Atopy in relationship with recurrent vaginal candidiasis in rabbits
R. A. Faaz
Coll.Vet.Med. Univ. of Basrah

Abstract
To determine whether there is an association between atopy and recurrent vaginal candidiasis (RVC) and to evaluate the type-2 immune response in rabbits with RVC. Evaluation of immediate hypersensitivity skin tests to aeroallergens, measurement of total IgE and Candida albicans specific IgE and levels of IL-5 in 10 rabbits (females) with RVC and 8 rabbits with sporadic vaginal candidiasis (SVC). Statistical analyses were performed by Mann–Whitney test and $X^2$ test with Yates correction. History of atopy (60%) and positive skin test (42.8%) were higher ($P<0.05$) in RVC than in rabbits with SVC. No significant difference was found in total IgE, C. albicans specific IgE and IL-5 levels. There was a strong association between atopy and RVC, but type-2 immune response to C.albicans antigen have residual mitogenic faction or epitope.

Introduction
As Candidiasis is an iatrogenic, nosocomial infection which is usually endogenous in origin many other clinical manifestation may occur, especially in the debilitated patient. Candida albicans can be isolated from the vagina of approximately 20% of the nonpregnant females of reproductive age$^{(1,2)}$. While approximately 75% of rabbits will experience a single episode of candidal vulvovaginitis in their lifetime, a significant proportion of these rabbits (5%) will subsequently experience recurrent Candida infections$^{(3)}$. The pathogenesis of recurrent vaginal candidiasis (RVC) remains controversial but evidence has been accumulated that a decrease in cell-mediated immunity occurs in these rabbits$^{(4,5)}$. Women with RVC have a decrease in delayed type hypersensitivity reaction to C. albicans antigens, but can react to other antigens$^{(4,6)}$. Also, we have previously demonstrated a lack of C.albicans specific interferon-γ production in vitro in RVC rabbits$^{(7)}$. It is also known that mucocutaneous candidiasis is associated with T cell impairment$^{(8)}$ and that C. albicans is an opportunistic infection in patients with Acquired Immuno Deficiencie Syndrome AIDS$^{(9,10)}$. There is association between vaginal candidiasis with a type 2 immune response has been well documented in experimental models of candidiasis. While in the mice, a type 1 immune response is generated with subsequent control of infection, other mice produce predominantly IL-4 and have recurrent infections$^{(11)}$. This may imply that type 1 immune reactivity is associated with resistance to candidal disease, while type 2 immune responses are associated with pathology of RVC. However, in spite of the evidence in experimental models of RVC, that candidal disease is associated with an enhancement of type 2 response, this is not clear in humans. While we and others, evaluating rabbits with RVC, have failed to document IL-4 and IL-5 in supernatants of lymphocyte cultures stimulated with C. albicans antigens$^{(6,7)}$, a few studies have associated RVC to allergy to C. albicans$^{(11,12)}$. The aim of the present study was to determine whether there was an association between atopy and RVC. Allergy was evaluated by previous history of atopy, skin prick test responses to aeroallergens and total and C. albicans specific-IgE. Moreover, levels of IL-5 were measured in supernatants of lymphocyte cultures stimulated with phytohemaglutinin (PHA) and C. albicans antigens.

Materials and methods
Participants of this study included 10 rabbits (females) with RVC. Was using some contraceptive method. Every rabbits had positive Candida albicans vaginal
cultures prior to study entry and the tests were performed during active disease. Swab specimens were placed on Sabouraud’s dextrose agar and subsequently identified at the species level with the use of the API 20C system rabbits were recruited sequentially, but were excluded from the study if the microscopical findings could not be confirmed by Candida culture or they had other infection. None had diabetes mellitus, anaemia, AIDS, autoimmune or thyroid diseases or were in use of immunosuppressive drugs or had receipted of antifungal agents in the previous four weeks. The severity of each sign and symptom was scored on a scale of 0 (absent or normal) to 3 (severe). The level of vulvovaginal discharge was not scored. Seven (7), nonpregnant, age-matched rabbits from the same clinic, who had history of successfully treated nonrecurrent candidal vulvovaginitis were chosen as the control group. They were recruited simultaneously with the case group. Successful treatment was defined as absence of symptoms during a 1-year follow-up. These rabbits had negative Candida culture at the beginning of the study. We used standard allergy and medical histories to obtain information about symptoms, the frequency and intensity of RVC episodes, rabbits underwent physical examination and skin prick test with different allergens. Atopic status was defined as the presence of allergic respiratory disease (rhinitis or asthma) in the preceeding 3 months and skin prick test positive for at least one antigen.

Skin prick test for immediate hypersensitivity (SPT):

SPT were performed on the right forearms of all individuals using a panel with 10 relevant local allergens: Pool of Molds (M. mucedo, P. pullulans), Candida albicans, dog and cat epithilia, according to standardized techique[14]. Histamine (1/1·000) and saline were used as positive and negative controls, respectively. The size of the reaction was determined by measuring the greatest transverse diameter of the papulae 15 min after implantation. The skin reaction was considered positive if the diameter was > 3 mm in experimental and positive control and < 3 mm in saline negative control.

Candida albicans antigen and mitogen for in vitro studies:

C. albicans isolated from a rabbits with RVC was cultured in Sabouraud’s media. The cultures were centrifuged at 3000 g for 30 min and the pellet was washed 3 times with PBS. The pellet was re-suspended in 0·5 N sodium hydroxide and then frozen (−70 °C) and thawed at 37 °C about 20 times. After the suspension was centrifuged at 3000 g for 30 min, the supernatant was collected and the pH was adjusted to 7·3. The protein content was determined by using the method of Lowry et al.[15]. The mitogen PHA was used also. Dose–response curves were performed and the optimal concentration for C. albicans antigen was 0·05 µg/ml and for PHA was 10 µg/ml.

Fig (1):- Candida albicans
Cell culture and cytokine assay
Peripheral blood mononuclear cells (PBMC) were isolated from heparin-treated venous blood by gradient centrifugation. After being washed 3 times in 0.9% NaCl, cells were resuspended in RPMI-1640 culture medium nutrient agar supplemented with 10% rabbits serum, 100 IU/ml penicillin and 100 IU/ml streptomycin. Cells were adjusted to (3×10^6 cells/ml), placed in 24-well plates and stimulated with C. albicans antigen (0.05µg/ml) or PHA (10 µg/ml) (fig 2). After 72 h of incubation at 37 °C, 5% CO2, supernatants were collected and stored at −70 °C. Levels of IL-5 were determined by ELISA results are expressed in pg/ml.
Specific and total IgE

Total IgE was determined by ELISA as previously described\(^{(16)}\). The IgE concentration in sera from controls and rabbits with RVC was determined by an antigen-capture ELISA, using an anti-rabbits IgE monoclonal antibody and a goat anti-rabbits IgE peroxidase conjugate. The results are expressed in International Units (IU). For determination of Candida albicans-specific IgE, polystyrene 96-well plates were coated with 40 μg/ml of C. albicans antigen in carbonate-bicarbonate buffer pH 9.6 and incubated overnight at 4 °C. After 6 washes, PBS-Tween-20 0.1% was added (100 μl/well) and incubated overnight at 4 °C. Sera were depleted of IgG prior to use by treatment with RF absorbent (Dade Bhering, Germany) and added to the plate. After washing, mouse IgG anti-human IgE antibody was added (100 μl/well) at a dilution of 1 : 100 in PBS-Tween 0.05% and incubated for 2 h at 37 °C. After a further sequence of washes, anti-mouse IgG peroxidase conjugate (1:3000 in PBS-Tween 0.05%) was added and the plates were incubated at room temperature for 30 min. TMB was then added (100 μl/well) and the reaction was stopped with H2SO4 (50 μl/well) within 30 min.

Statistical analysis

To compare the IL-5 levels and IgE levels between RVC and SVC rabbits Mann–Whitney test were used. To compare the frequencies of atopy by history and the proportion of skin prick test positivity in the two groups, chi-square
tests with Yates correction were performed.

Results

The mean age ± standard deviation of cases and controls. In the rabbits with RVC, the mean illness duration was 3 month. Data relating to a previous personal history of allergy, and the frequency of positive immediate hypersensitivity skin prick test in the experimental and control rabbits are shown in (Table 1)

Table 1:- History of atopy and skin test response to aeroallergens in rabbits with Recurrent Vaginal Candidiasis (RVC) and Sporadic Vaginal Candidiasis (SVC).

<table>
<thead>
<tr>
<th>Variables</th>
<th>RVC n (%)</th>
<th>SVC n (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Personal history of atopy</td>
<td>6/10(60%)</td>
<td>2/8 (25%)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Positive aeroallergens skin prick test</td>
<td>3/7 (42.8%)</td>
<td>1/4(25%)</td>
<td>0.02</td>
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</tbody>
</table>

The majority (6/10, 60%) of rabbits with RVC had a history of atopy versus only (2/8, 25%) in the control group (P<0·0001). The majority (3/7, 42.8%) of RVC rabbits with at least one skin prick test reported severe symptoms while only (2/4, 50%) of RVC rabbits without positive SPT had such symptoms (P<0·0001). Tests of immediate hypersensitivity to aeroallergens were performed in 10 rabbits with RVC and 7 controls (Table 2). The proportion of RVC patients with at least one positive skin prick test (3/7, 42.8%) was higher than that observed in the control group (1/4, 25%) (P<0·02).

Immediate hypersensitivity skin prick test to Candida allergen was negative in all rabbits tested (Table 2).

Table 2:- Proportion of rabbits positive for each one the allergens in the skin prick test.

<table>
<thead>
<tr>
<th>Aeroallergens</th>
<th>RVC patients n (%)</th>
<th>SVC patients n (%)</th>
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</thead>
<tbody>
<tr>
<td>D. pteronyssinus</td>
<td>3/7 (42.8)</td>
<td>1/4 (25%)</td>
</tr>
<tr>
<td>Blomia tropicalis</td>
<td>2/7 (28.5%)</td>
<td>1/4(25%)</td>
</tr>
<tr>
<td>Fungi pool</td>
<td>0/35 (0)</td>
<td>0/21 (0)</td>
</tr>
<tr>
<td>Candidina</td>
<td>6/7 (85%)</td>
<td>0/4 (0)</td>
</tr>
<tr>
<td>Dog epithelium</td>
<td>0/7(0)</td>
<td>0/4 (0)</td>
</tr>
<tr>
<td>Cat epithelium</td>
<td>0/7 (0)</td>
<td>0/4 (0)</td>
</tr>
</tbody>
</table>

*M. mucido, P. pullulans

Figure 1a shows the levels of total IgE in rabbits with in RVC and control groups. There was a tendency for higher median IgE levels in rabbits with RVC (239·5 IU) ranging from 13 to 4419 IU, as compared with the control group (80 IU), ranging from 13 to 697 IU, but this difference was not statistically significant (P>0·05). While the median of the optical density in the ELISA for Candida specific IgE (Fig.1b) in the RVC case group was 0·223 pg/ml (range 0·0600 to 0·3930 pg/ml), in the control group it was 0·1785 pg/ml (range 0·1390–0·5540 pg/ml) (P>0·05). Four RVC patients had very high levels of total IgE. All had history of atopy, but there was no evidence of association of IgE levels with severity of symptoms.
In order to discover if the RVC patients had an elevated type-2 immune response when compared with controls, the levels of IL-5 in PBMC cultures stimulated with *C. albicans* or PHA were determined (Fig. 2). The median of IL-5 levels in cultures stimulated with *C. albicans* antigen in the case group was 0 (range 0-1332 pg/ml) and in the control group was also 0 (range 0-580 pg/ml) (P= 0·7039). No statistically significant difference was found in the levels of IL-5 when the cultures were stimulated with PHA (RVC group: median 1691·5 pg/ml) (range 0–2995 pg/ml); SVC group: median 1357 pg/ml (range 143–2815 pg/ml) (P= 0·6714).
Discussion

RVC is an important problem of rabbits and the understanding of the pathogenesis of this disease may help to control and prevent the recurrence of vaginal candidiasis. We have previously shown that the majority of rabbits with RVC had impaired type-1 T cell response with inability to produce appropriate levels of IFN-γ when stimulated with C. albicans antigen in vitro\(^7,17\). Here we show that there is a strong association between atopy and RVC, however, the IL-5 production in response to Candida antigen was not observed. It remains to be established whether the synthesis of other type 1 and 2 cytokines were altered under other conditions.\(^{19}\). There is an antigen specific decreasing in IFN-γ production, while type 2 cytokines are increased. In this study we did not show higher IL-5 levels or higher C. albicans specific IgE antibody levels in rabbits with RVC when compared with women with SVC. A few reports have documented high titres of Candida specific IgE as well as eosinophils in vaginal washes suggesting that a compartmentalized immune response may occur.\(^{20,21}\). However, the percentage of rabbits with RVC with evidence of IgE in vaginal washes is much lower than the frequency of rabbits who report history of allergy or have evidence of atopy in this and in a previous study\(^{22}\). Although a lack of standardization of C. albicans antigen could be a bias in the results of the immune responses in these rabbits, the fact that both proteins and carbohydrates fractions of the yeast contain allergens.\(^{23}\) Moreover, the negative skin prick test to C. albicans antigen in all RVC rabbits also supports that strong type 2 immune responses to C. albicans is not an important finding in RVC. This is in agreement with our previous report that down regulation of type 1 immune responses to C. albicans antigen in patients with RVC was not associated with a strong type 2 immune response, but rather with high IL-10 levels, since neutralization of this cytokine enhanced in vitro IFN-γ production in lymphocyte cultures stimulated with C. albicans antigen\(^{26}\). Although a type 2 immune response to C. albicans antigen was not observed in rabbits with recurrent candidiasis, a strong association with atopy was documented. Moreover, pruritus, burning and vaginal discharge were worse in patients who had atopy, suggesting that allergy to other antigens may play a role in RVC. This observation gives support to previous studies that showed an association between
RVC and rhinitis and between RVC and positive immediate skin prick test to aeroallergens (23). This is also in agreement with the observation that vaginal itching as well as allergic vulvovaginitis may be manifestations of seasonal allergy caused by pollen (24,25). Vulva and vagina may be important routes for the entrance of allergens that induce local or even systemic reactions (26,27). For instance: seminal fluid components, medications, spermicide, sanitary napkins, latex, food particles and house dust mite can be introduced into vagina and may induce an allergic response. Based on this study, there is a strong association between atopy and RVC and little evidence of a strong type 2 immune response to C. albicans antigen. In such case, the allergic reaction to other antigens at the vaginal mucosa could facilitate the colonization or infection with C. albicans as a secondary event due to the break of the natural resistance to pathogens. Subsequently, this environment with a predominant type 2 immune response to other allergens or C. albicans antigen could block the development of a protective type 1 immune response. The observation that RVC is associated with both atopy and impaired type 1 immune response may have clinical implications. It is possible that enhancement of T cell response and decrease allergy may be a tool in the treatment of RVC.

References


علاقة الحساسية بداء المبيضات المتكرر في الأرانب

روى عدنان فائز
جامعة البصرة / كليّة الطب البيطري

الخلاصة

لغرض تحديد ما إذا كان هناك علاقة بين الحساسية وداء المبيضات المهبلي المتكرر والإشارة إلى النوع الثاني من الاستجابة المناعية في الأرانب مع داء المبيضات المهبلي المتكرر. تم تحديد قدرة الحساسية المناعية بفحص الجلد C. albicans ضد الأليلات الهوائية وقياس الضد المناعي E الكلي والضد المناعي كمستخلص ضد قطر مع قياس مستوى (5 – 11) في عشرة أرانب أما مصابة بداء المبيضات المهبلي المتكرر مع ثمانية أرانب انثى C. albicans (1L – 5L) والضد المناعي لكي مستوى (5 – 1L) P<0.01 هذه علاقة بين الحساسية وداء المبيضات المهبلي المتكرر ولكن الاستجابة المناعية من النوع الثاني ضد مستضد C. albicans بقایا لجزء أو طرف المايتونين.