**Artikel Ulasan/*Review Article***

# Selenium and Hepatocellular Carcinoma
(Selenium dan Karsinoma Hepatoselular)

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ABSTRACT

*While cancer is considered to be one of the leading causes of death worldwide, there is a growing scientific and public interests on selenium as a dietary and antioxidant of many diseases, in particular, cancer. Despite advanced technology and significant improvement of surgical, chemical, hormonal and radio therapies, hepatocellular carcinoma (HCC) is still common in Asia and Africa and is increasing in the developed countries. Prognosis of HCC at an early stage is still challenging. At the moment, combination of Alpha feto protein (AFP) and ultrasonography tests offers more accurate and sensitive results for the diagnosis of HCC. Selenium (also known as the moon element) has been recognized for almost 49 years as an antioxidant and anti cancer agent. The weight of evidence supports the position of selenium as an anti cancer agent for HCC but the molecular mechanism of how selenium inhibits HCC is still unknown. Numerous theories have been proposed and selenium induced apoptosis and cell cycle arrest is the predominant one so far.*

*Key words: Selenium, Hepatocellular Carcinoma, Deficiency, Toxicity, Alpha Fetoprotein, Tumour Markers*

ABSTRAK

*Kanser merupakan penyebab kematian utama di dunia. Sejajar dengan itu, terdapat minat yang memberangsangkan masyarakat dan kajian sains terhadap selenium sebagai sumber diet dan agen antioksidan bagi pelbagai penyakit, terutamanya kanser. Biarpun dalam zaman perkembangan teknologi dan perawatan seperti pembedahan, kimia, hormon dan radioterapi, namun karsinoma hepatoselular (HCC) masih merupakan jenis kanser yang kerap berlaku di Asia dan Afrika dan diperhatikan meningkat di negara-negara maju. Prognosis HCC pada tahap awal agak sukar dan mencabar. Ketika ini, gabungan ujian alfa fetoprotein (AFP) dan ultrasonografi menawarkan keputusan yang lebih tepat dan lebih sensitif untuk mendiagnosis HCC. Selenium (juga digelar sebagai unsur bulan) telah dikenal pasti sebagai agen*

*antioksidan dan antikanser bagi* HCC *sejak 49 tahun yang lalu. Namun begitu mekanisme molekul tindakan perencatan* HCC *oleh selenium masih belum diketahui dengan jelas. Pelbagai teori telah dicadangkan. Apoptosis aruhan selenium dan pemberhentian kitaran sel adalah antara mekanisme yang mendominasi setakat ini.*

*Kata kunci: Selenium, Karsinoma Hepatoselular, Kekurangan, Ketoksikan, Alfa Fetoprotein, Petunjuk Tumor*

## INTRODUCTION

The main cause of cancer is still to be determined. However, diet, chemicals, life style, family history, bacteria and viruses are important factors toward the formation of cancer. Carcinogenesis is a multi-step process resulting in a precancerous cell or cancer cell. Hepatocellular carcinoma (HCC), like other cancers, is often characterized by the up-regulated expression of one or more oncogenes (Feitelson 2004). It has been reported that chronic infection with hepatitis B and C are major risk factors for HCC worldwide (El-Serag & Mason 2000). Other risk factors include alcohol abuse, hemochromatosis, fatty liver disease, aflatoxin exposure and androgenic steroid use (Gurusamy 2007).

Despite significant improvement of surgical, chemical, hormonal and radio – therapies, there is no real cure for cancer, in particular, liver cancer. Cancer is considered to be one of the leading causes of death worldwide. In 2006, World Health Organization (WHO) has recorded a total of 58 million deaths worldwide and cancer accounted for 7.6 million of all deaths. In fact, there are more than 10 million new cancer patients per year (Greenlee et al. 2001; Parkin et al. 2001). After lung and stomach cancers, liver cancer is the most important cancers in the world, mainly affecting men, and resulting in more than 1 million patients per year (Jia – Guo et al. 2006) and over 662,000 deaths per year (WHO 2006). Worldwide, the male to female ratio among cases of HCC is between 4:1 in low incidence area, and 9:1 in high incidence area (Szmuness 1978). Israel and his colleagues (1989) reported that the male preponderance in HCC may be explained by a number of factors such as the male bias among hepatitis B carrier, genetic susceptibility, androgenic steroids, and higher body iron stones.

HCC is geographically variable. It is common in Asia and Africa and is also increasing in the developed countries such as USA, UK, France, Italy, Japan and Australia (Colombo 2000). This might explain why the majority of the HCC researchers are from China, Korea and Egypt. Probably there are two main reasons that make HCC difficult to first diagnose and then to cure. First, at an early stage, most types of cancers are asymptomatic. In the other words, abnormal cells grow silently and rapidly. Once symptoms started to appear, multi – nodular tumours within the liver are well present. Thus, the choice of treatment, at this

stage, is limited. Secondly, at the molecular level, oncogenes, which are responsible for HCC, are still unknown. Thus, worsen the cure of HCC.

Selenium, which is an essential micro-nutrient mineral, might not be necessary for healthy individuals on daily intake basis, however, as part of a preventive health strategy 'Prevention rather than treatment' selenium intake is essential to protect against many unknown diseases. The Recommended Dietary Allowance (RDA) for selenium has been generally set at 55 µg (microgram) per day for adults with adjustments for women who are pregnant or are lactating and for babies and children (Table 1). A tiny amount of selenium might save thousands of dollars and have longer life without stress and worry. Previous studies have shown that dietary intake of selenium at levels 1 – 5 ppm (parts per million), which is above the dietary requirement (0.1 ppm), can prevent certain cancers. (Medina & Morrison 1988; Clement 1998; Combs & Gray 1998). This review focuses on four main areas; the worldwide spread of HCC and possible early measuring tests such as AFP and ultrasonography, various scientific evidences of selenium as an anti cancer agent against HCC, stimulation of apoptosis and cell cycle arrest as a possible mechanism action of selenium and deficiency and toxicity of selenium.

TABLE 1. Recommended dietary allowances for selenium.

|  | µg per day |
| --- | --- |
| Adults | 55 |
| Pregnant women | 60 |
| Lactating women | 70 |
| Children |  |
| 1 – 3 years | 20 |
| 4 – 8 years | 30 |
| 9 – 13 years | 40 |
| 14 – 18 years | 55 |
| Infants |  |
| 0 – 6 months | 15 |
| 7 – 12 months | 20 |

*Source*, IOM (2000)

## HCC TUMOUR MARKERS

Tumour markers are widely used in prognosis, staging, and diagnosis of cancers. These markers are useful to localize the tumour burden, monitor therapeutic effectiveness, detect recurrence or localization of the tumour and screen the general population or groups who are at risk (Giannelli & Antonaci 2006). These markers, when are in use, should be easy to detect, specific, reliable, reproducible and cheap. There are hundreds of tumour markers ranging from enzyme source

to genetic mutation source, but none of them have been shown to have a role that is specific to known tumour. This might explain the necessity to use more than one marker to classify or identify doubtful or unknown tumours.

Despite significant improvements in techniques and instruments, prognosis of HCC at an early stage is still challenging. In 1960, Abelev described AFP as a useful tumour marker for HCC. Although AFP is widely used as a tumour marker for the diagnosis of HCC, there are two main drawbacks. First, limited sensitivity of AFP, ranging from only 39% to 65% (Sherman et al. 1995; Collier & Sherman 1998; Trevisani et al. 2001; Nguyen et al. 2002). Thus the diagnosis of HCC can not depend mainly on the measurement of AFP. Secondly, AFP is not specific for HCC. It has been reported that elevated AFP can also be associated with other cancers such as gastric carcinoma, biliary tract carcinoma and pancreatic cancer and others (Taketa 1990). There are other tumour markers for the identification of HCC such as AFP-L3 (AFP- Lens culinary), DCP (Des – gamma carboxy prothrombin), GP73 (Golgi protein 73), GPC 3 (Glypican -3) and SCCA (Squamous cell carcinoma antigen). However, these markers, with the exception of AFP-L3, are less used for HCC prognosis due to their inconsistent sensitivity and specificity. AFP-L3 has received a considerable attention as a marker for HCC due to its high specificity. Three studies have demonstrated that AFP-L3 could detect HCC less than 2 cm in diameter in high risk patients such as those with chronic hepatitis B and C and liver cirrhosis (Sato et al. 1993; Takeda et al. 1993; Zinkin et al. 2008). In addition, it has been reported that AFP-L3 is associated with the aggressiveness of HCC (Li et al. 2001). Recent study by Yamashita et al. (2008) showed that EpCAM (epithelial cell adhesion molecule) may serve as an early biomarker f HCC because its expression is highly elevated in precancerous hepatic tissues. They revealed that EpCAM – positive HCCs displayed genes with features of hepatic progenitor cell markers such as cytokeratin 19, c-kit and activated Wnt-B-catenin signaling, whereas EpCAM – negative HCCs displayed genes with features of mature hepatocytes. Jia et al. (2007) showed that the measurement of GPC3, PEG10, MDK, SERPIN11 and QP-C, using prediction analysis of microarray, may improve the diagnostic tool of HCC including those with normal AFP and smaller-sized tumors. Another study by Sun et al. (2007) revealed that the analysis of TFF3, IGF2, LPL and SPPI genes in an HBx transgenic mouse model was better for the detection of HCC as compared with AFP. Obviously, as shown above, more investigations are needed for these and other HCC markers.

Ultrasonography can be used more accurate and sensitive to identify nodules in doubtful cases of HCC. Two main features of successful ultrasonography results, the operator and the technological improvement of the instrument used. Giannelli & Antonaci (2006) have reported that the combination of ultrasonography with the measurement of serological marker AFP is the best choice to diagnose HCC at an early stage. Previously, Zhang & Yang (1999) tested serum AFP and ultrasonography to assess the validity and cost of a

screening test for primary liver cancer. In brief, they studied 9373 Chinese patients, aged 35 to 59, with either positive hepatitis B surface antigen or chronic hepatitis. They investigated many parameters, we are concerned with two main parameters, the detection rate and false positive rate. Combination of AFP test and ultrasonography showed 92% and 7.5% of the detection rate and false positive rate, respectively. Whereas AFP test alone revealed 69% and 5% of the detection rate and false positive rate, respectively. When ultrasonography was used alone the detection rate and false positive rate were 84% and 2.9%, respectively. Clearly, the combination of both screening methods (AFP and ultrasonography) resulted in a small increase in detection rate but a considerably higher false positive rate. These two scientists recommended the use of ultrasonography alone as the method of choice in China to measure primary liver cancer.

## SELENIUM AND HCC

Experimental, epidemiological and clinical studies all have shown that selenium can inhibit liver cancer. Many experimental studies have shown that selenium is an anti – liver cancer that prevents or reduces chemical hepatocarcinogenesis in animals (Dorado et al. 1985; Thirunavukkarasu & Sakthisekaran 2003; Katzenellenbogen et al. 2007).

Björkhem – Bergman and his colleagues (2005) have shown that daily dietary exposure of 1 to 5 ppm sodium selenite to rats treated with diethyl nitrosamine and promoted later with 2-AAF (2-acetylaminofluorene), showed that the carcinogenesis process could be prevented by selenium supplementation both during the promotion and the progression phases. They concluded that selenium is a potent cancer preventive agent. In vitro experiments, Cuello et al. (2007) investigated the potential effect of selenium methylselenocysteine (Se-MeSe Cys) against a chemical oxidative stress induced by ter-butyl hydroperoxide on human hepatoma HepG2 cells. Their results showed that Se-MeSe Cys was significantly able to protect HepG2 cells against an oxidative stress.

Epidemiological studies have also shown that cancer patients generally have lower selenium levels, on average, than healthy subjects (Combs 1997). For example, Yu and his colleagues (1985) measured the selenium content of blood stored in blood banks in 30 different regions of China, and used this data to classify the regions as high, medium or low selenium. They divided 10 regions per group. Then they looked at total death rates from cancer in the low, medium and high selenium groups of provinces. They found that the death ratio was 3 to 2 to 1, respectively, which means that the cancer death rate in the high selenium region was only one third that of the low selenium region. Another study in China, researchers demonstrated a significant inverse association between the incidence of primary hepatocellular carcinoma and plasma selenium levels (Li et al. 1995).

From the clinical point of view, the most important studies on selenium and liver cancer were the two studies of Yu and his team (1997) in Qidang (Shandong province in China), where 15% of adults carry the hepatitis B surface antigen and those people are 200 times more likely to develop hepatocellular carcinoma . The first study involved 2474 family members of people who had developed liver cancer, this group was randomized to receive either 200 µg of selenium- enriched yeast daily or placebo over a period of 2 years. They found that 13 of the 1030 controls developed liver cancer as compared to 10 of the 1444 subjects receiving selenium. The second study, which was over a 7 year period, involved the use of a table salt fortified with 15 ppm selenium as sodium selenite. Yu and his team reported that liver cancer incidence among villagers provided selenium fortified salt dropped from 54.8 to 34.5 per 100,000 cases whereas rates in control villagers remained high 54 – 65 per 100,000.

How does selenium inhibit HCC? The exact answer is still to be known. However, Taylor (2004) reported that selenium affects oxidative stress, DNA methylation, DNA repair, inflammation, apoptosis, cell proliferation, carcinogen metabolism, hormone production, angiogenesis and immune function. Clearly, as reported above, selenium has many anti cancer actions that it is difficult to establish which ones are predominant. However, the most compelling evidence of selenium effects as preventive agent come from observations demonstrating its effects on induced cell cycle arrest and stimulation of apoptosis. To date, there are as many as 25 selenoproteins have been identified so far (Gladyshev & Hatfield 2001). These selenoproteins include the family of glutathione peroxidase, thioredoxin reductase, iodothyronine deiodinases, selenoprotein P, selenoprotein W, selenoprotein R and others. Selenoproteins are enzymes that contain selenium at their active sites. They are responsible for selenium functions in biologic systems (Raymond 2001). Some of these selenoproteins have antioxidant functions, others have unknown functions. However, their role in cancer preventive is not yet known. There are many genes that have been reported to be involved in selenium induced apoptosis and cell cycle arrest such as cell cycle CDK2, CDK5, GADD 153, cyclin D1 and NF-κB (Nuclear factor-kappa B) (El-Bayoumy & Sinha 2005). The study of Martin and Dufour (2008) revealed that there are at least four possible genetic pathways that are involved in the formation of HCC: p53 pathway involved in DNA damage and response, pRB/p16[INK4A] pathway involved in cell cycle control, TGF-B1 pathway involved in growth inhibition and apoptosis, and B-catenin/Axin 1 pathway involved in morphogenesis and signal transduction. However, this review focuses mainly on the effects of p53 in cancer.

Under normal conditions, cells are protected from being cancerous if they have an intact p53. p53, also known as a tumour suppressor protein, is a transcription factor that induces cell cycle arrest (at G1 and/or G2 phase) or apoptosis (programmed cell death) (Jin & Levine 2001). Thus, p53 has dual

functions, first it acts as a highly regulated sequence – specific DNA-binding protein that in response to a wide variety of stress signals such as DNA damage and oncogene activation. Secondly, it acts as a master transcription regulator to induce the expression of many target genes (Dey et al. 2008). Dey and his colleagues also reported that p53 is under control by Mdm2, which is a protein that inhibits the transcriptional activity of p53 and promotes its degradation by the proteasome. The exact criteria that influence p53 to stimulate cell cycle arrest or apoptosis are not fully understood. However, there are several factors that influence the function of p53; these include expression level of p53, the type of stress signal, the cell type and the cellular context at the time of exposure to stress (Balint & Vousden 2001).

Abnormal or damaged cells that are at risk of becoming cancerous are either given time to repair the damage or are eliminated from the body. p53 is present at low levels in a latent state in most normal cells. In response to stress like DNA damage or oncogene activation, the p53 protein undergoes modifications that result in its stabilization, accumulation and activation as a transcription factor that stimulates the synthesis of RNA from DNA (Wilson et al. 1992). It has been reported that approximately 50% of human cancers bear p53 gene mutations and the majority of the remaining cancer cases, p53 activity is compromised by alternative mechanisms (Vogelstein et al. 2000). Cancer begins with DNA mutation, aberrant DNA methylation or defective cell cycle control. DNA is normally protected from cancer causing substances by methyl groups but selenium deficiency can result in decreased DNA methylation and therefore increased DNA damage and mutation (Davis 2000). Ganther (1999) reported that methylation is a major pathway for selenium metabolism in microbes, plants and animals. Methylation of selenium produces less toxic forms and subsequently monomethylated forms of selenium have emerged as a critical class of selenium metabolites having powerful effects on carcinogenesis (Ganther & Lawrence 1997; Clement 1998). Stable methylated selenium compounds such as selenobetaine or Se – methylselenocysteine serve as precursors, similar to a pro-drug, and release methylselenol or methylselenenic acid through the action of cysteine conjugate β- lyase or related lyases. The monomethylated compounds are effective in vitro at very low concentrations to give chemo-preventive effects (apoptosis and cell cycle arrest) (Lu et al. 1995; Sinha et al. 1996; Kaeck et al. 1997; Sinha & Medina 1997). It has been shown that the thioredoxin reductase promotes p53 induction of DNA repair enzyme (Seo 2002). In addition, cells exposed to selenomethioine have shown a 3-fold increase in p53 activity (Longtin 2003). It is important to note that apoptosis can be triggered by selenium independent of DNA damage and cells having a null p53 phenotype (Kaeck et al. 1997). Seo (2002) reported that selenium in the form of selenomethionine on H1299 cells could activate p53 by a redox mechanism independent of DNA damage.

## DEFICIENCY AND TOXICITY OF SELENIUM

When dietary intake of selenium is less than 0.1 µg /100 g body weight per day, Keshan disease is highly to be the outcome. Keshan disease, which was first identified in China in 1935, is a type of heart muscle disease (cardiomyopathy) that is caused by selenium deficiency and affects mainly young children and women. Usual symptoms include loss of appetite, weight loss, fatigue, cardiac insufficiency, cardiomegaly and congestive heart failure. From the histopathological point of view, the heart muscle of Keshan disease shows a multi-focal myocardial necrosis and fibrosis. Li-Qun and his colleagues (2004) reported that at present Keshan disease is rare in China due to the government's application of sodium selenite to growing crops. In fact, Yang & Xia (1995) reported that areas in China, where dietary intakes of selenium are 20 µg /day or more, are free of Keshan disease in children. It is important to note that very low levels of selenium might not be the only cause of Keshan disease. More than one study has reported that Keshan disease may be the result of several interacting causes including a dominant selenium deficiency, vitamin E, polyunsaturated fatty acids and an infectious agents such as coxsackie-virus B3 (Levander & Beck 1997; Li-Qun et al. 2004).

Kachin-Beck disease is a bone and joint disease (osteoarthropathy) that has also been linked with selenium deficiency. It is an endemic in certain areas of China, Siberia and North Korea (Sokoloff 1989; Allander 1994) and also has been found in areas around Lhasa, Tibet (Moreno-Reyes et al. 2003). Clinical symptoms of Kachin-Beck disease include joint necrosis, persistent pain, restricted mobility, deformity of the knees, ankles, fingers and shoulders, and growth retardation (Ge & Yang 1993). Apart from selenium deficiency, Kachin-Beck disease has also been linked with fungal contamination (Chasseur et al. 1997). Being an endemic and rare, scientists worldwide have not studied Keshan and Kachin-Beck diseases in more details. In addition to Keshan and Kachin-Beck diseases as a result of selenium deficiency, selenium deficiency has also been reported to be associated with cancer, coronary heart disease, liver necrosis (Burk et al. 1980; Salonen et al. 1991; Suadicani et al. 1992; Clark et al. 1996; Allan et al. 1999) loss of immuno – competence (Field et al. 2002) and impairment of cell – mediated immunity and B-cell function (McKenzie et al. 1998).

Selenium is not different from other minerals, it has both negative and positive issues. There is a considerable debate about the reference range of selenium toxicity in man, and to a lesser extent, in animals. Jacobs and Frost (1981) reported that selenium becomes toxic at levels of 8 – 10 ppm. On the other hand, another study showed that selenium consumption at 1510 µg per day did not show any toxicity (Yang et al. 1983). One study revealed that an intake of 3200 – 5000 µg selenium per day resulted in toxicity (Reid et al. 2004). However, most studies would recommend that 500 µg selenium per day is the maximum dose for safe consumption in humans. Selenium toxicity (selenosis) was first discovered in

animals in 1930s in South Dakota, USA. At the time, two diseases, Blind Staggers and Alkali diseases, were noticed in livestock feeding on plants containing high selenium soil. Blind staggers is a chronic selenosis that occurs in animals feeding on plants containing 100 to 10,000 ppm selenium (Fan & Kizer 1990). The signs of this disease include blindness, ataxia, anorexia, disorientation, weight loss, and in a later stage, generalized paralysis and death might occur due to failure in respiratory system. Alkali disease is another form of chronic selenosis among livestock resulting from continuous consumption of plants containing 20 to 50 ppm selenium (Fan & Kizer 1990). The signs of this disease include dermatologic changes such as alopecia and hoof necrosis (Combs & Combs 1986).

Worldwide, selenium toxicity in humans is not common but there have been a few cases associated with (Kerdel-Vegas 1964; Yang et al. 1983). For example, in Australia, a 3 year old boy accidentally drank a liquid gun bluing preparation containing 1.8% monohydrated selenium dioxide. A few hours after ingestion, he showed bradycardia, hyper salivation, garlic breath and finally death (Carter 1966). Symptoms of selenium toxicity depend on the severity of toxicity. Selenium toxicity can be acute or chronic. Symptoms of acute selenium toxicity in humans include headache, dizziness, dyspnea, fatigue, nausea, vomiting, garlic breath, a bitter taste in the mouth and irritation of the mucous membranes of the eyes and upper respiratory tract. Whereas the symptoms of chronic selenium (ingesting large amount of selenium over the long term) include depression, nervousness, giddiness, emotional liability, dermatitis, garlic breath, sweat, gastrointestinal disturbances, excess dental caries (Fan & Kizer 1990) and, in extreme cases, loss of hair and fingernails (Yang et al. 1983). The quantity and the chemical form of selenium are the major factors for selenium toxicity. Thus, selenium in organic form, such as selenium-methylselenocysteine, is less toxic than inorganic selenium form such as selenite. In addition to the above, other factors must be kept in mind when measuring the selenium toxicity such as age, species, physiological state, nutrition and dietary interactions and the route of administration (Tinggi 2003). Serum selenium is measured as short term indicator for selenium status whereas hair and nail selenium are measured as long term indicators (Robinson & Thomson 1983; Yang et al. 1989). Hadjimarkos and Shearer (1973) were the first to publish results from studies that used toenail clippings to assess selenium intake and status. Since then, several studies have been reported relationships between toenail selenium concentration and selenium intake (Morris et al. 1983; Hunter et al. 1990; Swanson et al. 1990).


CONCLUSION

HCC remains to be a major life threating disease. Apart from AFP and ultrasonography tests, other serological HCC tumour markers are needed to assess the prognosis and diagnosis at an early stage of HCC. Despite the

successful scientific evidences of selenium as anti-cancer agent against HCC, more researches are needed to explore the actual mechanism of selenium as an anti – cancer agent and also to get closer at the molecular aspects of selenium. The level range of deficiency and toxicity of selenium is narrow thus selenium dietary intake should be well monitored.

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