Effect of H.pylori and Cag-A on the infertility among males
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Abstract: Among infertility factors, the immunological factor that associated with humoral immunity against sperm antigens is one of the causes of human infertility. A hypothesis for the induction of anti-sperm antibodies (ASA) is the cross-reactivity of spermatozoa antigens and exogenous antigens including; bacteria, viruses, fungi and allergens. The presence of anti-sperm Ab, anti-H.pylori and Cag-A and its effect on infertility among male and female have been found. In the current study, 129 male and 38 females (18-59 years) participated. Indirect Enzyme-Linked Immuno Sorbent Assay (ELISA) used for detection of ASA, anti-H.pylori Ab and Cag A. The seminal fluid examination test was performed according to an examination and processing of human semen. H.pylori infection can be observed among both of infertile male and female who participated in this study. A significant difference in mean values was observed between the presence of ASA, anti-H.pylori Ab and Cag-A (P-value < 0.000). P-value < 0.000 also observed between Cag A and ASA according to the trauma of testes. The result of the current study showed that about one of a third of males who participated in this study have a history of a microbial infection that can lead to the destruction of testes barrier thus result in the production of auto-antibodies against sperms.

Key words: Anti-sperm Ab; anti-H.pylori Ab; Cag A; immunological infertility.

Introduction
Infertility is a worldwide problem. It’s affecting one couple out of each six couples (Kazemijaliseh et al.,) and is defined as failure to conceive after one year of unprotected intercourse in the reproductive age. Primary infertility affects about 15% of couples, while male factor infertility accounting for fifty percent of cases. In more than 20% of the cases, the causes of infertility stay behind unexplained (Ali A. Al-Fahham.; Deepali Thaper; Jun Fu). A theory for the initiation of anti-sperm antibodies (ASA) which is one of the humoral immunity (Brunner-Agten et al.; Bobak I.; Zhao et al.) is the cross-reactivity of spermatozoa antigens and exogenous antigens (Ag). Common antigenicity has been recognized between human sperm and bacteria, viruses, fungi and allergens (Nowicka-Bauer et al.,). Indeed, the similarity between certain epitope of bacteria and part of the structure of sperms leads to the cross-reaction and damage of sperms in the male and female body (Deepali Thaper; Hiroaki Shibahara). These antibodies are directed to various sperm antigens and implicated in sperm dysfunction (V. Zodinsanga; Ali A. Al-Fahham.; Azizi). Some researchers noticed that the presence of ASA was significantly higher (42.5%) among patients with unexplained and persistent infertility (Ali A. Al-Fahham.).

A number of possible mechanisms have been suggested for the formation of ASA in women. Isoimmunization may happen following phagocytosis of sperm by immunocompetent cells in the female reproductive tract and the subsequent appearance of spermatic antigens to the immune system. This mechanism is not yet fully clear. The cross-reactivity, cervix uteri surgery, continual traumatization of the cervix, inflammation, and sexually transmitted infections are other causes of formation of ASA (Bobak I.; Bubanovic; Cui et al.,). Recently, advanced special laboratory investigation has been found for detecting ASA, including; IgA, IgG, and IgM. But the last one seems to have no clinical impact because it is rarely detected alone or combined with IgA or IgG (Sandro C. Esteves).

Recently, the correlation between the presence of anti-H.pylori Ab and the damage of sperm was demonstrated in both of cervical mucus (Deepali Thaper; Collodel G). Furthermore, cytotoxin-associated gene A (CagA) positive (strain of H.pylori) was showing the potential effect on the sperm activity in both of male and females. While depending on the molecular mimicry theory Vacuolating cytotoxin A (VacA) could bind with the tail of sperms and reduce its activity or even can kill it (El-Garem, El-Sawy and Mostafa; Collodel G; Ambrosini G). This study aims to use ELISA Antisperm antibody, cytotoxin-associated gene A Cag A assay and seminal fluid analysis of serum and seminal plasma as a complementary test in the evaluation of male infertility.
Materials and Methods

Study population: In this study, the ethical approval and permission to perform the work was granted by the local scientific committee of Hawler Health Technology College. Furthermore, consent was obtained from each participant prior to the study. For hundred and twenty-one women comprising 129 male and 38 women, aged between 18 years and 59 years, were selected randomly for this study. All men and women who have a problem in conception were selected randomly. Peripheral blood samples were collected by intra-venous puncture and aspiration from the cubital vein. Anti H.pylori, anti Cag-A, and Brucellosis. Investigations were done by using Vitek Immuno Diagnostic Assay System (VIDAS). While, data for G.C, Mumps and Syphilis were obtained through interview and questionnaire. The interpretations of tests were performed at the Department of Medical Laboratory Technology, Erbil Health Technology College/Erbil University Polytechnic.

Seminal fluid examination: After absence 3 days of sexual intercourse, the seminal fluid examination test was performed according to an examination and processing of human semen (World Health Organization). During history taking and sample collection, all information that related to the current study was recorded.

Antisperm antibody test: Test principle: Anti-spermatozoa antibodies exert heterogeneous effects on the ability of spermatozoa to fertilize. The inhibiting effect of anti-spermatozoa antibodies on the motility of spermatozoa by binding to the surface and by aggregating processes is well-known. The Sperm Antibody ELISA (Enzyme Linked Immuno Sorbent Assay) is a solid-phase sandwich-enzyme-immunnoassay for the quantitative determination of anti-spermatozoa antibodies in human serum (ALPCO).

Anti CagA antibody test: Test principle: The microtiter plate provided in this kit has been pre-coated with an antibody specific to CagA. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for CagA and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB (3,3', 5' tetramethyl-benzidine) substrate solution is added to each well. Only those wells that contain CagA, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm±2nm. The concentration of CagA in the samples is then determined by comparing the O.D. of the samples to the standard curve (EIAlab).

Result

The result in (Table 1) below shows the category of men and women who participated in this study. According to the age of participators, the age group of men was between 18-59 years, while for women were 18-44 years. In the current study and depending on the fertility of participators, there are three types of male participators: fertile: 38 (29.5%), primary infertile 49 (38.0%), and secondary fertile 42 (32.6%). While, the number and percentage of women were: fertile 35 (51.5%), primary fertile 18 (26.5%), and secondary fertile 15 (22.1%). According to the information that obtained during questionnaire and sample collection for the current study, there are 37 (18.8%) of male who participated in this study informed that they had sexually transmitted disease in the past of their life, while only 4 (5.9%) of women who participated informed about sexually transmitted disease in the period of their life. Finally, 76 (58.9%) of males participators have a positive result of Anti-H.pylori Ab – IgG, while 31 (45.6%) of females recorded a positive result about the presence of Anti-H.pylori Ab – IgG.

Table 1: The demographic of male and female participators.

<table>
<thead>
<tr>
<th>Age</th>
<th>Male n=129</th>
<th>Female n=38</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-59 Years</td>
<td>38 (29.5%)</td>
<td>35 (51.5%)</td>
</tr>
<tr>
<td>Fertility</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary infertile</td>
<td>49 (38.0%)</td>
<td>18 (26.5%)</td>
</tr>
<tr>
<td>Secondary infertile</td>
<td>42 (32.6%)</td>
<td>15 (22.1%)</td>
</tr>
<tr>
<td>History of STD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>37 (18.8%)</td>
<td>4 (5.9%)</td>
</tr>
<tr>
<td>No</td>
<td>92 (71.3%)</td>
<td>64 (94.1%)</td>
</tr>
<tr>
<td>Anti H.pylori Ab</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>76 (58.9%)</td>
<td>31 (45.6%)</td>
</tr>
<tr>
<td>No</td>
<td>53 (41.1%)</td>
<td>37 (54.4%)</td>
</tr>
</tbody>
</table>

Moreover, (Table 2) shows the number and percentage of participators who have positive results for Anti-H.pylori Ab IgG test based on their status of infertility. The table shows that 18 (13.9%) of fertile males have positive results for Anti-H.pylori Ab (IgG) test compared 20 (15.50%) of the negative result. While, for primary infertile participators 31 (24.03%) have positive of Anti-H.pylori Ab IgG test and 18 (13.9%) have negative, and for secondary infertile 27 (20.93%) of them have a positive result for Anti-H.pylori Ab IgG but 15 (11.62%) on the other hand, table 2 shows that 15 (11.62%) of fertile females have positive results for Anti-H.pylori Ab IgG test and 8 (6.20%) have negative, and for secondary infertile 6 (4.65%) of them have a positive result for Anti-H.pylori Ab IgG but 9 (6.97%).
Furthermore, (Table 3) below shows the mean±SD of ASA according to the presence of Anti-\( \text{H.pylori} \) Ab (IgG) in the sera of participators. The result of the statistical analysis shows that the mean±SD of males who have a positive result of the presence of Anti-\( \text{H.pylori} \) Ab (IgG) and positive of ASA was 76 (1.2368±0.42797) and 53 (1.0189±0.13736) have a negative result. On the other hand, the mean±SD of women who have positive results for both of Anti-\( \text{H.pylori} \) Ab (IgG) and ASA 31 (1.3548±0.48637) and 37 (1.0270±0.16440) recorded a negative result with a highly significant (P value= 0.000).

As far as the (Table 4) below is concerned, it shows mean±SD of Anti-\( \text{H.pylori} \) Ab (IgG) and Cag A according to clumping of sperm during seminal fluid analysis. The results show that the mean±SD of the positive result of Anti-\( \text{H.pylori} \) Ab (IgG) among males who have clumps of sperm during SFA test was 85 (1.4824±0.50265) and for the negative result was 44 (1.2727±0.45051) with a highly significant (P value= 0.000). Furthermore, the mean±SD of Cag A who have clumps of sperm during SFA test was 88 (13.0148±10.83124) and 41 (7.8146±4.94492) with a highly significant (P value= 0.000).

Table 5 shows the number and percentage of participators who have ASA positive with the different type of infection that can destroy the barrier of testes. The number and percentage of participators who had not infections in the past period that can lead to the destruction of the barrier of the testes were 20 (15.50%). The result shows that the participators who had an infection of \( \text{Gonorrhea coci} \) (G. C), mumps, brucellosis, and syphilis respectively, were 1 (0.77%), 2 (1.55%), 3 (2.32%), and 0 (0%).

Figure 1 shows that there is a strong correlation between the presence of Cag A and the result of ASA test. The statistics, results show that the mean±SD of Cag A for participators was 11.36±9.65, and ASA positive was 33.22±26.97 with a highly significant (P value= 0.000).
The mean±SD of the participators who have ASA positive and exposed to the trauma in the past period of their life was 73 (39.0274±30.92148) and mean±SD for men who haven’t any history of trauma for their tests was 56 (25.6607±18.42358) and P value =0.000.

Table 6: The mean±SD of Cag A and ASA according to the trauma that exposed testes of males during the past period of their life.

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Mean±SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cag A n=129</td>
<td>Positive</td>
<td>73 (56.38%)</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>56 (43.41%)</td>
<td></td>
</tr>
<tr>
<td>ASA n=129</td>
<td>Positive</td>
<td>39.0274±30.92148</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>25.6607±18.42358</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

The findings of this study from the demographic point revealed the following: Infertility is one of the problems that face Erbilian couples. It is important to be mentioned that the patients who participated in this study were 18 -59 y for males years and 18 – 44 years for females and they were having problems in conception including primary and secondary infertility. This implies that couples in Kurdistan suffer more from infertility and there is no significant difference in the spread of infertility between the genders. This finding agrees with (Taha) results, that the primary infertility is more common problems that couples suffer in Erbil city. The findings of this study show that sexually transmitted infections can be seen among infertile couples and it's one of the causes of infertility (Apari, de Sousa and Müller). Furthermore, the table of demographic of the current study shows that H.pylori infection can be observed among both of infertile male and females who participated in this study. This agrees with the result that reported by (Eusebi).

The result of the current study showed that there are about half of males have a positive result for anti- H.pylori Ab test, while the positive result among females is 1/4 (Deepali Thaper; Collodel G). The relationship between the presences of anti-sperm antibodies is one of the findings of the current study. The result of the current study showed that there are about near of half males and 1/4 of females have a positive result for anti-H.pylori Ab test. Thus, results of this study. Conforms with similar results of the study, which provided that the presence of H.pylori leads to forming antibodies can attack sperms in the male's body, follicular fluid, and vaginal secretions that lead to infertility (Figura et al.). This finding supports the result of other studies that reported the possibility of cross-reactivity with spermatozoa of anti-H.pylori (Figura et al.). Furthermore, the explanation of the presences of the cytotoxin-associated gene A (CagA) antibodies in the sera of participants related with the low activity of sperms in the of body of both of male and females with presence of infertility among them is the logical cause between molecular mimicry theory, this type of Ab could bind with the tail of sperms and reduce its activity or even can kill it (El-Garem, El-Sawy and Mostafa; Collodel G; Ambrosini G). The result of the current study showed that about one of a third of males who participated in this study have a history of a microbial infection that can lead to the destruction of testes barrier thus result in the production of auto-antibodies against sperms. This result supports by Deepali Thaper, and his team in 2014, when they reviewed about the relationship between microbial infection and the formation of anti-sperm antibodies (Deepali Thaper). Furthermore, the microbial infection can cause a formation of anti-sperm antibodies among male and female (Bobak).

Conclusion

As far as the results are concerned, the presences of anti- H.pylori Ab and Cag A were related to primary and secondary infertility among male and females. They support the researchers who believe that the cross-reactivity between spermatozoa antigens and exogenous antigens is one of the causes of infertility.

References


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