Liver Cancer in the Middle East

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For my daughters: Ophira and Feridey

"Everything should be made as simple as possible, but not simpler."

- Albert Einstein

"Truth does not become more true by virtue of the fact that the entire world agrees with it, nor less so even if the whole world disagrees with it."

"The physician should not treat the disease, but the patient who is suffering from it."

- ben Maimon (Rambam)

"Scientific knowledge is in perpetual evolution; it finds itself changed from one day to the next."

"What we see changes what we know. What we know changes what we see."

- Jean Piaget

"The knowledge of anything, since all things have causes, is not acquired or complete until it is known by its causes."

- Ibn Sina (Avicenna)

"The easiest method of acquiring the habit of scholarship is through acquiring the ability to express oneself clearly in discussing and disputing scholarly problems. This is what clarifies their import and makes them understandable."

- Ibn Khaldun

"We should not be ashamed to acknowledge truth from whatever source it comes to us. One must not be afraid of new ideas, no matter the source."

- Al-Kindi

"A hair divides the false and true; yes, and a single aleph were the clue-could you but find it- to the treasure house, and peradventure to the Master too."

- Omar Khayyam

"Judge a man by his questions, rather than his answers."

- Voltaire

Preface

Primary liver cancer is the fifth most common cause of cancer globally and the second most common cause of death from cancer. The ratio of death to incidence is about 0.9, since most patients who are diagnosed with it, die from it. The global burden of this disease is predominantly borne by less-developed countries and its causes are mainly known. The major cause is chronic infection with hepatitis B (HBV) and the majority of patients are in Asia. Although there are several types of primary liver cancer, approximately 90% are due to hepatocellular carcinoma (HCC), and this book focuses exclusively on this.

The Middle East comprises many countries with a huge range in income per capita, national wealth and its distribution, as well as hygiene and its practices. The causes of HCC and especially the cofactors are also varied. Some countries, such as Egypt and Saudi Arabia, have a very high percent of HCV-based HCC, while others, such as Turkey, Iran, and Kuwait, have a high percentage HBV-based HCC. As in the Western world, obesity and its associated liver diseases is increasingly becoming a cause of morbidity and HCC in the Middle East. Much of the cause of HCC in the Middle East is preventable, as elsewhere, the major approaches being HBV neonatal vaccination and lifestyle changes for obesity prevention and horizontal transmission of hepatitis C (HCV). Unlike other parts of the globe, alcoholism is a lesser cause.

In much of medicine in general and in HCC in particular, the major approaches to decreasing the disease burden and thus mortality depend on prevention (when the causes are known, as for HCC), early diagnosis via surveillance of patients who are known to be at risk (cirrhosis from any cause), and treatment of limited stage tumors (as a result of early diagnosis). Unlike most other tumors, the vast majority of HCC patients actually have two diseases, namely their HCC and an underlying liver disease that was the precursor to the HCC development. Both diseases interact bidirectionally, the liver disease influences HCC incidence and severity, and the HCC growth impairs residual liver function. Thus, consideration of both these co-existent diseases and their severity necessarily informs rational individual patient management decisions.

There is a large body of knowledge about HCC causes, pathophysiological mechanisms, and associated biology and biochemistry. Despite all this, too many patients present for medical care when their disease is at an advanced stage, when surgical therapies (resection, ablation, liver transplantation) with curative intent are no longer feasible. The next series of therapeutic options

consist of the loco-regional therapies, chemo-embolization (TACE), and radio-embolization (TARE), for non-metastatic disease patients. Thereafter come an increasing large choice of systemic therapy options, consisting of recently approved tyrosine kinase inhibitor drugs and immune checkpoint inhibitor drugs, both of which have recently been shown to greatly increase survival in this group of patients.

This book is divided into 4 parts. The first part (chapters "Biological Aspects of HCC", "Changing Etiology and Epidemiology of Human Liver Cancer", "Hepatocarcinogenesis Induced by Environmental Exposures in the Middle East", "Obesity and Hepatocellular Carcinoma: Epidemiology and Mechanisms", "Epidemiology of Hepatitis B Virus in the Middle East", "Hepatocellular Carcinoma in the United Arab Emirates", and "Overview of Clinical HCC and Its Management") considers the causes of HCC and clinical syndromes associated with HCC. The second part gives a descriptive overview of clinical HCC and describes the treatment modalities, with a chapter on treatment selection for individual patients, including settings where choices of therapies are less available (chapters "Cost-Effective Therapies for HCC: Resection and Ablation", "Transarterial Radioembolization in Hepatocellular Carcinoma", "Intra-arterial Chemotherapy and Transarterial Chemoembolization in Hepatocellular Carcinoma", "Radiotherapy for Hepatocellular Carcinoma", "Liver Transplantation in the Middle East", "Individual Patient Assessment and Therapy Decision-Making in a Live Donor-Based Liver Transplant Institute", and "Hepatocellular Cancer in Iran"). The third part gives a series of Middle Eastern country-specific chapters on local clinical HCC experience and practice (chapters "Hepatocellular Carcinoma in Kuwait", "Insights on Hepatocellular Carcinoma in Saudi Arabia", "Hepatitis C-Induced Hepatocellular Carcinoma in the Middle East", "Hepatocellular Carcinoma in the Middle East: An Overview", "Current HCC Clinical and Research in Egypt", "Hepatocellular Carcinoma in Turkey: A Review of Disease Epidemiology and Treatment Outcomes", "Targeting c-Met and AXL Crosstalk for the Treatment of Hepatocellular Carcinoma", "Hepatocellular Carcinoma in Morocco", "Hepatocellular Carcinoma in Lebanon and Its Association with Thalassemia", "An Overview of Hepatocellular Carcinoma (HCC) in Lebanon: A Focus on Hepatitis B- and Thalassemia-Related HCC", "Hepatocellular Carcinoma in Pakistan: An Update", and "Future Directions"). A final part (chapter "The Need for Region-Wide HCC Collaborations" and 27) considers what the next needs are for our subject and proposed useful HCC collaborations across our region.

Malatya, Turkey Brian I. Carr
March 2021

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Part I

Medical Science

Biological Aspects of HCC

Brian I. Carr

1 Etiology: Risk Factors for HCC and Presumptive Causes

The most common risk factors for developing HCC include cirrhosis from any cause, chronic hepatitis B or C viral infection, chronic alcohol consumption, fatty liver disease caused by obesity, and eating foods that have been contaminated by cancer-causing fungal toxins, as depicted in the list of cancer-causing and cancer-preventing substances of Table 1.

2 Biological Characteristics of Human HCC

The prognosis and management of HCC are influenced in most patients by the concurrence of two separate but related and interacting liver diseases, namely, hepatitis or cirrhosis from any cause and HCC. It is likely that each influences the other (i.e., cirrhosis is a precursor to most HCCs and growing HCC can destroy liver parenchyma and thus worsen liver function), and the selection of HCC therapy cannot take place without considering the limitations imposed by the concurrent liver disease. Thus, HCC is "a tale of two diseases."

3 Primary Drug Resistance to Cytotoxic Cancer Chemotherapeutic Agents

For most other cancers that have been studied, after a given number of chemotherapy treatments, the tumors can adapt and become resistant to the cytocidal actions of the cancer chemotherapy. This is called secondary or acquired resistance and is similar to the resistance seen in bacteria after exposure to antibiotics or in insects after exposure to insecticides. HCC is different in that it has primary resistance to a large array of toxins and most chemotherapeutics, without prior exposure to these agents. Work done several decades ago showed that cells that develop in a chronic toxic/carcinogenic milieu acquire a pan-drug resistance phenotype (pleiotropic) as they develop cancers. This is called primary resistance. Thus, trying to overcome this resistance with high doses of chemotherapeutic agents, especially in the presence of chronic liver damage, is often futile at best and dangerous for the liver at worst. Perhaps this is why such a large number of cancer chemotherapy clinical trials failed to produce any meaningful survival advantage for patients with HCC and could usually only be done in selected patients.

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Table 1 Substances of natural origin in the human diet that can cause or prevent cancer

A. *Substances that can cause cancer (carcinogens)*
1. [a]Aflatoxins – fungal contamination of stored rice and grains; ochratoxin A
2. Nitrosamines – fried bacon, cured meats
3. Hydrazines – found in edible mushrooms (false morel)
4. Safrole – found in sassafras plant and black pepper. Oil of sassafras in "natural" sarsaparilla root beer is 75 % safrole
5. Pyrrolizidine alkaloids – found in herbs, herbal teas, and occasionally in honey (e.g., senkirkine [coltsfoot], symphyline [comfrey])
6. Estrogens – from wheat germ, unpolished rice, forage crops
7. Bracken fern carcinogen
8. Methylazoxymethanol or cycasin (cycad plants)
9. Carrageenan – from red seaweeds
10. Tannins – from tea, wine, and plants
11. Ethyl carbamate in some wines, whiskey, and beers
B. *Carcinogens from molds and bacteria in food*
1. Aflatoxins (*Aspergillus flavus*)
2. Sterigmatocystin (*Aspergillus versicolor*)
3. Microcystins – from *Cyanobacteria* in drinking water in China
C. *Tumor antagonists in the diet*
1. Selenium
2. Coffee
3. Antioxidants
4. Phytochemicals, including polyphenols (curcumin from turmeric; resveratrol from red wine)
5. Vitamins A, K, and D. Vitamin A analog (polyprenoic acid, an acyclic retinoid)
6. Flavonols
7. Fish consumption
8. Vitamin K or polyprenoic acid (an acyclic retinoid analog of vitamin A)

[a]Only aflatoxins have strong epidemiologic evidence of association with human HCC. Reproduced with permission from © John Wiley and Sons, 2014; Carr (1985)

4 Vascular Characteristics

There are two different and unrelated vascular characteristics of HCC.

Vascularity: Firstly, it is one of the most vascular of tumors, and HCC has distinctive features on the arterial phase of computed tomography (CT) and magnetic resonance imaging (MRI) scan images. Unlike other organs, approximately a large proportion of the oxygenated blood of the normal liver comes from the portal vein. In contrast, around 80% of oxygenated blood that feeds HCCs comes from arterial outgrowths from hepatic artery branches. This was noted 30 years ago in Japan to offer a potential means for delivering drugs/chemotherapy moderately selectively to the HCC by injecting them into the hepatic artery and thus minimizing the exposure of the underlying diseased liver to drug toxicities. However, the liver is only partially protected, because in cirrhosis there is often hepatic arterial-venous blood shunting and direct intrahepatic arteriovenous connections open up.

PVT: Secondly, HCC has the propensity to invade radicals of the portal vein and grow in its lumen. When the portal vein is occluded by HCC, a characteristic enlargement and vascular enhancement of the portal vein are seen on CT. This is called macrovascular portal venous invasion (PVT). By contrast, microvascular venous invasion is only seen on biopsy or in HCC pathology specimens from liver resection/transplantation. Because the tumor cells are now in a vein, they can/do get carried by the blood stream around the circulation, with the increased possibility of forming distant metastases. Macrovascular invasion very often results in post-liver transplant recurrences and is thus considered a contraindication to transplantation surgery. Microvascular invasion does not seem to carry such a great risk. The reasons are unclear, as the cells are also within the venous lumen. Main branch PVT is considered to be a relative contraindication to trans-arterial chemoembolization (TACE), as HCC cells have blocked the portal vein and the TACE/chemoembolization therapy (transiently) blocks the artery, so the affected liver lobe loses its blood supply and can be severely damaged. Often, if only one of the two major portal vein branches is blocked by the tumor (branch PVT), then TACE therapy can still be safely given to the hepatic artery branches that feed the HCC.

5 HCC Growth Rates

HCCs have been reported to have a wide range of doubling times (growth rates), from 1 month to a year. Without repeated scans over several months, it is difficult to calculate the tumor growth rate of HCC in an individual patient. A newly diagnosed patient could have had a slow-growing 5 cm HCC for 3 years; but another patient with the same size tumor on the first clinic visit might have had only a 2 cm tumor 6 months ago and will thus have an aggressively behaving and rapidly growing HCC. On that first clinic visit, without the knowledge of prior scans, it would have been impossible to know the HCC growth rate. Thus, patients are heterogeneous with respect to their tumor biology, growth rates, and other characteristics. In fact, there is now evidence that HCCs change and evolve as they grow. If true, then a single baseline biopsy might be insufficient for rational patient management decisions, and multiple (liquid) biopsies over time may be a solution.

Size alone may not be so important, as many large HCCs with >8 cm diameter can arise in noncirrhotic liver and are thus quite resectable. Thus, although size is widely seen as a negative prognostic factor, it really depends on the clinical context. A study of platelets (a surrogate for cirrhosis) has shown that very large HCCs grow in a normal platelet environment (low or absent cirrhosis), whereas most patients with smaller and multifocal HCCs have thrombocytopenia. Thus, the cirrhotic and inflammatory context likely influences the ability of the liver to support the growth of an HCC to large size without liver failure due to parenchymal destruction.

Faster-growing tumors are often associated with several "satellite" lesions likely because they invade the surrounding liver. However, there is another mechanism for multifocality, as portal venous invasion by HCC is also a means for tumor spread within the liver (more common than distant metastases). This has significance for resection surgery, where up to 40% of patients have recurrence within 4 years after apparently curative surgery. Such recurrences are observed to be "early" within a few months or "late" after a year or more, which may have different causes. Early recurrence tends to be near the resection site and close to where the removed tumor was located; it is thought to be due to direct tumor extension from microscopic cells that could not have been seen at surgery or on the pre-resection scan. Late recurrences are often in other parts of the liver and may be new primary HCCs. These may occur in cirrhosis because there are millions of proliferating cirrhotic nodules, all being potentially premalignant, and eventually one or more of the nodules develop into new HCCs.

6 The Inflammatory Background

More than 80% of patients with HCC also have disease of the underlying liver that often profoundly affects HCC patient management choices. Most commonly HCC is associated with chronic inflammation (from HBV, HCV, or alcoholism, or their various combinations, or from obesity-associated liver disease), which may lead to cirrhosis, depending on the duration and intensity of the inflammation. Such inflammation may also lead to liver failure, for which only liver transplantation is an effective treatment. Depending on the severity of the underlying liver damage (inflammation/fibrosis/cirrhosis), the ability to perform resection or ablation therapies beyond that needed for a minimal size tumor could be thwarted by the risk of subsequent liver failure after the contemplated surgery. This can also be true for any potentially hepatotoxic medical therapy, such as regional cancer chemotherapy, TKIs, or ICIs. Since many chemotherapeutics also damage the bone marrow where granulocytes and platelets are produced, this combination can produce clinical toxicities. Furthermore, cirrhosis is often associated with bleeding tendencies from failure of the liver to produce sufficient coagulation proteins, in addition to low blood platelet counts thought to be due to splenic destruction of platelets from the back pressure resulting from liver fibrosis. In summary, the fragility of the underlying liver can limit the safety of many therapies other than liver transplantation.

7 HCC Microenvironment

For several decades, it has been thought that tumors arise because one or more growth pathway genes become mutated and are expressed or otherwise activated in a way that leads to excessive stimulation of the growth control pathways of the cell; this is known as the oncogene hypothesis. There is much experimental support for this hypothesis. However, in recent years, it has become clear that the activity of genes is often affected by other factors, either by controls on the gene involved (epigenetic factors), such as methylation, or by not yet well-understood factors in their microenvironment. Thus, both oxygenation and nutrients can affect how a given gene might behave within a cell, including oncogenes. Recent support for this "seed" (gene) and "soil" (cell environment) idea (a hypothesis originally developed for metastases by Stephen Paget (1889): "When a plant goes to seed, its seeds are carried in all directions; but they can only live and grow if they fall on congenial soil. … While many researchers have been studying 'the seeds,' the properties of 'the soil' may reveal valuable insights into the metastatic peculiarities of cancer cases." Support for this idea has come from molecular clinical studies in which it has been found that the behavior of HCC can be predicted from knowledge of the pattern of genetic changes (molecular signature) to be found in the nontumorous part of the liver (microenvironment). This environmental influence may have relevance in at least two HCC circumstances:

1. Prediction of the behavior of an individual patient's tumor, such as the likelihood of recurrence after resection
2. The reason for an expected benefit of virus hepatitis therapy (of the "soil") as part of HCC therapy in chronic virus carriers

8 Tumor Microenvironment Systems

Immune and inflammatory mediators: interleukins, chemokines, reactive oxygen molecules, PDL-1

Tumor angiogenesis/vascularization factors: VEGF, PDGF, FGF, and TGF alpha

9 Tumor Microenvironmental Mediators

Cells: hepatic stellate cells, cancer-associated fibroblasts (CAFs), lymphocytes, Kupffer cells, endothelial cells, platelets, tumor-associated macrophages (TAMs), dendritic cells, stem/progenitor cells

10 Noncellular Components

Growth factors (EGF, TGFα, FGF, PDGF, VEGF, HGF, IGF), TGFβ

Proteolytic enzymes: MMPs
Extracellular matrix proteins: laminins, integrins, heparan sulfate proteoglycans
Inflammatory cytokines: IL-6, IL-1, TNFα

11 Platelets and HCC Growth

A significant association has been found in HCC patients, between thrombocytosis and larger tumor volume, high AFP levels, and poor survival. By contrast, thrombocytopenia is also considered as a prognostic factor in HCC. Studies involving clinical parameters of patients with small or large HCCs have shown that along with other factors such as AFP, tumor size is correlated with platelet counts. HCCs associated with thrombocytosis are often found in noncirrhotic liver and tend to be larger-sized tumors. However, HCCs associated with thrombocytopenia are associated with small tumor size, lower blood albumin, and impaired liver function and a fibrotic background. The relationship between platelets and cancer cells is bidirectional, since tumor cells stimulate platelet aggregation, whereas platelets stimulate the growth of tumor cells and promote their metastasis through activation and secretion of several molecules. As tumor cells activate platelets, activated platelets in turn contribute to sev-

eral steps of carcinogenesis. These include the secretions by platelet granules containing (1) growth factors (IGF-1, EGF, VEGF, HGF, transforming growth factor-β (TGF-β), FGF, PDGF, etc.), (2) coagulation factors (prothrombin, fibrinogen, factor V, and factor VIII), (3) pro-angiogenic and anti-angiogenic factors (angiopoietin-1, angiostatin, etc.), (4) MMPs and tissue inhibitor of metalloproteinases (TIMPs) (MMP-1, MMP-2, MMP-3, MMP-9, MT1-MMP, MMP-14, TIMP-1, and TIMP-2), (5) pro-inflammatory mediators (C-X-C motif chemokines, such as CXCL4, CXCL7, and CXCL12), and (6) immunologic molecules (C1 inhibitor and IgG) [12,21,70,71]. Recent data identifying the effects of platelet extracts on HCC cell lines have shown that platelets and platelet-derived factors increase cell proliferation, invasion, and migration, whereas they decrease apoptosis and cell AFP levels, through JNK signaling. Secretory platelet granules also trigger angiogenesis by cytokines VEGF, PDGF, TGF-β, IGF-1, and endostatin. Nevertheless, since tumor cells grow without new blood vessels up to 1–2 mm^3, pro-angiogenic factor stimulation is necessary for tumor cells to grow further, which is also provided by platelets. Platelets help the tumor cells to adhere to the blood vessel wall through expressions of P-selectin (CD62P) and αIIbβ3 and enhance both intravasation and extravasation. The "education of platelets by tumor cells" is another recently described and potentially important observation. Studies show that platelets take up pro-angiogenic cytokines, proteins, and RNA which are secreted by tumor cells.

12 Growth Factor Receptors and their Inhibitors (TKIs)

Numerous cellular functions that include tumor cell differentiation, growth, apoptosis, and angiogenesis are mediated via signals from membrane-bound tyrosine kinase receptors. They include EGFR, IGFR, FGFR, Met (HGF receptor), VEGR, IGFR, and PDGFR and they transduce intracellular signals, often via the Ras/MEK/ERK pathway to the nucleus, that often result in transcription factor activation. Two major kinase types are dysregulated in HCC, namely, the tyrosine kinases (TKs) and cyclin-dependent kinases (CDKs), and they are each targets for the treatment of HCC via TKI inhibitors.

Some FDA-approved tyrosine kinase inhibitor (TKI) drugs that inhibit signaling associated with the above growth receptors include sorafenib, lenvatinib, regorafenib, cabozantinib, and ramucirumab. Other drugs, such as bevacizumab, are antibodies that also target growth receptors.

13 Immune Checkpoint Inhibitors (ICIs) and the Liver

The immune checkpoint inhibitors (ICIs) are compounds that target the regulatory signals between T lymphocytes and target cells, as well as other immune cells. T lymphocytes recognize specific antigens on target cells through major histocompatibility complex (MHC) proteins through their T-cell receptors and can induce apoptosis of target cells.

Immune checkpoint proteins, including PD-1/PD- L1 and cytotoxic T lymphocyte antigen 4 (CTLA-4), suppress T-cell inflammatory activity to prohibit overactivation of the immune system and promote self-tolerance. Immune checkpoint inhibitors (ICIs) suppress immune inhibition (suppress the suppressor) induced by PD-1/PD-L1 or CTLA-4 and thereby reactivate T cells to promote their cytotoxicity to tumor cell targets.

ICIs thus prevent the association of programmed cell death protein-1 (PD1) with its ligands, programmed death ligand1 (PD-L1) and 2 (PD-L2), enhancing the T-cell response toward HCCs, and have recently come into clinical use for many tumor types, including HCC. When used in various combinations, recent clinical trials have shown that they greatly enhance HCC responses by shrinkage, with associated increase in patient survival. Examples include nivolumab, a monoclonal antibody against PD-1; pembrolizumab, also a humanized monoclonal antibody against PD-1; atezolizumab, a monoclonal antibody against PD-L1; and ipilimumab, a monoclonal antibody against cytotoxic T lymphocyte

antigen-4 (CTLA-4), a receptor that also functions as an immune checkpoint, to downregulate immune responses. The ICIs appear to have the possibility of enhancing the lifespan of many HCC patients and are perhaps the most exciting development in HCC work in the last 10 years, as of end-2020.

14 Clinically Useful HCC Serum Biomarkers

Alpha-fetoprotein (AFP) is a glycoprotein produced in the embryonic liver and a form of fetal albumin, the synthesis of which is turned off at birth. Hence, an older name is oncofetal antigen, which, like CEA and glypican-3, is resynthesized postnatally in some tumors. It is frequently used and inexpensive and is a simple blood test to perform, but its blood levels are elevated in only 50% of patients with HCC. AFP is not a sensitive enough marker for screening for small, new HCCs but is extremely useful if elevated, when monitoring the HCC response to therapy. The biological function of AFP is still speculative, though there is some evidence for its role in apoptosis. Since it is the fetal form of albumin and albumin has some growth control properties, it may be that AFP has a functional role in the loss of growth control which characterizes the HCC phenotype. Recently, more HCC-specific tests have come into general clinical practice, such as a glycosylated form of AFP (itself, a fetal form of albumin) called AFP-L3.

Des-gamma carboxy prothrombin (DCP) or protein induced by vitamin K absence (PIVKA-2) is an HCC blood biomarker, and US Food and Drug Administration (FDA)-approved kits for measuring it are readily available to clinical labs. Several studies have shown that elevated DCP is commonly elevated in the presence of portal vein thrombosis (PVT). The molecule is really interesting, as it is an immature form of the coagulation protein, prothrombin. The enzyme responsible for catalyzing the immature to the mature form of prothrombin has an absolute requirement for vitamin K. This highlights an important role for loss of vitamin K function in HCC development and suggests that some vitamin K-dependent protein or vitamin K itself might be important in HCC migration, given the association of DCP with PVT. Several attempts have been made to assess the value of high doses of vitamin K in suppressing DCP (it does) and thus suppressing clinical HCC growth. The experimental evidence is good, but the one big randomized clinical trial fell short.

A diagnostic model hepatocellular carcinoma (HCC) has been proposed that incorporates the levels of each of the three biomarkers, AFP, AFP-L3%, and DCP, along with patient sex and age, into the gender, age, AFP-L3%, AFP, and DCP (GALAD) model, but awaits validation for screening.

Glypican-3 is another oncofetal glycoprotein that appears to have prognostic significance as an HCC serum biomarker and is being investigated both for imaging and as a potential target in HCC therapeutics.

15 Clinical Context Is Key

For all HCC parameters, context is key. For a newly presenting HCC patient, we normally do not know at what point the patient is on his/her disease trajectory. Thus, the total clinical context has to be understood to make rational patient management decisions. Tumor size alone is less important, unless we know about the presence of PVT or residual parenchymal liver function. That is why all modern classification systems employ parameters of both tumor aggressiveness (maximum tumor dimension, number of tumor nodules, presence of PVT, and often tumor biomarker levels), as well as liver function parameters. In this approach, two important papers showed that HCC microenvironmental factors may be as important as tumor factors, or more so. Hoshida et al. (2008) showed that gene-expression profiles of tumor tissue failed to yield a significant association with survival. In contrast, profiles of the surrounding nontumoral liver tissue were highly correlated with survival. Utsonomiya et al. (2010) showed that molecular signatures of noncancerous liver tissue can predict the risk for late recurrence of hepatocellular carcinoma.

It has recently been shown that the high rates of recurrence after HCC resection can be significantly reduced by anti-hepatitis viral therapy. Thus, the viral-mediated inflammation must influence the HCC behavior. In summary, there are at least two types of molecular signatures (patterns of genetic changes) and clinical prognostic factors in HCC: those of the tumor and those of the underlying liver.

It has become increasingly clear in recent years that the behavior of a given HCC, and thus the treatment approaches for a patient with HCC, depends on more than just the clinically observed tumor characteristics. This was anticipated in the 1985 staging system of the Japanese hepatologist Kunio Okuda, who brought attention to the need to consider both tumor and liver characteristics in prognosis and therapy. More recently, this approach has been greatly expanded by advances in HCC biology, biochemistry, and molecular understanding. As a result, a fuller understanding of HCC behavior needs to consider genes and gene alterations, tumor stroma (the underlying tissues), tumor neovasculature (the growth of new blood vessels that is necessary to support the increasing mass of the growing tumor), inflammation, supporting liver parenchyma (cells in the liver that support the specialized hepatocytes), and gene/molecular signatures (patterns of genes and their expression through proteins). Although much of this is still in the research realm (at least for the vasculature, inflammation, and molecular signatures), there are rapidly advancing clinical applications. For example, new knowledge of the growth factors that encourage new blood vessel growth has led to the development of several new cancer drugs that target this vasculature, such as bevacizumab or sorafenib. Another example is the use of anti-hepatitis therapy to decrease HCC recurrences after successful tumor resection. The recognition of the importance of the inflammatory microenvironment has had two recent consequences. One is the use of HCC clinical inflammatory markers in patient prognostication. Examples include use of blood levels of C-reactive protein (CRP), Glasgow Index (CRP plus albumin), neutrophil-lymphocyte ratio (NLR), and platelet-lymphocyte ratio (PLR). A second use is the finding that patients being treated with anti-inflammatory agents, such as aspirin for cardio-preventive purposes, seem to have lower incidence of some GI cancers, including HCC. These results point to the possibility of using aspirin or NSAIDs in HCC prevention, possibly as an adjunct to resection. As explained above in the section on HCC growth, absence of cirrhosis likely permits the growth of larger tumors. Counterintuitively, these may be easier to manage with better resultant prognosis, due to absence of the cirrhosis and associated inflammation, rendering hepatic resections safer.

16 Circulating Tumor Cells (Liquid Biopsy)

Precision oncology is becoming increasingly important in the diagnosis and management of patients with various cancers, and liquid biopsy has shown promise as a minimally invasive technique for diagnosis, detection of actionable (therapy) mutations, in the monitoring of tumor evolution and in making rational treatment decisions. Liquid biopsy depends on the observation that many patients with solid tumors shed tumor cells and tumor cell DNA (in addition to a vast amount of nontumor circulating DNA) or its fragments into their circulating blood.

There are several liquid biopsy analytes, including circulating tumor RNA, cell-free micro RNA, exosomes, circulating tumor cells (CTCs), and circulating tumor DNA (ctDNA). This approach permits the use of a minimally invasive means for obtaining clinically useful tumor information without invasive tissue biopsies. Furthermore, since they are based on peripheral blood samples, they can be repeated during the course of a patient's disease at the same time as other routine clinical bloods are drawn for standard tests. However, there is not yet a standardized platform for such testing. Despite this, several blood tests have already been FDA-approved as accompaniments to rational patient selection for several new molecularly targeting therapies, so far in non-HCC tumors. In addition to therapy, some uses of liquid biopsy include the

potential for diagnosis or assessment of postsurgical residual disease and presence of micrometastases. For HCC, measurement of methylation profiles of ctDNA appears to be a promising surveillance tool. Given that AASLD and EASL guidelines do not recommend HCC biopsy for the diagnosis of most patients with a vascular liver mass in a cirrhotic liver, there is a dearth of HCC tissue to examine unless the tumor has been removed by resection or transplantation. Circulating tumor cells offer the potential for repetitive molecular analysis of HCCs over the course of an individual patient's disease.

Further Readings

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Changing Etiology and Epidemiology of Human Liver Cancer

John D. Groopman

1 Introduction

Collectively liver cancer, including hepatocellular carcinoma (HCC) and cholangiocarcinoma, accounts for 8.2% of all reported cancer deaths in men and women, and it is the third/fourth most common cause of cancer mortality worldwide, tied with stomach cancer [1, 2]. Globally, the incidence rates and age of diagnosis of liver cancer vary enormously, and unfortunately the burden of this nearly always fatal disease is much greater in the less economically developed regions of Asia, Central America, and sub-Saharan Africa (Fig. 1) [3]. HCC, perhaps due to a changing pattern of risk factors, is also the most rapidly rising solid tumor in the USA and Central America and is overrepresented in minority communities, including African-Americans, Hispanic/Latino-Americans, and Asian-Americans [4–6]. This increase in the USA may portend a resurgence of this disease in the more economically wealthy countries. Currently, there are more than 840,000 new cases of this nearly always fatal cancer each year and nearly 370,000 deaths annually in the People's Republic of China (PRC) alone [3]. The combined age-standardized rate of mortality from liver cancer for men and women worldwide was 8.5 per 100,000 in 2018 [1]. Further, there are striking sex differences in the age-standardized rate of liver cancer deaths for men and women which was 12.7 and 4.6 per 100,000 people in 2018, respectively. The countries that traditionally are considered as part of the Middle East span two World Health Organization (WHO) regions: Eastern Mediterranean and Europe. Of these countries, Egypt has the second highest age-standardized rate of mortality of liver cancer, 49.0 and 16.7 per 100,000 for men and women, respectively, in the world [1]. Indeed, this mortality rate is only exceeded globally by Mongolia, and the unique circumstances of this liver cancer burden will be discussed in the following sections. While Egypt has the largest population in the Middle East, the next six countries with populations ranging from 20 million to 80 million people (Yemen, Syria, Saudi Arabia, Iraq, and Turkey) all have age-standardized rates of liver cancer deaths for both men and women less than the global average (8.5 per 100,000 people), but as will be discussed later in this chapter, these statistics are likely to change in the next few decades.

For a cancer such as liver cancer that has such a poor prognosis, less than a 15% 5-year survival, the age of diagnosis has a major impact on society [7]. In contrast with most common cancers in the economically developed world where over 90% of cases are diagnosed after the age of 45, in many high-risk regions for liver cancer, onset begins to occur in both men and women by

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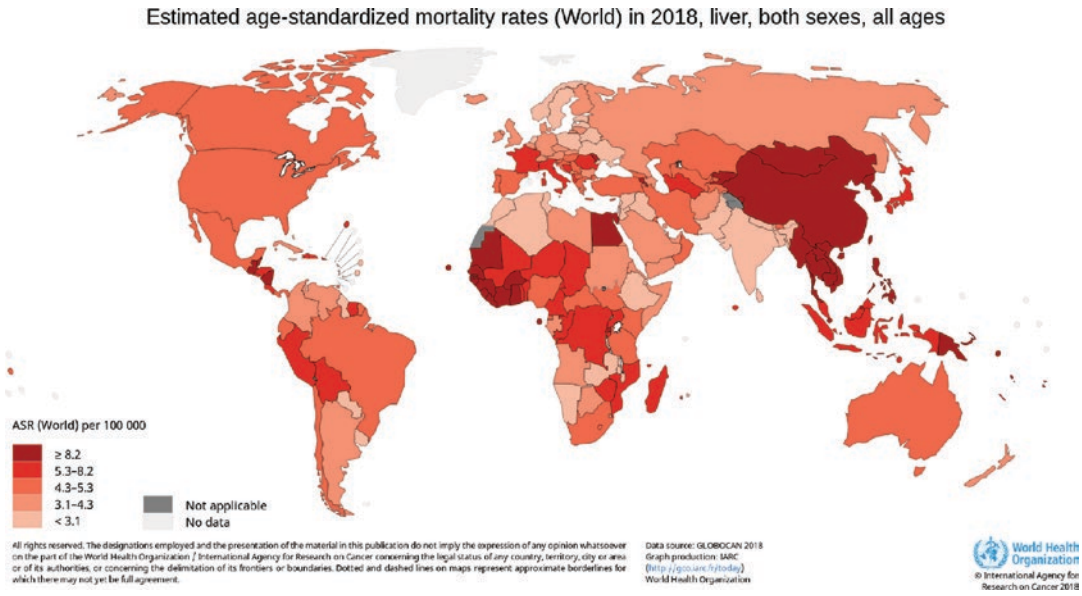

Fig. 1 Age-standardized mortality of liver cancer in men and women worldwide [1, 2]

20 years of age and peaks between 40 and 49 years of age in men and between 50 and 59 years of age in women [8, 9]. This earlier onset of HCC might be attributable to exposures that are both substantial and persistent across the life span and starting early in life. As mentioned above, sex differences in liver cancer incidence have also been well described, and worldwide the number of cases among men was 596,000 and 244,000 among women in 2018 [1, 2, 7]. These human epidemiologic findings are also reflected in experimental animal data for one potent liver carcinogen linked to human HCC, aflatoxin, where male rats have been found to have an earlier onset and higher incidence of cancer compared to female animals [10]. Thus, the consistency of the experimental animal and human data points to the important role that environmental exposures play in sex differences in HCC risk.

This chapter will review the significant data that links exposures to specific environmental toxicants, host factors, and biological agents with the etiology of liver cancer and with a specific commentary for counties in the Middle East. The epidemiologic studies revealing these etiologic factors have been made possible by devising biomarkers reflective of exposure, dose, and risk. The translation of these basic science findings to an understanding of the etiology of HCC has also provided guidance for the development of preventive interventions in high-risk populations. A number of these major investigations will be reviewed, to provide an overview of this very active field of research. Taken together, the etiology of many liver cancers diagnosed today is well understood, and when the underlying genetic diseases of hemochromatosis, alpha-1-antitrypsin deficiency, and copper overload disease are included, probably greater than 90–95% of the risk factors causing today's liver cancers have been identified [11–13]. With the emergence of fatty liver disease as a risk factor for liver cancer, a number of genetic risk factors have been identified including mutations in PNPLA3 that is more common in Hispanic populations [14, 15]. This knowledge base has been actively translated into effective screening tools, and prevention strategies that should continue if implemented effectively mitigate this cancer. Of great concern is the hypothesis that the emergence of new risk factors such as obesity and type 2 diabetes is changing the landscape of liver cancer etiology. With nearly 70% of the US population being

overweight or obese and over 400 million people worldwide being type 2 diabetic, there is an emergent likelihood of dramatically rising hepatic morbidity and mortality [16]. This problem is especially emergent in the countries of the Middle East where a majority of adults are now obese and the rates are rising [17].

1.1 Currently Identified Etiologic Agents for Liver Cancer

Prior prospective cohort studies conducted during several decades across many countries and populations have revealed the critical role that hepatitis B virus (HBV), hepatitis C virus (HCV), alcohol, and dietary aflatoxins play, often interacting with each other, in high-risk settings for liver cancer [6, 18]. The identification of these critical risk factors and others such as vinyl chloride and cigarette smoke has often been the result of developing validated biomarkers reflecting these exposure situations. Further, there have been a number of underlying genetic predispositions that have also been linked to increased liver cancer susceptibility.

1.2 The Promethean Liver

The liver has remarkable self-repair mechanisms that can lead to recovery at almost every stage of the progressive etiopathogenesis to cancer including fibrosis and cirrhosis. While Greek mythology poetically captured this biology in the stories of Prometheus, experimental studies have documented this remarkable repair and recovery process [19]. Rodents that undergo 70% partial hepatectomy recover a fully functioning liver within 2–4 weeks following surgery. Some studies from the 1960s demonstrated that rats could be subjected to biweekly partial hepatectomies for a year (20–25 surgeries) and still recover complete liver function [20, 21]. While not as dramatic, humans have also been found to have remarkable hepatic regrowth properties following injury or surgeries. In many respects, it is not surprising that the tissue which is the first line of response to the remarkably diverse number of compounds and nutrients absorbed from the gut into the portal vein would have this type of repair proficiency. A recent review has documented the recovery after several months from fatty liver disease and fibrosis in morbidly obese individuals following weight loss [22]. Thus, quantitative assessments of liver status through the use of biomarkers would be extremely valuable for designing interventions that facilitate this liver repair.

Biomarkers detected from blood samples have become important tools for cancer prevention and control. Many of these biomarkers fall under the rubric of the liquid biopsy. In the case of liver cancer diagnosis, α-fetoprotein (AFP) has been studied as an early diagnostic measurement since elevated levels are detected in patients having liver cancer. Unfortunately, AFP has not proven to be an effective early detection tool in a number of prevention investigations. Other tools such as FibroScan are capable of detecting relatively advanced but not early-stage liver disease [23]. Current liquid biopsy strategies have already been deployed for the early detection of tumor recurrence following initial therapeutic interventions. In a number of studies, mutant tumor suppressor and oncogenes known to have occurred in the primary tumor have been detected as DNA fragments in blood samples. Using the modern tools of nucleic acid detection, these mutant fragments can be readily measured. The analytical challenge of these strategies results from the relatively low number of these DNA fragments emerging in circulation, and this leads to the necessity of collecting large volumes (5–40 ml) of blood in order to have enough fragments of DNA measurement following PCR amplification. Hence, this technology is most applicable to the clinical setting and is not currently practicable for prospective cohort and other environmental exposure studies where much smaller volumes of blood were and are obtained from participants. Given the volumes of blood available in these investigations of healthy individuals, current strategies and technologies are focused on the more abundant proteins and their nucleophilic targets in circulation.

1.3 Hepatitis B Virus

In the case of identifying a role for HBV in liver cancer was the identification of a major biomarker the specific antigen (HBsAg) in blood samples partnered and grounded with cohort investigations [24]. Thus, one of the first breakthroughs in defining a biological agent in the etiology of this disease occurred with a series of studies describing a role for the hepatitis B virus (HBV) in HCC pathogenesis. Historically, a number of investigations found that chronic carriers of HBV, as indicated by sequential hepatitis B surface antigen (HBsAg) positivity at 6-month intervals, were at increased risk of developing HCC [3, 25]. Further, the age of initial infection by this virus was directly related to the prevalence of the chronic carrier state and subsequent accelerated risk for HCC. Approximately 90% of HBV infections acquired in infancy or early childhood become chronic infections, whereas only 10% of infections acquired in adulthood become chronic, and less than 50% of chronic carriers progress to HCC [26–29]. The global burden of HBV infection varies widely, and historically China, Southeast Asia, and sub-Saharan Africa had some of the highest rates of chronic HBV infection in the world, with a population prevalence of over 10% [30]. The public health significance of HBV as a risk factor for HCC is staggering with the consideration that there are still, despite the availability of an effective preventive vaccine, over 400 million viral carriers and between 10% and 25% of these individuals are likely to develop HCC [18, 31, 32]. The biology, serology, mode of transmission, and epidemiology of this viral infection continue to be actively investigated and have been recently reviewed [24, 33, 34]. Collectively, this work directly led to the research that resulted in a vaccine effective against HBV. Indeed, this vaccine has been reported to reduce HCC incidence by up to 70% in a cohort of young vaccinated children in Taiwan that have been followed for up to 30 years [35–40].

Historically, many studies across the globe that explored the relationship between HBV infection and HCC calculated risk estimates ranging from 3 to 30 in case-control studies and from 5 to 148 in cohort studies [41]. For example, an early small hospital-based case-control study from northeast Thailand showed an adjusted odds ratio (OR) of 15.2 for the presence of HBsAg among HCC patients [42]. An adjusted OR of 13.5 was reported from a case-control study in the Gambia [30]. The risk of HCC among HBsAg-positive individuals in Korea from a prospective cohort study of government workers was 24.3 among men and 54.4 among women, adjusted for age, smoking, alcohol use, and diabetes [43]. A similar prospective study from Taiwan found men positive for HBsAg were 223 times more likely to develop HCC than men negative for HBsAg [28]. All of these investigations were grounded and successful because of the measurement of a validated biomarker of high sensitivity and specificity that tracked with the development and risk for HCC. Further, these studies benefited by the high-throughput for the measurement of this biomarker that in turn permitted the recruitment of large numbers of individuals facilitating sufficient power for the study.

The contribution of HBV to the pathogenesis of liver cancer is multifactorial and is complicated by the identification of mutant variants in HBV that modulate the carcinogenesis process [24]. The HBV genome encodes its essential genes with overlapping open-reading frames; therefore, a mutation in the HBV genome can alter the expression of multiple proteins. In many cases of HCC in China and Africa, a double mutation in the HBV genome, an adenine to thymine transversion at nucleotide 1762, and a guanine to adenine transition at nucleotide 1764 ($1762^{T}/1764^{A}$) have been found in tumors [44–46]. This segment of the HBV genome contains an overlapping sequence for the base core promoter and the HBV X gene; therefore, the double mutation in codon 130 and 131 of the HBV X gene reported in human HCC is identical to the 1762 and 1764 nucleotide changes [47]. The increasing occurrence of these mutations has been also associated with the increasing severity of the HBV infection and cirrhosis [45, 46]. This acquired mutation following HBV integration into hepatocytes was originally characterized in

HBV e antigen-negative people [48]. The $1762^{T}/1764^{A}$ double mutation occurs more frequently in people infected with the genotype C strain of HBV, which is the most common genotype found in East Asian patients [49–51]. This double mutation tracks with an increased inflammatory response that becomes stronger as the progression of liver damage transits through chronic hepatitis and into a cirrhosis stage [52]. The underlying mechanism of the effects of HBV e antigen on the biology of inflammation and cirrhosis is still unclear, but there are substantial data that point to modulation of the immune surveillance system and immune tolerance in the presence and absence of this protein [52–54]. The $1762^{T}/1764^{A}$ double mutation has also been demonstrated to affect an increase in the rate of HBV genome synthesis in cellular models [55, 56]. In cellular studies, the $1762^{T}/1764^{A}$ double mutation increased the replication of the viral genome twofold, and in the case of some of rarer triple mutations, an eightfold increase in genome replication was found [54, 56]. A matched case-control investigation of 345 men who died of HCC and 625 controls were nested within a cohort of male hepatitis B surface antigen (HBsAg) carriers from Qidong, China found the HBV $1762^{T}/1764^{A}$ over twice as frequently in cases (81%) as compared with controls. The matched preserving OR of 6.72 (95% CI: 4.66 to 9.68) strongly indicated that cases were significantly more probably than controls to have the mutation. Plasma levels of DNA harboring the HBV mutation were on average 15-fold higher in cases compared with controls ($P < 0.001$). Most strikingly, the level of the mutation in the 20 controls who later developed and died of HCC was on average 274-fold higher than controls who did not develop HCC. Thus, within this cohort of HBsAg carriers at high risk of developing HCC, individuals positive for the HBV $1762^{T}/1764^{A}$ mutation at enrollment were substantially more probably to subsequently develop HCC, with a higher concentration of the mutation in plasma enhancing predisposition for cancer development [57].

Collectively, over 50 years of biomedical research have unequivocally established a role for HBV in the etiology of human liver cancer, and with the availability of an effective vaccine, the impact of this virus can be dramatically reduced. It will take many decades for this to occur because of the need to vaccinate children prior to infection that still occurs very early in life. To those individuals who become infected and therefore not eligible for vaccination, therapeutic drugs are being developed constantly. Hopefully, these therapeutic strategies will become both cost-effective and have minimal adverse effects to accelerate the elimination of HBV as a human carcinogen.

1.4 Aflatoxin

As shown earlier, HCC is among the leading causes of cancer death in most parts of the economically developing world with the unequal distribution of this disease depicted in Fig. 1 [4, 58]. Since the burden of HCC is also coincident with regions where aflatoxin exposure is high, many efforts starting over 50 years ago examined this possible association [59]. These initial studies were hindered by the lack of adequate data on aflatoxin intake, excretion and metabolism in people, the underlying susceptibility factors such as diet and viral exposure, as well as the incomplete statistics on worldwide cancer morbidity and mortality. Despite these deficiencies, early investigations did provide data illustrating that increasing HCC rates corresponded to increasing levels of dietary aflatoxin exposure [60]. The commodities most often found to be contaminated by aflatoxin were common human food staples including peanuts, cottonseed, maize, and rice [61]. The requirements for aflatoxin production are relatively nonspecific since molds can produce these toxins on almost any foodstuff and the final levels in the grain product can vary from micrograms to tens of milligrams [62]. Strikingly in a case of aflatoxin-related deaths in rural villages in Kenya, daily exposures were estimated to be over 50 milligrams [63]. Because contamination of foodstuffs is so heterogeneous, the measurement of human exposure to aflatoxin by sampling foodstuffs or by dietary questionnaires

is extremely imprecise [64]. The development of aflatoxin-specific biomarkers based upon its metabolic activation and subsequent binding to nucleophilic sites in DNA and serum albumin has been validated and used to demonstrate a significant role for this dietary contaminate in HCC across many countries [65].

Many published case-control studies have examined the relation of aflatoxin exposure using various biomarkers and HCC. Compared with cohort studies, case-control studies are both cost- and time-effective. Unfortunately, case-control studies are often initiated long after exposure has occurred, and it cannot be assumed that the exposure has not appreciably changed over time. Additionally, such studies involve assumptions in the selection of controls, including that the disease state does not alter metabolism of aflatoxin. Thus, matching of cases and controls in a specific biomarker study is much more difficult than in a case-control study involving genetic markers [59]. Data obtained from cohort studies have the greatest power to determine a true relationship between an exposure and disease outcome because one starts with a healthy cohort, obtains biomarker samples, and then follows the cohort until significant numbers of cases are obtained. A nested study within the cohort can then be designed to match cases and controls. An advantage of this method is causation can be established (due to the longitudinal nature of cohort studies, there is no temporal ambiguity) and selection bias is minimized. A major disadvantage, however, is the time needed in follow-up (often years) to accrue the cases, especially for chronic diseases such as HCC. This disadvantage can be overcome in part by enrolling large numbers of people (often tens of thousands) to ensure case accrual at a reasonable rate.

Essential to the designation by IARC of the aflatoxins as a Group 1 known human carcinogen were two major cohort studies with aflatoxin biomarkers that have demonstrated the important role of this carcinogen in the etiology of HCC [66]. The first study, comprising more than 18,000 men in Shanghai, examined the interaction of HBV and aflatoxin biomarkers as independent and interactive risk factors for HCC. The nested case-control data revealed a statistically significant increase in the adjusted relative risk (RR) of 3.4 [95% CI: 1.1–10.0] for those HCC cases where urinary aflatoxin biomarkers were detected. For HBsAg-positive people, only the RR was 7 [95% CI: 2.2–22.4], but for individuals with both urinary aflatoxins and positive HBsAg status, the RR was 59 [95% CI: 16.6–212.0] [67, 68]. These results strongly support a causal relationship between the presence of the chemical- and viral-specific biomarkers and the risk of HCC.

A subsequent cohort study in Taiwan has substantially confirmed the results from the Shanghai investigation. Wang et al. [69] examined HCC cases and controls nested within a cohort and found that in HBV-infected people, there was an adjusted odds ratio of 2.8 for detectable compared with non-detectable aflatoxin-albumin adducts and 5.5 for high compared with low levels of aflatoxin metabolites in urine. In a follow-up study, there was a dose-response relationship between urinary aflatoxin metabolite levels and risk of HCC in chronic HBV carriers. Similar to the Shanghai study, the HCC risk associated with AFB_1 exposure was more striking among the HBV carriers with detectable aflatoxin-DNA adducts in urine. The use of aflatoxin biomarkers as efficacy endpoints in primary prevention trials in West Africa has been reported [70]. This study assessed postharvest measures to restrict aflatoxin contamination of groundnut crops. Six hundred people were monitored, and in control villages, mean aflatoxin-albumin concentration increased postharvest (from 5.5 pg/mg [95% CI 4.7–6.1] immediately after harvest to 18.7 pg/mg [17.0–20.6] 5 months later). By contrast, mean aflatoxin-albumin concentration in intervention villages after 5 months of groundnut storage was much the same as that immediately postharvest (7.2 pg/mg [6.2–8.4] vs. 8.0 pg/mg [7.0–9.2]). At 5 months, mean adduct concentration in intervention villages was less than 50% of that in control villages (8.0 pg/mg [7.2–9.2] vs. 18.7 pg/mg [17.0–20.6], $p < 0.0001$). Thus, primary prevention maybe an effective means to reduce HCC burden, especially in areas where single foodstuffs such a groundnuts are major components of the diet.

Recent data utilizing the cancer registry in Qidong, China, has provided some very exciting insights into the role of aflatoxin in liver cancer. Utilizing the availability of serum samples collected over a 30-year period, aflatoxin exposure patterns have been documented. In China, major agricultural reforms in the 1980s led to diminished maize consumption, a major source of aflatoxin contamination. The population-based cancer registry in Qidong, China, has documented a more than 50% reduction in HCC mortality rates occurring across birth cohorts from the 1960s to the 1980s for Qidongese less than 35 years of age although all were born before universal vaccination of newborns. Median levels of the aflatoxin biomarker decreased from 19.3 pg/mg albumin in 1989 to undetectable (<0.5 pg/mg) by 2012. A population attributable benefit of 65% for reduced PLC mortality was estimated from a government-facilitated switch of dietary staple from maize to rice; 83% of this benefit was in those infected with HBV. Food policy reforms in China thus resulted in a dramatic decrease in aflatoxin exposure, which, independent of HBV vaccination, reduced liver cancer risk *[71]*. Now that an extensive HBV vaccine coverage is in place, this augurs even greater risk reductions in the future [72].

Biomarker development in HCC has been further advanced by the molecular biological studies on the TP53 tumor suppressor gene, the most common mutated gene detected in human cancer [73, 74]. Many studies of *p53* mutations in HCC occurring in populations exposed to high levels of dietary aflatoxin have found high frequencies of guanine to thymine transversions, with clustering at codon 249 [75, 76]. In contrast, no mutations at codon 249 were found in *p53* in HCC from Japan and other areas where there was little exposure to aflatoxin [77, 78]. The occurrence of this specific mutation has been mechanistically associated with AFB_1 exposure in experimental models including bacteria [79] and through demonstration that aflatoxin-8,9-epoxide could bind to codon 249 of *p53* in a DNA plasmid in vitro [80]. Mutational analysis of the *p53* gene in human HepG2 cells and hepatocytes exposed to AFB_1 found preferential induction of the transversion of guanine to thymine in the third position of codon 249 [81] [82, 83]. In summary, studies of the prevalence of codon 249 mutations in HCC cases from patients in areas of high or low exposure to aflatoxin suggest that a G-T transition at the third base is associated with aflatoxin exposure, and in vitro and mutagenesis data would seem to support this hypothesis [84].

The remarkable increase in the throughput and cost-effectiveness of DNA sequencing has propelled the analysis of mutations in DNA from tumors at every organ site. When these data are combined with new bioinformatics tools, there has been a revolution in the determination of mutational signatures found in human cancer [85, 86]. Data from a variety of studies have explored these mutational signatures in liver cancer and has revealed a number of insights that are consistent with the etiologic factors that have been identified from epidemiologic studies [87]. Experimental models have also demonstrated a consistency between aflatoxin exposure and a unique set of mutational signatures and liver tumors [88, 89]. With the further development of these technologies, the opportunity for using this mutational signature data for the early detection of liver cancer is an evident opportunity in the near future.

1.5 Hepatitis C Virus

Hepatitis C virus (HCV) is a positive strand RNA virus, and its linkage to liver cancer has been extensively reviewed [90]. People who are chronically infected by HCV represent the high-risk group for its carcinogenic impact. There are upward of 75 million people worldwide who are infected with HCV, and this is primarily due to blood-borne transmission especially among intravenous drug users or in medical situations where insufficient sterilization and handling of needles have occurred. For HCV, the detection in blood of the HCV nucleic acid became an essential biomarker for risk reduction and prevention [91]. From a historic perspective, an HCV-specific test only became available in the early 1990s, and prior to this technology, the attributable etiologic