The Polymorphism of the Glucose-6-Phosphatase Dehydrogenase Enzyme (G6PD) in Hepatocellular Carcinoma

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ABSTRACT
Glucose-6-phosphatase dehydrogenase is an enzyme with an important biochemical role. Its deficiency may be related to cell growth suppression, metastasis and tumor generation. Offering an expectation of protection against the development of some carcinomas, such as hepatocellular carcinoma (HCC), the focus of this study. This is a literature review, articles searched on digital platforms and consolidated literature. Reviewing the pathophysiology of the development of HCC, seeking to understand how G6PD and its deficiency could be related.

Keywords: G6PD deficiency, Hepatocellular carcinoma, Glucose-6-phosphatase dehydrogenase.

1 INTRODUCTION

The discovery of glucose-6-phosphatase dehydrogenase (G6PD) deficiency dates back to the 19th century, when pediatricians began studying children who underwent intense hemolysis from the consumption of fava beans (João Pedro L. 2021). Since then, several researches have been carried out on the subject. This made it possible to establish not only the genetic role and how hemolysis occurred, but also other comorbidities that could be related.

Among them, some diseases presented G6PD deficiency as an aggravating factor, while others showed how it could act positively, preventing the disease and aggravations, as in the case of malaria (Hoffbrand, P.A.H.; Moss, J.E. Pettit, 2013).

Along these lines, some studies point out that G6PD deficiency is related to suppression of cell growth, metastasis, and tumor generation (Dore M.P. at al, 2018). This could, in theory, protect against the development of severe forms of some carcinomas, such as hepatocellular carcinoma.

Hepatocellular carcinoma (HCC) has its development mainly related to viral infections (Hepatitis B and C) and toxic lesions (Aflatoxin, alcohol), and metabolic syndrome associated with diabetes mellitus and non-alcoholic fatty liver disease is also of great importance as a risk factor. (Abbas, A.K.; Kumar, V.; Mitchell, R.N. 2010)

Some studies suggest the possibility that in the face of these conditions that predispose to the development of hepatocellular carcinoma, a person with G6PD deficiency would have a lower chance of developing the carcinoma when compared to a person without a deficiency. However, there are other studies that dispute this statement, and claim that there is no relationship between G6PD deficiency and the development of neoplasms.

The objective of this research was to review previous studies that present both points of view and to evaluate the possibility that G6PD deficiency is considered a protective factor against the development of more severe forms of hepatocellular carcinoma.

Using as a theoretical basis, in addition to articles, published and reputable textbooks, which can offer a basis for understanding the pathophysiology of both comorbidities, and how they are related. Functioning as a basis for understanding the articles published on the subject.

2 MATERIAL AND METHODS

A literature review was carried out, based on the search for published articles on platforms such as Pubmed, Scielo, Periódico Capes, Fapesp, CNPq, google scholar. And reputable textbooks.

The reading of literature published in books served as a basis for understanding the pathophysiology and possible relationship between comorbidities, enabling the development of clinical reasoning.

An intensive search of articles was then carried out on the platforms mentioned above, and all the articles that, upon reading the title and abstract, we considered would add value to the study, were selected.

The selected articles were read and included in the study those that correctly addressed the topics in their content, and those that dealt with subjects other than GCPD deficiency and hepatocellualar carcinoma were excluded.

Thus, the articles were separated by type of study and reread together. To then start writing the present study.

3 RESULTS AND DISCUSSION

Glucose-6-fophosphate dehydrogenase (G6PD) is an enzyme responsible for reducing nicotinamide adenine dinucleotide phosphate (NADP) in NADPH and oxidizing glucose-6-phosphate. NADPH, in turn, plays an important role in the conversion of oxidized glutathione into reduced glutathione, which will help protect against oxidative damage, since it participates as a cofactor in reactions that neutralize compounds such as H2O2. (Abbas, A.K.; Kumar, V.; Mitchell, R.N. 2010)

G6PD deficiency is a sex-linked recessive disease. Because it is related to the X chromosome, males have the highest risk of symptomatic disease (Abbas, A.K.; Kumar, V.; Mitchell, R.N. 2010). Women who carry the mutation have about half the mean erythrocyte G6PD values (Hoffbrand, P.A.H.; Moss, J.E. Pettit,

2013). There are more than 400 variants identified, most of which cause point mutations, leading enzymes to show lower activity than normal (Hoffbrand, P.A.H.; Moss, J.E. Pettit, 2013), among them, the Mediterranean G6PD and G6PD- variants, cause the majority of clinically significant hemolytic anemias, and G6PD B is the most commonly found. (Abbas, A.K.; Kumar, V.; Mitchell, R.N. 2010).

G6PD deficiency is remembered to cause episodic hemolysis. Related to events that predispose to oxidative stress (Abbas, A.K.; Kumar, V.; Mitchell, R.N. 2010), such as infections, acute diseases (such as diabetic ketocytosis), medications, some foods, and other substances, which lead to rapidly progressive intravascular hemolysis. In the inter-crisis period, the patient may be asymptomatic and have a normal blood count. In children, it may be related to neonatal jaundice, which is usually not related to hemolysis per se, but to impairment of normal neonatal liver function. It is rarely associated with nonspherocytic congenital hemolytic anemia. (Hoffbrand, P.A.H.; Moss,J.E. Pettit,2013)..

Due to its importance, G6PD deficiency in Brazil is among the neonatal screening tests evaluated by the heel prick test.

Within medicine, there are several studies that relate both the role of glucose-6-fophosphate dehydrogenase and its deficiency to different comorbidities, such as neoplasms, infections, endocrinological diseases, etc. Relating both severe and asymptomatic disease presentations. There is, for example, evidence that both G6PD deficiency and women carrying the mutation (heterozygous) are resistant to infection by Plasmodium falciparum, which causes malaria (Hoffbrand, P.A.H.; Moss, J.E. Pettit, 2013). Among the comorbidities listed in the studies is hepatocellular carcinoma.

Hepatocellular carcinoma (HCC) is mainly related to viral infections (Hepatitis B and C) and toxic lesions (Aflatoxin, alcohol), and metabolic syndrome associated with diabetes mellitus and non-alcoholic fatty liver disease is also of great importance as a risk factor. Two initial mutational events are known to be the most common, beta-catenin activation and p53 non-activation. (Abbas, A.K.; Kumar, V.; Mitchell, R.N. 2010).

There is evidence that points to the pathogenesis of HCC, the importance of cell death and regeneration cycles in chronic inflammatory states, which increase the risk of mutations in hepatocyte regeneration. There are also studies that point to some role of IL-6, which would suppress the differentiation of hepatocytes and promote their proliferation, through the regulation of the transcription factor HNF4-alpha. (Abbas, A.K.; Kumar, V.; Mitchell, R.N. 2010).

One of the characteristics of cancer cells is to have their metabolism altered. An important component of this metabolism is the pentose phosphate (PPP) pathway (Lu M. at al,2018), it consists of two irreversible oxidation reactions, followed by reversible glucose-phosphate interconversions. The reversible oxidation reactions begin with the oxidation of glucose-6-phosphate, for each oxidized molecule there is the formation of ribulose-5-phosphate, CO2 and two NADPH molecules. Glucose-6-fophosphate

dehydrogenase is responsible for catalyzing the irreversible oxidation reaction of glucose-6-phosphate, and is therefore the main regulator of the pentose-phosphate pathway (Pamela C. Champe, Richard A. Harvey, Denise R. Ferrier, 2009).

G6PD is elevated in many cancers, and the formation of ribose-5-phosphate contributes to tumor growth. Although its role in hapatocellular carcinoma has not been very well established (Lu M. at al,2018).

A study investigating tissue samples from HCC patients found that an increased expression of G6PD was significantly associated with metastases and poor prognosis. They also observed that in vitro Knocdown of G6PD inhibited the proliferation and migration and invasion of HCC cells (Lu M. at al, 2018).

There is also a study that points to the action of G6PD as an inhibitor of ferroptosis in the HCC cell (Cao F. at al, 2021). Ferroptosis is "a type of unscheduled cell death, which involves the participation of iron ions and has an essential component of lipid peroxidation. Iron catalyzes lipid peroxide decomposition reactions, generating lipid derivatives that are highly reactive and toxic to the cell, such as the lipid radicals alkoxyl and peroxyl, and electrophilic lipid derivatives, such as aldehydes and ketones, among others, capable of promoting modifications in biomolecules." (Maria Celia Wider).

Ferroptosis has been studied in recent times, and several studies have tried to understand its role in tumorigenesis. G6PD inhibits ferroptosis in HCC cells through POR (cytochrome P450 oxidoreductase) (Cao F. at al, 2021). Cytochromes P450 are a superfamily of related monoxygenase enzymes, which contain a heme group and participate in a wide variety of reactions (Pamela C. Champe, Richard A. Harvey, Denise R. Ferrier, 2009)., and would promote ferroptosis through peroxidation of saturated phospholipids in the cell membrane (Cao F. at al, 2021). It is proposed that G6PD deficiency suppresses cell growth, metastasis, and tumorigenesis through POR upregulation. (Cao F. at al, 2021) However, it is not yet well defined how POR promotes ferroptosis and how ferroptosis is regulated in tumors.

Thinking about this possibility of G6PD activity as an aggravating factor for hepatocellular carcinoma, there are currently studies that seek compounds capable of pharmacologically inhibiting G6PD activity, and believe that they are a class of safe drugs for the treatment of hepatocellular carcinoma, emphasizing the Chinese similax root extract. (Kanwal L at al, 2022)

Another study shows that the mechanism of action behind Similax is not well known. However, its use in the study, as well as other medications that reduce the action of G6PD, in mice demonstrated anticancer potential (Cocco P, 1987).

The study by MingLu, at al, 2018, reviewed tissue samples from patients with HCC and HCC cell lines. It was shown that increased expression of G6PD was significantly related to the occurrence of metastases and worse prognosis. They also revealed that the inactivation of G6PD *in vitro* inhibits the proliferation, migration, and invasion of HCC cell lines.

On the other hand, the case-control study by Maria Pina Dore at al, 2017. It had a sample of 11,143 clinical records, of which 11.5% (1,280) had G6PD deficiency. It showed an inversely proportional relationship between hepatocellular carcinoma and G6PD deficiency, showing a 55% lower risk of HCC in a patient with G6PD deficiency, even after adjusting for gender, age, marital status, liver disease, and diabetes.

This study did not perform genotyping of patients with G6PD deficiency, thus not differentiating homozygous from heterozygous women.

Liver and bile duct neoplasms are the seventh most prevalent cancer in the world. Of which hepatocellular carcinoma accounts for 70% to 85% of primary hepatic neoplasms. (Flávia Arichelle Cavalcante dos Santos at al, 2019). Table 1 shows statistics on mortality from liver and bile duct neoplasms from 2010 to 2020.

Table 1: Unadjusted proportional mortality due to cancer of the LIVER AND INTRAHEPATIC BILE TRACT, men and women, Brazil, between 2010 and 2020

YEAR	OBITO TOTAL	OBITO CANCER	PERCENTAGE
2010	1136947	7721	0,68
2011	1170498	8100	0,69
2012	1181166	8790	0,74
2013	1210474	8772	0,72
2014	1227039	9170	0,75
2015	1264175	9711	0,77
2016	1309774	9786	0,75
2017	1312663	10201	0,78
2018	1316719	10551	0,8
2019	1349801	10902	0,81
2020	1556824	10764	0,69

Source: MS/SVS/DASIS/CGIAE/Mortality Information System – SIM MP/Brazilian Institute of Geography and Statistics Foundation – IBGE MS/INCA/Conprev/Surveillance Division

Based on these data, it is possible to understand the clinical importance of hepatocellular carcinoma in Brazil. It is a disease of considerable prevalence, with an unfavorable prognosis. In which few cases are amenable to potentially curative surgical intervention. And that when no interventions are performed on the tumor, life expectancy is about 10 months. (Flávia Arichelle Cavalcante dos Santos at al, 2019)

With this in mind, and the following facts: patients who have glucose-6-phosphatase dehydrogenase (G6PD) deficiency are 55% less likely to develop hepatocellular carcinoma (a value higher than other factors considered protective against HCC, such as statins, coffee, white meat and fish, omega-3 fatty acids, vitamin E, and vegetable consumption (Maria Pina Dore, at al, 2017). And increased expression of G6PD is related to a higher occurrence of metastases and worse prognosis (Lu M. at al,2018). This topic opens the discussion

to possible therapies that involve inhibition of G6PD, aiming to interrupt the progression of the disease or even its onset when strong risk factors are identified.

MingLu at al, 2018 cites in their article that "when the genetic sequence of a given gene is known, it is possible to inactivate it by synthesizing an "antisense" oligonucleotide complementary to the messenger RNA (mRNA) produced by that gene (Callegari et al., 2015)". Thus, a point loss-of-function mutation in the G6PD gene may be a proposal for future therapy.

4 CONCLUSION

Considering the pathophysiology of both G6PD deficiency and the development of hepatocellular carcinoma, the relationship in which the enzyme glucose-6-phosphatase dehydrogenase (G6PD) somehow interferes with the progression of HCC seems plausible.

This hypothesis is strongly reinforced by some of the experimental studies presented during the article. However, there is still a need for further studies, both prospective and in vitro, which reinforce not only the hypothesis that patients with G6PD deficiency are less likely to develop hepatocellular carcinoma, but also that if it were possible to block the enzyme in patients with HCC, this would slow or stop the progression of the disease, preventing metastases and early death in those patients who could not benefit from potentially curative therapies.

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