of 46–55 years, but tending to already reach a plateau of 70% as soon as 20 years of age [28]. This situation is primarily due to the consumption of raw, fermented, or brined cyprinoid fish *padek* infected with OV metacercaria in a context where absence of latrines facilitates the perpetuation of the parasite cycle by the daily shedding of parasites eggs in stools of infected patients.

3.2.2. Other Helminths

Furthermore, multiple parasitism with other helminths and protozoa is common in populations of southern Laos and might play some role in liver disease progression [29]. In this context, *Schistosoma mekongi* (Sm), responsible for hepatomegaly, liver fibrosis, and portal hypertension is present in the same region where OV prevalence is high and OV/Sm co-infestation was shown to be synergistic in liver pathology [30].

Overall, at a given time point, it is considered that more than 1% of OV-infected patients present liver lesions compatible with cholangiocarcinoma CCA [30–32].

3.3. Chemical/Environmental Risk Factors

It is well known that cancer is primarily a genetic disease characterized by the accumulation of mutations in a cell clone. Recent genomic data indicate that liver infections, rather than being mutagenic *per se*, instead create a tissue microenvironment that is favorable for tumor development [33]. Infected liver tissue becomes the target for decades of inflammatory infiltrates that kill both infected and uninfected cells. The resulting regeneration and DNA replication that occur on a daily basis as an emergency response to cell death are associated with intrinsically poor genome maintenance due to lack of time. During this accelerated tissue repair process, concomitant presence of mutagenic substances such as polycyclic aromatic hydrocarbons (PAH) produced by tobacco combustion, aldehydes, or carbamates present in alcoholic beverages, nitrosamines generated during fish fermentation (an important component of Laotian cuisine), aristolochic acid found in herbal teas or aflatoxin B1 (AFB1) present in cereals or nuts will invariably lead to accumulation of mutations that promote tumor processes [34–37]. As mentioned above, many of these mutagens are prevalent in Laos, such as PAH produced by tobacco combustion in a country where the majority of men and a substantial proportion of women are smokers [2]. Other mutagenic contaminants may be found in unrecorded spirits. Lao-Lao rice whiskey and Lao Hai rice wine consumption were studied only from an anthropological point of view and chemical contaminants in these home-made alcoholic beverages (Figure 2) are unknown [38,39]. It is known, however, that spirits resulting from rice fermentation may contain traces of carcinogenic substances such as ethyl carbamate, acetaldehyde, and/or arsenic [40–42].



Figure 2. Traditional alcohol production device still in use in the Sayaboury province of Laos.

Other important compounds, nitrosamines, are mutagens produced during fermentation of fish. Nitrosamines directly ingested with food or endogenously produced by OV-infected patients have been long suspected to play an important role in cholangiocytes transformation [43–46].

Another of the mutagens potentially present in Laos is AFB1, a carcinogenic mycotoxin produced by *Aspergillus* species. Unfortunately, there are no publications about AFB1 in Laos. However, the toxin has been found in food from all neighboring countries, Thailand, Cambodia, Vietnam, Myanmar, and China [47–50]. It seems, therefore, reasonable to assume that AFB1 is present in Laos as well. AFB1 is known for its molecular fingerprints in liver cancer, as it was shown to generate mutation due to transversion of G to T at codon 249 of *TP53* gene leading to the replacement of an ARG by a SER. Such mutations, clonally present in developing HCC, have already been found in neighboring Thailand [51].

Finally, it is worth briefly mentioning the issue of Agent Orange. Agent Orange (AO) was the principal herbicide derived from chlorinated phenoxyacetic acid used by the US Army during the Vietnam war (1955–1975). It was sprayed to remove the forest cover that was hiding North Vietnamese military units particularly along the Ho Chi Minh trail linking North to South Vietnam and occasionally passing through Laotian territory. Laos was thus affected to some extent essentially in the Savannakhet province. It was estimated that 1.8 million liters of AO were sprayed on Laos [52]. AO turned out

to be contaminated by dioxin (in fact tetrachlorodibenzodioxin, TCDD), an environmentally-stable non-mutagenic tumor promoter [53]. Recent surveys conducted in Laos on small series of breast milk, blood, and food samples observed only very low levels of contamination with dioxin [54,55]. US government acknowledges the fact that AO exposure is associated with some tumors in US veterans (lymphoma, soft tissue sarcomas, and possibly prostate cancer). Some Asian and European researchers found an epidemiological association of AO exposure with PLC development, while surveys conducted on US Army veterans did not reach similar conclusions [56–59]. It seems that the liver tumor-promoting activity of dioxin or phenoxyacetic acid derivatives develops primarily when combined with chronic HBV infection, a condition obviously more frequent in Indochinese populations than among US Army veterans [57]. We consider that molecular studies, rather than epidemiological ones, are warranted to uncover hypothetical fingerprints of dioxin exposure in Laotian patients with PLC.

3.4. Non-Communicable Liver Diseases

Chronic liver disease with an infectious origin or exposure to mutagens are not the only threats for liver health in Lao PDR. Non-communicable diseases (NCD) such as alcoholic liver disease (ALD) or non-alcoholic fatty liver disease (NAFLD) both characterized by the presence of significant steatosis in liver tissue emerged in the last decades as a major threat at a global level [60].

There is, actually, a dearth of data concerning both ALD and NAFLD in Laos. However, NAFLD is considered the most frequent chronic liver disease in Southeast Asia, with a 40% prevalence according to a recent meta-analysis [61]. Abdominal ultrasound assessment of the liver is the most convenient screening method to evaluate the consequences of *O. viverrini* infection. A recent survey observed a 12% prevalence of fatty liver changes in a series of 431 patients from southern Laos [31]. Consistent rates are also observed in similar patients from Thailand (12–19%) [62,63].

NAFLD is primarily associated with metabolic diseases (obesity, type 2 diabetes, metabolic syndrome) linked to excessive nutritional intake, lack of physical activity, and aging. The Laotian population is, however, far to have reached the affluence and food security observed in some of its neighboring countries and there is in the country a double nutritional burden [64]. A significant subset of the adult population is affected with malnutrition (underweight, body mass index, BMI < 18.5, 10%) while another segment is on the contrary affected by obesity with substantial variations in prevalence (6–25.2%) depending on which criteria (WHO or Asian-specific) are retained [2,65]. Overall, the prevalence of obesity in Laos is one of the lowest in the Western Pacific region [66]. Likewise, the prevalence of diabetes is mild in Laos (5.7%) [2,67]. As a consequence, the proportions of NAFLD only-associated deaths from liver cirrhosis (12–19%) or PLC (10%) can be considered as still limited in Laos [68,69].

The medical literature concerning ALD is similarly scarce in Laos. However, as most countries in Southeast Asia, alcohol per capita consumption increased significantly in Laos in the last decade. The annual consumption of alcohol by Laotians is 10.6 L/year, the highest in Southeast Asia. As a consequence, with a rate of 81/100,000, Laotian population is affected by the heaviest alcohol-attributable burden of death in the region [70]. According to a recent review, the alcohol-attributable fractions of death is 41% for liver cirrhosis and 39% for PLC, i.e., the highest proportions in Southeast Asia [68,69].

Overall, ALD represents the largest NCD affecting the liver and is currently a more worrisome health problem than NAFLD in Laos.

4. Conclusions

In conclusion, populations in Laos suffer from a heavy but imprecisely quantified burden of PLC. This situation is due to the pressure applied on Laotian populations by highly prevalent and deleterious risk factors such as chronic infections, either with hepatitis viruses or with carcinogenic trematodes from *Opisthorchis* genus, responsible for HCC and CCA, respectively (Figure 3). The disturbingly high level of alcohol intake *per capita* in Laos represents an additional contributor to the situation. The proportions of cancer cases, which can be attributed to each agent are unknown, just as are their

respective implications in liver cirrhosis or severe periductal fibrosis prevalence among Lao citizens. Likewise, virus or parasite subtypes/strains potentially endowed with an increased carcinogenic potency are unknown in Laos.

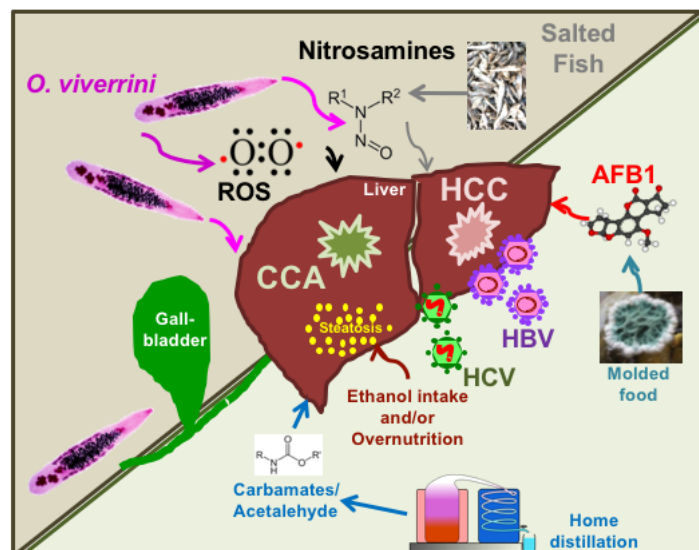


Figure 3. Schematic representation of the liver carcinogenic risk factors strongly prevalent in Laos. *O. viverrini*: *Opisthorchis viverrini*, ROS: radical oxygen species, HCC: hepatocellular carcinoma, CCA: cholangiocarcinoma, HBV: hepatitis B virus, HCV: hepatitis C virus, AFB1: aflatoxin B1.

Finally, carcinomas are invariably multifactorial, and the high incidence of PLC in Laos is also due to the exposure of infected patients to mutagens, e.g., aflatoxins, nitrosamines present in foodstuffs and acting synergistically with infectious agents to trigger liver tumorigenesis. Molecular fingerprints left by these chemicals in DNA have never been characterized in Laos.

Overall, the issue of severe liver diseases among Lao people could be tackled through a holistic approach gathering expertise from different fields. The production of data through sound clinical and translational studies is a crucial and mandatory milestone to design and implement prevention policies that will benefit the youngest and future generations of Laotian citizens.

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Conflicts of Interest: The authors declare no conflict of interest.

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