DISCUSSION
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The present study is an attempt to establish the average immunoglobulin concentrations in cervical mucus of normal women during various phase of menstrual cycle, pregnant women and women with various cervical pathologies including endocervicitis cervical erosion and cervical dysplasia in cases which were histologically proved.

1. **Age of patients**

   The average age of control group was 21.2 years (ranged 18 to 25 years). The pregnant group ranged from 18-29 years (average 21.9 years). The average age of patients with cervical pathology was 25.1 years (range 20-33 years). Thus the average age of control and pregnant group was almost identical but those in cervical pathology group were a little older. Weldmen et al (1971) have reported decrease in percentage of IgA and increase in that of IgG, in cervicovaginal secretions with increasing age. Present study didnot shown any correlation of these levels with age. In another study of influence of aging on external secretions, Alford (1968) found a decrease in nasal wash.

   IgA concentrations with increasing age.

2. **Nature of antibodies in cervical mucus**

   The human endocervix is lined with secretory columner epithelium which produces mucus shown to contain immunogl-
obulins (Moghissi and Newhous, 1962; Schumacher et al 1965; Herve et al 1965, Elstein and Pollard, 1968). Specific antibodies in cervical mucus have been demonstrated against spermatozoa (Solish et al 1961; Strauss, 1965), blood group antigens, E. Coli and C. albicans (Parish et al, 1967). Local formation of antibody is suggested by finding of higher titres in mucus than in serum (Parish et al, 1967), the detection of secretory piece and lactoferrin in endocervical epithelium by immunofluorescence (Masson et al, 1968; Hulka and Omran, 1969; Tourville et al 1970), and the location of IgA and IgG containing plasma cells in the underlying stroma by the same method (Masson and Ferrin, 1969). Moreover, Hutcheson et al (1974) have incriminated local antibody in the aetiology of infertility, they demonstrated increased numbers of IgA + containing plasma cells in cervical biopsies from patients with unexplained infertility.

In the female reproductive tract local antibody production was first investigated in heifers by Kerr and Robertson (1941) who showed that vaginal secretions contained agglutinins to trichomones foetus when locally infected by these organisms. This has been subsequently confirmed in heifers (Kerr and Robertson 1943), in
cattle (Hughes 1953; Kerr and Robertson 1953; Kan 1955) cows (Pierce 1946). Pierce (1946) also studied Ig in serum besides mucus and observed higher titres in cervical mucus of cases infected by trichomonas foetus than serum. Batty and Warrack (1955) and Bell and Wolf (1967) found similar results in rabbit and straus (1961) in humans.

In the present study, simultaneous studies of IgG and IgA levels were not done in serum. Yet the results obtained from cervical mucus showed that there was local antibody production. Cervical mucus IgG/IgA ratio of 2.2 in normal controls 2.6 in pregnant women and 1.8 in women with cervical pathology is quite different from that in serum 5:1 to 8:1 Coughlan and Skinner (1977) also obtained an IgG/IgA ratio of 2:4. Similarly Behrman and Lieberman reported a ratio of 2:3. Thus present findings are similar to those of above two studies. Findings of Firemans et al (1963) are also in agreement with the findings of this study. However, these findings are contrary to those of Chodirker and Tomasi (1963), Tomasi and Bienestock (1963) and 1968) and Masson Ferrin (1969) who reported relatively high concentration of IgG.

Since IgG/IgA ratio is significantly lower than that found in serum this provides indirect evidence of local antibody production in cervical mucus.
While there is a good evidence that IgA is produced locally in the cervix (Waldman et al 1972; Ogra and Ogra, 1973, Rebells et al 1975), Coughland and Skinner (1977) are not clear about the origin of IgG in cervical mucus. Von Kaulla et al (1957) believe that mucus IgG originates by passive transudation in serum. Coughlan and Skinner (1977) reported no positive correlation between serum and mucus IgG concentrations. Basing on positive correlation between cervical mucus IgG and IgA concentration they argued that there was a possibility that influences controlling IgA production may also be involved in part at least in local IgG production.

Amino et al (1978) observed gradual decreases in serum concentration of IgG, IgA and IgM during normal pregnancy. In the present study cervical mucus no such decrease in IgA and IgG levels with progress of pregnancy (Table-III) was observed suggesting an independent production of IgG and IgA into cervical mucus for reproductive tract.

In the present study the levels of IgG and IgA were very high in cervical mucus of patients with cervical pathology (mean IgA 167.3±93.9; mean IgG 299.2±124.5) as compared to normal controls (mean IgA 49.0±35.3; mean IgG 107.2±59.4). These observations might suggest a
selective loss of selective serum/mucus transudation in abnormal cervices (Coughlan and Skinner, 1977). Schumacher et al (1965) also suggested similar mechanism. But this does not explain higher level of IgA (IgG/IgA ratio of 1.8) in diseased cervix as compared to that in normal controls (IgG/IgA ratio 2.2). This observation suggests the possibility of local response to abnormal epithelial cells or to a prolonged antigenic stimulus. Bhatia et al (1981) conclusively showed that increased production of IgG and IgA at local level in such conditions when they could not find a simultaneous local increase in complement (C₃ & C₄), albumin and transferrin.

To summarize, present study suggests a local production of IgA and IgG into cervical mucus and not merely a transudation of serum proteins, a review held by previous workers like Von Koulla (1957), Tomasi and Bienestock (1963 & 1968) and Masson and Ferrin (1969).

3. Immunoglobulin levels in normal controls (during various phases of menstrual cycle):

As shown in table II mean values of IgG ± SD (mg/dl) in premenstrual, mid cycle and post menstrual phases were 171±46.9, 44.3±34.3 and 106.3±15.3 respectively. On the other hand, mean IgA values ± SD (mg/dl) were 81.1±2.8, 27.3±27.5 and 38.9±17.6 in premenstrual mid cycle and postmenstrual phases, respectively. Thus the average IgG value ± SD (mg/dl) in normal controls
(n=21) was 107.2±59.4 while IgA value ± SD (mg/dl) was 49.0±35.3.

It is evident from above data that Ig levels were lowest during mid cycle phase and highest during premenstrual phase. These findings are in agreement with Elstein and Pollard (1968) who found detectable immunoglobulin in pregestational phase only. Tourville et al (1970) also reported increase in immunoglobulin IgG and IgA in secretory phase of menstrual cycle. Elstein (1970) also reported similar increase in immunoglobulin level in premenstrual phase.

Schumacher (1973), Davis et al (1983), Bhatia et al (1981) who measured IgG and IgA in mucus also found mid cycle nadir of these Ig's and peak in their levels in premenstrual phase. Coughlan and Skinner (1977) failed to demonstrate mid cycle decrease but did shown increase of IgG and IgA in premenstrual phase.

Harve et al (1965) on the contrary reported a mid cycle peak of immunoglobulin which they attributed to follicular fluid as it did not occur in anovulatory cycle. The present study included only fertile women with regular menstrual cycles. It is unlikely that most if not all women included in this study had anovulatory cycles. However, it may be pointed out that patient
No. 45 did not have any detectable immunoglobulin of either class at mid cycle. But this may be due to some technical error in dilution/storage of cervical mucus rather than anovulatory cycle.

These findings of present study do not confirm to observations of a number of workers viz Schumacher (1965) Solish et al (1961), Moghissi and Neuhaus (1966), Waldman et al (1971), Chipperfield and Evans (1972) and Eissa et al (1985) who could not demonstrate any variation in immunoglobulin levels of either class with the phases of menstrual cycle.

It is evident from above studies that there is a lot of controversy regarding changes in Ig levels with various phases of menstrual cycle. It may be due to difference between method of collection or storage of cervical mucus or technique employed for estimation of immunoglobulin levels. But in present study the increase in IgG as well as IgA levels in premenstrual phase was highly significant ($P \leq 0.01$) when levels were compared with those in post menstrual phase.

Though the predominant immunoglobulin was IgG yet the increase in premenstrual phase was more evident in IgA (108.3\%) as against IgG (61.3\%). Similar findings were observed by Bhatia et al (1981) who showed relatively more increase in IgA level in premenstrual phase.
than IgG though the predominant Ig was still IgG. Coughlan and Skinner (1977) also found relatively greater increase in IgA. But Schumecher (1973) found relatively greater increase in IgG. Present findings are in contrary to Waldman et al (1971) and Chipperfield and Evans (1972) who reported IgA as predominant immunoglobulin of cervical mucus.

<table>
<thead>
<tr>
<th>Study</th>
<th>Mean IgG/ range in normal control</th>
<th>Mean IgA/ range in normal control</th>
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<tbody>
<tr>
<td>Waldman et al (1971)</td>
<td>12 mg/dl</td>
<td>22 mg/dl</td>
</tr>
<tr>
<td>Hulka &amp; Omran (1969)</td>
<td>9-456 mg/dl</td>
<td>2.8-90 mg/dl</td>
</tr>
<tr>
<td>Eissa et al (1985)</td>
<td>420±7 mg/dl</td>
<td>21±4 mg/dl</td>
</tr>
<tr>
<td>Coughlan and Skinner (1977)</td>
<td>131.8±11.7 mg/dl</td>
<td>47.1±7.2</td>
</tr>
<tr>
<td>Masson et al (1969)</td>
<td>45 mg/dl</td>
<td>9 mg/dl</td>
</tr>
<tr>
<td>Bhatia et al (1985)</td>
<td>Mean 60.2 mg/dl</td>
<td>Mean 32.9/dl</td>
</tr>
</tbody>
</table>

If we have a look over table No. VI, than it is evident that levels found in present study are quite similar to those of Coughlan and Skinner (1977), Hulka and Omran (1969). But levels quoted by Eissa et al (1985) Masson et al (1969) and Waldman et al (1971) were quite low.

In only other study from India (Bhatia et al.) IgG and IgA values were almost half to 2/3 that of present
study. But that study used immuno-electrophoretic method (Holbrow and Johnson technique) for estimation of Ig unlike present study using radial immunodiffusion. Moreover there was difference in method of collection, dilution and storage of cervical mucus. These differences might explain this dissimilarity between two different Indian studies. Similar differences also existed in western studies.

Coughlan and Skinner (1977) did not find any difference in IgG/IgA ratio during various phases of menstrual cycle. Bhatia et al (1981) also did not show any significant change in ratio during various phases. But present study, like Hulka and Omran (1969) found a relative decrease in IgG/IgA ratio (1.6 midcycle as against 2.2 and 2.1 in post and premenstrual phase). Hulka and Omran (1971) found a mid cycle decrease reaching nadir at the day of ovulation. Present study though did not make a record of day of ovulation yet it confirms to the findings of these authors.

4. **Immunoglobulin levels in cervical mucus during pregnancy**

As shown in table (III) mean values of IgG±S.D. (mg/dl) observed in first second and third trimesters of pregnancy were 89±58.9, 114.9±26.4 and 119.5±71.6 respectively; while respective values for IgA were
31.1±18.6, 43.4±15.7 and 53.4±32.9. Thus there was a gradual increase in immunoglobulin levels with the progress of pregnancy. But this increase was not statistically significant when results were compared using student 't' test.

The percent increase in IgA was more as compared to IgG, with progress of pregnancy. IgA showed a 71.7% increase while IgG only 23% from first trimester to third trimester. In the present study IgG was the dominant immunoglobulin which is in agreement with Bhatia et al (1981). In both the studies increase in IgA was more prominent as compared to IgG.

Relatively higher levels of immunoglobulins were detected in present study as compared to those reported by Bhatia et al (1981).

Present observations are not in agreement with those of Waldman et al (1971) who showed no change in immunoglobulin levels with the progress of pregnancy. Levels observed in their studies were as such quite low (IgG 9 to 11 mg/dl and IgA 1.3-25 mg/dl). Predominant immunoglobulin in their study was IgA not IgG. These results might be so due to difference in technique of collection and storage of cervical mucus.

Amino et al (1978) reported a decrease in serum immunoglobulin levels with the progress of pregnancy.
which they attributed to mainly immunosuppression. Present study over cervical mucus shows opposite trend. This observation suggests production of local antibodies by human female genital tract which is governed by an independent mechanism.

Marculis et al (1971) observed that concentration of serum IgG diminished with each successive trimester whereas concentration of other two immunoglobulin IgM and IgA were either unchanged or had no constant trend. Similar were the observations of Tiselius (1937).

Brown et al (1954), Gudson (1989) and Gangun et al (1989) who besides a fall in IgG levels also observed a fall in IgM levels, with no change occurring in IgA levels.

As far as this present study is concerned we have found a progressive increase in immunoglobulin levels of IgG and IgA, though the dominant immunoglobulin is IgG the percentage increase of IgA was 71.7% as compared to IgG which was only 23%. Hence we can now say according to the present study the cervix play a role in the production of local antibodies, independent of the levels of serum.

5. Immunoglobulin levels in women with cervical pathology

As evident from table V and VI both IgG and IgA were quite high in cervical pathology group as compared to
normal controls. Results were significant for cervical erosin/endocervicitis group. But they were more significant in cervical dysplasia group. Corresponding percent increased in IgG and IgA reflected the same trend. In cervical erosion/endocervicitis group percent increase in IgG was 111.1% and in IgA was 119.2%. In cervical dysplasia group it was 301.5% for IgG and 461.2% for IgA. Schumacher et al (1965) have also reported increase in immunoglobulin level in cervical mucus of patients with non malignant abnormal cytology. Like present study Bhata et al (1981) and Coughlan Shimmer (1977) noted quite significant increase in IgG and IgA in patients with abnormal cytology. Bhata et al (1981) who differentiated between cervical pathologies found more increase in patients with malignant group as compared to non malignant group. They also noted a predominant increase of IgA. On the other hand Waldman et al (1971) found no significant difference with cervicitis.

According to Schumacher et al (1965) this relative increase in IgG and IgA may be due to loss of selective serum/mucus transudation in abnormal cervixes, in patients with non abnormal cytology. But this does not, however, explain relatively greater increase in
IgA. Concentrations in patients with abnormal cytology, and it is possible that increased immunoglobulin concentrations may represent a local response to abnormal epithelial cells or to a prolonged antigenic stimulus from an exogenous or endogenous infections agent (Coughlan and Skinner, 1977). To refute the theory of loss of selective barrier between serum and mucus as profounded by Schummacher et al (1965). Bhatia et al (1981) made a simultaneous study of cervical mucus $C_3$ and $C_4$ albumin and transferrin levels in patients with cervical pathology. These levels were not suggestive of loss of any such barrier strengthening the suggestion of Coughlan and Skinner (1977) that this might be due to prolonged antigenic stimulation by endogenous or exogenous agent. In the view of Coughlan and Skinner (1977) type 2 herpes simplex virus a known associate of cervical carcinoma (Skinner et al 1971, Skinner 1976) and an agent capable of establishing latent infection in the human subject (Sterens et al 1972 and Baringer, 1974). Would seem a reasonable candidate.

Relatively more increase of IgA in present study with female genital tract infection (endocervicitis) is also supported by previous studies (Chipperfield and Evans 1972; Waldman et al 1972; Ogra and Ogra 1973 etc.).
Chipperfield and Svan (1972) however, did not find any relation between cervical erosion and immunoglobulin level.

Present study reemphasizes the suggestion of Bhatia et al (1981) that estimation of immunoglobulin level particularly of IgA and IgG should be done as an early diagnostic aid for cervical malignancies. It is agreed that investigation of cervical mucus for neutralizing antibody or any other antibody the type two herpes simplex virus as suggested by Coughlan and Skinner (1977). But in places where such advance facilities are not available this simple estimation of mucus IgA and IgG besides cytological examination might prove quite helpful in early diagnosis of cervical dysplasia and other premalignant or malignant conditions.