RESEARCH ARTICLE

Efficacy of Glucose Starvation of Cancer Cells in the Progress of Oral Squamous Cell Carcinoma Induced in Hamster

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Abstract

Background and aim of the study: Oral squamous cell carcinoma (OSCC) is the most common form of oral cancer, showing poor prognosis and high mortality. Meanwhile, cancer metabolism is an essential contributor to its progression and response to treatment. This research aims to investigating the effect of a glucose-rich and glucose-free diet on the progress of oral squamous cell carcinoma induced in hamsters. **Materials and Methods:** forty Syrian Hamsters were incubated in two groups. The first one consisted of twenty hamsters, in which the carcinogenic material (DMBA) was applied in the buccal pouch of the hamster three days per week with a glucose-rich diet). The second one was composed of twenty hamsters, in which the carcinogenic material (DMBA) was applied in the buccal pouch three days per week with a glucose-free diet). Hamsters in both groups were sacrificed in groups of five hamsters at a time and at intervals (two weeks, six weeks, ten weeks, and Fourteen weeks). A histological study was performed after conventional staining with hematoxylin and eosin was done. **Results:** After two weeks of the experiment hyperplasia, mild dysplasia, and moderate dysplasia were recorded in hamster buccal pockets with a glucose-rich diet, and after six weeks moderate dysplasia, severe dysplasia, and carcinomas in situ were recorded, after ten weeks severe dysplasia, carcinomas in situ, and OSCC, after fourteen weeks OSCC were recorded. While with a glucose-free diet Hyperkeratosis, hyperplasia, and mild dysplasia were observed after a two-week the experiment, after six weeks, mild dysplasia, moderate dysplasia, and severe dysplasia were recorded, after ten weeks, moderate dysplasia, severe dysplasia, and carcinoma in situ, after fourteen weeks Severe dysplasia, carcinoma in situ, and OSCC were reported. **Conclusion:** our results showed that a glucose-free diet slightly prevents oral squamous cell carcinoma, It may be a supportive treatment in addition to conventional cancer treatment.

Keywords: Oral squamous cell carcinoma (OSCC)- Syrian Hamsters- cancer metabolism- carcinogenic material -DMBA

Introduction

In normal cells, aerobic glycolysis means the conversion of glucose via pyruvate to acetyl-CoA and its complete oxidation which generates 38 molecules of ATP for each molecule of glucose. In contrast, aerobic glycolysis in tumor cells implies the conversion of glucose into pyruvate which generates only two ATP molecules per molecule of glucose and subsequently into the waste product lactic acid (Kroemer and Pouyssegur, 2008). In 1956, Otto Warburg reported that cancer cells exhibit high rates of glucose uptake and lactic acid production, even in the presence of oxygen (Tran et al., 2020). Such acidic conditions support tumor invasion and suppress immunity against cancer (Gatenby et al., 2006). The Warburg effect is generally believed to confer growth advantages to cancer cells including a rapid supply of ATP, which may be essential in cell proliferation (Lemasters, 2021). Warburg effect then has been used in introducing a more

effective treatment for cancer (Mankoff et al., 2007; Elf and Chen, 2014). Many clinical studies were achieved based on the Warburg effect using drug compounds and dietary changes (Tran et al., 2020). Although previous studies have linked intake of sugar with the incidence of cancer and other chronic diseases such as diabetes, cardiovascular disease and non-alcoholic fatty liver disease, its association with mortality remains unknown (Bray, 2012; DiNicolantonio and Lucan, 2014; Tasevska et al., 2014; Cantley, 2014; DiNicolantonio et al., 2015). Oral Squamous Cell Carcinoma represents more than 90% of all oral cancer. OSCC is considered a complicated process that depends on multiple risk factors such as long-term use of tobacco and excessive consumption of alcohol (Givony, 2020). This study aims to investigate the effect of glucose metabolism in oral squamous cell carcinoma and pre-cancerous disorders to better explain the complex process of oral carcinogenesis.

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Materials and Methods

The effect of diet was studied synchronous with the application 7, 12-dimethylbenz[a]anthracene (DMBA) induced oral cancer. DMBA, an aromatic hydrocarbon manufactured by SEGMA, was used. They were used after mixing 1 gram of them in 200 ml of mineral oil with heating and stirring until homogeneity was achieved, and special masks were used in order to avoid toxic fumes resulting from the preparation process and kept in a brown bottle to be used in research (Singh et al., 2021).

The study sample consisted of forty Syrian hamsters. They were incubated in the incubators of the Faculty of Pharmacy - Damascus University. They were weighted and ranged between 50_80 grams under constant conditions (22°C temperature, 12/12 h light/dark cycle). The study was carried out on two main groups, the first consisted of twenty hamsters, in which the carcinogenic material was applied within the buccal pouch three days per week with a glucose-rich diet, while the second one consisted of twenty hamsters in which DMBA was applied within the buccal pouch three days per week with a glucose-free diet (Pourshahidi et al., 2019). we used a size 4 wooden paintbrush to apply the substance to the buccal pouch of the hamster, an average of three times a week, taking into account the change of the brush every two weeks and attention to the change in its number according to the change in the size of the hamsters over time.

The hamsters were sacrificed and the left cheek sinus was removed, to which the carcinogen was applied. Then it was prepared for histological study with hematoxylin and eosin dye.

Hamsters in both groups were sacrificed into groups of five hamsters at a time and at intervals (two weeks, six weeks, ten weeks, and fourteen weeks), In order to study the changes occurring in the hamster's pocket with the progression of time and to study the effect of a sugar-rich diet or sugar-free diet on the histological behavior of the development of squamous cell carcinoma.

Results

Histopathological findings

The histopathological examination of the hamster buccal pouch in the first group (glucose-rich diet) revealed the presence of two cases of hyperplasia, one case of mild dysplasia, two cases of moderate dysplasia (Figure 1-a) after two weeks, while six weeks after the experiment, two cases of moderate dysplasia, one case of severe dysplasia (Figure 1-b), and two carcinomas in situ were recorded.
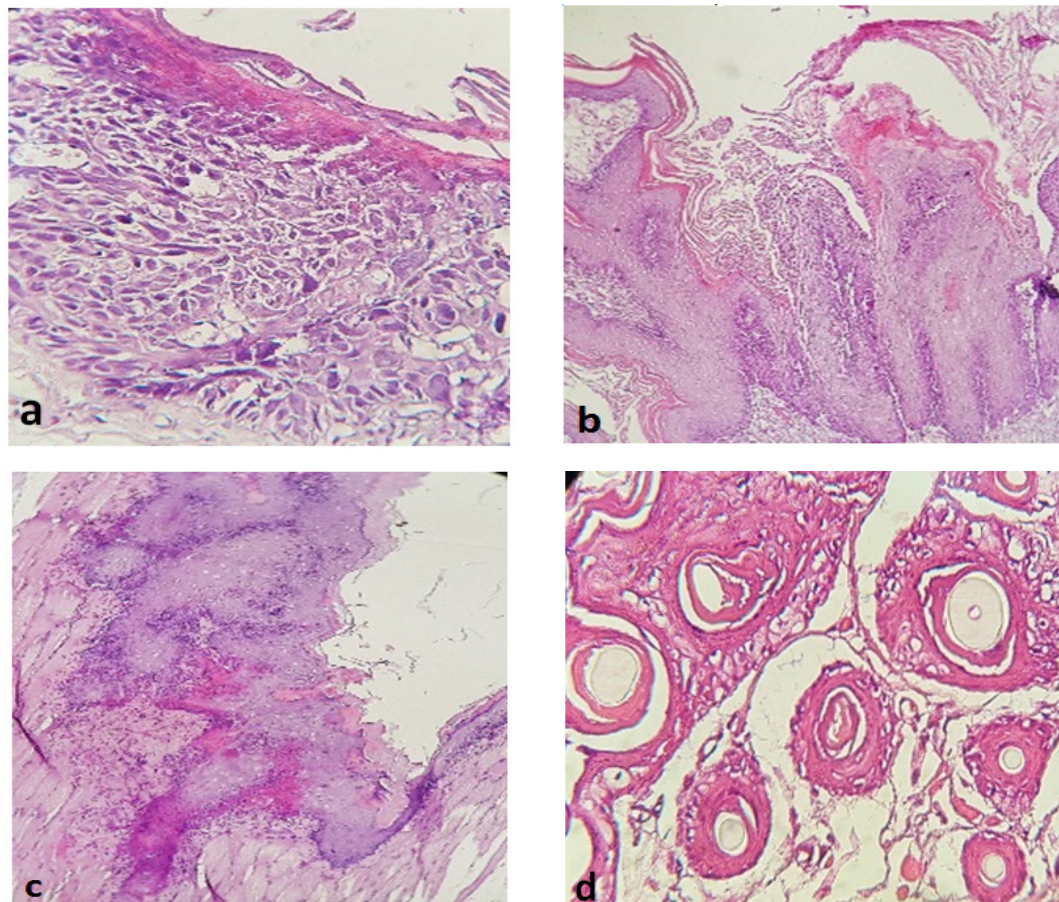

Figure 1. Photomicrographs of the Cheek Pocket Tissue Showing Hematoxylin and Eosin-Magnification 40x) in the First Group (group in which the carcinogenic material was applied within the cheek pocket with a diet glucose-rich): a, the case of moderate dysplasia after two weeks after the experiment in the first group; b, the case of severe dysplasia after six weeks after the experiment in the first group; c, the case of carcinomas in situ after ten weeks after the experimentin the first group; d, the case of oral squamous cell after fourteen weeks after the experiment in the first group.

Table 1. Mann-Whitney Test has been Used to Investigate a Binary Comparison.

The time period	groups	number	U value	P value	Differentiation
After two week	DMBA + diet (glucose-rich)	5	50	0,100	No Significant differences
	DMBA + diet (glucose-free)	5			
After six weeks	DMBA + diet (glucose-rich)	5	75	0,281	No Significant differences
	DMBA + diet (glucose-free)	5			
After ten weeks	DMBA + diet (glucose-rich)	5	65	0,178	No Significant differences
	DMBA + diet (glucose-free)	5			
After fourteen weeks	DMBA + diet (glucose-rich)	5	75	0,134	No Significant differences
	DMBA + diet (glucose-free)	5			

The results were computed and subjected to statistical analysis using the; Mann-Whitney test: Statistical analysis indicates to be statistically not significant between the groups

After ten weeks, two cases of severe dysplasia, two cases of carcinomas in situ (Figure1-c), and two cases of squamous cell carcinoma were recorded, After fourteen weeks of the experiment, five cases of squamous cell carcinoma with keratin pearls were recorded (Figure 1-d).

The histopathological examination of the hamster buccal pouch in the second group (glucose-free diet) revealed the presence of two cases of hyperkeratosis, two cases of hyperplasia (Figure 2-a), and one case of mild dysplasia, while six weeks after the experiment, one case of hyperplastic, one case of mild dysplasia (Figure 2-b, two cases of moderate dysplasia, and one case of severe dysplasia were recorded. After ten weeks, A case of moderate dysplasia, two cases of severe dysplasia, and two cases of carcinoma in situ (Figure 2-c) were recorded. After fourteen weeks of the experiment, one case of Severe dysplasia, two cases of carcinoma in situ, and two squamous cell carcinomas (Figure 2-d) were reported in the second group.

Statistical analysis

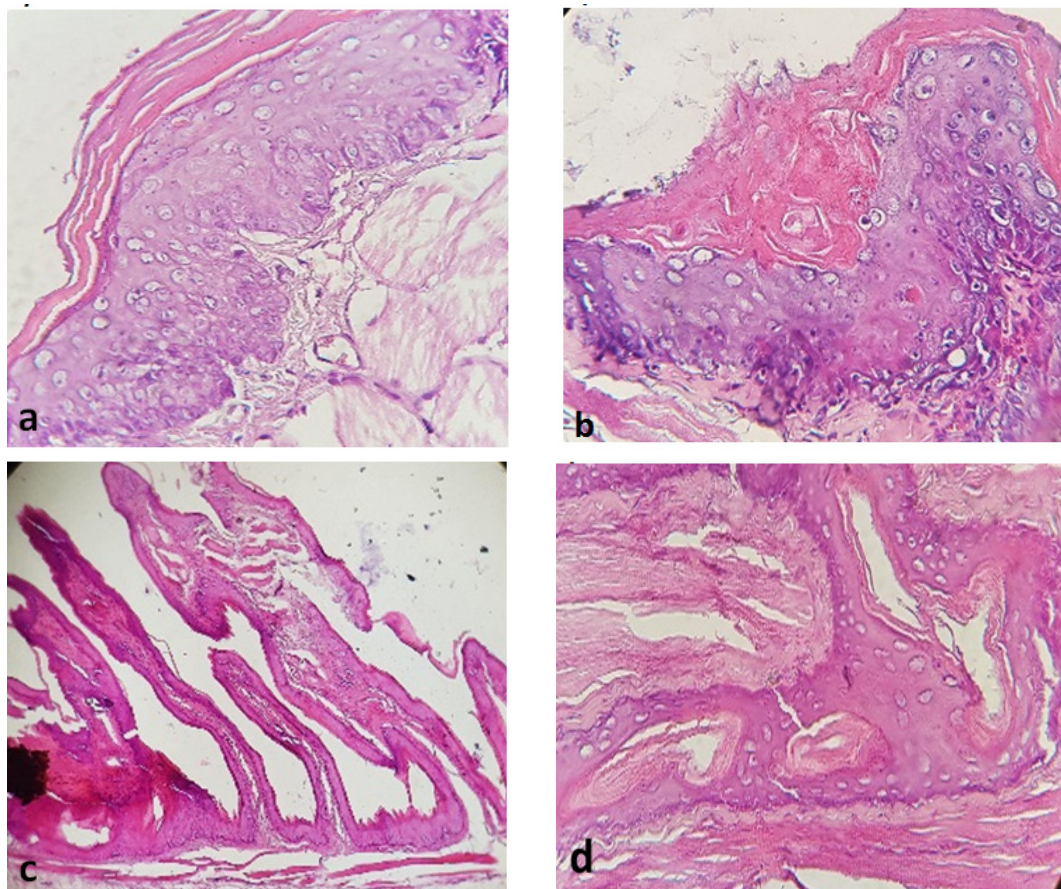The results were computed and subjected to statistical

Figure 2. Photomicrographs of the Cheek Pocket Tissue Showing Hematoxylin and Eosin-Magnification 40x) in the Second Group (group on which the carcinogenic material was applied within the cheek pocket with a diet glucose-free. a, the case of hyperplasia after two weeks after the experiment in the second group; b, the case of mild dy dysplasia after six weeks after the experiment in the second group; c, the case of carcinoma in situ after ten weeks after the experiment in the second group; d, the case of oral squamous cell carcinomas after fourteen weeks after experiment in the second group.

analysis using the Mann-Whitney test (Table1):

It is noted in the above table that the value of the significance level is much greater than the value of 0.05 regardless of the time period studied, that is, at the 95% confidence level, Statistical analysis indicates to be statistically not significant between the groups.

Discussion

Metabolism is an essential process for all cellular functions. For decades, there has been increasing evidence of a relationship between metabolism and the proliferation of malignant cells. Unlike differentiated normal cells, cancer cells reprogrammed their metabolism in order to meet their energy needs (Li et al., 2021).

Cancer cells display important alterations in several metabolic pathways, such as glycolysis and glutaminolysis, which include the tricarboxylic acid (TCA) cycle, the electron transport chain (ETC), and the pentose phosphate (PPP) pathway (Chen and Russo, 2012). Since the discovery of the Warburg effect, the metabolism of cancer cells has been shown to play an important role in cancer survival and growth. Recent research indicates that glutamine's involvement in cancer metabolism is more important than previously thought Glutamine, a non-essential amino acid with both amine and amide functional groups (Li et al., 2021), provides a continuous source of nitrogen for the de novo synthesis of nucleotides and proteins in cancer cells (Sharma et al., 2022). Aerobic glycolysis is an effect and not the cause of OxPhos dysfunction in cancer (Liberti and Locasale, 2016).

Cancer cells cannot proliferate or grow without carbons and nitrogen for the synthesis of metabolites and ATP. Glucose carbons are necessary for metabolite synthesis through the aerobic glycolysis and pentose phosphate pathways (PPP) while glutamine nitrogen and carbons are necessary for the synthesis of nitrogen-containing metabolites and ATP through the glutaminolysis pathway (Seyfried and Chinopoulos, 2021). Head and neck squamous cell carcinoma are considered the sixth most common species of cancer in the world. The majority of patients present advanced-stage disease and have poor survival. Therefore, it is important to search for new biomarkers and new alternative and effective treatment options In addition to traditional treatment. Most cancer cells rely on aerobic glycolysis to generate energy (ATP) and metabolic intermediates. This phenotype is a hallmark of cancer, characterized by an increase in glucose consumption and the production of high amounts of lactate. Consequently, cancer cells need to up-regulate many proteins and enzymes related to glycolytic metabolism (Simoes-Sousa et al., 2016). Schroeder and colleagues' study found decreased lactate concentrations in tumor tissue after following a ketogenic diet (a glucose-free diet) in head and neck squamous cell carcinoma (Schroeder et al., 2013). In a study based on an investigation of an association exists between diet and oral squamous cell carcinoma (OSCC) in a Brazilian population, this population-based study investigated food groups intake by means of a quantitative food frequency questionnaire, 665 individuals were included, being 133 cases of OSCC, selected from reference hospitals for cancer in Paraíba and 532 being part of a control group, Data of the research suggested that the ingestion of refined carbohydrates were associated with OSCC cases (de Oliveira Bezerra et al., 2017).

Although many questions about the metabolic features of OSCC have already been answered in the metabolic studies, further validation and improvement are still required to translate these findings into clinical applications. The current study was conducted to estimate the effect of a glucose-rich diet or glucose-free diet on OSCC in a hamster model by histological stages.

In our study, histological examinations were analyzed in hamsters. There are no statistically significant differences in the results of traditional staining, and this indicates that oral squamous cell carcinoma cells cannot be starved by glucose deprivation only due to a lack of targeting of glutamine. In the study of Ogawa et al, to characterize the metabolic system of oral squamous cell carcinoma (OSCC) by metabolome analysis in (32 patients) using capillary electrophoresis and a time-of-flight mass spectrometer, Their results indicated that the aerobic glycolysis in OSCC stemmed from the combined enhancement of glucose consumption and glutaminolysis, and may provide new strategies to control the clinical behavior of OSCC based on metabolism (Ogawa et al., 2014).

Ketogenic diets (KD) are high in fat and low in carbohydrates, forcing cells to take advantage of fatty acid oxidation in mitochondria for energy production. As cancer cells show increased mitochondrial oxidative stress relative to normal cells. KD might selectively enhance metabolic oxidative stress in head and neck cancer cells, and sensitize them to radiation and platinum chemotherapy without causing increased toxicity in surrounding normal tissues. This hypothesis was tested in preclinical murine xenografts in a previous study, mice bearing human head and neck cancer xenografts were fed either standard mouse chow or (a glucose-free diet) and exposed to ionizing radiation, Mice-bearing xenografts that received radiation and a glucose-free diet showed a slight improvement in tumor growth rate and survival compared to mice that received radiation alone (Ma et al., 2021), This is consistent with the conclusion of our research that showed that a glucose-free diet slightly prevents oral squamous cell carcinoma.

In another study, ketogenic a glucose-free diet and a glutaminase inhibitor (DON) (6-diazo-5-oxo-l-norleucine) were used to treat glioblastoma in vitro (Mukherjee et al., 2019), The keto diet restricts glucose availability while elevating ketone bodies and thus induces competition between normal cells and tumor cells for glucose, Ketone bodies, and fatty acids are non-fermentable. Ketone body elevation under fasting (nutritional ketosis) can allow blood glucose to reach extremely low levels without adverse effects (Drenick et al., 1972). Glucose restriction will inhibit the growth of cancer cells, as the glucose carbon is essential for

the synthesis of growth metabolites through the pentose phosphate and glycolysis pathways. Hence, simultaneous restriction of glucose and glutamine, while on a ketogenic diet, will reduce the acidity in the tumor microenvironment and will target both the glutamine and glutamine degradation pathways essential for tumor cell growth and survival (Seyfried et al., 2019).

Although reducing carbohydrates to varying degrees is a popular and controversial dietary trend, the potential long-term effects on health and cancer are largely unknown (Nilsson et al., 2013). Reduction in the consumption of sugar can substantially reduce the burden of non-communicable diseases. Public health interventions to facilitate this behavioral change must be instituted and encouraged (Gupta et al., 2018). Adopting a healthy lifestyle may be the most effective way to reduce the risk of cancer development. These include regular exercise, eating a healthy diet, staying away from smoking, and not drinking too much alcohol (Yeung, 2019).

The conclusion of our research showed that a glucose-free diet slightly prevents oral squamous cell carcinoma, It may be a supportive treatment in addition to conventional cancer treatment. and oral squamous cell carcinoma cells cannot be starved by glucose deprivation only due to a lack of targeting of glutamine.

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Author Contribution Statement

All authors contributed equally in this study.

Acknowledgements

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