Association of sperm DNA fragmentation (SDF) with different parameters that may be related to fertility and reproduction

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ABSTRACT

Aim of study to see the association of the presence of DNA fragmentation in males with smoking, low sperm motility, infertility, and recurrent pregnancy loss. Sperm DNA integrity is an important factor in normal fertilization and embryo development. Test for SDF seems to be a good option for the husband of females with unexplained history of recurrent miscarriages or infertility. DNA damage can be induced by environmental mechanisms like smoking. 40 males referred to our genetic laboratory for DNA fragmentation test for different causes, we calculate the incidence of SDF among them and evaluate their conditions regarding association with infertility, recurrent abortions, and also we study their age, smoking and sperm count and motility. 27 patients had positive results, so the incidence of DNA fragmentation was 67.5%. 8 of 27 their age were more than 35 years (29.6%), 12 of 27 were smokers (44.4%), 15 of 27 had sperm count less than 15 million (55.5%). 20 patients of these 27 had a history of infertility (74%), 7 patients of 27; their wife had a history of recurrent pregnancy loss (25%). DNA fragmentation associated with low sperm count and motility, smoking, and high incidence of infertility and recurrent miscarriages.

INTRODUCTION

Male infertility may be responsible for about 20% of cases, and in 30–40% combined factors are found in both males and females (Thonneau et al., 1991). Evaluation of male infertility was dependent on the seminal fluid analysis (SFA) according to World Health Organization (WHO) guidelines (World Health Organization, 2010). As seen in Figure 1.

This test is based on manual and visual estimation of sperm count, motility, and morphology using light microscopy, so it is difficult to be performed reliably. (Pacey, 2010). As seen in Figure 2.

Therefore DNA integrity testing of sperm has been proposed to have a good reliable potential to complement the standard SFA.

Although, we can say that semen analysis was the cornerstone of male fertility assessment despite its poor predictive value on fertility potential, and there is an extensive overlap in conventional semen parameters result between fertile and infertile males (Guzick et al., 2001).

While the release of the first edition of WHO guidelines for semen analysis over three decades ago (World Health Organization, 1980), SDF testing is becoming an important tool for infertility specialists.
WHO 2010

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1992</th>
<th>Lower Reference Limit 2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semen volume</td>
<td>2 ml</td>
<td>1.5 ml</td>
</tr>
<tr>
<td>Sperm concentration</td>
<td>20 M</td>
<td>15 x 10⁶/ml</td>
</tr>
<tr>
<td>Total sperm number</td>
<td></td>
<td>39 x 10⁶/ejaculate</td>
</tr>
<tr>
<td>Progressive motility</td>
<td>&gt;50 %</td>
<td>32 % A</td>
</tr>
<tr>
<td>Total motility</td>
<td></td>
<td>40 % A+B</td>
</tr>
<tr>
<td>Vitality (live sperms)</td>
<td></td>
<td>58 %</td>
</tr>
<tr>
<td>Sperm morphology</td>
<td>&gt;15 %</td>
<td>4 %</td>
</tr>
<tr>
<td>pH</td>
<td>≥7.2</td>
<td>≥7.2</td>
</tr>
<tr>
<td>Leucocyte</td>
<td>&lt;1 M</td>
<td>&lt;1 x 10⁶/ml</td>
</tr>
<tr>
<td>MAR/Immunobead test</td>
<td>&lt;10 %</td>
<td>&lt;50 %</td>
</tr>
</tbody>
</table>

Figure 1: Seminal fluid analysis WHO 2010

Figure 2: Seminal fluid analysis normal and abnormal parameters

Figure 3: Fragmented and non-fragmented sperm
DNA in sperm can be damaged by the followings,

1. Apoptosis which can occur during spermatogenesis;
2. Strand breaks happen when chromatin remodeling occurs and during spermiogenesis;
3. DNA fragmentation which happens Post-testicular results from oxygen free radicals during passage through the reproductive tract;
4. Endogenous endonucleases can cause fragmentation in sperm DNA;
5. Radiotherapy and chemotherapy in male with cancer,
6. Environmental causes or factors as smoking or air pollution (Robinson et al., 2012a).

The difference in sperm morphology during testing for fragmentation is shown in Figure 3

Many studies had concluded the adverse effect of DNA damage in sperm on fertility or conception, and it can be associated with,

Failure to conceive (Evenson and Wixon, 2008),

1. Long-time infertility (Spanò et al., 2000),
2. Bad outcome after intrauterine insemination (Duran, 2002; Bungum et al., 2007),
3. Impaired embryo growth (Morris, 2002),
4. Higher abortion or miscarriage rates (Robinson et al., 2012b),
5. Higher risk of fetal loss after in vitro fertilization (IVF) and also after intracytoplasmic sperm injection (ICSI) (Zini et al., 2008),

Spontaneous miscarriage can occur in about 10–15% of pregnancies in normal fertile couples, but the percentage is higher in sub fertile families.

Many studies assessed the relationship between high DNA damage and pregnancy rates showed an effect on pregnancy rates with conventional IVF and little or no effect with ICSI (Spanò et al., 2000).

There is a relation between high SDF and impaired embryo quality (Bungum et al., 2007), low or no implantation rate (Zini et al., 2008), higher miscarriage rate (Brahem et al., 2011), and a higher risk of pregnancy loss (Robinson et al., 2012a).

Methods for chromatin integrity evaluation can find the percentage of cells with damaged DNA only and are based on an idea that the higher the fragmentation rate, the greater the chance that the sperm contamination will be pathological (Zini and Sigman, 2009).

No definite threshold of DNA damage beyond which semen can be considered pathological has been agreed, and many studies provided different ideas and gave different results (Simon et al., 2010).

Significant impact after either IVF or less after ICSI, the degree of sperm DNA fragmentation on embryo growth (blastocyst) and on miscarriage rate, utilized the TUNEL technique. While using SCSA, variable results have been obtained (Tamburrino et al., 2012).

Tests for sperm DNA fragmentation seems to be a reliable option for a couple with unexplained recurrent pregnancy loss history, and referral of husband to a genetic counselor if results are positive (Kohn et al., 2016), it is difficult to know or predict the exact risk of the unfavorable outcome if positive results presents from other available tests.

Couples can get benefit in understanding the reasons for pregnancy losses, and for the diagnosis of a male factor can help them to consider an alternative better reproductive options.

Clinicians can currently counsel couples with unexplained recurrent pregnancy losses and tell them that; chance of live birth in a future pregnancy is approximately 75% (American Society for Reproductive Medecine, 2012)

MATERIALS AND METHODS

From 40 males referred to our genetic laboratory for DNA fragmentation test for different causes, we calculate the incidence of DNA fragmentation among them and evaluate their conditions regarding association with infertility, recurrent abortions, and also we study their age, smoking state and sperm count and motility.

A fresh semen sample was collected in a sterile recipient and by using halosperm G2 kit for detection of halosperm DNA fragmentation rate, as follows,

1. Melt the agarose screw tube in a water bath at 95-100 C0 for 5 minutes.
2. Dilute the sperm sample with PBS until a maximum of 20 million sperm / milliliter.
3. Transfer 50 ml of this sperm sample to the agarose screw tube (appandrofe tube) then mixed well.
4. Place a drop (8mL) of the cell suspension over the center of the sample well and covered by a coverslip.

5. Put in the fridge at 4°C for 5 minutes.

6. Remove the coverslip gently.

7. Cover the well with solution 1 and incubate for a period of 7 minutes then remove the reactive by tilting.

8. Apply solution 2 on the well and incubate for 20 minutes then remove the reactive by tilting.

9. Wash the slide by distal water then tilt the slide until dry completely.

10. Dehydrated by flooding with 70% ethanol, then incubate for about 2 minutes drain and apply 100% ethanol for about 2 minutes, then drain and allow to dry.

11. Apply solution 3 on the well and incubate for 7 minutes, remove the stain by tilting and dry the slide.

12. Apply solution 4 on the well for about 7 minutes, remove the stain by tilting and allow its drying.

13. Visualized under the light microscope.

14. We calculate sperm with fragmented DNA percentage according to the equation,

\[ SDF\ percentage = \frac{\text{degraded sperm} + \text{fragmented sperm}}{\text{total sperm count}} \times 100\% \]

**RESULTS AND DISCUSSION**

From 40 patients send for DNA fragmentation test, we found the followings,

27 patients had positive results, so the incidence of DNA fragmentation was 67.5%, as shown in Table 1.

1. 8 of 27 their age were more than 35 years (29.6%)

2. 12 of 27 were smokers (44.4%)

3. 15 of 27 had sperm count less than 15 million (55.5%)

4. 22 of 27 had sperm motility (a+b) less than 20% (81.4%)

5. 20 patients of these 27 had a history of infertility (74%)

As we said, DNA fragmentation is defined as the separation or breaking of DNA strands into smaller pieces. It can be caused or done by laboratory personnel or by cells also can occur spontaneously; in both situations, fragmentation can gradually accumulate inside a cell. It can be assessed or measured by the Comet assay or by the TUNEL assay.

Male with motility defects of sperms often associated with high levels of SDF (Bello et al., 2014).

Some said that it is not reasonable to support the hypothesis that SDF is an important factor in recurrent miscarriage or recurrent implantation failure, nor that tests of SDF have predictive value in the prospective identification of women at risk of recurrent pregnancy loss or implantation failure. However, studies provide important points into the use of available test kits use for the measurement of SDF and the impact of sperm preparation ways such as density centrifugation method (Pacey et al., 2015).

Systematic reviews have examined the impact of SDF on IUI/IVF/ICSI clinical outcomes, but they failed to reach a strong evidence or conclusion. The latest review –Simon, 2016– found a significant adverse effect of SDF on clinical pregnancy rate, which varied according to the type of assay used.

Other systematic studies confirmed a relation between SDF and abortion (Robinson et al., 2012b), but a number of further studies have showed no evidence for this relation (Pacey et al., 2015), and others (Kirkman-Brown and Jonge, 2017) support the findings of Robinson systematic review (Kirkman-Brown and Jonge, 2017).

Previous studies to examine SDF in recurrent miscarriage couples have shown mixed results, as the use of acridine orange staining for a male with a history of recurrent abortions showed a significant difference when compared with the sperm of fertile men (Bhattacharya, 2008).

The use of TUNEL assay with a semen sample from a family with unexplained recurrent miscarriage has a different result from semen obtained from fertile men and randomly selected unscreened men from the general population (Carrell et al., 2003).

On the other hand, when chromatin integrity of sperm was examined on motile sperm isolated from men with previous recurrent abortions and fertile men and find that the results from the Sperm Chromatin Structure Assay –SCSA had no significant value in the test (Gil-Villa et al., 2010).
On the other hand, micro deletions of Y chromosome, SDF, and oxidative stress of sperm were assessed as a reason for recurrent miscarriage of unknown etiology, and it was found that SDF lacked a strong predictive value to be used as a dependent test for recurrent spontaneous abortions (Bellver et al., 2010).

In our study, we found that 25% of cases with SDF was associated with a history of recurrent abortions.

In about 16 studies, a meta-analysis found that there was a significant rise in miscarriage rate in men with high DNA damage compared with those with lower DNA damage (Ribas-Maynou et al., 2012).

In contrast, there are few studies which considered SDF in men with recurrent implantation failure following IVF. For example, embryos from couples undergoing pre-implantation diagnoses PGD were examined, and it was concluded that although the semen samples with increased DNA fragmentation, there was no correlation of DNA fragmentation with aneuploidy rate, also in fresh or processed sperms (Bronet et al., 2012).

The DNA fragmentation also found to be more in smoker patients probably because of oxidative stress that results from the cadmium and nicotine in cigarette smoke which usually associated with an inflammation of the accessory glands as well as the testis, which can cause an alteration in sperm function, that is characterized by reduced integrity of acrosome and mitochondrial activity, and also by higher DNA fragmentation of nucleus (Antoniassi et al., 2016).

Other studies found that cigarette smoking affected all semen parameters negatively in addition to condensation of chromatin and viability of sperm. These abnormalities increased according to the amount or number of cigarettes smoked / day and to the duration or time history of smoking (Mostafa et al., 2017).

In our study, we found that 44.4% of men with SDF were smokers, so nearly half of the males with positive SDF were heavy smokers, so stopping smoking may be of benefit to improve fertility.

CONCLUSIONS

Sperm DNA fragmentation is associated with low sperm count and motility.

Sperm DNA fragmentation is found to be associated with smoking and found more in males with a history of infertility and recurrent miscarriages.

We suggest the SDF test to be one of the important tests in the assessment of couples presented with infertility or recurrent miscarriages.

Smoking could be a cause for SDF, so better for us to elevate patient awareness about this fact.

REFERENCES


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