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COMPARISON BETWEEN CERVICAL CYTOLOGY (PAPANICOLAOU SMEAR) INTERPRETATION AND HPV HR CERVISTA (THIRD GENERATION) IN NILM CASES: A TERTIARY CARE LEVEL LABORATORY EXPERIENCE FROM NORTH INDIA.

Pathology

Ashish Gupta* Consultant- Department of Hematopathology and Molecular biology, Bhagwan Mahaveer Cancer hospital and research centre, Jaipur, India *Corresponding Author

Sonam Singh Trainee, Department of Molecular Biology, Quest diagnostics India limited, Gurgaon-122001, Haryana, India.

ABSTRACT

According to (world health organization) WHO, cervical cancer is among the top 5 commonly diagnosed cancer in women and is the leading cause of mortality among females and is responsible for 2,70000 deaths annually.

Most consequential factor behind the high incidence and mortality rate in underdeveloped countries in comparison to developed is the absence of more sensitive diagnostic tool. Though introduction of Papanicolaou (pap) cervical smear test has helped preventing the disease but in our study where we have compared Pap screening with Cervista HPV invader screening and it was seen that pap cerivcal smear results are not always definitive and needs further corroboration with other sensitive molecular techniques as hybrid capture technique, real time or conventional PCR for HPV detection or with HPV cervista technique based on DNA invader technology to catch the HPV infection which may be reported as false negative by standard pap cervical smear test which is based on morphological interpretation and has element of both inter and intra observer biasing.

KEYWORDS

Comparison, cervical cytological interpretation, Papanicolaou cervical smear test, HPV Cervista, NILM.

Introduction-:

Human papillomavirus is double stranded, small, iscosahedral and non-enveloped virus [1, 5]. More than 100 types of strain have been recognized till now which infect cutaneous and mucosal sites in human[2]. Majority of HPV types are only associated with the benign cutaneous lesion and are classified under non-genital types. Among the genital HPV types - 6,11,42,43 and 44 are characterized as the low risk type and 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 are associated with increased risk of cervical neoplasia and designated as high risk type[1,3].The cervical lesions which lead to the cervical neoplasia are detectable in pap cervical smear test screening. The abnormal cervical smear test result shows the following cervical intraepithelial neoplasia types: NILM (negative for cervical intraepithelial lesion), ASCUS (Atypical Squamous Cells of Undetermined Significance), LSIL (Low-grade Squamous Intraepithelial Lesion), HSIL (High-grade Squamous Intraepithelial Lesion) and SCC (Squamous cell carcinoma).

In the following study we compared the pap screening reports which were negative for cervical intraepithelial lesion (NILM) which includes the majority of population, with the 'Cervista HPV HR DNA Invader technology'. Cervista HPV HR (invader technology) is an automated system which includes an internal DNA control due to which the low cellularity and false-negatives results are lowered which is an advantage over the other technology of same generation like hybrid capture [1, 4]. This is a first study from India comparing results of cervical cytology with Cervista HPV HR technology. The study was done on female subjects with a sample size of 416 cases followed for a period from June 2014 to June 2016.The female subjects with a age range of 20-60 years were included in the study. The study was designed and performed in department of Molecular Biology at Quest diagnostics India limited which is a tertiary care level diagnostic laboratory situated at Gurgaon, Haryana, India.

Materials and methods-:

Cervicovaginal test samples were received in Quest Diagnostics India Private Limited, Gurgaon for cytological examination and HR-HPV DNA testing in the thin Prep vials containing Cytyc'sthin PrepPreserCyt medium.

Liquid based cytology- PAP (Papinacolaou test)-:

Cervicovaginal specimens were subjected to cytological examination. Single layer slides were made using the thin Prep 2000 automated processor and Papinacolaou staining of smeared slides were done at Department of Cytopathology, Quest Diagnostics, Gurgaon. Finally slides were microscopically analyzed by cytotechnologist and Cytopathologist.

HPV-HR (Cervista) DNA testing-:

Nucleic acids were extracted and qualitative assay was performed from the same cervical cytological specimens whose pap cervical smear test was already done. Cervista HPV HR test is a qualitative and in-vitro test for detecting high risk HPV types. The Cervista assay platform utilizes the DNA invader chemistry for detecting specific nucleic acid sequences. In the Invader assay (Third Wave Technology) the conformational change in the target DNA sequence is recognized by the Cleavage enzyme which can even detect single base pair change.

This method utilizes the two type of isothermal reaction, in primary reaction a probe and invader oligonucleotide binds to target DNA and as they overlap with the targeted sequence the invasive structure is formed which is recognized by the cleavage enzyme as a substrate, the probe is cleaved at 5´ end of the probe. This cleaved 5´ ends then binds to universal hairpin fluorescence resonance energy transfer (FRET) oligonucleotides which again form a substrate for cleavage enzyme and this enzyme cut the FRET oligonucleotides between fluorophore and quencher molecule and produce quantifiable fluorescent signal[6].

Table 1: Comparison of Cervista HPV HR results with Pap cervical smear results (Age range: 20- 60 years old)

	Cervista HPV HR Result		
Cervical Cytology Result-Pap smear	Positive	Negative	Total
Positive	0	0	0
Negative(NILM)	31	385	416
	31	385	416

Results-:

In the present study we have compared the results of cervical pap smear (cytological screening) with HPV HR DNA screening. Out of the total sample size of 416 female subjects, 385 subjects were NILM cases which subsequently turned out to be negative on HPV HR also hence there was 92.5% concordance (true negative) between cervical smear cytology and HPV HR results however 31 NILM cases turned out to be positive by HPV HR assay hence 7.45% of cervical cytological samples which were negative for intraepithelial region (NILM) in cytology were found to be positive in HPV HR screening. The results are depicted in tabular fashion in Table 1.

Discussion-:

Our studies has shown that Cervista HR HPV Testing is more sensitive assay for Human papilloma virus and thus making it more reliable for patient undergoing screening for cervical neoplasia.The inclusion of an internal control further makes it more desirable by avoiding false negative results. All the females undergoing cervical cytology screening should be accompanied by a cervical HPV HR screening to catch those NILM cases which could be undertreated due to lower sensitivity and intra/inter observer subjective variation in interpretation of cervical cytology results. Our study clearly indicates

that there is 92.5% concordance rate for true negativity between cervical smear results and HPV HR results but 7.45% NILM cases can be misdiagnosed as false negative if relied up on basis of cervical cytology results alone. Hence all NILM cases diagnosed by cervical cytology should always be cross checked with a molecular assay having higher sensitivity of detection as HPV HR cervista, Hybrid capture 2 or Real time PCR detection for HPV. This data suggests a higher positive percentage of agreement between two methods and a lower negative percentage of agreement. As compared to hybrid capture 2(HC2) which is designed to detect 13 high risk HPV sub types, HPV HR cervista has the ability to detect in addition to 13 high risk sub types a 14th HR-HPV(HPV type 66)[3]. We have preferentially selected the NILM cases because under treatment of these cases would have the most significant clinical impact especially in younger age group of females and in Indian scenario where HPV infection has a high prevalence rate in low socio economic group females which may go undetected and pose serious health consequences for reproductive life of such females.

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