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The Seminal Fluid Isoenzyme LDH-C_4 in Infertile Men

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Abstract: In order to determine the seminal activity of LDH-C4 and to investigate its correlation with other fertility indexes, we studed total LDH, total CK avtivities, and LDH-C4 in relation to sperm count, motility and sperm motility index (SMI). SMI is the measurement of electro-optical fluctuations caused by motile sperm. Total LDH and total CK were determined with a Technicon RA 1000 analyser, and LDH isoenzymes with Helena Lab. isoenzyme kits. The results in 23 infertile men showed that LDH-C4 activity was either absent (4 cases), or decreased (19 cases). LDH-C4 showed a positive correlation with total LDH, sperm count and SMI ($p<0.01$ $r=0.5529$, $p<0.05$ $r=0.5025$ and $p<0.05$ $r=0.475$, respectively). In addition, there were significant correlations between % C4 and total LDH activity, SMI,motility and sperm count ($p<0.01$ $r=0.5529$, $p<0.05$ $r=0.475$, $p<0.05$ $r=0.503$, and $p<0.01$ $r=0.528$, respectively). On the other hand, LDH-C4 activity/sperm count showed no correlation with any other index (sperm count, motility and SMI).

On the basis of these results we suggest that isoenzyme LDH-C4 and/or % C4 activity would be a simple and reliable indicator(s) in testing fecundatory potentials.

Key Words: LDH-C4, SMI, seminal fluid, infertility.

Introduction

Mature human testes and spermatozoa possess a unique lactate dehydrogenase isoenzyme, LDH-C4. Synthesis of this isoenzyme is restricted to active spermatogenesis and the isoenzyme of lactate dehydrogenase with catalytic properties fulfils the specialised functions for the metabolic requirements of mature spermatozoa. Thus, a direct relationship between the LDH-C4 isoenzyme and sperm count is a well established fact (1). The LDH-C4 isoenzyme can be determined in seminal plasma owing to the outward diffusion of the enzyme from the spermatozoa or to their destruction (2).

The objective of the present study was to investigate the relationship between the LDH-C4 isoenzyme and sperm parameters of infertile men to determine whether it may serve as an indicator of fecundatory potential, and also between % C4 activity (3) and the parameters mentioned above. Therefore, a plan was made, to investigate the correlation with other fertility indexes, e.g., total LDH and total CK activities, and to study the LDH-C4 isoenzyme and % C4 activity in relation to sperm count, motility and sperm motility index (SMI).

Material and Methods

LDH-C4 isoenzyme and % C4 activity in seminal fluid were determined in 23 men attending the infertility unit at the Department of Obstetrics and Gynaecology at the Teaching Hospital of Dokuz Eylül University. All subjects had been living in childless marriages for several years and all had normal testicular sizes. Samples were collected after 4 days of abstinence. After liquefaction, the parameters of the seminogram (density, motility) were first studied using the standard methods (4). SMI was measured by SQA (Sperm Quality Analyzer). After centrifugation at 1500 rpm for 15 minutes, total LDH and total CK activities were assessed by Technicon RA 1000 analyser using Boehringer Mannheim kits and LDH isoenzymes were assessed with Helena Lab. isoenzyme kits. Five microliters of each sample was applied to the agarose gel and 15 minutes of 100-volt current was applied for electrophoresis. After incubation with the

Table 1. Means and Standard Deviations

Sperm counts (Spermx10^6)	Motility	Motility Index (SMI)	CK levels (U/L)
Mean 25.048	24.318	54.70	657.473
SD 19.709	16.033	51.67	670.212
LDH (U/L)	LDH C4 (U/L)	%C4	LDHC4/counts (U/Spermx10^6)
Mean 3348.43	451.692	12.48	20.95
SD 1768.322	313.94	10.13	20.24

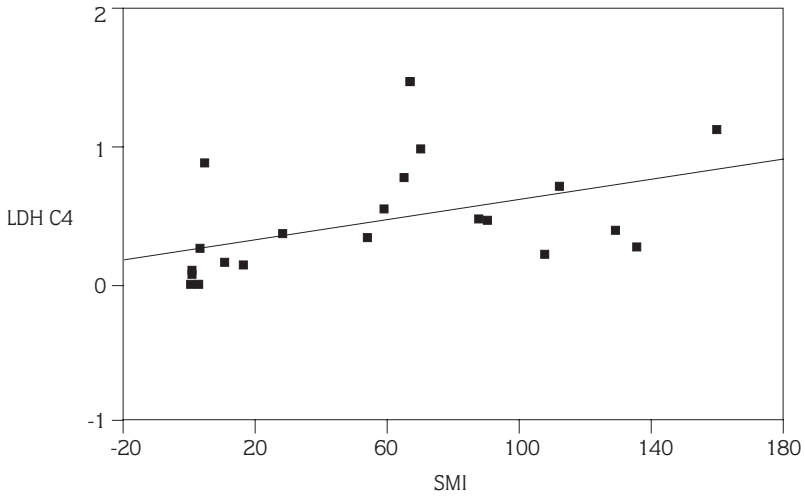

Figure 1. Relation between LDH C4 and SMI

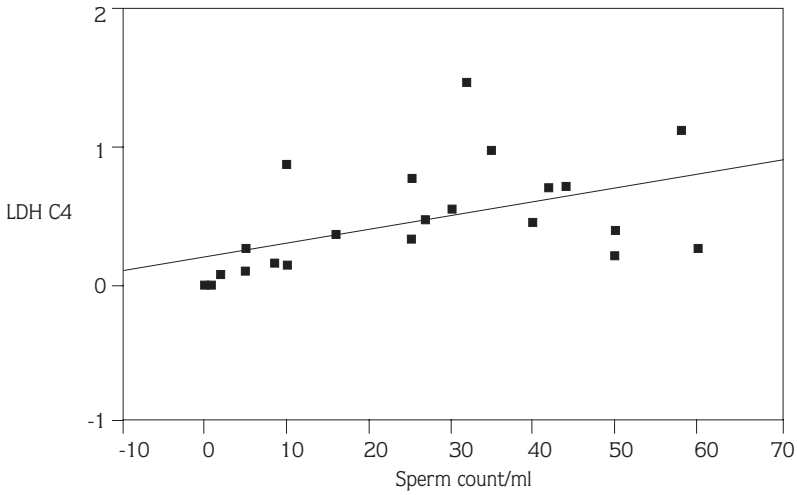

Figure 2. Relation between LDH C4 and Sperm count/ml

appropriate dye, gels were dried and analysed with an automatic densitometer Cliniscan 2 of Helena Laboratories at 595 nm. C4 activity was calculated from the C4 peak area by the densitometer as IU/L.

Correlation studies were done on a Machintosh Statworks Cricket Graph sofware using regression analyses (simple linear).

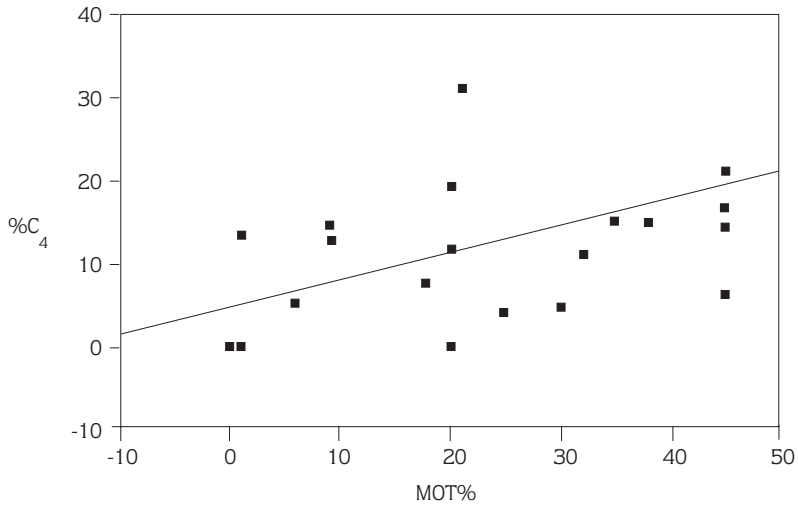

Figure 3. Relation between %C4 and % motility

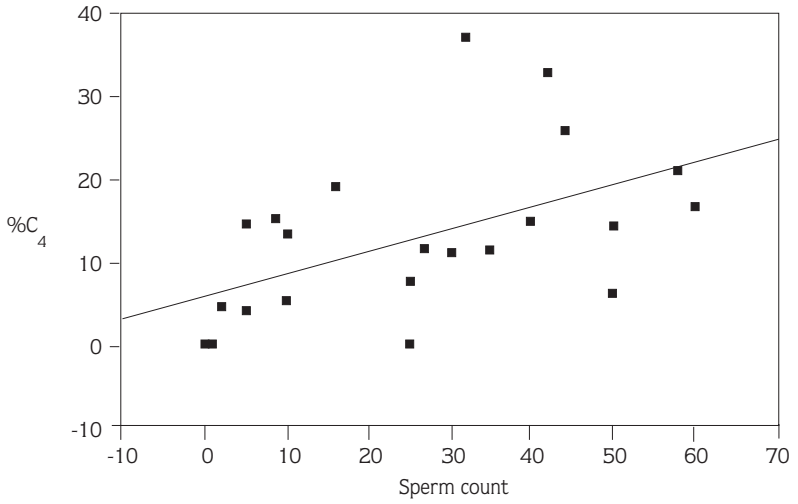

Figure 4. Relation between %C4 and sperm count

Results

Results in 23 infertile men showed that the LDH-C4 isoenzyme activity was either absent (4 cases), or decreased (19 cases). LDH-C4 isoenzyme showed positive correlations wih total LDH activity, sperm ocount, and SMI ($p<0.01$, $r=0.5529$, $p<0.05$, $r=0.5025$, and $p<0.05$, $r=0.475$, respectively. In addition, there were significant correlations between %C4 and LDH activity, SMI, motility and sperm count ($p<0.01$ $r=0.5529$, $p<0.05$ $r=0.475$, $p<0.05$ $r=0.503$, and $p<0.01$ $r=0.528$, respectively. The LDH-C4/sperm count ratio showed no correlation with any other fertility index (sperm count, motility and SMI).

Discussion

Although sperm count is an important factor in determining the fecundatory potential of men, it is not the sole factor in determining infertility (5). The presence of lactate dehydrogenase activity in the seminal fluid of the azospermic subjects suggest that the prostate gland and the seminal vesicles contribute to the secretion of that enzyme. The fact that normospermic subjects display higher LDH activity in semen indicates that both testicles and spermatozoa are important sources of LDH (6). Garzia et al. (6) have identified the estimation of the isoenzymes of LDH as a marker of seminal quality and perhaps of the fencudation potential of the human spermatozoa, and none were found useful for differentiating excretory from the secretory azospermias.

However, the LDH-C4 isoenzyme is one of the many regulatory mechanisms involved in the metabolic processes needed for fertility; encoded by a third gene, synthesis of LDH-C4 is required for active spermatogenesis (1). To date, the importance of LDH-C4 and other lactate dehydrogenase isoenzymes in the

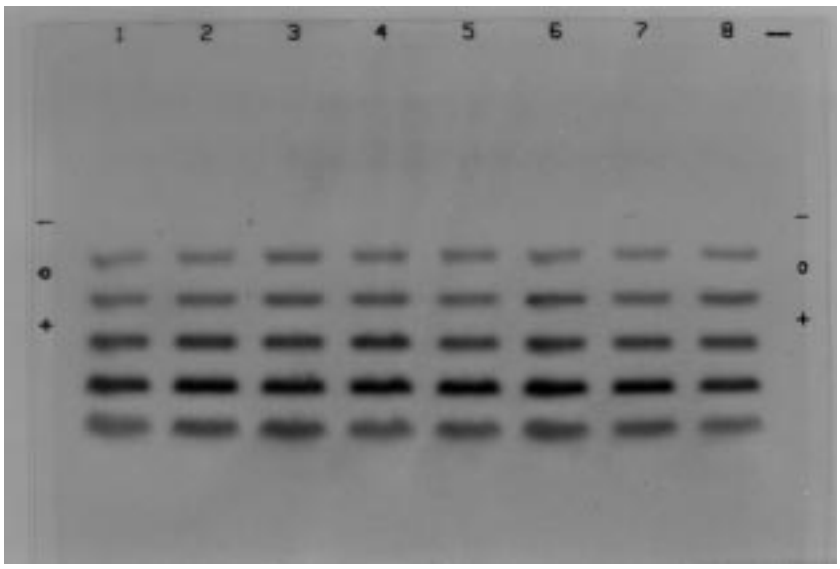

Figure 5. Photograph of a serum LDH electrophoretogram

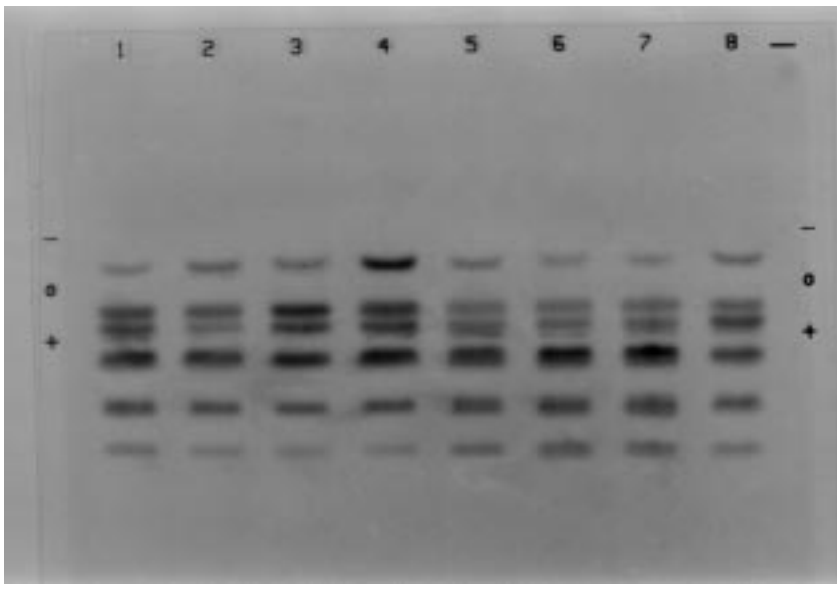

Figure 6. Photograph of a seminal LDH electrophoretogram

seminal fluid of infertile men are still controversial. However, Steinberg et al. (7), detected the presence of LDH-C4, which they had designated as "Band X", in the seminal fluid of men with immotile and abnormal spermatozoa, and pointed out the fact that LDH-C4 could be also affected by defective spermatozoa and speculated that LDH-C4 might be related to a qualitative defect of the sperm cell (8). An electron microscopic study disclosed that the abnormality was probably related to the absence of the mitochondrial sheath (9). According to Jutte et al. (10), lactate secreted by the Sertoli cells is essential for the metabolic activities of he spermatozoa, and the LDH-C4 isoenzyme in the germ cells catalyses lactate oxidation. In the absence of LDH-C4, no lactate utilisation can take place, which leads to reduced motility and sperm survival. LDH-C4 activity disappears earlier than the sperm count in vasectomised men, which confirms that the isoenzyme is important for both germinal activity and spermatozoid quality (11).

Previously, it was shown that LDH-C4 deficiency is associated with impaired motility, decreased forward progressive motility and immotile or dead spermatozoa (12). We correlated LDH-C4 with different parameters of the seminogram and obtained significant relations with the total LDH activity, sperm count and SMI. The simple linear relations were ($p<0.01$ $r=0.5529$, $p<0.05$ $r=0.5025$, and $p<0.05$ $r=0.475$, respectively). % C4

activity also correlated well with SMI, motility and sperm count ($p<0.05$ $r=0.475$, $p<0.05$ $r=0.503$, and $p<0.01$ $r=0.528$, respectively). Thus it was shown that LDH-C4 and %LDH-C4 activity are both involved in the mechanism of sperm motility and survival.

We also correlated LDH-C4 with total LDH and CK and found a simple linear relationship with LDH ($p<0.01$, $r=0.5529$) but none with CK. However, the LDH-C4 activity: sperm count ratio, which was previously reported to have a high correlation with the "vital variables" of the spermatozoon (11), did not show any correlation with any of the parameters (SMI, sperm count and motility) in the present study.

In conclusion, regardless of the cause, LDH-C4 isoenzyme and % C4 activity deficiency are seen to be associated with diminished sperm count, markedly reduced motility and SMI, and accordingly, with the reduced fecundatory potential of spermatozoa.

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