



## Pyruvate kinase and haemoglobin levels in breast carcinoma patients

 Ravi Babu.Birudu<sup>1,\*</sup> and M.Jagadish Naik<sup>2</sup>
<sup>1</sup>Department of Biotechnology, Acharya Nagarjuna University, Nagarjuna Nagar-522510, Guntur, Andhra Pradesh, India.

<sup>2</sup>Department of Zoology & Aquaculture, Acharya Nagarjuna University, Nagarjuna Nagar-522510, Guntur, Andhra Pradesh, India.

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### ABSTRACT

Breast cancer is the second leading cancer among the women in the world. India is the one of the country having the highest breast cancer patients. In breast cancer patients the glycolysis rate is very high when compare to normal glycolysis rate in normal women. The enzymes like pyruvate kinase, hexokinase, phosphofructokinase levels are different concentrations among the breast cancer patients. The high rate of glycolysis observed in cancer at anaerobic conditions is known as Warburg effect. The Warburg effect is the enhanced conversion of glucose to lactate observed in tumor cells, even in the presence of normal levels of oxygen. The pyruvate kinase level in tumors are high optimum density values when compared to normal tissues values. The mean values of pyruvate kinase enzyme is 0.278 in tumor tissue and 0.200 in normal tissues. There is a significant effect of haemoglobin on pyruvatekinase values. There may be some significant effect of pyruvate kinase on haemoglobin and vice versa.

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### Introduction

In breast cancer patients the glycolysis rate is very high when compare to normal glycolysis rate in normal women. The enzymes like pyruvate kinase, hexokinase, phosphofructokinase levels are different concentrations among the breast cancer patients. The high rate of glycolysis observed in cancer at anaerobic conditions is known as Warburg effect. The Warburg effect is the enhanced conversion of glucose to lactate observed in tumor cells, even in the presence of normal levels of oxygen. Otto Heinrich Warburg demonstrated in 1924 that cancer cells show an increased dependence on glycolysis to meet their energy needs, regardless of whether they were well-oxygenated or not. Converting glucose to lactate, rather than metabolizing it through oxidative phosphorylation in the mitochondria, is far less efficient as less ATP is generated per unit of glucose metabolized.

Breast cancer is the most commonly occurring female cancer in the world with an age standardized incidence rate (ASR) of 39.0 per 100,000, which is more than double that of the second ranked cancer (cervical cancer ASR=15.2 per 100,000(1.2). Breast cancer accounts for 23% of all newly occurring cancers in women worldwide and represents 13.7% of all cancer deaths (6). It is the most frequent cancer in both developed and developing regions (estimated 690,000 new cases in each region) as well as the most frequent cause of cancer death in these regions (280,000 deaths in developing countries) of the world (2). Although incidence rates are higher in the West, the disability adjusted life years (DALY's) show the highest burden for breast cancer in middle-income countries (3,144,000 vs. 1,856,000 in high-income and 1,626,000 in low-income countries), where there are increasing incidence rates and a higher proportion with late stage of disease at diagnosis (3).

India is a country with wide ethnic, cultural, religious, economic diversities and variations in the health care infrastructure. The health care facility pattern is heterogeneous, with numerous regions where the benefits of breast cancer awareness, early diagnosis and multidisciplinary treatment

programs have not yet reached(4) Global breast cancer incidence increased from 641,000 (95% confidence intervals 610,000—750,000) cases in 1980 to 1,643,000 (1,421,000—1,782,000) cases in 2010, an annual rate of increase of 3.1% (5). For women aged 15—49 years, twice as many breast cancer cases were recorded in developing countries than in developed countries. This variation in incidence may be due to multiple factors, including geographic variation, racial/ethnic background, genetic variation, lifestyle, environmental factors, socioeconomic status, the presence of known risk factors, and utilization of screening mammography, stage of disease at diagnosis and the availability of appropriate care( 6) India has a huge population base of more than 1 billion people, who are genetically, culturally and socio-economically diverse. It is this diversity which global biopharmaceutical companies wish to leverage to accelerate recruitment in their clinical trials. Patient recruitment and site data taken from the Clinical Trial Registry of India shows that in the years 2009-11, each site was able to recruit an average of 9.7 patients22. Hereditary susceptibility to breast cancer has led to the identification of several susceptibility genes, including BRCA1, BRCA2, TP53, PTEN/MMAC1 genes. Pyruvate kinase promotes the Warburg effect in cancer cells (7) PK catalyses the phosphoenol pyruvate to pyruvate in glycolysis. Lowering PK activity expected to produce less pyruvate or prevent complete conversion of glucose to pyruvate.1 molecule of glucose produce molecules of pyruvate in the presence of pyruvate kinase enzyme.PK consist of four is forms(8). Pyruvate kinase M2(PKM2) is shown to be vital for cancer metabolism and critical to tumor growth (9). Out of the four isoforms of pyruvate kinase L, R, M1 and M2, proliferating embryonic and tumor cells predominantly express M2. This isoform switch is a pre-condition for aerobic glycolysis to occur.

**Phospho(enol) pyruvate + ADP Pyruvate Kinase> Pyruvate + ATP**

Present study includes the estimation of the pyruvate kinase from the fresh tissues collecting from the breast after

mastectomy. The estimation includes the normal tissue cells and the breast carcinoma.

Anaemia is a common symptom and complication in patients with solid malignant tumours (10). In these patients anaemia is multifactorial and may occur as either a direct effect of the cancer (blood loss, bone marrow infiltration or nutritional deficiencies), as a result of the cancer treatment itself, or due to chemical factors produced by the cancer (11). Anaemia can lead to a wide array of symptoms that could negatively affect patients' physical status and functional capacity and subsequently impair their quality of life (QOL). Notable among these symptoms are fatigue, dyspnoea, palpitations and other cardiovascular complications, cognitive dysfunction, depression, nausea, sexual/reproductive. Dysfunction and impaired immune function (12, 13). Cancer related anaemia has been related to a deterioration of patients' QOL, through a higher rate of fatigue and impairment in cognitive function (14). The incidence of tumor-related anaemia and its clinical value has been investigated in various human malignancies including breast cancer and gynecological cancer (15,16). The treatment of tumour-related or chemotherapy-induced anaemia with supplemental iron therapy or erythropoietic growth factor has been increasing in an attempt to improve the QOL of patients with cancer (17). Adjuvant endocrine and/or combination chemotherapy remain the mainstay of systemic treatment for patients with early-stage breast cancer; (18). Anaemia is a common occurrence in patients with breast cancer; its prevalence depending on the extent of the disease and type and duration of anticancer therapy (19, 20).

Breast cancer patients undergoing adjuvant therapy commonly suffer from a decline in hemoglobin (Hb) concentration, which in cancer patients has been related to higher rates of fatigue and depression, combined with a deterioration in physical functioning (21), quality of life(22), and survival (23). Kirshner and colleagues (24) reported a ~40% increase in the incidence of moderate to severe anemia (Hb levels <10 g/dl) in stage II and III breast cancer patients undergoing adjuvant doxorubicin and cyclophosphamide chemotherapy. Treatment of severe anemia may require erythrocyte transfusion, but this carries the risk of transfusion reactions and infectious disease transmission. Erythropoietic supportive agents (ESA) are only moderately effective and their use in cancer-related anemia increases thromboembolic complications and hypertension, and has been associated with adverse effects on survival (25-27). Furthermore, recent evidence suggesting tumor-stimulating effects of ESAs are of particular concern in the curative-intent setting of adjuvant breast cancer chemotherapy (28).

#### Materials and Methods

**Estimation of pyruvate kinase enzyme form the breast tumors:** Pyruvate kinase (PK) is a key enzyme in glycogen metabolism. Mammalian pk of different distinct ,their characteristics being related to tissue metabolic requirements (10). To estimate the pyruvate kinase activity we use the pyruvate kinase activity assay kit (catalog #K-709-100:store kit at -200C) from BioVision. The assay kit is useful for the measuring pyruvate kinase activity in various biological samples such as blood, tissues, and culture cells ect. In the assay phosphoenol pyruvate and ADP were catalyzed by PK to generate pyruvate and ATP. The generated pyruvate is oxidized by pyruvate oxidize to produce color at 570 nm. since the increase in color or fluorescence intensity is proportional to the increase in pyruvate amount ,the PK activity can be accurately measured. The kit detects 0.1mU pyruvate kinase. As we used

the kit contains the PK assay buffer, OxiRed probe, PK Enzyme mix, PK Substrate mix, PK Positive control, pyruvate standard (100ul). Firstly we measure the protein concentration of the normal breast tissue cells and the breast tumor tissue cells.

#### Preparation of reagents:

- OxiRed Probe: Ready to use as supplied. Allow to come to room temperature before use to melt frozen DMSO and stored at -200C.
- PK substrate mix, PK Enzyme mix: dissolve with 220ul diH<sub>2</sub>O. Pipette up and down to completely dissolve and stored at -200C.
- Pyruvate kinase positive control: Dissolve with 100 ul of diH<sub>2</sub>O. Pipette up and down to completely dissolve and stored at -200C.

#### Experimental design:

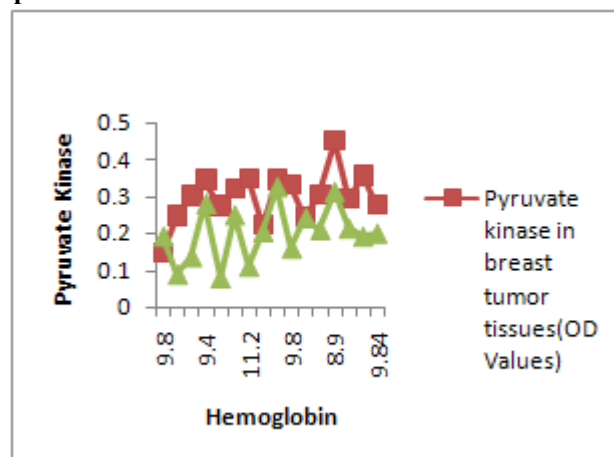
50 mg of tissue collected different breast cancer patients, (15 samples of normal and 15 samples of tumor tissues). To 50 mg of tissue we add 200µl of pyruvate kinase assay buffer, then centrifugation done at 12,000 rpm for 15 minutes and then collect the 5 ml of supernatant. For 5ml of supernatant added 95µl of reagent A and 100µl of water and 800µl of reagent B, then incubated for 10 minutes. The solution's optimum density (OD) values are taken from spectrophotometer at 750nm

#### Haemoglobin Estimation

The blood samples were collected from Bommidala cancer hospital, Guntur. The samples are collected in between date of admission to date of discharge from hospital. it includes the time of diagnosis, mastectomy, and chemotherapy. 15 women breast cancer patients blood samples are collected from the hospital and the estimation of hemoglobin is obtained from diagnosis laboratory. The values obtained from the laboratory are analyzed with compared to breast cancer. The cell count instrument is used for the analysis of blood.

**Result & discussion:** The haemoglobin and pyruvate kinase values of the breast cancer patients are compared. The haemoglobin values between 9.1 to 10.2 have the pyruvate kinase values are having the average levels of pyruvate kinase. haemoglobin at 8.9 shows the highest level of pyruvate kinase in normal and tumor tissues. all over the haemoglobin values above the 9 are showing highest pyruvate kinase when compared to normal tissues. At 11.2 haemoglobin level, the tumor pyruvate values are nearly three times higher than the normal values. at 8.1 haemoglobin level shows same as the 11.2 level. The mean value of the haemoglobin 9.84 shows the higher pyruvate kinase levels in tumor tissues and low levels in normal tissues.

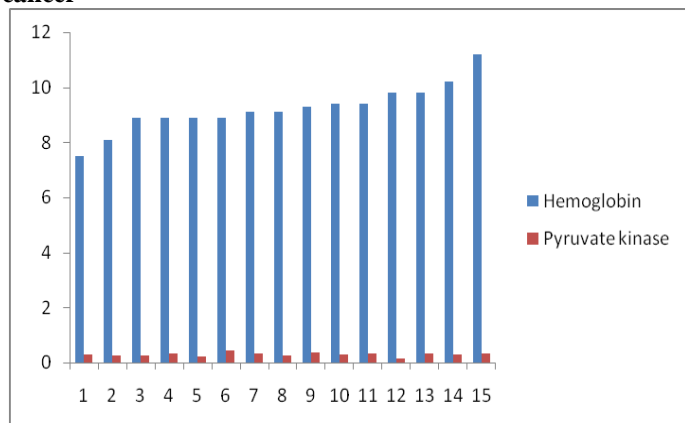
#### Graph 1



### Haemoglobin and pyruvatekinase levels in breast cancer patients (Table.1 &graph1)

Serial Number	Haemoglobin (grams/dl)	Pyruvate kinase in breast tumor tissues(OD Values)	Pyruvate kinase in normal breast tissues(OD Values)
1	9.8	0.146	0.192
2	8.9	0.246	0.090
3	7.5	0.300	0.137
4	9.4	0.344	0.280
5	8.1	0.273	0.080
6	8.9	0.321	0.250
7	11.2	0.347	0.111
8	8.9	0.222	0.205
9	9.1	0.344	0.325
10	9.8	0.329	0.160
11	9.1	0.245	0.242
12	9.4	0.305	0.210
13	8.9	0.450	0.312
14	10.2	0.294	0.214
15	9.3	0.355	0.192
Mean Values	9.84	0.278	0.200

### Haemoglobin and tumor pyruvate kinase levels in breast cancer



**Conclusion:** The present study shows that there is a significant impact of haemoglobin on the pyruvate kinase in breast cancer development. The mean values show, there is greater difference among the pyruvate kinase levels at same haemoglobin levels, but the haemoglobin levels are anaemic in all patients. Pyruvate kinase enzyme may be reason for the anaemic conditions and breast tumor development.

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