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Review and Meta-analysis

A Review on Cervical Dysplasia: Etiology, Risk Factors, Diagnostic Biomarkers and Possible Nutritional Association

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Objectives: Cervical dysplasia which is the precursor or premalignant form of cervical cancer is prolonged; hence its diagnosis is essential for the early detection and inhibiting the development of cervical cancer. This review briefs the extensive studies conducted globally to gain knowledge about the development of cervical dysplasia along with the risk factors associated, role of human papilloma virus (HPV), potential diagnostic biomarkers and association with various micronutrient levels.
Materials and method: All these data were collected through extensive literature review.
Result: Based on the review, it can be stated that HPV virus (HPV 16 most commonly) is the most important etiological agent for the process of cervical carcinogenesis. However, HPV infection solely does not cause cervical cancer. There are various factors which act synergistically to develop cervical dysplasia and cancer. Smoking was found to be an important independent risk factor. There are studies which showed conflicting results regarding oral contraceptive intake association with cervical dysplasia. There are quite a few biomarkers like HPV DNA, p16INK4a, telomerase, and microRNA expression which have been identified as effective in diagnosing cervical dysplasia. Chromosome 3q mutation has been reported to be present in early dysplastic lesions; hence, it can be used in screening early lesions. Various micronutrient studies highlighted the facts that high plasma concentrations of several carotenoids and Vitamin C are inversely proportional to the degree of cervical dysplasia. Low red cell folate levels n plasma increases the risk of cervical dysplasia association. Low dietary intake of Vitamin A is also associated with increased risk of cervical dysplasia.
Conclusion: Finally, it can be stated that more extensive studies relating to nutritional and serum markers level need to be conducted with larger cohorts so that an appropriate nutrition plan can be implemented for these patients.

Introduction

Cervical dysplasia is considered as a fence between benign and malignant lesions [1]. Cervical cancer is the most common cancer of developing countries. Around 80 % of the global cervical cancer cases are reported from developing countries [2]. The lesions which show characteristics of cervical dysplasia are known as squamous intraepithelial lesions (SIL) or cervical intraepithelial neoplasia (CIN).

Human papilloma virus (HPV) is considered as the most important etiology for development of cervical carcinogenesis; the initial idea of which was provided by Zur Hausen (1976) [3]. Various studies have stated that this HPV infection is pretty common, especially in young women [4, 5]. So, getting infected with HPV virus does not necessarily mean that the women will develop cervical dysplasia. A persistent and prolonged HPV infection is the cornerstone of cervical dysplasia which was put forward by many studies [6-8]. Fife et al., (2001) [9] put forward the fact that infection by multiple HPV types could have a possible role in growth of cervical dysplasia. Another study [10] stated that HPV 16 and HPV 18 are the commonest high risk strains associated with cervical dysplasia.

According to yet another study, along with HPV 16 and 18, other strains like 31, 33, 35, 45, 52 and 58 constituted about 90% of cervical lesions [11].

There are several other risk factors which contribute to the development of cervical dysplasia and cancer with HPV infection. Among these risk factors; smoking, use of oral contraceptive pills (OCP) and nutritional deficiency of various ingredients are the most studied ones to the best of the author's knowledge.

Many studies have indicated that the degree of cervical dysplasia is directly proportional to the intensity of smoking [12-14].

Correlation of OCP use with cervical dysplasia has been subjected to different views by various studies. Some authors reported that cervical dysplasia is associated with oral contraceptive use [15,16] whereas, some other authors concluded that the development of cervical dysplasia is independent of contraceptive use [17,18].

Many studies have correlated nutritional levels of various components with cervical dysplasia. Various studies were carried out to correlate folate and Vitamin C level with occurrence of cervical dysplasia [19-21]. Role of Vitamin A has also been widely studied by many authors. Butterworth Jr. et al., (1992) also evaluated the role of folic acid supplementation in the progression of cervical dysplasia [22].

Several biomarkers have also been studied through many years for early detection of cervical dysplasia and its development to cervical cancer. The markers which have been highlighted in this review are HPV DNA, p16INK4a, Ki-67, survivin, microRNA, mRNA, and telomerase.

There are studies which associated p16 expression in cervical dysplasia with HPV infection [23-26].

There are also studies which were conducted to correlate HPV DNA level expression in patients with cervical dysplasia [27-30].

Li et al., (2010), and Gocze et al., (2015) carried out studies to assess the correlation between microRNA expression in cervical tissues which show dysplasia or are HPV infected [31,32].

Authors have carried out studies to correlate mRNA expression profile in cervical dysplastic lesions [33,34]. Telomerase have also been considered as an essential biomarker in detecting dysplastic lesions. Riethdorf et al., (2001) [35] and Anderson et al., (2009) [36] evaluated the expression of telomerase in HPV positive cervical dysplastic lesions and evaluated the telomerase gene amplification in liquid based cytology samples of histologically conformed cervical dysplasia cases respectively.

Chromosome 3q genetic alteration; a common genetic alteration in cervical carcinomas have been evaluated in cervical dysplasia as well [37-40]. This review attempts to compile the findings of these studies and many more and provide an insight to the research update on cervical dysplasia (in the above mentioned scopes) till date and suggests what further research is necessary.

Pathogenesis and Association with HPV

The association of human papilloma virus (HPV) infection with cervical carcinogenesis was projected by Zur Hausen (1976) [3]. A subgroup of the HPV types, known as the high risk papillomaviruses (HR-HPVs), mostly infect the mucosal epithelium of the genital tract; and they are considered the major cause of cervical cancer. HPV 16 and 18 are known to be highly associated with cervical cancer [10]. HPV 16 is consistently the most common high risk HPV type, irrespective of study design and topographical area [41]. Other common high-risk HPV types include: 31, 33, 35, 45, 52 and 58. These along with HPV 16 and 18 constitute for about 90% of cervical lesions [11].

Women infected with high risk-HPVs have a multifold risk of getting affected by a high grade cervical lesion paralleled with non-infected individuals. The nucleic acids of the high risk-HPVs can be readily identified in virtually all high grade cervical lesions [10],[42]. Therefore, detection and diagnosis of CIN is extremely necessary to prevent its further development to cervical cancer. Various epidemiological studies have discovered that high risk- HPV infections are very common and are easily detected, especially in young women [4, 5]. Mostly, the infection is self-limited and heals naturally without significant cervical pathologies. Only part of these infections continue to develop into cervical dysplastic lesions.

A study indicated that HPV DNA occurrence declines with age and increases with sexual activity and cigarette smoking [43]. It is not related to the use of oral contraceptives, condoms or infection with sexually transmitted diseases.

Ho et al., (1995) [6] conducted a study to associate persistent HPV infection as a risk factor for persistent cervical dysplasia in patients with histologically diagnosed CIN II. It was concluded that type specific persistent HPV infection, particularly with a high viral load, may lead to a chronic cervical lesion which may further progress to cancer rather than spontaneous regression.

HPV testing is very helpful to screen patients with abnormal cervical smears diagnosed on cytology [44].

A study attempted to determine if infection with multiple subtypes of HPV was associated with higher risk of cervical dysplasia by using cervicovaginal lavage [9]. This study supports a possible role for multiple HPV types (16, 51, 52, 56 and 58) in the development or progression of cervical dysplasia. The limitation of this study was the use of cervicovaginal lavage which made it impossible to isolate the HPV types detected in cervix and vagina.

Some other studies have assessed risk for development of intraepithelial lesions in the setting of persistent HPV infections [7, 8]. These studies indicated that multiple infections confer a four times increase in risk for persistent HPV infection and subsequent intraepithelial lesions.

Associated Risk Factors

While persistent HPV infection is the cornerstone of cervical dysplastic lesion formation and eventual development to cervical cancer, HPV infection alone does not cause cervical dysplasia. There are an array of risk factors which have been studied from time to time for assessing the risk associated with cervical dysplasia independently and in conjunction with HPV infection.

A prospective study was carried out to associate the correlation between steroid contraceptive use and rate of progression of cervical dysplasia [15]. It was observed that there is a surge in severity of dysplasia and oral contraceptive pill users when compared with patients using other methods of contraception like barrier method.

Association of cigarette smoke with cervical dysplasia in a dose-response manner was studied by many researchers. These studies included the period of smoking as well as the number of cigarettes smoked [12-14], [43]. La Vecchia et al ., (1986) reported higher risk estimates associated with longer periods of cigarette use [13], and Trevathan et al., (1983) showed a dose-response relationship for pack-years of cigarette use with increasing severity of cervical dysplasia [12].

Coker et al., (1992) found in their study that oral contraceptive use is not associated with cervical pre invasive lesion [17].

A case controlled study [45] was carried out to ascertain the risk factors for cases which was histologically confirmed as mild, moderate and severe dysplastic lesions. The incidence of cervical dysplasia was found to be greater in women who were smokers and those who took oral

contraceptives rather than barrier methods.

Kjellberg et al., (2000) [46] concluded that after taking HPV infection in account, smoking proved to be the most significant risk factor for cervical dysplasia/neoplasia.

There are studies which stated that hormonal contraceptives are associated with a moderately increase in risk of cervical dysplasia, especially for HPV positive women suggesting that hormonal contraceptives act as a promoter in cervical carcinogenesis [47,48].

According to the WHO collaborative study, there is an elevated risk for oral contraceptives users that increased with duration of more than four years [49].

There is no increased risk in women who used hormonal contraceptives for less than 5 years; however, risk became obvious after 10 years [50].

In a case control study it was concluded that, hormonal contraceptive use is associated with some increase in the rate of cervical dysplasia which can be reduced by using barrier method during sex [16].

There are studies which indicated that there is a strong correlation between factors like multiparity, early age of marriage, early age of childbirth and lack of awareness/ education to cervical carcinogenesis [51-53].

Conflicting data are observed when OCP intake was considered as risk factor for cervical dysplasia development. Hence, further extensive studies are needed to determine the clear association between these.

Association of Cervical Dysplasia with Various Micronutrient Levels

Koss (1979) [19] pointed out that folic acid deficiency produces changes in cervical smear which are very similar to cervical dysplasia. Therefore, patients with such early lesions should undergo colposcopic evaluation; considering that the cervix should be normal in folic acid deficiency and abnormal in cervical dysplasia. To the best of the author’s knowledge, a detailed study supporting this theory is yet to be carried out.

Wassertheil-smoller et al ., (1981) [20] pointed out in a study that low Vitamin C intake is an independent contributor to risk of severe cervical dysplasia and it is important to explore a bit more about the protective role of supplementary Vitamin C for women at high risk of cervical cancer.

In another study, the red cell folate concentrations in OCP users with dysplasia was lower that the non OCP user controls. The dysplastic changes improved with folic acid therapy [54]. However, whether the correction of dysplasia was due to the pharmacological response or nutritional effects of folic acid needs to be studied further. The protective effect of β carotene was established by

La Vecchia et al.,(1984) [55]. Later on, Potischman et al. (1991) [56] supported it by serological indicators.

Wylie Rosett et al., (1985) [57] directed a case control study to define the dietary intake of Vitamin A in women having abnormal cervical cytology. This study demonstrated that women with less dietary intakes of total Vitamin A or β-carotene are more likely to have severe dysplasia than women with a higher intake of these food nutrients.

Butterworth et al., (1992) [58] conducted a study to associate any nutritional deficiency with cervical dysplasia. It was concluded from this study that low red blood cell folate levels (at or below 660 nmol/l) increase the effect of other risk factors for cervical dysplasia, in particular, that of

HPV-16 infection. In this study, an increased tendency of dysplasia was found among HPV positive women with lowest folate level even though no treatment effect was observed in the study.

In another study by Butterworth Jr. et al., (1992) [58] it was concluded that although low folic acid levels increased the incidence of cervical dysplasia, oral folic acid supplementation did not alter the course of progression.

A theory was formulated that folate deficiency may act at the initiation stage [21,22] [58], while other vitamin deficiencies, such as Vitamin A, may act as promoters [59].

The laboratory and clinical studies in synergy provide a sturdy justification for testing retinoids in the regression of CIN of the cervix [60].

Goodman et al., (2000) [61] carried out a case control study to estimate the association of plasma levels of folate, Vitamin B12, homocysteine and cysteine in the various stages of cervical dysplasia. A very little or insignificant association of plasma levels of folate, homocysteine, or Vitamin B12 was found with the estimated risk of cervical dysplasia. However, a possible inverse relation of plasma cysteine levels with cervical dysplasia was brought forward, but a further study into this fact is required.

Biomarkers for Detecting Cervical Dysplasia

Several methods and markers were employed in the detection of cervical dysplasia over the years.

p16 and HPV DNA

Many studies indicated increased expression of p16INK4A, as a strong indicator of cervical neoplasia [23,24].

Klaes et al., (2001) [25] and Sano et al., (1998b) [62] found greater p16 expression on biopsy samples of high grade cervical dysplastic lesions.

A study was conducted to associate relationship between p16 expression by immunocytochemistry and HPV cytopathic effect in liquid based specimens [26]. The findings of this study is suggestive of the fact that p16INK4A expression is a vital tool for HPV cytopathic detection in cervical samples, especially the ones which are admixed by a background of inflammation and organisms. MUC4, a mucin gene, has increased expression in cervical dysplasia; however, it has been found to be less sensitive. Thus this requires further research.

Viral DNA detection is associated with cancer cell lysis as well as with micro metastases shed from cancer cervix [27-29].

Hwang and Shroyer, (2012) [63] published a review article on the potent biomarkers of cervical dysplasia and carcinoma which can be useful for screening. They concluded that HPV DNA is the most extensively used biomarker. Randomized large studies have put forward the fact that when HPV testing is integrated in primary screening, an additional 50% to 70% premalignant cervical lesions is diagnosed [64,65].

Cocuzza et al., (2017) [30] directed an analysis to assess the quantitative and qualitative presence of circulating HPV DNA in patients with a recent history or cervical dysplasia and also to correlate between the plasma and the cervical level of HPV DNA taking into account seven high risk HPV types (16, 18, 31, 33, 45, 51 and 52) prevalent in that geographical area with the use of type-specific real-time quantitative PCR assays. In this study, contemporary detection of the seven high risk-HPV types inspected in cervical as well as plasma samples increased proportionately with the

intensity of the lesion. This study reported that the quantification and detection of HPV DNA is possible in the plasma of women with a recent HPV infection as well as a low grade dysplasia of cervix.

However, longitudinal studies need to be carried out to further assess the role of detection of HPV DNA in the blood sample of patients whose infection is not yet symptomatic or in patients with early stage of cervical neoplasia.

p16INK4a and Ki-67

p16INK4a and Ki-67 are also considered useful markers for cervical dysplasia detection. Diffuse immunohistochemical positivity of p16INK4a has been seen in almost all cases of CIN II and CIN III. However, the drawback of p16INK4a is that it can sometimes also be expressed in normal endocervical lining cells, in cervical endometriosis, and tuboendometrial metaplasia [66]. A specific pattern of CIN1+ lesions is intense p16INK4a expression in the lower third of the squamous mucosa.

p16INK4a immunohistochemistry may also aid in detecting CIN1 lesions which are accompanying HR-HPV types. The lesions which are positive possess an elevated risk for evolution to high-grade dysplasia or cancer [67]. p16INK4a has also proven to be a sensitive as well as a specific diagnostic aid for underlying CIN2+ lesions in specimens collected for cervical cytology (68). Denton et al., (2010) [69] demonstrated that the use of p16INK4a in immunocytochemistry provides considerably higher specificity than HR HPV particularly for the ASC-US and LSIL cases diagnosed on cytology.

Ki67 expression by itself does not differentiate between HPV-induced dysplasia and benign reactive proliferating cells; therefore, the solo role of Ki67 to diagnose cervical dysplasia is limited. However, studies from Europe and United States, demonstrate that a dual staining approach for p16INK4a and Ki67 in cervical cytology sample can be used. This method provides a greater specificity than that of HR-HPV testing [70-72]. Adding p16INK4a immunostaining increased the diagnostic accuracy of high grade CIN significantly [73].

MicroRNA expression

De-regulation of microRNAs, in connection with malignant transformation, is well known.

According to few studies, miRNAs are more effective than mRNA for differentiating between disease states [74].

Li et al., (2010) [31] established that the expression of miR-34a is considerably lower in HR HPV-infected tissues. This confirms that miR-34a acts as a tumour suppressive miRNA in HPV mediated cervical lesion transformation.

Gocze et al., (2015) [32] conducted a study to analyze the role of microRNA expression tissues of patients with known positive HPV status and histologically diagnosed cervical lesions. According to this study, in HPV positive cases, miR-27a showed upregulation and miR34a showed downregulation with increasing grades of cervical lesion.

Thus, assessment of miRNA expression can prove to be helpful for differentiating various cervical lesions and may help in the estimation of HPV infection outcome.

mRNA

Tests for detecting E6/E7mRNA have been established; centred on the fact that E6/E7 manifestation results in an uncontrolled cell cycle proliferation due to degradation of tumour

suppressor genes like p53 and Rb [33].

Molden et al., (2005) [34] conducted a comparative study of mRNA and HPV DNA by PCR on women with a preliminary cytology diagnosis of ASC-US or LSIL. It was concluded that although HPV mRNA is as sensitive as HPV DNA, it is more specific in detecting underlying high grade cervical dysplasia.

Telomerase

Telomerase has been put forward as an additional markers that can triage patients to avoid overtreatment and not to overlook dysplastic lesions.

Telomerase activity appears to increase proportionately with the increase in cervical cytology abnormality. Although Gorham et al., (1997) [75] and Wisman et al., (2000) [76] found telomerase activity in only a relatively small proportion of cases of high grade lesions, other studies have detected the telomerase activity bulk of them [77,78]. Telomerase can be utilised as a diagnostic supplement for the triage of patients with ASC-US or LSIL in Pap smears for consequent colposcopic examination and biopsy. The ultimate role for telomerase analysis may be to adjourn the treatment of patients who do not require further evaluation, instead of prevailing as a method to elevate the already high level of sensitivity of the Pap smear.

In a study by Riethdorf et al., (2001) [35] the strength of telomerase expression correlated with the intensity of HPV 16/18, as determined by in situ hybridization.

Andserson et al., (2009) [36] evaluated the human telomerase gene, TERC amplification in liquid based cytology (LBC) samples of histologically confirmed diagnoses of cervical lesions. TERC amplification study in cytological smears had the highest sensitivity and specificity among all markers used in this study (p16, MYC, HPV mRNA expression) to distinguish low grade cervical dysplasia from high grade dysplasia and cancer.

Two other studies applying the TERC FISH probe as a diagnostic marker on cervical Thin prep samples were published which were in agreement that TERC positivity can detect high-grade lesions with high sensitivity [79,80].

Chromosome 3q

Studies have shown that acquisition of extra copies of chromosome 3q is a common genetic alteration in cervical carcinomas, and is less frequently associated with pre malignant lesions [37-39].

Heselmeyer - Haddad et al., (2003) [40] concluded that visualization of aneuploidy of chromosome 3q can detect dysplastic cells. They stated that the acquisition of additional copies of 3q may represent an early event in malignant transformation, which could provide a useful biomarker for screening of cervical dysplasia. The drawback of this study was that patient follow up and HPV status was not available.

In conclusion, cervical cancer is the fourth most common cancer among women globally [81]. Although, substantial studies and sizable research had been conducted in the field of the above discussed topics of cervical dysplasia, there are few zones which have loopholes and further studies are required in those sectors.

There has been conflicting data regarding association of oral contraceptive use and development of cervical dysplasia. Studies with larger sample size should be implemented to shed some light into this conflict.

Cervical smear changes due to folic acid deficiency was found to be similar to the changes by cervical dysplasia. Further investigations regarding this and obtaining a clear cut differentiation needs to be carried out.

The fact that Vitamin C is an independent risk factor for cervical dysplasia needs to be further addressed and additional studies need to be carried out. Low serum folic acid level and low Vitamin A level were also found to be associated with increased cervical dysplasia; further studies are required to ascertain this finding and to outline a proper nutritional management plan for cervical dysplasia and attempt for preventing it. The inversely proportional relation of plasma cysteine level with cervical dysplasia, needs to be confirmed by further studies.

MUC4, a mucin gene, is found to have increased expression in cervical dysplasia; however, it has been found to be less sensitive. Thus this requires further research.

Longitudinal studies need to be carried out to further assess the role of detection of HPV DNA in blood of patients whose infection is asymptomatic and in patients with early stage of cervical dysplasia.

MiRNAs are considered to be a better choice for expression studies, since they are more precise for differentiating disease states than mRNA.

It was concluded in different studies , that although, HPV mRNA is as sensitive as HPV DNA but it is also more specific in detecting underlying high grade cervical dysplasia.

Telomerase activity appears to increase proportionately with the increase in cervical cytology abnormality in many studies. Most of these studies conducted used cervical samples. To the best of the author’s knowledge, there is meagre literature about the serum/plasma estimation of telomerase activity in detecting cervical intraepithelial lesions. Such studies if carried out in future and successful results produced, it will be an excellent mode of early diagnosis and that too from patient’s blood sample. In the future, one can think of a screening strategy in which HPV-positive Pap smears are triaged with the help of the TERC marker that would contribute to a more evidence-based clinical management and patient categorization.

It is expected that the points highlighted in this review, will be helpful for the future investigators for carrying out further research in this field.

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