Original Article

Evaluation of CD52 positive sperms in subfertile human semen samples: Is there any relationship with main semen parameters?

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Abstract

Background: Sperm maturation and sperm membrane integration are the most important elements in male fertility. CD52 is one of the antigens. CD52 is a GPI (glycosylphosphatidylinositol) anchored that express on lymphocytes and epididymal cells. This antigen bind to sperm membrane during transition sperm from epididymal duct as well as its relationship with semenogelins in human seminal plasma. The aim of this study was to obtain any association between the percentage of CD52 positive sperms with main semen parameters such as percentage of motile sperms, percentage of sperm with normal morphology, and the presence of normal viscosity.

Materials and Methods: Semen samples from subfertile men were analyzed, the samples totally were 45 that divided according to their motility into three groups, first one, more than 40%, second one 10-40%, and the third one under 10% total motility. Fifteen samples in each group were evaluated by semen analysis according to WHO 2010 guidelines for infertility laboratory. Sperms were washed by Ham's F-10 and immunostaining with the monoclonal antibody CAMPATH-1G and then analyzed by flow cytometry. We compared each of the groups based on their motility and the data were analyzed by SPSS 20.

Results: Correlation between CD52 labeling and sperm motility was negatively significant, in the second group ($r = –0.592$, $P = 0.020$) and in the third group ($r = –0.805$, $P = 0.00$).

Conclusion: Our results showed that the correlation between CD52 labeling and sperm motility was negatively significant, but we did not observe any relation with other semen parameters, such as sperm normal morphology, sperm concentration, and semen viscosity.

Key Words: CD52, infertility, sperm parameters

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Received: 05.07.2013, **Accepted:** 15.09.2013

Access this article online	
Quick Response Code:	Website: www.advbiores.net
	DOI: 10.4103/2277-9175.133201

INTRODUCTION

Sub fertility is present in 14-15% of couples, although the 40-50% of this disorder is due to the male factor. Sperm disorders include quantity and quality dysfunction or structural abnormality.[1,2]

First step to recognize the status of semen samples is semen analysis (SA) that is performed in any infertility

How to cite this article: Aboutorabi R, Mazani F, Rafiee L. Evaluation of CD52 positive sperms in subfertile human semen samples: Is there any relationship with main semen parameters?. Adv Biomed Res 2014;3:124.

laboratory.[3,4] In the SA schedule, the quality of semen couples is evaluated by these parameters: Clear, viscosity, agglutination and PH, and the semen about the quality, these parameters will be assessed: volume, concentration, motility, and morphology.[1,4,5]

Sperm motility has a vital effect on male fertility and Categories of sperm movement including: Progressive motility (PR), nonprogressive motility (NP), and immotility (IM) according to WHO (2010) guideline.[3,5,6]

In order to obtain a successful fertilization, we need many molecular and genetically sperm maturation. This maturation could be occurred along sperm passing epididymal duct, and this duct has an important role in sperm maturation and capacitation.[2,7,8]

One of the specific proteins which are produced by epididymal ductal cells is CD52. CD52 is a human GPI-anchored antigen and is produced by single gene on chromosom1 and is expressed in lymphocytes and epididymal epithelium human cells.[9-12]

Some of CD52 functions are in complement regulation and its antibody has cytotoxic effects. Therefore, its antibody has could be used in bone marrow and other organ grafts.[13,14]

The presence of CD52 on the sperm membrane is the cause of electro negativity of sperm. Therefore, normal sperm morphology and its capacitation should be associated with the CD52 presence on the sperm surface. Hence, the presence of CD52 is good index for sperm selection with high quality for assisted reproductive technologies (ARTs).[15,16]

Soluble CD52 in seminal plasma has a function in clot formation and liquefaction of semen in male reproductive tract.[17]

CD52 is the sperm membrane attached tightly to semenogelins. Type 1 and Type 2 of semenogelins are secreted by seminal vesicle and these are major components of human semen fluid. These clots could be trapped sperms at the time of ejaculation and will be broken up to 30 min, after ejaculation by PSA (prostate specific antigene) that is secreted by prostate. This process is nominated as liquefaction of semen. In this process, the sperm will be free to swim in a liquefied semen fluid.[18-20]

As mentioned earlier, the production and expression of CD52 is the responsibility of epididymal ductal cells. These cells secreted the vesicles of CD52 and other proteins by mode of apocrine secretion and then this protein binds to sperm surface. Hence, the presence of CD52 in the sperm membrane will affect the motility of sperms in semen fluid.[7,17,21]

There is one report that revealed inhibitory role for CD52.[18] Another study showed that positive correlation between the presence of CD52 with any main semen parameters include motility.[22]

In literature review, there are no adequate study on the importance presence of CD52 and its probably relationship with motility.

So the aim of this study was to clarify the association between the percentage of CD52 positive sperms and sperm parameters include the percentage of motility.

MATERIALS AND METHODS

Study population

The study population totally were 45 that divided according to the motility to three groups, first group with sperm motility more than 40%, second group with sperm motility between 10-40%, and the third group under 10%; there were 15 semen samples in each group.

Semen samples

Semen samples were obtained from 45 subfertile men referring to Beheshti Hospital fertility and infertility center in Isfahan, Iran by nonprobability convenience method.

Semen samples were obtained by masturbation after 2 or 3 days of sexual abstinence.

Semen analysis were performed after liquefaction of samples after 30 min according to the manual guideline of the world health organization (WHO 2010)

Sperm preparation and analysis by flowcytometry

Equal volume Ham's F-10 added to the semen sample. Then the samples were centrifuged at 3000 g for 5 min at room temperature. Sperm pellets were washed with 1 ml Ham's F-10 medium to obtain a sperm concentration of 20×10^6/ml.

One μ/lit of the monoclonal antibody campath1-G against CD52 (of rat IgG, from AbD Serotec) was added to 100 μ/lit sperm suspension to a final dilution of 1:100.

Rat serum IgG was added at an equivalent protein concentration to another sperm aliquot, which was processed in parallel as a negative control for background subtraction.

After incubation for 20 min at 37°C in 5% CO_2, the sperm were washed twice with 0.5 ml Phosphate, Buffered Saline (PBS, containing BSA 4 mg/ml), in all washing steps in the immunostaining procedure. Sperm were pelleted by centrifugation at 3000 g for 5 min at room temperature.

Staining with FITC-conjugated rabbit antirat, secondary antibody was added to a final dilution of 1:200. Then the mixture was incubated 30 min at room temperature in the dark. The semen samples were washed once in 0.5 ml PBS containing 4 mg/ml BSA.

We added Propidium Iodide (PI) to a final concentration of 5 mg/ml for the staining of nonviable

cells after 5 min in the dark, each sample were analyzed by flowcytometry.

Flow cytometric analysis

We analyzed each sample by flow cytometry (FACSCalibur flow cytometer CellQuest pro software) using laser light excitation at 488 nm and with forward and side scatter signals to set ratings for sperm cells. Fluorescence signals were detected from gating sperm at wavelengths for FITC (550DL dichroic filter and 525BP band pass filter) and PI (620BP filter).

RESULTS

This is descriptive–analytical study. Semen samples were obtained from 45 subjects referring to the Isfahan Beheshti Hospital fertility and infertility center. Then samples were divided based on sperm motility in to three groups.

As shown in Table 1, three groups have significant differences in morphology and normal head.

The mean and SD of normal head is high is group with motility lesser than 10% and is lower than in motility more 40%.

According to ANOVA test, the expression of CD52 in different groups are not same (P = 0.010).

LSD post-hoc indicated that the difference between first and third groups is significant (P = 0.005). Also this difference observed between second and third groups (P = 0.014) [Table 1 and Figure 1].

Significant negative correlation were found between the percentage of CD52-positive sperm and sperm motility which this correlation in third group (with motility <%10) stronger than other two groups. And also there was no significant correlation in first group (with motility >%40) [Table 2].

The correlation CD52 expression and other parameters in Table 3 are shown. The correlation between CD52-positive and normal morphology in third group not only were significant but also showed a negative correlation, but they were not significant in other groups. There were not observed significant

Table 1: Comparison of sperm concentration, normal morphology and normal head between three group individuals

	Mean±SD (n=15)			P
	Group 1 motility >%40	Group 2 %10< <%40	Group 3 <%10	
Normal morphology	33.73±14.11 (%)	27.13±10.042	11.43±9.964	0.005
Normal head (%)	64.13±12.03	70.93±10.50	74.80±31.34	0.000
Concentration (×10^6/cc)	111.07±42.21	74.53±74.38	44.64±40.31	0.35
Viability(%)	21.29±14.47	18.58±16.77	45.39±28.09	0.001

cc=Cubic centimeter

Table 2: Correlation between sperm motility and expression of anti-CD52 antibody

	Coefficient	P
Motility (%)		
>40	0.308	0.26
10-40	−0.592	0.020
<10	−0.805	0.00

Table 3: Correlation between semen parameters and expression of anti-CD52 antibody

	Group 1 P value coefficient	Group 2	Group 3
Morphology	0.476	0.138	0.044
Concentration	−0.199	−0.402	−0.525
Viscosity	0.933	0.214	0.075
Head	−0.024	−0.341	−0.473
	0.0624	0.396	0.265
	0.0138	0.273	0.307
	0. 372	0.087	0.449
	0.248	0.456	0.212

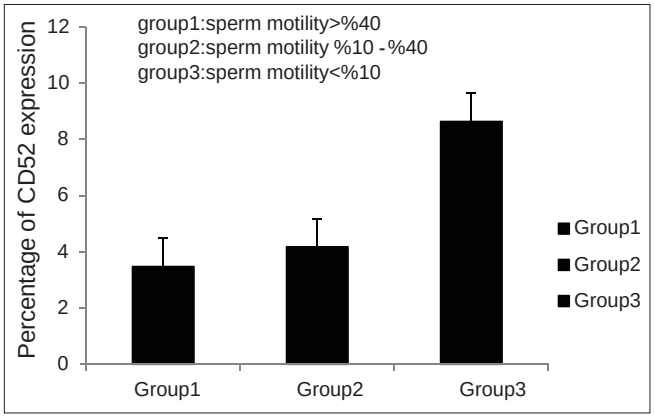

Figure 1: Assessment of CD52 -positive sperms by flow cytometry in 3 groups

correlation between CD52-positive sperm and other sperm parameters including: Semen viscosity and sperm with normal head.

DISCUSSION

CD52 is the most abundant antigen among the sperm glycoproteins and it is acquired during transition of epididymal duct, and is necessary for fertility.[10] CD52 is related with sperm maturation.[11] This study was performed to find the relationship between expression of CD52 and sperm motility. We assessed the presence of CD52 on the sperm surface and sperm motility to discover the relationship between them. Our results demonstrated inverse correlation in subgroups of low and medium sperm motility. On the other hand increases in amount of CD52 inversely decreases sperm motility.

A study performed in 1997 has presented positive correlation between the expressions of CD52 with sperm motility. Although, amount of CD52 are lesser in oligo and asthenospermia in comparison with normal population, this protein should be an effective factor in creating normal morphology and fertility of sperm.[22] Since this glycoprotein is structurally flexible so could have impact on people fertility or infertility status, its epitope probably express by some supportive factors. In this circumstance, this structure identified by antibodies, this controversy between studies might be due to shortage or lack of these supportive factors. Studies with larger sample size will demonstrate concise results.

Finding of a study that has performed by Japanese urologic center supports our results. Semenogelin proteins are as a compound of the semen plasma clotting factor. They has pointed that a part of semenogelin entitled seminal plasma motility inhibitor (SPMI) remains on the surface of sperm in people with asthenospermia. They concluded that CD52 is a factor for attachment of SPMI to sperm surface. We could explain that in people with lower sperm motility. there are more amount of SPMI and CD52 which facilities connection between SPMI and sperm surface.[18,23]

The findings of *in-vitro* studies indicated the absorbsion rate of CD52 by immature sperms in corpus of epididymis is lesser than caudal. This secretory production of epididymis should be accessible for sperms, and this capably depends on availably of proteins and sperm maturity. Probably disability in absorbsion of CD52 by sperms could be due to unsuitable condition or the absence of preparation to receive the factors.[9,22,24]

Our results were showed a negative correlation between amount of CD52 and other sperm parameters including: normal morphology, concentration, and viscosity.

In assisted reproduction technique (ART) interesting results are found. CD52 and other glycoproteins are the cause of negative charge on the sperm surface and increased sperm quality. They have stated that, this negative charge is an important factor for development of sperm with normal morphology.[16,25-28]

We found that, there is no correlation between CD52 and sperms with normal morphology. We could justified may be there are some other glycoproteins, that has more effective role on negative charging.

Other study has demonstrated that, the amount of CD52 are same in fertile and infertile males.[29]

In animal study on the mice sperms verified that sperms with CD52 mutant have fertility ability; hence, mouse sperms are protected by vaginal plug that is created post coitus. The same study is performed on humans.[10]

CONCLUSION

This study revealed that the CD52 plus sperms have negative association with sperm total motility in asthenospermia, but it seems that we need more investigation for clarifying this protein role on fertility. In addition, we did not observe any correlation between CD52 plus with other sperm parameters such as sperm normal morphology, sperm concentration, and semen viscosity.

ACKNOWLEDGMENTS

The authors are thankful to Dr. Z. Farajzadegan Associated Professor of Community Medicine for editing the manuscript and giving some valuable suggestions.

This work was supported by Isfahan University of Medical Science and supported by Department of Physiology, Physiology Research Center, School of medicine, Isfahan University of Medical Science, Isfahan, Iran.

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Source of Support: Nil, **Conflict of Interest:** None declared.