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COMPARISON OF STABILITY OF BLOOD LACTATE IN F/OX AND PLAIN BULB STORED AT 2- 8^{0}C AFTER 24 HOURS AND 48 HOURS.

Biochemistry

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ABSTRACT

SUMMARY : This study was conducted in the clinical laboratory in the department of biochemistry, Government medical college, Jalgaon for a period of 3 months . A total of 30 subjects were taken randomly and 2 ml blood was collected in each of the F/OX bulb and plain bulb by phlebotomy after a rest of 30 minutes.The blood lactate was measured at 0 hrs (within 15 minutes), 24 hrs and 48 hrs from both F/OX bulb and plain bulb.For the Quantitative measurement of plasma lactate, enzymatic lactate oxidase-peroxidase method was employed .We observed a significant change in blood lactate level in plain bulb preserved in 2-8oC after 24 hours and 48 hours as compared to F/OX bulb preserved in 2-8oC. The stability of lactate in F/OX preserved sample was maintained for 48 hours.Thus we conclude that F/OX bulb should be preferred over plain bulb to measure the lactate level.

KEYWORDS

F/OX bulb, blood lactate, anaerobic glycolysis.

Lactate, the end product of anaerobic oxidation of glucose through glycolysis, is formed by the reduction of pyruvate with the help of the enzyme lactate dehydrogenase. It is the final product of anaerobic glycolysis. It is formed in vigorous contracting muscle[1]. The formation of lactate is the major fate for pyruvate in lens and cornea of the eye, kidney medulla, testes, leukocytes and red blood cells, because these are all poorly vascularized and/or lack mitochondria[2]. It is also formed inside tumour cells where the main energy source for tumour growth is anaerobic glycolysis[3]. The measurement of blood lactate is used to access the metabolic or circulatory impairment. The blood lactate is increased in condition like shock, sepsis, severe anaemia, liver disease, alcoholic ketoacidosis, pancreatitis, lymphoma, lung cancer, metabolic disorders like von gierke's disease, pyruvate carboxylase deficiency and drug intake like biguanides, β adrenergic agents, salicylates etc[4].

Traditionally lactate estimation is done within 15 minutes of acquisition of sample in a plain bulb or it is to be centrifuged within 15 minutes and separated of the blood cells. This is because, the anaerobic glycolysis continues in the red blood cells present in the plain bulb leading to the formation of lactate and falsely elevating the blood lactate level[5]. Many times, it is not possible to estimate blood lactate level within 15 minutes. To overcome this difficulty we have studied the use of sodium fluoride/potassium oxalate (F/OX) bulb (which is routinely used to measure blood glucose) for measuring the stability of blood lactate stored in the temperature of 2- 8^{0}C. We have compared this with lactate measurement in plain bulb stored at 2- 8^{0}C. It is known that fluoride present in the F/OX bulb inhibits the glycolytic enzyme enolase and thus prevent the decrease in the level of glucose present in the blood[6]. We have used same anti-glycolytic agent to prevent the conversion of glucose to lactate.

AIM AND OBJECTIVES:

To find out the stability of blood lactate in F/OX and plain bulb stored at 2- 8^{0}C after 24 hours and 48 hours.

MATERIAL AND METHODS:

The study was conducted in the clinical laboratory in the department of biochemistry, Governtment medical college, Jalgaon for a period of 3 months. A total of 30 subjects were taken randomly and 2 ml blood was collected in each of the F/OX bulb and plain bulb by phlebotomy after a rest of 30 minutes. Informed consent was taken from all subjects. For the preparation of F/OX bulb 5 gm sodium fluoride and 15 gm potassium oxalate was added in 500 ml distilled water and 0.5 ml of this solution was used for each F/OX bulb preparation. The blood lactate was then measured at 0 hrs (within 15 minutes), 24 hrs and 48 hrs from both F/OX bulb and plain bulb. In between the measurements both sample were kept in the refrigerator at 2-8^{0}C. For the Quantitative measurement of plasma lactate, enzymatic lactate oxidase-peroxidase method was employed. In this method lactate is oxidized by lactate oxidase (LO) to hydrogen peroxide and pyruvate, which under the influence of Peroxidase (POD), 4-aminophenazone and 4-cholorophenol form a red quinine compound. The intensity of the color is measured at 505 nm in a colorimeter against the standard (10 mg/dL) and water blank. The plasma which is measured should be free of hemolysis[5].

Before taking the blood care was taken that the patient had not done any strenuous exercise before the blood is withdrawn. Patients on intravenous injections of epinephrine, glucose, bicarbonate or other infusions that modify the acid – base balance, causing elevation of lactate were not selected for the study[5].

RESULTS AND OBSERVATION:

The following measurements were observed in both plain bulb and F/OX bulb at 0 hr, 24 hr and 48 hr. At '0' hours, the mean value of blood lactate level was same in both bulbs.

Table 1.The mean value of lactate in plain bulb after '0' hours and '24' hours.

At '0' hours (mg/dl)	Plain Bulb at '24' hours (mg/dl)	Paired 't'test
19.14	30.5	p<0.0001

The mean value of lactate in plain bulb at '0' hours and '24' hours was found to be 19.14 mg/dl and 30.5 mg/dl respectively. Paired t-test was applied on the data on table 1 by comparing the mean value of lactate at '0' hours and '24' hours in plain bulb respectively. There was a significant difference in the mean values of lactate in plain bulb at 24 hours compared with values at '0' hours (p<0.0001).

Table 2.The mean value of blood lactate in F/OX bulb after '0' hours and '24' hours.

At '0' hours (mg/dl)	F/OX Bulb at '24' hours (mg/dl)	Paired 't' Test p value
18.03	18.64	p=0.1002

The mean value of blood lactate in F/OX at '0' hours and '24' hours was found to be 18.03 mg/dl and 18.64 mg/dl respectively. Paired t-test was applied on the data on table 2 by comparing the mean value of blood lactate at '0' hours and '24' hours in F/OX bulb respectively. There was no significant difference in the mean values of lactate in plain bulb at 24 hours compared with values at '0' hours (p=0.1002).

Table 3. The mean value of lactate in plain bulb after '0' hours and '48' hours.

At '0' hours (mg/dl)	Plain Bulb at '48' hours (mg/dl)	Paired 't'test
19.14	37.2	P<0.0001

Also, the mean value of lactate in plain bulb at '0' hours and '48' hours was found to be 19.14 mg/dl and 30.5 mg/dl respectively. Paired t-test was applied on the data on table 3 by comparing the mean value of lactate at '0' hours and '48' hours in plain bulb respectively. There was a significant difference in the mean values of lactate in plain bulb at '48' hours compared with values at '0' hours (p<0.0001).

Table 4.The mean value of blood lactate in F/OX bulb after '0' hours and '48' hours.

At '0' hours (mg/dl)	F/OX Bulb at '48' hours (mg/dl)	Paired 't'test
18.03	18.7	P=0.06

The mean value of blood lactate in F/OX at '0' hours and '48' hours was found to be 18.03 mg/dl and 18.7 mg/dl respectively. Paired t-test was applied on the data on table 4 by comparing the mean value of blood lactate at '0' hours and '48' hours in F/OX bulb respectively. There was no significant difference in the mean values of lactate in plain bulb at '48' hours compared with values at '0' hours (p=0.06).

DISCUSSION:

A significant change in blood lactate level was observed in plain bulb preserved at 2-8°C after 24 hours and 48 hours as compared to F/OX bulb preserved at 2-8°C. The stability of lactate in F/OX preserved sample was maintained for 48 hours. In glycolysis enzyme Enolasecatalyzes the formation of phosphoenolpyruvatefrom 2-phosphoglycerate. The mechanism of the enolase reactioninvolves an enolic intermediate stabilized by Mg^{2+}.The enzyme is strongly inhibited by fluoride ion in the presence of phosphate. Inhibition arises from the formation of fluorophosphate, which forms a complex with Mg^{2+} at the active site of the enzyme[6]. Thus sodium fluoride present in F/OX inhibits the enzyme enolaseand inhibits glycolysis and prevents the conversion of glucose to pyruvate in aerobic condition and to lactate in anaerobic condition present in F/OX bulb. Also keeping the blood at 2-8°C significantly decreases the enzyme activity and glycolytic process.

CONCLUSION:

Thus F/OX bulb can be used to measure the lactate by collecting blood in it. The stability of the lactate in F/OX bulb was found to be around 48 hours when stored in 2-8° C, as compared to plain bulb which showed significant change in 24 hours.

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