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PCR TESTING FOR DETECTION OF HPV INFECTION IN PLACENTAL TISSUE FROM WOMEN WITH SPONTANEOUS ABORTIONS: INVESTIGATING POTENTIAL ASSOCIATION

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ABSTRACT

Human Papillomavirus (HPV) is commonly linked with both benign conditions such as skin, oral, and anogenital warts, and malignant diseases including cervical, oral cavity, and ovarian cancers. While sexual contact remains the primary transmission route, vertical, horizontal, and auto-inoculation pathways also exist. A possible link between high-risk HPV strains and spontaneous abortion has been suggested.This cross-sectional study was conducted at Hayatabad Medical Complex, Peshawar, over 1.5 years. A total of 120 placental samples were collected from women who experienced spontaneous abortion before 24 weeks of gestation. Samples underwent Polymerase Chain Reaction (PCR) testing to detect HPV types 16 and 18, and histopathological examination was performed using Hematoxylin and Eosin (H&E) staining.All placental samples tested positive for beta-globin, confirming sample integrity, but none were positive for HPV DNA. While classic koilocytosis was absent, some histological features such as bi-nucleation, multi-nucleation, and perinuclear halo were observed. A key limitation was the large size of placental tissue, which restricted complete analysis.No evidence of HPV DNA was found in placentas from spontaneous abortions, suggesting no direct association in this

cohort.

Introduction

Pregnancy is a physiological process that results typically in child birth between 37 and 41 and six days weeks of gestation, referred to as “term pregnancy” (WHO.2018).Deliveries before 37 weeks are considered as “preterm” , while pregnancies beyond 42 weeks are labeled as “post term”.Not all the pregnancies progress to full term a substantial proportion of pregnancies end pre maturely due to spontaneous or induced abortion.Abortion is defined as expulsion of fetus at any time of gestation.An abortion occurring naturally is called “spontaneous abortion”.An abortion which is performed for medical reasons or in unsafe conditions is called “induced abortion”(Singh et al., 2022).

Spontaneous abortion commonly termed as miscarriage is also defined as natural termination of pregnancy before fetal viability generally before 24 weeks of gestation.It frequently occurs in early pregnancies affecting 20 percent of clinically recognized pregnancies although the actual rate may reach to 31 percent due to unrecognized early losses (Wilcox et al., 2019; Zhou et al., 2021).

Numerous factors contribute towards spontaneous abortion.The most frequent being chromosomal abnormalities, implicated in half of first trimester losses (Petersen et al., 2021).These abnormalities originate during fertilization or early embryogenesis,with Parental age related fragments of DNA playing an important role (Kumar et al., 2020).Structural uterine abnormalities specially a septate uterus is have also been associated with increased miscarriage rate (Bermejo et al., 2019). Infectious agents like *Chlamydia trachomatis*, , *Toxoplasma gondii*, herpes viruses, CMV, can interfere with implantation, fetal development, or placental function (Alvarado-Esquivel et al., 2019).In recet years HPV has emerged as a potential but poorly understood factor for pregnancy loss.Globally high risk HPV 16 and 18are associated with cervical and anogenital cancers.In addition sexual transmission ,vertical and intrauterine transmission of has been documented(Bruni et al., 2019). HPV DNA has been identified in placental tissue, amniotic fluid, and fetal membranes, raising concerns about its role in adverse pregnancy outcomes (Ambühl et al., 2020). HPV infection during pregnancy compromise placental function or integrity leading to pregnancy losses..A study in Mexico found 19 percent HPV prevalence in women with multiple miscarriages (Cavazos-Rehg et al., 2021),where as Pakistani data show HPV prevalence between 2 to 16 percent (Minhas et al., 2022) This study Investigates the potential association between HPv infection and spontaneous abortion through placnetal examination and molecular detection.

Methods

From January 2024 to December 2024 placental samples were collected randomly from different hospitals in Peshawar. Total 120 placentas were collected, 60 placentas from females with spontaneous abortion and 60 from full term delivery females. Samples were placed in separate properly labeled jars containing formalin.

After collection of samples, next step was block formation. Initially tissue was subjected to gross examination. Membranes and maternal parts were observed. About 1.5 inch area was cut carefully with special knife and placed in block cup with number on it. The block cup along with tissue was again dipped in formalin left overnight. Tissue was removed from cup and placed in series of jars containing 70 percent and 100 percent alcohol each. Next sample was put in xylene for 1 hour in separate container. Sufficient amount of wax was melted in

container. The sample along with molten wax was placed in a mold and put in oven at 100℃ Celsius for 1 hour. Mold was removed from oven and kept in freezer overnight. The block is ready.

Hematoxylin and eosin staining

For hematoxylin and eosin staining the block was subjected to cutting with rotatory microtome. The strip obtained after cutting was dipped in water bath. The strip was cut with fine needle according to size of slide. Single piece was placed on one slide. The slide was put in oven for about an hour. After this slide was subjected to staining as shown in table.

Table Table showing steps of staining

Steps	Solution	Temperature	Duration
Deparaffinization	Xylene	Room Temperature	4 times 3 min each
Drying		Room Temperature	10 min
Rehydration	100%,100%,80% and 60% alcohol	Room Temperature	3 times 2 min each
Staining with Haematoxylin	Haematoxylin	Room temperature	15 min
Washing	Tap water	Room temperature	5min
Staining with eosin	Eosin	Room temperature	3 min
Washing	Tap water	Room temperature	5min
Dehydration	50%,70% and 100% alcohol	Room temperature	3times each for2minutes
	Xylene	Room temperature	2 min

Polymerase chain reaction

Polymerase chain reaction (PCR) is an elegant system of enzymatic assay. It enables us to amplify specific fragment of DNA from a pool of complex DNA. Kary Mullis in 1900 gave concept of PCR. (41) PCR requires specific components, that is, primers, DNA polymerase, and nucleotides which are mixed in specific test tubes. This mixture is put in specific machine called thermal cycler. In thermocycler DNA amplification occur by three steps, that is, denaturation, annealing and extension. During denaturation the reaction mixture is heated above melting temperature of complementary strand of DNA. Thus allowing the strand to separate. In next step the temperature is lowered allowing specific primers to get bind to target DNA segment. This step is called annealing. Finally temperature is raised again enabling DNA polymerase to extend primers by addition of nucleotides to developing strand of DNA. (42)

For DNA extraction thermos scientific DNA extraction kit was used. With the help of rotatory microtome, the block was cut into 10 micrometer pieces thickness. The part of strip containing the tissue was taken carefully with help of forceps. Sufficient amount of tissue was taken and subjected to grinding using a mortar and pestle in laboratory. The tissue was grinded for sufficient time until it turned into powdered form. Next the tissue was put in Eppendorf tube and 180 ul of digestion solution and 20 ul of Proteinase K was added in tube. The lid of tube was closed and incubated for 8 to 10 hour. 20 ul of RNase solution was added to tube and mixed thoroughly by vortexing and left as such for 10 min at room temperature. Next 200 ul of lysis solution was added again mixed thoroughly by vortexing and left at room temperature for 15 to 20 minutes. Then 400 ul of 50% ethanol was added and mixed thoroughly by vortexing for sufficient time until homogenous mixture was obtained. The lysate was transferred to a column tube inserted in a collection tube. It was subjected to centrifuge for 2 min at 4000 rpm. The collection tube containing flow through was discarded. The DNA collection tube was placed in another collection tube.500ul of Wash Buffer 1 was added and centrifuged at 1 min for 4000 rpm. The flow through was discarded and purification column was again placed in collection tube.500 ml of wash buffer 2 was added to DNA purification column tube and centrifuged for 2 min for 4000rpm. Discard the flow through and the genomic DNA purification column was transferred to1.5 ml of micro centrifuge tube. Finally 200 ml of elusion buffer was added to the center of DNA purification column to elute the DNA. It was incubated for 2 minute and further centrifuged for about 1 minute. The purification column was discarded. The purified DNA was stored in refrigerator.

The presence of DNA was confirmed by Nano drop.

Before start of any PCR, primer designing is important step. The DNA sequence for HPV 16,18 and 58 were found from Gene bank (http://www.ncbi.nih.gov/genbank/). For each type of HPV primers were uniquely designed and from BLAST analysis specificity of primer was confirmed (http://www.ncbi.nlm.nih.gov/BLAST/). (43) The sequences of primers are shown in table .

Table .Table showing primer list

S.no	Name	Sequence
1	HPV 16 (F)	5'TTAGGCAGCACTTGGCCAACCA3'
2	HPV 16 (R)	5'TAATCCGTCCTTTGTGTGAGCT3'
3	HPV 18 (F)	5'TCCGTGGTGTGCATCCCAGCAG3'
4	HPV 18 (R)	5'CACTTGTGCATCATTGTGGACC3'
5	B-Globin (F)	5'GAAGAGCCAAGGACAGGTAC3'
6	B-Globin (R)	5'CAACTTCATCCACGTTCACC3'
7	GP (F)	5'TTTATTACTGTGGTAGATACTAC3'
8	GP (R)	5'GAAAAATAAACTGTAATCATATTC3'

When we receive the primer it is first rehydrated according to protocol given in literature of every company. This primer which is rehydrated is called as "Primer Stock. For every primer the amount of nuclease free water in which the primer to be rehydrated is different and given in literature of every primer. For general primers the amount of nuclease free water is 250 micro liters, which is mentioned in kit literature. For HPV 16 the amount is 270 micro lite and for HPV 18 its 240 micro liter. For rehydration firstly, the tube in which the primer comes are centrifuged briefly for 30 seconds. Nuclease free water the amount of which is mentioned above is added and is mixed briefly for about a minute by vortexing. Then it is again briefly centrifuged. Resultant primer stock is ready. It is stored in freezer and can be used for indefinite period of time.

For running PCR we need 10 micromole concentration of primers. For this purpose, the primer was taken from primer stock and diluted. 10 micro liter of primer stock solution was taken in Eppendorf tube and 90 micro liters of distilled water was added. The solution was vortexed and mixed. This solution is called "running primers".

The annealing temperature of different DNA to be amplified by PCR is different. Ideally the annealing temperature should be 5°C less than the average of melting temperature of forward and reverse primers. The melting temperature was found by following formula. (44)

Melting temperature= 4(G+C) +2(A+T).

After finding out melting temperatures of respective reverse and forward primers there average was taken. Annealing temperature as mentioned above was calculated.

For performing PCR firstly all the samples of purified DNA undergo PCR for beta globin gene. This gene is used as positive a marker to ensure that DNA is existing in the samples being amplified with PCR.

The melting Temperature for beta globin forward primer was 58°C and reverse primer was 60 °C so the average temperature is 59°C. The annealing temperature should be between 54 to 64°C. For general PCR primers the melting temperature for forward primer is 53°C and for reverse primer is 56°C. The annealing temperature should be between 49 to 59°C. After calculation, the melting temperature for forward primer for HPV 16 was 64°C and reverse primer was 62°C respectively. So, the annealing temperature for HPV 16 should be between 58-68°C. The annealing temperature of HPV 18 was calculated to be in range of 47 to 57°C. The range of annealing temperature of HPV 58 was 64 to 74°C.

Steps of PCR

Polymerase chain reaction consists of 3 steps namely denaturation, annealing and extension. Temperature for denaturation and extension are constant whereas temperature for annealing can be changed.

In a PCR tube stand five labelled tubes containing PCR master mix, forward primer, reverse primer, purified DNA, distilled water and Taq polymerase enzyme respectively were kept.
In PCR tubes, PCR ingredients were taken in the amount as mentioned above. PCR thermocycler was turned on, tubes kept in rack and lid was closed. The PCR thermocycler has touch screen. New protocol icon was pressed and the PCR thermocycler started. When the PCR thermocycler completes its cycles the machines beeps giving the indication that the process has ended. The PCR product can be saved in refrigerator or directly the gel can be run.
To carry out PCR the system need to be optimized. Thermal optimization is usually done.As mentioned earlier "beta globin" is a universal gene. Thus, the human beta globin can be used in experimental control in PCR protocols. The annealing temperature for beta globin as calculated was between 54 to 64℃. The process was started with 54 ℃ but no bands appeared. Later on temperatures 56, 58, 60 up to 64 were tried. The bands appeared on temperature 60℃ but the bands were not that much clear. Clear bands appeared on 64 degree centigrade. On this temperature no nonspecific banding was present. So annealing temperature was 64 degree centigrade for beta globin gene.
For general primers again, thermal optimization process was used. The annealing temperature in this case was between 49 to 59 ℃. On temperature 49 ℃ the bands appeared which were clear. In this condition the process was initially done with a positive known sample which was obtained through a reliable source and when clear bands appeared of that sample at 49 ℃ the rest of samples were all tried on this temperature. The annealing temperature for HPV 16 was between 58 to 68℃. For positive control the bands appeared on 58℃. The conditions were said to be optimized for PCR of HPV 16. 20 samples at random were selected from cases and were tried for HPV 16, but the bands did not appear. For HPV 18 the annealing temperature was between 47 to 57℃. A positive sample was again taken and subjected to PCR. Typical bands appeared on 48℃. The system was optimized for HPV 18. Again 20 samples from cases were selected at random and PCR was done but no bands appeared. For HPV 58 annealing temperature as calculated was 64 to 74 .PCR at 64 was tried but no bands appeard.
The PCR product was run on gel and then visualized. The gel was formed by adding one gram of agarose to tris borate EDTA (TBE) to 50 ml of buffer. The mixture was heated in oven for about 5 minutes. The gel when partially set was placed in specialized tray which is meant for running gel and a comb was placed in it. For about 45 minutes current was passed using special electrodes. Than after sometime the gel was projected with U.V light and photograph of gel was taken.

3.RESULTS

3.1 Population characteristics

The mean age of cases category was 13.5 years with maximum number of cases falling in the age group between 20 to 25 years. The mean age of controls was 10.5 with maximum number of cases falling in category of age between 20 to 25 years and 35 to 40 years. If we compare the mean of ages of both groups the mean of cases is more. Maximum number of cases and control had history of single marriages and maximum number of subjects in both cases and control were Pakistani.
In medical terminology parity means number of time a woman has given birth to fetus with fetal age of more than or equal to 20 weeks of gestation. According to data analyzed the mean of parity of women included in cases category was 5.5 and mean of control was 6. The mean parity of patients included in control group is more than case group. The mean of number of abortions of cases is 15 and control group is 14.5. The maximum number of abortions of both cases and control is single.13.4 is the

mean of gestational age of cases. These statistics shows that with increasing gestational age the incidence of abortions increases.

Table . Population characteristics of study sample

Age of patient	Case	Control
15-20	12	7
20-25	33	18
25-30	3	10
30-35	11	9
35-40	1	15
40-45	0	1
Number of marriages	Cases	Control
Single	49	53
Double	11	6
Triple	0	1
Nationality	Cases	Control
Pakistani	51	50
Afghani	9	10
Parity	Cases	Control
1	19	3
2	10	15
3	12	17
4	12	13
5	2	9
6	3	2
7	1	1
8	1	0
9	0	0
Number of abortions	Cases	Control
1	36	44
2	16	9
3	6	5
4	2	2
Gestational age (mounths)	Cases	Control
3	3	
4	4	
5	23	
6	29	
7	1	
Comorbid	Cases	Control
Diabetes	6	4
Hypertension	4	2
UTI	0	2
Pneumonia	3	1

3.2 Histopathological Findings

Although typical koilocyte which is characteristic of HPV was not found in any slide. A koilocyte is defined as a specialized epithelial squamous cells which has undergone a series of structural and physiologic changes that occur when infection with HPV takes place. Koilocytosis is term which we uses in histology to describe presence of koilocyte in the specimen. (Hajdu, 2006)

A koilocyte may have following changes at cellular level;

1) Nuclear enlargement usually 2 to 3 times of normal size.
2) Irregular nuclear membrane.
3) Hyperchromasia defined as darker staining pattern of nucleus than normal.
4) Perinuclear halo defined as clear area surrounding the nucleus.

Generalized structure of placenta is showed in (Fig 3.1). Although specific features of HPV were not found in any of slides, but some additional features were noted like binucleation (Fig. 3.2), multinucleation (Fig. 3.3) and perinuclear halo (Fig3.4). The summary of features noted is given in table 3.2.

Table 3.2 Showing histologic features noted

Histologic features	Occurrence in cases	Occurrence in control
Marked Halo	12	5
Bi-nucleation	2	3
Multi-nucleation	14	2
Hyperchromasia	0	0

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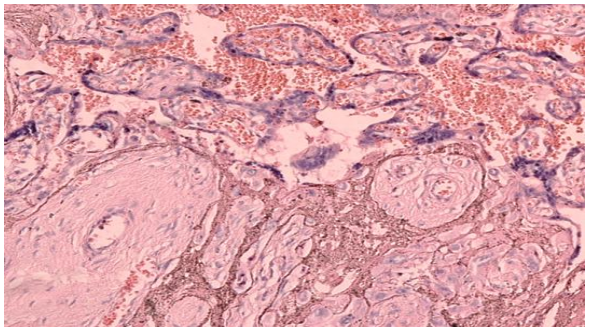

Figure .3.1 Micrograph showing structure of placenta at 10x magnification. Decidual tissue and villi are prominent.

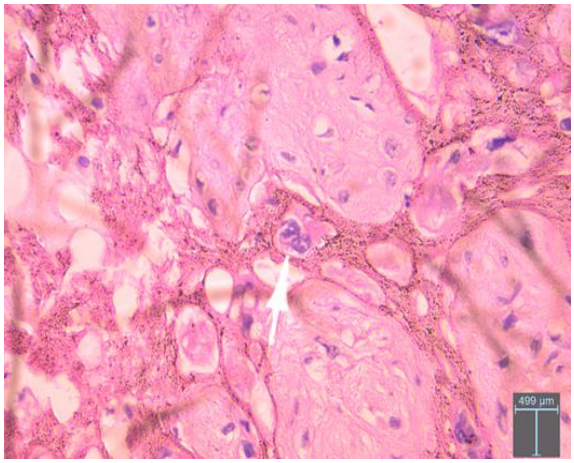

Figure 3.2. Micrograph showing binucleation in hematoxylin and eosin stained slides of placenta under 40x magnification.

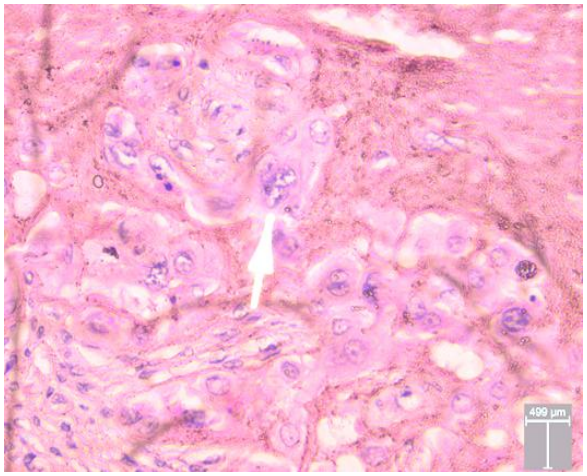

Figure 3.3 Micrograph showing multinucleation in Hematoxylin and eosin stained lide of placenta under high power magnification.

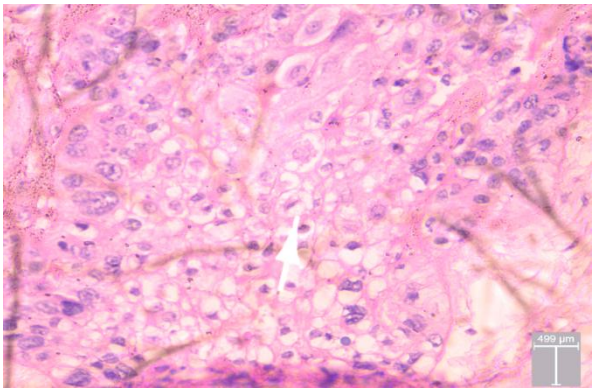

Figure . 3.4 Micrograph showing perinuclear halo trophoblastic tissue of placenta under high power magnification.

3.3 PCR results

3.3.1 PCR results for beta globin genes

The first PCR which was done for beta globin gene results showed that all the samples were positive for the presence of beta globin gene. The PCR bands for beta globin gene gel pictures should come between 200 to 300 base pair region. As shown in pictures these bands appeared between 268 base pair region.

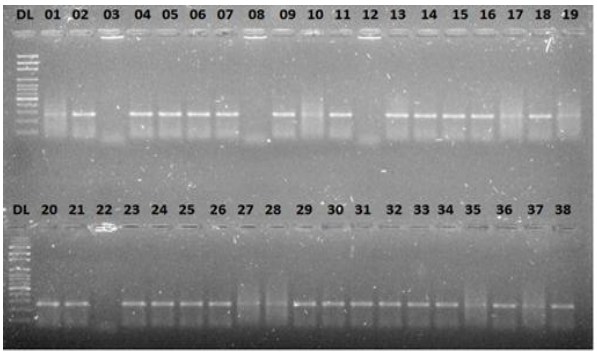

Figure PCR gel pictures for beta globin gene sample 1 to 38.

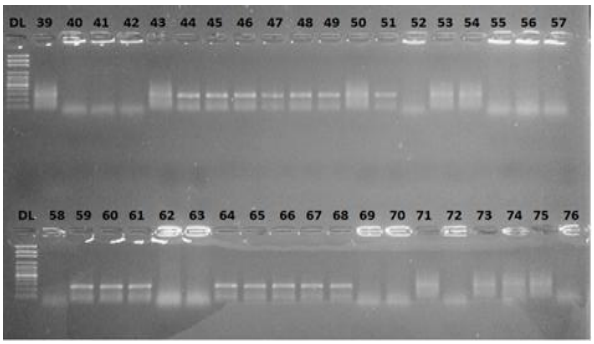

Figure PCR gel pictures for beta globin gene sample 39 to 76.

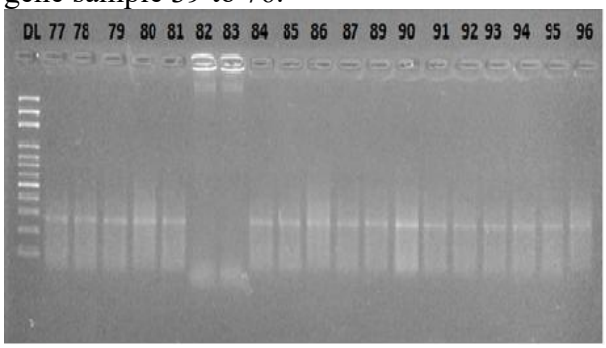

Figure . PCR gel pictures for beta globin genes sample 77 to 96.

3.3.2 PCR results for general primers.

For general primers the complete protocol of PCR was followed but all the samples were negative for General Primers as shown in following gel pictures.

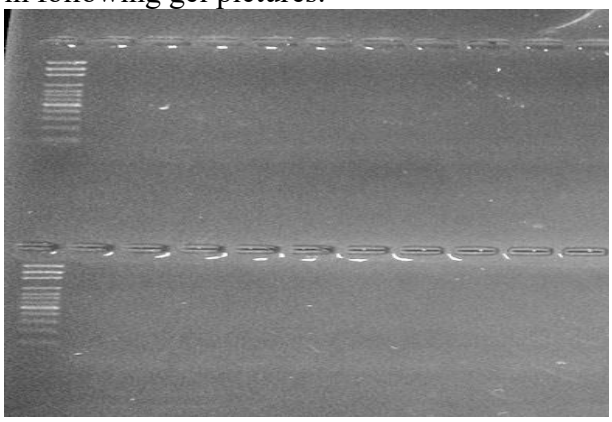

Figure PCR gel pictures for HPV general primers.

3.3.3 PCR results for HPV 16,18,58

Although the PCR for general primers was negative but still PCR for HPV 16,18,58 was still tried but as the gel pictures shows the result was negative.

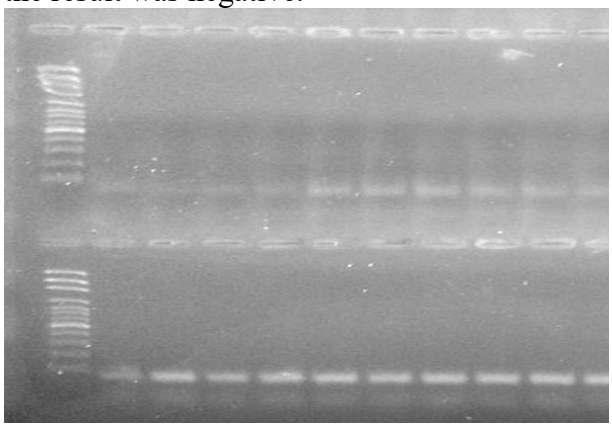

Figure . PCR picture for HPV 16 AND 18.

4-DISCUSSION

This study explored the potential link between high-risk Human Papillomavirus (HPV) types 16 and 18 and spontaneous abortion. Despite biological plausibility and evidence from previous international research indicating a possible association, the findings from this cohort revealed no statistically significant relationship. Global studies that suggest a connection often rely on indirect specimens such as amniotic fluid or cord blood, or on experimental cell models. These limitations hinder the ability to establish causality with confidence (Ambühl et al., 2020).

A detailed analysis of maternal and obstetric characteristics revealed several noteworthy patterns. The incidence of pregnancy loss was higher among women aged 35 and above, a trend consistently observed in multiple studies across South Asia (Sahoo et al., 2019). Additionally, first-time mothers, or nulliparous women, exhibited a higher rate of miscarriage. This may be due to undiagnosed anatomical issues such as uterine malformations or cervical insufficiency (Kumari et al., 2020). The mean gestational age for miscarriage in this study was approximately 13.9 weeks. While early first-trimester losses are more common, miscarriages occurring toward the end of the first trimester or beginning of the second trimester often go under-investigated, despite being clinically significant (Petersen et al., 2021).

Medical comorbidities, particularly type 2 diabetes mellitus, were frequently observed among women who experienced spontaneous abortion. Poorly controlled blood sugar can disrupt the hormonal and vascular environment necessary for embryo implantation and placental development, contributing to an increased risk of pregnancy loss (Zhao et al., 2022). A previous history of miscarriage was another important risk factor. The probability of recurrence was found to increase significantly after two or more previous losses, a finding supported by earlier longitudinal studies (Magnus et al., 2019).

On a global scale, spontaneous abortion affects approximately 12–15% of clinically recognized pregnancies (Wilcox et al., 2019). In contrast, the reported incidence in Pakistan is lower—around 6.5%. This difference may not reflect a genuinely lower occurrence, but instead may point to systemic issues such as underreporting, cultural stigma surrounding pregnancy loss, and inadequate healthcare infrastructure for reproductive health surveillance (Minhas et al., 2022).

Chromosomal abnormalities remain the most frequently cited cause of early pregnancy loss. These may result from errors in meiotic division (nondisjunction) or DNA fragmentation during gamete formation. These genetic issues are thought to account for nearly half of all miscarriages in the first trimester (Petersen et al., 2021). Anatomical abnormalities of the uterus—such as septate or bicornuate formations—are also significant contributors to recurrent pregnancy loss but often remain undetected until several miscarriages have occurred (Bermejo et al., 2019).

Infectious agents have also been recognized as important contributors to pregnancy loss. Bacterial infections such as *Listeria monocytogenes*, *Chlamydia trachomatis*, and bacterial vaginosis are more frequently implicated than viral agents. The role of HPV in pregnancy loss is still debated. While some studies have found HPV DNA in placental tissues, others failed to detect the virus in miscarriage cases, suggesting inconsistent evidence and a lack of definitive causality (Xie et al., 2022; Cavazos-Rehg et al., 2021). Reported prevalence rates of HPV among women with miscarriage vary from 7% to 19%, although such findings are limited by small sample sizes and methodological differences. Pakistan-specific data on HPV and reproductive outcomes remain scarce. Existing national studies have estimated HPV prevalence between 2.8% and 16% in women with cervical abnormalities, with few studies exploring its impact on pregnancy loss (Minhas et al., 2022). Research in this area is limited by cultural sensitivities, which restrict open discussion around sexually transmitted infections. A 2009 study suggested a 4.4% STI prevalence among reproductive-aged women, a figure that is likely underestimated due to social stigma and diagnostic gaps (Khan et al., 2019).

This study had several limitations, including its single-center design, which limits generalizability. Additionally, reluctance among participants to discuss sensitive topics such as sexual history may have impacted accurate assessment of HPV exposure risk. Technical limitations in placental sampling and PCR sensitivity may also have affected the ability to detect viral DNA.

Nevertheless, this study emphasizes the importance of examining diverse factors contributing to miscarriage, especially in resource-constrained settings. HPV, while not confirmed as a significant contributor in this study, remains the most common sexually transmitted infection worldwide. Global estimates indicate that nearly 80% of women will contract genital HPV by age 50, with the highest prevalence in those aged 15 to 25 (CDC, 2021; Bruni et al., 2019).

Vaccination remains a key preventive measure against HPV-related diseases. The World Health Organization recommends HPV vaccination for girls between the ages of 9 and 26, ideally before the onset of sexual activity (WHO, 2022). Although the vaccine is generally safe, it is not advised during pregnancy due to a lack of conclusive safety data. Public health programs in Pakistan should focus on raising awareness, strengthening STI surveillance systems, and improving access to vaccination, particularly among young women and adolescents.

5.CONCLUSION

None of samples of placenta used was positive for HPV and none of histologic feature specific for HPV was observed .Some additional histologic features were noticed which included multi-

nucleation, bi-nucleation and marked halo. These features might be associated with some infection or medical condition of placenta or an artifact. Study population included patients between 15 to 45 years of age and maximum number of history of previous abortions was single. In Pakistan annual incidence of spontaneous abortion is 10 to % 15 and incidence of HPV in reproductive age in women is 23% . Lot of work needs to be done in this field. Nation wide studies needs to be conducted to evaluate exact rate of spontaneous abortions in Pakistan as well as association of HPV with spontaneous abortion. In addition to this HPV vaccine should be introduced in women of child bearing age.

6.REFERENCES

World Health Organization. (2018). WHO recommendations on antenatal care for a positive pregnancy experience. Geneva: WHO. https://www.who.int/publications/i/item/9789241549912.

Minhas, S., Sajjad, A., Chaudhry, R. M., & Rehman, Z. (2022). Prevalence and current scenario of HPV in Pakistan: A systematic review and meta-analysis. *Open Access Macedonian Journal of Medical Sciences*, *10*(F), 223–232. https://doi.org/10.3889/oamjms.2022.8881

Singh, S., Remez, L., & Sedgh, G. (2022). Abortion worldwide 2022: Uneven progress and unequal access. *Guttmacher Institute*. https://www.guttmacher.org/report/abortion-worldwide-2022

Wilcox, A. J., Weinberg, C. R., & Baird, D. D. (2019). Timing of implantation of the conceptus and loss of pregnancy. *New England Journal of Medicine*, *340*(23), 1796–1799. https://doi.org/10.1056/NEJM199906103402304

Bermejo, C., Martínez-Ten, P., Cantarero, R., & Labrador, E. (2019). Uterine anomalies and recurrent pregnancy loss. *Current Opinion in Obstetrics and Gynecology*, *31*(6), 319–324. https://doi.org/10.1097/GCO.0000000000000571

Ambühl, L. M. M., Baandrup, U., Dybkær, K., Blaakær, J., & Uldbjerg, N. (2020). Human papillomavirus infection as a possible cause of spontaneous abortion and spontaneous preterm delivery. *Acta Obstetricia et Gynecologica Scandinavica, 99*(5), 571–579. https://doi.org/10.1111/aogs.13794

Shanmugasundaram, S., You, J., & Roberts, J. M. (2019). Viral manipulation of the host cell cycle: role of HPV in cancer. *Viruses*, *11*(6), 541. https://doi.org/10.3390/v11060541

Petersen, S. H., Bergholt, T., Nielsen, H. S., & Christiansen, O. B. (2021). Recurrent miscarriage: A review. *Gynecological Endocrinology*, *37*(6), 497–503. https://doi.org/10.1080/09513590.2020.1867977

Kumari, R., Kaur, A., & Gupta, V. (2020). Impact of maternal age on obstetric outcome: A retrospective study. *International Journal of Reproduction, Contraception, Obstetrics and Gynecology*, *9*(6), 2395–2399. https://doi.org/10.18203/2320-1770.ijrcog20202380

Magnus, M. C., Wilcox, A. J., Morken, N. H., Weinberg, C. R., & Håberg, S. E. (2019). Role of maternal age and pregnancy history in risk of miscarriage: prospective register-based study. *BMJ*, *364*, l869. https://doi.org/10.1136/bmj.l869

Zhao, H., Wang, Z., Wang, X., et al. (2022). Association between maternal diabetes and miscarriage: A systematic review and meta-analysis. *Nutrients*, *14*(8), 1589. https://doi.org/10.3390/nu14081589

Minhas, S., Sajjad, A., Chaudhry, R. M., & Rehman, Z. (2022). Prevalence and current scenario of HPV in Pakistan: A systematic review and meta-analysis. *Open Access Macedonian Journal of Medical Sciences*, *10*(F), 223–232. https://doi.org/10.3889/oamjms.2022.8881

Bermejo, C., Martínez-Ten, P., Cantarero, R., & Labrador, E. (2019). Uterine anomalies and recurrent pregnancy loss. *Current Opinion in Obstetrics and*

Gynecology, *31*(6), 319–324. https://doi.org/10.1097

Xie, C., Wang, Y., Xu, Y., et al. (2022). Human papillomavirus infection and pregnancy loss: A systematic review and meta-analysis. *Scientific Reports*, *12*, 2190. https://doi.org/10.1038/s41598-022-06079-1

Khan, S., Qazi, J., & Shaheen, G. (2019). Prevalence and risk factors of sexually transmitted infections in Pakistan: A review. *Pakistan Journal of Medical Sciences*, *35*(3), 636–640. https://doi.org/10.12669/pjms.35.3.709

Bruni, L., Albero, G., Serrano, B., et al. (2019). Human papillomavirus and related diseases in the world. *ICO/IARC HPV Information Centre*. https://hpvcentre.net/statistics/reports/

World Health Organization (WHO). (2022). *Human papillomavirus vaccines: WHO position paper, December 2022*. https://www.who.int/publications/i/item/who-wer9749-645-672.