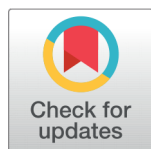


# Lactate dehydrogenase C4 (LDH-C4) is essential for the sperm count and motility: A case-control study

Baraa A. Saeed<sup>1</sup>, Rayah S. Baban<sup>2</sup> and Usama S. Alnasiri<sup>2</sup>

<sup>1</sup>Department of Biology, Al-Farabi University College, Baghdad, Iraq

<sup>2</sup>Department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University, Baghdad, Iraq



Received 01-07-2021

Revised 21-08-2021

Accepted 24-08-2021

Published 30-08-2021

## Corresponding Author

Rayah S. Baban

[baban.rs9@nahrainuniv.edu.iq](mailto:baban.rs9@nahrainuniv.edu.iq)

Department of Chemistry and  
Biochemistry, College of  
Medicine, Al-Nahrain University,  
Baghdad, Iraq

DOI [https://doi.org/10.47419/  
bjbabs.v2i03.65](https://doi.org/10.47419/bjbabs.v2i03.65)

Pages: 152-165

Distributed under  
the terms and conditions of the  
Creative Commons  
Non-Commercial 4.0  
International License (CC BY-NC  
4.0), which permits unrestricted  
use, distribution, reproduction  
and adaptation in any medium  
and for any purpose provided that  
it is properly attributed.

**Copyright:** © 2021 Baraa A.  
Saeed, Rayah S. Baban, Usama  
S. Al-Nasiri

## ABSTRACT

**Background:** The lactate dehydrogenase C4 (LDH-C4) isoenzyme is an important enzyme involved in metabolic processes that are needed for spermatogenesis and sperm motility.

**Objectives :** This study aims to assess the activity and kinetic parameters (maximum velocity,  $V_{max}$  and Michaelis constant,  $K_m$ ) of LDH-C4 in fertile and infertile (azoospermia and oligospermia) men in Baghdad City, Iraq.

**Methods :** A total of 120 participants (80 infertile and 40 healthy fertile men) were included in the current study. The patients were sub-grouped into: 40 infertile men with oligospermia, and 40 infertile men with azoospermia. The oligospermia patients were further subdivided into subgroups based on sperm count and motility. Semen samples were obtained by masturbation after 3-5 days abstain for seminal fluid analysis. The microscopic test included the assessment of the count, motility and morphology of the sperms. In addition, the coulometric assay was used for measuring the activity and kinetic parameters of LDH-C4 enzyme.

**Results:** The activity of LDH-C4 is significantly higher in fertile men when compared with infertile subjects (fertile:  $403.13 \pm 189.90$ , oligospermia:  $110.01 \pm 58.13$ , azoospermia:  $39.06 \pm 28.15$ ;  $p \leq 0.01$ ). Statistically significant differences in LDH-C4 activity were also noted among patients with oligospermia based on sperms count and motility. Based on sperms' count in patients with oligospermia, a significantly higher LDH-C4 activity ( $p \leq 0.01$ ) was noted in those with higher sperm count (10-15 million/ml) when compared to others who have lesser count. Significant elevation in enzyme activity ( $p \leq 0.01$ ) was also observed in oligospermia patients with higher percentages of motile sperms when compared with others who have fewer percentages of motile sperms. Moreover, the highest  $V_{max}$  value (0.483 mmol/L.min) and the lowest  $K_m$  value (0.39 mmol/L.min) were recorded in fertile men. While, the lowest  $V_{max}$  value (0.174 mmol/L.min) and the lowest  $K_m$  value (0.75 mmol/L.min) were detected in azoospermia patients.

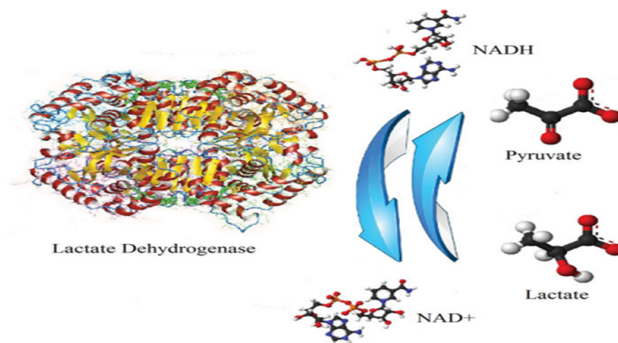
**Conclusions :** Our results suggest that LDH-C4 is essential for the count and motility of sperm and may be considered as a therapeutic approach for infertility.

**Keywords** azoospermia,  $K_m$ , lactate dehydrogenase C4, oligospermia,  $V_{max}$

## OPEN ACCESS

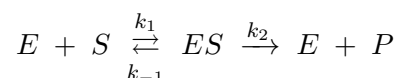
## INTRODUCTION

Testis specific lactate dehydrogenase C4 (LDH-C4), is one of the LDH isozymes that stimulate the terminal reaction of pyruvate to lactate in the glycolytic pathway.<sup>1,2</sup> LDH-C4 is an oxidoreductive enzyme of LDH (EC 1.1.1.27) family, which is present abundantly in testes, spermatocytes, spermatids and sperms.<sup>3,4</sup> The *Ldhc* gene expressed in male germ cells with the last functionally protein known as the LDH C4. LDH C4 constitutes more than 80% of the total LDH in mature spermatozoa. In contrast to spermatogenic cells, spermatids and spermatozoa exhibit high levels of glycolysis and contain high levels of LDH-C4.<sup>5</sup> The LDH-C4 can be determined in seminal plasma and spermatozoa due to the outward diffusion or leakage of the enzyme from sperm cell and spontaneous destruction of sperm cells.<sup>4,6</sup> It also plays an important role in maintaining glycolysis and ATP production in the flagellum during sperm capacitation, therefore essential for male fertility.<sup>6,7</sup> The final step of glycolysis process, which is essential for continued production of ATP, is the transformation of pyruvate to lactate accompanied via oxidation of NADH to NAD<sup>+</sup> (Figure 1). This reaction is stimulated by LDH-C4.<sup>8</sup> On the other hand, the sperm motility depends on the amount of energy produced by anaerobic glycolysis, aerobic glycolysis, and beta-oxidation of endogenous substrates.<sup>9,10</sup> Fructose is hexose secreted from seminal vesicles that can be used as an energy substrate for spermatozoa in anaerobic glycolysis to release lactic acid by the LDH activity.<sup>4,7</sup>



**Figure 1** Scheme of mutual transformation of pyruvate and lactate catalyzed by LDH .<sup>9</sup>

The basic concept of enzyme-kinetics is expressed via the Michaelis Menten equation, which is derived from the generally accepted assumption that enzyme catalyzed reactions involve the formation of an enzyme-substrate complex.<sup>9</sup> Leonor Michaelis and Maude Menten suggested a simple model for most of the features of enzyme stimulates reactions.<sup>11</sup> In this model, the enzyme reversibly joins with its substrate to form an enzyme-substrate (ES) complex then yields product and regenerate the free enzyme.<sup>12</sup> The model contains one substrate molecule is represented in figure 2.



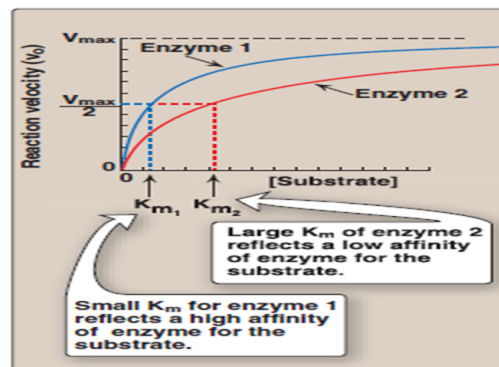
Where: S is the substrate, E is the enzyme, ES is the enzyme-substrate complex, P is the product,  $k_1/k_{-1}/k_2$  are rate constants.

The Michaelis-Menten equation describes how reaction's speed change with substrate concentration.<sup>13</sup>

$$V_0 = \frac{V_{max} [S]}{K_m + [S]}$$

Where:  $V_0$  is the initial reaction velocity,  $V_{max}$  is the maximal velocity,  $K_m$  is Michaelis constant =  $(k_{-1} + k_2)/k_1$ ,  $[S]$  is the substrate concentration.

According to Michaelis theory, the formation of enzyme substrate complex is a reversible reaction, while the collapse of the complex to enzyme product is irreversible. Speed in Y axis is extrapolated to the corresponding point on axis, which gives the numeral value of  $K_m$  (figure 2).<sup>14-16</sup>



**Figure 2** The effect of substrate concentration on reaction velocities for two enzymes: enzyme 1 with a small  $K_m$ , and enzyme 2 with a large  $K_m$ .<sup>16</sup>

The present study is aimed to evaluate the activity and kinetic parameters ( $K_m$  and  $V_{max}$ ) of LDH-C4 isoenzyme in seminal fluid of fertile and infertile (oligospermia and azoospermia) men from Baghdad city.

## MATERIAL AND METHODS

### Subjects and study design

This case-control study was approved by the institutional review board of the College of Medicine, Al-Nahrain University (No.:1, Date: 12-1-2020) and carried out between September 2019 and December 2020. After obtaining written informed consent from each participant, a total of 80 infertile men (40 with oligospermia and 40 with azoospermia) and

40 fertile men as controls were included in the current study. The staff of High Institute of Fertility Diagnosis, Al-Nahrain University, conducted the clinical diagnosis. The laboratory work was carried out at the Chemistry Laboratory, Department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University (Baghdad, Iraq).

The infertile men with oligospermia were subdivided into four subgroups depending on the sperm count (group 1: 10-15, group 2: 5-10, group 3: 1-5, and group 4: <1 million/ml) and motility (group 1: 30-40%, group 2: 20-30%, group 3: 10-20%, and group 4: <10%), each group included ten patients.

## Collection of samples and experimental work

Semen samples were collected by masturbation after 3-5 days abstain. They were preliminarily evaluated for volume, viscosity, and color within 1 hour after ejaculation. The microscopic test included the count, motility and morphology of the sperms. In addition to the routine test of semen, it was also centrifuged for 15 min at 4000 rpm and then divided into small aliquots for measuring the activity and kinetic parameters (maximum velocity,  $V_{max}$  and Michaelis constant,  $K_m$ ) of LDH-C4 enzyme.

## Coulometric assay

### Enzyme activity

The coulometric assay was used for assessing the enzyme activity quantitatively. The initial rate of the nicotinamide adenine dinucleotide (NAD) + hydrogen (H) NADH oxidation is directly proportional to the catalytic LDH-C4 activity. The UV Spectrophotometer (Apple, Japan) was used for detecting the absorption (decrease in absorbance) of seminal fluid of each sample at 340nm. The LDH kit (Spectrum, Egyptian company for biotechnology, Reference range of enzyme activity for adult is 240-480 U/L) was used in this study. The reagents were used; Reagent 1 (R1 Buffer: Tris buffer with pH 7.5, Pyruvate and Sodium Azide) and Reagent 2 (R2 Coenzyme: NADH and Sodium azide). The working solution was prepared by mixing 4 volumes of R1 and 1 volume of R2. For measuring the enzyme activity, 1 ml of working solution was mixed with 20 $\mu$ l of specimen and then was put in the cuvette. The initial absorbance was recorded after 30 seconds at 340nm. The absorbance was recorded again at different time points (1, 2 and 3 minutes). The mean absorbance changes per minute ( $\Delta A/\text{min}$ ) was calculated. The LDH-C4 activity was calculated based on the following formula:

$$LDH - C4 \text{ activity (U/L)} = 8095 \times (\Delta A \text{ at } 340 \text{ nm}) \quad (1)$$

### **Kinetics parameters ( $V_{max}$ and $K_m$ )**

For measuring kinetics parameters ( $V_{max}$  and  $K_m$ ), several substrate concentrations (0.4, 0.8, 1.2, 1.6, and 2 mmol/L) were prepared from a stock substrate (2.4 mmol/L) by using the serial dilution method. The solution was completed to the desired volume (1ml) with distilled water. One ml of substrate, which was previously prepared, was added into the cuvette and the absorbance at 340nm of each substrate concentration was recorded. The standard curve was plotted and the slope equation was written. Then 20  $\mu$ l of seminal fluid that contain LDH-C4 enzyme was added to the cuvette that contains 1 ml of substrate. The absorbance was recorded at 340nm after 1 min. The addition of 20  $\mu$ l of seminal fluid and recording the absorbance were repeated at each substrate concentration. The rate or velocity of reaction  $V$ , mmol/l. min (the change in substrate concentration over a time period) was calculated according to the following formula by El Seoud et al (2016):<sup>17</sup>

$$V = -\frac{\Delta[S]}{\Delta t} = \frac{[S]_2 - [S]_1}{t_2 - t_1}$$

Where:  $[S]_1$  is the substrate concentration before enzyme addition,  $[S]_2$  is the substrate concentration after enzyme addition,  $t_1$  is the initial time (= 0),  $t_2$  is the period time (1 min).<sup>17</sup>

The concentration of substrate after adding the enzyme was estimated from the standard curve. The velocity of reaction ( $V$  mmol/L.min) was plotted against the concentration of substrate (mmol/L) (Michaelis-Menten plot) and then the kinetic parameters of enzyme ( $K_m$  and  $V_{max}$ ) were estimated for control, oligospermia, and azoospermia groups. The value of  $K_m$  was estimated from Michaelis-Menten curve which is equal to the substrate concentration when the rate of reaction is equal to  $1/2V_{max}$ .

### **Statistical analyses**

The Statistical Analysis System (SAS) 2012 software was used to evaluate the data statistically. One way analysis of variation (ANOVA), followed by the post-hoc least significant difference (LSD) test, was used to assess the difference between means in this study. A p-value of equal to or less than 0.05 was considered statistically significant, while a p-value of equal to or less than 0.01 was considered highly significant, statistically.

## **RESULTS**

### **LDH-C4 activity**

The results showed a highly significant difference between the fertile (control) and infertile (oligospermia and azoospermia) men in LDH-C4 as shown in table 1. A high significant

rise ( $p \leq 0.01$ ) was detected in LDH-C4 activity in fertile men compared with infertile group (oligospermia and azospermia). While, the highest significantly increment ( $p \leq 0.01$ ) in LDH-C4 activity was noted in oligospermia group compared to the azospermia group and a significant decrease compared to the fertile group. On the other hand, the azospermia group showed a significant decrease in LDH-C4 activity compared to the control and oligospermia groups (Table 1).

**Table 1** LDH-C4 activity in controls (fertile) and patients (infertile).

Group	Fertile men (n=40)	Infertile men (oligospermia, n=40)	Infertile men (azospermia, n=40)	<i>p</i>
LDH-C4 (U/L)	403.13±189.90	110.01±58.13	39.06±28.15	0.0001

The oligospermia group was divided into four groups depending on sperm count (million/ml). According to the table 2, the results showed a significantly higher rise ( $p \leq 0.01$ ) in group 1 (10-15 million/ml) oligospermia compared with other groups in LDH-C4 activity and a significant decrease compared with control group. While, group 2 (5-10 million/ml) oligospermia showed a significant decrease in LDH-C4 activity compared with group 1 (10-15 million/ml) oligospermia and non-significant rise compared with group 3 and 4. Further, a significant decrease was appeared in group 3 and 4 compared with group 1 and control (Table 2).

**Table 2** Comparison between control and oligospermia groups in LDH-C4 activity depending on sperm count.

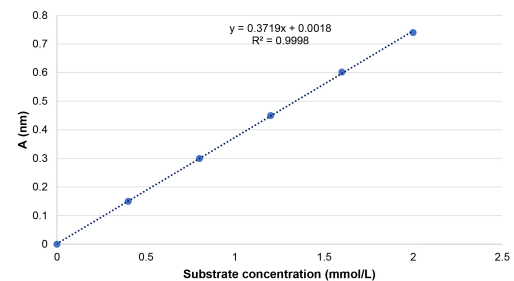
Groups	LDH-C4 activity (U/L)
Control >15 million/mL	428.75 ±149.79 <sup>a</sup>
Group 1 (10-15 million/mL) Oligospermia	195.90 ±29.51 <sup>b</sup>
Group 2 (5-10 million/mL) Oligospermia	100.37 ±11.57 <sup>c</sup>
Group 3 (1-5 million/mL) Oligospermia	80.95 ±14.77 <sup>c</sup>
Group 4 (< 1 million/mL) Oligospermia	54.23 ±34.14 <sup>c</sup>
<b>P-value</b>	<b>0.0001</b>

The oligospermia group was also divided into other four groups depending on sperm motility percentages. The results showed a higher significant difference between control and oligospermia groups (sperm motility groups) in LDH-C4 activity. A significantly higher rise ( $p \leq 0.01$ ) of LDH-C4 activity was detected in group 1 (30-40 %) oligospermia compared with the other groups (group 2, 3, and 4) and a significant decrease compared with control group. Additionally, the group 2 with motility 20-30% of oligospermia men showed a significant decrease in LDH-C4 activity compared with group 1 and controls. Also, there was a non-significant different in LDH-C4 compared with group 3 and 4. While, a significant decrease was detected in groups 3 and 4 compared with control and group 1 and non-significant difference was seen when compared with group 2 in LDH-C4 activity (Table 3).

**Table 3** Comparison between control and oligospermia groups in LDH-C4 activity depending on the sperm motility percentages.

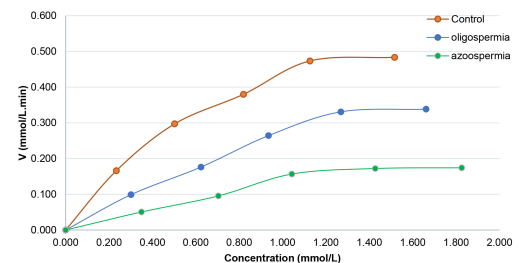
Group s	LDH-C4 activity (U/L)
Control > 40%	438.75 ± 149.79 <sup>a</sup>
Group 1 30-40% Oligospermia	193.90 ± 30.61 <sup>b</sup>
Group 2 20-30% Oligospermia	100.27 ± 11.36 <sup>c</sup>
Group 3 10-20% Oligospermia	79.95 ± 14.66 <sup>c</sup>
Group 4 <10% Oligospermia	50.23 ± 28.16 <sup>c</sup>
<b>P-value</b>	<b>0.0001</b>

Figure 3 shows the absorbance (A) in nanometers (nm) as a function of substrate (pyruvate) concentration (mmol/L).

**Figure 3** The standard curve.

### Kinetic study of LDH-C4 enzyme (Michaelis-Menten)

The reaction rate (velocity), mmol/L.min is plotted as a function of substrate concentration, mmol/L as shown in figure 4 (Michaelis-Menten plot) for control, oligospermia, and azoospermia groups. As presented in figure 4, the velocity (reaction rate) increases sharply at low substrate concentrations of control, oligospermia, and azoospermia. When the substrate concentration more increases, the reaction rate begins to increase less until it reaches maximum and then converts into a flat line.

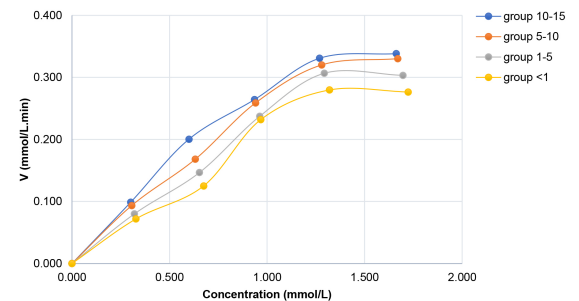
**Figure 4** Michaelis-Menten plot of control, oligospermia and azoospermia.

The  $V_{max}$  and  $K_m$  values of control, oligospermia and azoospermia are shown in table 4.

Groups	$V_{max}$ value (mmol/ L. min)	$K_m$ value (mmol/L)
Control	0.483	0.39
Oligospermia	0.338	0.58
Azoospermia	0.174	0.75

The results showed the highest value of  $V_{max}$  and the lowest value of  $K_m$  in the control group compared with oligospermia and azoospermia groups. While, a lower value of  $V_{max}$  and a higher value of  $K_m$  were detected in oligospermia men compared with fertile men (control). A higher  $V_{max}$  value and a lower  $K_m$  value were appeared in oligospermia group compared with azoospermia men. On the other hand, the azoospermia group appeared the lowest value of  $V_{max}$  and the highest value of  $K_m$  compared to the control and oligospermia groups as shown in table 4.

Figure 5 represents the Michaelis-Menten plot (velocity mmol/L.min versus substrate concentration mmol/L) of oligospermia groups that depending on the sperm count (million/ml).



**Figure 5** Michaelis-Menten plot of oligospermia groups according to sperm count (million/ml).

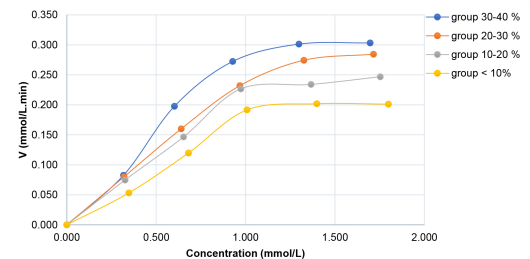
represents the values of  $K_m$  and  $V_{max}$  of LDH-C4 isoenzyme for the oligospermia groups depending on the sperm count (million/ml).

Oligospermia groups	$V_{max}$ (mmol/ L.min)	$K_m$ (mmol/L)
Group 1 (10-15 million/ml)	0.338	0.49
Group 2 (5-10 million/ml)	0.330	0.59
Group 3 (1-5 million/ml)	0.303	0.68
Group 4 (<1 million/ml)	0.276	0.71



In group 1 (10-15 million/ml) of oligospermia, the results appeared the highest value of  $V_{max}$  and the lowest value of  $K_m$  compared to other groups. While, in group 4 (<1 million/ml) of oligospermia, the result showed the lowest value of  $V_{max}$  and the highest value of  $K_m$  compared to other groups as shown in table 5.

Figure 6 explains the Michaelis-Menten plot (velocity mmol/L.min versus substrate concentration mmol/L) of oligospermia groups that depending on the sperm motility%.



**Figure 6:** Michaelis-Menten plot of oligospermia groups according to sperm motility (%).

represents the values of  $K_m$  and  $V_{max}$  of LDH-C4 isoenzyme for the oligospermia groups depending on the sperm motility (%).

**Table 6** Values of  $K_m$  and  $V_{max}$  of LDH-C4 enzyme of oligospermia groups depending on sperm motility (%)

Oligospermia groups	$V_{max}$ (mmol/L.min)	$K_m$ (mmol/L)
Group 1 (30-40%)	0.303	0.48
Group 2 (20-30%)	0.284	0.55
Group 3 (10-20 %)	0.247	0.57
Group 4 (< 10)	0.201	0.6

In group 1 (30-40%) of oligospermia, the results found that the highest value of  $V_{max}$  and the lowest value of  $K_m$  compared with other groups. Whereas, in group 4 (<10%) of oligospermia, the result showed the lowest value of  $V_{max}$  and the highest value of  $K_m$  compared to other groups as shown in table 6.

## DISCUSSION

### LDH-C4 activity

LDH-C4 is a highly soluble enzyme that can seeps into semen.<sup>18</sup> LDH-C4 is thought to be a highly specialized enzyme, involved in metabolic processes that produce the energy to the germ cells.<sup>16</sup> So, the detection of LDH-C4 activity in seminal fluid very useful for determining the ability of sperm to promote pregnancy.<sup>13</sup> In the current study, the statistical difference in LDH-C4 activity of healthy individuals, oligospermia, and azoospermia

patients were proven. In addition, the present results demonstrated a relationship between LDH-C4 activity and the count and motility of sperms among studied participants.

The LDH-C4 isoenzyme is the most important enzyme which has regulatory roles in mechanisms of metabolic processes that required for spermatogenesis and sperm motility.<sup>16</sup> The oligospermia sub-groups, depending on the sperm count, showed a statistical difference in LDH-C4 activity when compared with control group. The high activity of LDH-C4 indicates high seminiferous epithelial activity in the control group that means normal rate of spermatogenesis. Therefore, a higher significant increment of LDH-C4 activity was noted in fertile men compared with oligospermia and azospermia groups. Whereas, the reduction of activity LDH-C4 may indicate less seminiferous epithelial activity in the oligospermia group and, thus; this may negatively influence the rate of spermatogenesis that cause reduction in sperm count a lower-than-normal. Therefore, a low LDH C4 activity was observed in oligospermia groups compared with fertile men. The results showed lower activity of testicular isoenzyme of LDH (LDH-C4) in azospermia group compared with control and oligospermia groups that reduced the activity of seminiferous epithelial of testes and may adversely affect the rate of spermatogenesis leading to a lack of sperm (i.e. zero). This study's findings are in agreement with those of Gerez de Burgos et al. (1979),<sup>14</sup> Hashemi et al. (2016),<sup>6</sup> and Velasco et al. (1993)<sup>19</sup>.

The results also showed a statistical difference in LDH-C4 activity of oligospermia groups, depending on the sperm motility, and control group. The glycolysis pathway in living organisms is the main source of ATP molecules that essential for motility of sperm.<sup>15,16</sup> The final step in the glycolysis process, which is necessary for the production of ATP, is the conversion of pyruvate to lactate accompanied by the oxidation of NADH to NAD<sup>+</sup>. This reaction is catalyzed by lactate dehydrogenase C4.<sup>15,20</sup> So, a high LDH-C4 activity was detected in fertile group compared with oligospermia groups, depending on the motility % of sperms. Our results are in line with previous findings of Velasco et al. (1993),<sup>19</sup> Gavella and Cvitković (1985),<sup>21</sup> McCabe et al. (2014),<sup>22</sup> and Piomboni et al. (2012)<sup>23</sup>.

There are several structural defects that may cause reduced sperm motility, and it is probably the LDH-C4 deficiency suggest a blemish in the mitochondria where LDH-C4 is located. Mitochondrial aberrations in the region where LDH-C4 is located may cause low activity of LDH-C4 that negatively influence the count and motility of sperms.<sup>24</sup> The aberrations can be related to the defect of the middle segment, that is, the lack of a characteristic mitochondrial sheath in sperm with low motility.<sup>23</sup> The Sertoli cells excreted the lactate, which is necessary for the metabolic pathways that occur in the sperm. LDH-C4 isoenzyme stimulates the lactate oxidation in germ cells. In the case of decrease the activity or deficiency of LDH-C4 in spermatocyte, lactate is not used, and this leads to reduce the metabolic activity and decrease in sperm viability.<sup>25</sup> Also, inactivation of the C gene that essential for the synthesis of LDH-C4 isoenzyme led to decrease of LDH-C4 activity in germ cells, causing reduction in the count and motility of sperms.<sup>26</sup>

The reduction in LDH-C4 activity result in negative effects on the final stage of glycolysis pathway, which is necessary for ATP production, leads to decrease in the ATP which is necessary for the count and motility of sperms.<sup>9</sup> Regardless of the cause, low activity of

LDH-C4 in oligospermia and azospermia linked with a significant decrease in count and motility of sperm, and thus; reduced the ability to fertilization, indicates the biological needs of LDH-C4 for sperm metabolic activity.<sup>26</sup>

## The kinetic of LDH-C4

Michaelis constant ( $K_m$ ) is a feature of an enzyme and its specific substrates, and mirror the affinity of the enzyme for that substrate.<sup>12</sup> The  $K_m$  does not change with the concentration of enzyme.<sup>27</sup> The  $K_m$  value is equal to the substrate concentration at which of the reaction rate is equal to  $1/2V_{max}$ .<sup>13</sup> A large value of  $K_m$  reflects a low affinity of enzyme for substrate that means low saturation of enzyme by substrate.<sup>5,12</sup> In contrast, a small  $K_m$  value reflects a high affinity of the enzyme for substrate, that means high saturation of enzyme by substrate.<sup>5,12,27</sup> While, the maximal velocity ( $V_{max}$ ) is the substrate molecules number transformed to product per unit time. The velocity of an enzyme-catalyzed reaction rises with substrate concentration until a  $V_{max}$  is arrived.<sup>12</sup> The results revealed a difference between fertile men, oligospermia and azospermia groups in the values of  $V_{max}$  and  $K_m$ . In the same way, the results showed a different in  $V_{max}$  and  $K_m$  values in oligospermia sub-groups, based on counts and motility of sperms.

These findings lead as to assume a relationship between  $V_{max}$  values,  $K_m$  values and LDH-C4 enzyme activity. The control group has a high value of  $V_{max}$  and a low value of  $K_m$  that reflects a high affinity of LDH-C4 enzyme for substrate, so the enzyme has high activity compared with oligospermia and azospermia groups. This leads to activate the reversible reaction (pyruvate to lactate or lactate to pyruvate) and produce NADH or  $NAD^+$  molecules providing the necessary amount of ATP molecules (3 molecules of ATP for each NADH molecule in electron transport chain in mitochondria) for normal motility of sperm and for activation of seminiferous epithelial of testes to increase the rate of spermatogenesis processes.<sup>4,13</sup>

While, a low value of  $V_{max}$  and high value of  $K_m$  of LDH-C4 enzyme in oligospermia and azospermia groups mean low affinity of LDH-C4 enzyme for substrate. Therefore, a lower activity is recorded compared with control group, that leads to inactivate the reversible reaction (pyruvate to lactate or lactate to pyruvate) and production of NADH or  $NAD^+$  molecules is decreased providing no amount of ATP molecules that are necessary for normal motility and count.<sup>4,13</sup>

The first hypothesis that explaining low affinity of the LDH-C4 enzyme to its substrate in the oligospermia and azospermia groups compared with control group is that a defect in the gene encoded for LDH-C4, mutation that may alter the sequence of amino acids on the active side of enzyme that results in a negative influence reduce the binding of substrate to the active site of enzyme which causes the enzyme do not fit into the substrate.<sup>18</sup> The second hypothesis states that the substrate may have a defect which makes it unsuitable for the enzyme's active site.<sup>13,20</sup> These findings propose that LDH-C4 is necessary of glycolysis pathway in sperm which has a key role in producing enough ATP for sperm motility, and it

is related to LDH-C4 kinetics properties.

## CONCLUSIONS

In conclusion, there is a relationship between LDH-C4 enzyme activity and kinetic parameters ( $V_{max}$  and  $K_m$ ). The activity of LDH-C4 is high in fertile subjects when compared with infertile ones (oligospermia and azoospermia). Also, the fertile men have a highest  $V_{max}$  and lowest  $K_m$  compared with infertile (oligospermia and azoospermia) men. The LDH-C4 is thought to be a highly specialized enzyme and it is necessary for glycolysis pathway in sperm which has a great role in producing enough ATP for sperm motility, and it is related to LDH-C4 kinetics properties effecting of fertilization capacity. The activity and kinetic parameters of LDH-C4 enzyme can be considered as biomarkers to indicate the causes that lead to reduced sperm's count and motility of oligospermia and azoospermia patients.

## ACKNOWLEDGEMENTS

Special thanks from authors to Prof. Dr. Ula Alkawaz, High Institute of Fertility Diagnosis and ART, Al-Nahrain University, Iraq for the kind assistance in the practical part of this research.

## DECLARATIONS

### Authors' contributions

All the authors have equally contributed to this work, read and approved the final revision of the manuscript before publication.

### Conflict of interest

Authors have no conflict of interest.

### Ethical approvals

This project was approved by the College of Medicine, Al-Nahrain University (Baghdad, Iraq) and informed consent was obtained from all participants before enrolling in the current study.

### Data availability

All data associated with the current paper can be requested from the corresponding author.

### Funding resources

This work didn't receive any fund.

## REFERENCES

1. Jialal I, Sokoll LJ. Clinical utility of lactate dehydrogenase: a historical perspective. *Am J Clin Pathol.* 2015;143(2):158–159. Available from: [10.1309/AJCTP0FC8QFYDFA](https://doi.org/10.1309/AJCTP0FC8QFYDFA).
2. Armstrong AJ, George DJ, Halabi S. Serum lactate dehydrogenase predicts for overall survival benefit in patients with metastatic renal cell carcinoma treated with inhibition of mammalian target of rapamycin. *J Clin Oncol.* 2012;30(27):3402–3407. Available from: [10.1200/JCO.2011.40.9631](https://doi.org/10.1200/JCO.2011.40.9631).
3. Wang Y, Wei L, Wei D, Li X, Xu L, Wei L. Testis-Specific Lactate Dehydrogenase (LDH-C4) in Skeletal Muscle Enhances a Pika's Sprint-Running Capacity in Hypoxic Environment. *Int J Environ Res Public Health.* 2015;12(8):9218–9236. Available from: [10.3390/ijerph120809218](https://doi.org/10.3390/ijerph120809218).
4. Satyanarayana U, Chakrapani U. *Essentials of Biochemistry.* and others, editor; 2008.
5. Sawane MV, Kaore SB, Gaikwad RD, Patil PM, Patankar SS, Deshkar AM. Seminal LDH-C4 isoenzyme and sperm mitochondrial activity: a study in male partners of infertile couples. *Indian J Med Sci.* 2002;56(11):560–566.
6. Hashemi M, Tabandeh A, Tajari H, Behnampour N, Joshaghani HR. Evaluation of LDH activity and its relationship with fructose levels in seminal plasma of normospermic and asthenospermic males. *Med Lab J.* 2016;10(4):18–23. Available from: [10.18869/acadpub.mlj.10.4.18](https://doi.org/10.18869/acadpub.mlj.10.4.18).
7. Goldberg E, Rcd. Inhibiting Human Lactate Dehydrogenase-C for Male Fertility Control; Initial Hits. *J Pharm Drug Deliv Res.* 2014;(02):3–3. Available from: [10.4172/2325-9604.1000125](https://doi.org/10.4172/2325-9604.1000125).
8. Forkasiewicz A, Dorociak M, Stach K, Szelachowski P, Tabola R, Augoff K. The usefulness of lactate dehydrogenase measurements in current oncological practice. *Cell Mol Biol Lett.* 2020;25:35–35. Available from: [10.1186/s11658-020-00228-7](https://doi.org/10.1186/s11658-020-00228-7).
9. Tang P, Xu J, Oliveira CL, Li ZJ, Liu S. A mechanistic kinetic description of lactate dehydrogenase elucidating cancer diagnosis and inhibitor evaluation. *J Enzyme Inhib Med Chem.* 2017;32(1):564–571. Available from: [10.1080/14756366.2016.1275606](https://doi.org/10.1080/14756366.2016.1275606).
10. Heinová D, Kostecká Z, Csank T. Separation of turkey lactate dehydrogenase isoenzymes using isoelectric focusing technique. *Electrophoresis.* 2016;37(2):335–338. Available from: [10.1002/elps.201500407](https://doi.org/10.1002/elps.201500407).
11. Rogers A, Gibon Y. *Enzyme Kinetics: Theory and Practice.* Springer; 2009. p. 71–103. Available from: [10.1007/978-0-387-78745-9\\_4](https://doi.org/10.1007/978-0-387-78745-9_4).
12. Champe PC, Harvey RA, Ferrier DR. *Biochemistry.* 5th ed. and others, editor. Lippincott Williams & Wilkins; 2010.
13. Talaiezhadeh A, Shahriari A, Tabandeh M, Fathizadeh P, Mansouri S. Kinetic characterization of lactate dehydrogenase in normal and malignant human breast tissues. *Cancer Cell Int.* 2015;15(1):19–19. Available from: [10.1186/s12935-015-0171-7](https://doi.org/10.1186/s12935-015-0171-7).
14. Burgos NMGD, Burgos C, Coronel CE, Camusso ABD, Pigini T, Blanco A. Correlation of lactate dehydrogenase isoenzyme C4 activity with the count and motil-

- ity of human spermatozoa. *J Reprod Fertil.* 1979;55(1):107–111. Available from: [10.1530/jrf.0.0550107](https://doi.org/10.1530/jrf.0.0550107).
15. Mukai C, Okuno M. Glycolysis plays a major role for adenosine triphosphate supplementation in mouse sperm flagellar movement. *Biol Reprod.* 2004;71(2):540–547. Available from: [10.1095/biolreprod.103.026054](https://doi.org/10.1095/biolreprod.103.026054).
  16. Odet F, Gabel SA, Williams J, London RE, Goldberg E, Eddy EM. Lactate dehydrogenase C and energy metabolism in mouse sperm. *Biol Reprod.* 2011;85(3):556–564. Available from: [10.1095/biolreprod.111.091546](https://doi.org/10.1095/biolreprod.111.091546).
  17. Seoud OAE, Baader WJ, Bastos EL. Practical Chemical Kinetics in Solution. In: Wang Z, et al., editors. *Encyclopedia of Physical Organic Chemistry*. John Wiley & Sons, Inc.; 2016. p. 1–68. Available from: [10.1002/9781118468586.epoc1012](https://doi.org/10.1002/9781118468586.epoc1012).
  18. Sa AB, Ni AL, Hazzaa SA. Purification of lactate dehydrogenase-C4 from seminal plasma of male infertility. *Basrah J Vet Res.* 2018;17(3):726–737.
  19. Velasco N, A J, Zapata T, I, Hernandez M, Albacete PP, et al. Lactic dehydrogenase-C4 activity in seminal plasma and male infertility. *Fertil Steril.* 1993;60(2):56107–56107. Available from: [10.1016/s0015-0282\(16\)56107-x](https://doi.org/10.1016/s0015-0282(16)56107-x).
  20. Williams AC, Ford W. The role of glucose in supporting motility and capacitation in human spermatozoa. *J Androl.* 2001;22(4):680–695. Available from: [10.1002/j.1939-4640.2001.tb02229.x](https://doi.org/10.1002/j.1939-4640.2001.tb02229.x).
  21. Gavella M, Cvitković P. Semen LDH-X deficiency and male infertility. *Arch Androl.* 1985;15(2-3):173–176. Available from: [10.3109/01485018508986907](https://doi.org/10.3109/01485018508986907).
  22. McCabe MJ, Bancalari RE, Dattani MT. Diagnosis and evaluation of hypogonadism. *Pediatr Endocrinol Rev.* 2014;11(2):214–229.
  23. Piomboni P, Focarelli R, Stendardi A, Ferramosca A, Zara V. The role of mitochondria in energy production for human sperm motility. *Int J Androl.* 2012;35(2):109–124.
  24. Ruiz-Pesini E, Díez-Sánchez C, López-Pérez MJ, Enríquez JA. The role of the mitochondrion in sperm function: is there a place for oxidative phosphorylation or is this a purely glycolytic process? *Curr Top Dev Biol.* 2007;77:3–19. Available from: [10.1016/S0070-2153\(06\)77001-6](https://doi.org/10.1016/S0070-2153(06)77001-6).
  25. Jutte N, Grootegoed JA, Rommerts F, Molen HJVD. Exogenous lactate is essential for metabolic activities in isolated rat spermatocytes and spermatids. *J Reprod Fertil.* 1981; Available from: [10.1530/jrf.0.0620399](https://doi.org/10.1530/jrf.0.0620399).
  26. Odet F, Duan C, Willis WD. Expression of the gene for mouse lactate dehydrogenase C (*Ldhc*) is required for male fertility. *Biol Reprod.* 2008;79(1):26–34. Available from: [10.1095/biolreprod.108.068353](https://doi.org/10.1095/biolreprod.108.068353).
  27. Vaidyanathan K. *Textbook of Biochemistry for Medical Students. and others, editor;* 2016. Available from: [doi:10.5005/jp/books/13014](https://doi.org/10.5005/jp/books/13014).