ISSN : 2350-0743

www.ijramr.com

International Journal of Recent Advances in Multidisciplinary Research
Vol. 04, Issue 01, pp.2166-2170, January, 2017

REVIEW ARTICLE

CONTRIBUTIONS OF EPIDEMIOLOGICAL AND LABORATORY METHODS TO THE ESTABLISHMENT OF THE CAUSAL ASSOCIATION OF HUMAN PAPILLOMAVIRUS WITH CANCER OF THE CERVIX

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ARTICLE INFO

Article History:
Received 27th October, 2016
Received in revised form
25th November, 2016
Accepted 06th December, 2016
Published online 30th January, 2017

Keywords:

Cancer of the Cervix,
Etiology,
Human Papillomavirus,
HPV,
Cause,
Cervical Cancer,
Epidemiological Methods.

ABSTRACT

For many decades, the epidemiological finding of females who have cervical cancer was identified as being suggestive of a sexually transmitted process and many biological agents were proposed as being responsible. In order to understand the contributions of epidemiological and laboratory methods to identifying cervical cancer etiology, this paper aimed to provide an historical review of the accumulation of laboratory and epidemiological evidence on the causal association of human papilloma virus (HPV) and cancer of the cervix, from early descriptive studies to the recent development of an effective intervention protocol with emphasis on the use of different epidemiological study designs. Research about the cause of cancer of the cervix has made a tremendous impact in the last two decades both scientifically and clinically. The epidemiological research was very crucial in establishing the relationship between the risk factors and cancer of the cervix and also to describe the type of the association. The advent of effective preventive vaccines prove the etiological role of persistent HPV infections in cancer of the cervix development, and it has taken clinical, laboratory and several designs of the epidemiological study to arrive at this point. Epidemiological research must be embraced and encouraged because this type of research can lead to the discovery of new medicines, vaccines, and other medical products that can improve the health and health outcomes of individuals in the community.

INTRODUCTION

Cervical cancer is the third most commonly diagnosed cancer in women worldwide (Ferlay *et al*., 2015; Odekunle, 2017) and perhaps the second commonest malignancy among women in the developing countries with approximately 527,000 new cases occurring globally every year with 80% in underdeveloped countries (Ferlay *et al*., 2015; Jemal, 2010; Odekunle, 2017; Siegel *et al*., 2010). Two hundred thousand deaths occur as a result of cervical cancer and one hundred and sixty thousand (80%) deaths also occur in less developed areas (Galani & Christodoulou, 2009). Although cervical cancer is known to be preventable, it remains one of the leading causes of cancer related deaths in females under the age of 60 (Galani & Christodoulou, 2009; Odekunle, 2017). There are different histological types of cervical cancer. Carcinoma of the cervix is the commonest form of genital cancer (Agboola, 1988). Squamous cell carcinoma (SCC) accounts for 80% of all cervical cancer; adenocarcinoma accounts for most of the remaining 20%. One type of adenocarcinoma is clear cell carcinoma, which is caused by exposure to utero diethylstilbesterol (DES). Very rarely a sarcoma or lymphoma of the cervix is found (Callahan & Caughey, 2013).

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HPV is a member of the papovaviridae family that is capable of infecting humans. HPV is a non-enveloped virus made of two stranded closed circular DNA genome. More than two hundred papillomaviruses strains have been discovered and about 50 percent of these viruses cause human infection and are thus known as HPV (Munoz, 2006). Out of these 100 types of HPV, 40 – 60 types mainly cause genital infections, which are further divided into two classes: the oncogenic group (high-risk group: 16, 18, 31 and 33), which is the most important group and a low-risk group: 6,11,42 and 44 (Munoz, 2006). HPV types 16 and 18 are most commonly implicated in cancer of the cervix, other subtypes are 31, 33, 35 45,51,52,58 and 59 (Harven, 1999). Human papilloma virus is stable regarding the genetic constitution. It does not change strains like influenza. Genetics findings show that the biology of HPV has not changed for over two hundred thousand years (Lehtinen & Paavonen, 2003). Laboratory methods for identifying HPV include immunologic assay, viral culture, DNA assay, and polymerase chain reaction among other methods.

Systematic Review of Epidemiological and Laboratory Methods in Establishing the Causal/ Etiological Relationship between HPV and Cancer of the Cervix

For centuries, the etiology of cancer of the cervix remained unknown, and there were several attempts by past scientists to

establish the cause of cervical cancer without much success. However, the earliest breakthroughs came in the 1930s while Dr. Richard Shope of the Rockefeller University was working on wild rabbits that had developed "horn", which upon further analysis, was caused by a virus that could be transmitted. This discovery played a crucial part in the subsequent studies by Dr. Zur Hausen (Cummings, 2011). According to Moghissi and Mack (1968), one of the earliest epidemiological studies to probe into the relationship between sexual intercourse and cancer of the cervix involved prostitutes in prison in the U.S whose exceptional social peculiarities bordered on sexual intercourse with multiple partners. This epidemiological study concluded by identifying early exposure to coitus and multiple sexual partners as the primary etiologic basis for the development of cancer of the cervix. In the classic research by Gagnon involving the study of 13,000 Canadian Nuns, he found no single case of cervical cancer (Fenoglio, 1982). This was a significant finding in that it concretized the association between sexual exposure and cancer of the cervix thereby paving the way for further descriptive studies to investigate the relationship. There was also rising clinical, laboratory and social interest in the diagnosis of cancer of the cervix, and pap smear having been established as a reliable screening test (van Nagell 1973), readily assumed this role. This led Neumann *et al* (1975), after their mass screening study, to call for a well organized mass screening system to cut down the incidence of cervical cancer.

While researching on the relationship between sexual exposure and cancer of the cervix in the middle of the 1970s, zur Hausen postulated a hypothesis, after extensive research, that cervical cancer may arise from infections with the virus found in condylomata acuminata. He later found HPV DNA in cervical cancer (zur Hausen 1976, cited in Galani *et al*. 2009).A lot of researches and studies ensued, which aimed to investigate the epidemiological links between HPV and the development of cervical cancer. The early 1980s witnessed the publication of overwhelming evidence linking cervical cancer to a sexually transmitted virus –HPV. One of such papers was by Stanbridge *et al*. (1981) which demonstrated the presence of papillomavirus particles in cervical and vaginal scrape materials in a study involving ten cases. Early descriptive studies also include a blind comparative survey by Reid *et al*. (1982) aimed at investigating the prevalence of subclinical papillomavirus infection (SPI) in a female sample that had been treated for preinvasive cervical as well as invasive neoplasm. The result of this controlled study, which showed that 91% of the study group had HPV infection, in contrast to 12.5% in the control group, added to the mounting evidence connecting HPV to cervical cancer.

In the 1980s following the development of a laboratory technique to detect the presence of HPV DNA at the cellular level, the two major HPV strains implicated in cancer specimens were isolated by zur Hausen'group and these are HPV-16 and HPV-18 (Boshart *et al*.1984). In1985, Grussendorf-Conen *et al*. published their results that demonstrated the presence of the HPV genome in cervical carcinoma cell nuclei. The development of a laboratory test for detection of HPV DNA led to more refined studies which sought to identify the virus in case-control studies. One of such studies, according to Reeves *et al*. (1987) was a pilot case-control study conducted in Panama, Costa Rica, and Columbia. The results of the study showed a strong association between HPV 16 and 18 to cervical cancer and highlighted age at first intercourse as the most significant factor associated to with them. Additionally, Bosch *et al*. (1993) noted in a two-country case-control study of cervical intraepithelial neoplasia grade III (CIN III) involving 525 and 512 women in Columbia, and Spain respectively that the estimated fraction of CIN III attributable to HPV were 72.4% in Spain and 60.3% in Columbia. In Spain the odds ratio (OR) was 295.5 (Confidence Interval (CI) 44.8-1946.4), and in Colombia, the OR was 27.1(CI 10.6-69.5) which indicated a very strong association. The HPV 16 was found to be the most predominant viral type with the strongest association to CIN III. The study equally identified Polymerase chain reaction (PCR) as the method of choice for epidemiological studies in this regard and concluded by stating that their data support the viral origin of CIN III (Bosch *et al*., 1993).

The International Agency for Research on Cancer (IARC) multicentre also conducted 9 case-control studies in several parts of the world, especially in countries with high risk and HPV DNA laboratory testing was done in two main research centers using the MY09/11 and the general primer (GP) GP5+/6+ polymerase chain reaction(PCR) testing system (Bosch *et al*., 2002). "ORs were between 50 to 100 fold for HPV DNA and attributable fractions (AF) for the entire study greater than 95%"(Bosch *et al*. 2001cited in Bosch *et al*. 2002). The authors further stated that the ORs results are very high which indicated that they are significant and this means there a strong etiological or causal relationship between HPV and cervical cancer. AF of greater than 95% indicates that more than 95% of cervical cancers are due to HPV infection (Bosch *et al*., 2002).

The late 1980s and early 1990s had witnessed the emergence of mounting and somewhat irrefutable evidence associating HPV to cervical cancer. In fact, the 1990s saw the largest series of cases of invasive cervical cancer investigated by the IARC in 22 countries around the world involving about 1000women with histologically verified cancer of the cervix (Munoz *et al*., 2006). HPV-DNA was detected in 99.7% of the tumors leading to the conclusion that HPV is a necessary cause of cervical cancer. The almost established nature of this hypothesis encouraged more studies into other related epidemiological factors involved, such that parity, early sexual debut, multiple sexual partners, low socioeconomic status, oral contraceptives and other factors featured prominently in different studies. (Bosch *et al*., 1993; Liaw *et al*., 1995; Schiffman *et al*., 1993). The epidemiological approach of these studies at the same time had started accumulating evidence linking HPV to other anogenital cancers. Also, accumulated case-control and cohort studies' results had progressed to the stage of delineating different strains of HPV and establishing their association to cervical cancer and other malignancies with HPV 16 and HPV 18 featuring prominently in the case of cancer of the cervix (Bosch *et al*., 1993; Liaw *et al*., 1995). Palpably, "in 1995, the IARC monograph working group concluded that there was sufficient evidence for the carcinogenicity of HPV-16 and HPV-18 and limited evidence for carcinogenicity of HPV-31 and HPV-33" (IARC 1995, cited in Munoz *et al*. 2006). Evidently, in the 1990s the relationship between HPV and cervical neoplasia was confirmed. According to Bosch *et al*., the 1990s produced the key results of case-control and cohort

studies, and witnessed an increasing number of results of the clinical uses of HPV-DNA testing in screening and triage (Bosch *et al*., 2002) and, as Liaw *et al*. (1995) noted in their case control studies, it was also becoming apparent that those with multiple HPV infections have a higher risk of developing cancer of the cervix. With the changing dimension of research studies having confirmed that HPV was a necessary cause of cancer of the cervix, epidemiological studies advanced to associating different strains of HPV to various anogenital pathologies, categorizing different histological forms of cervical cancer in relation to HPV strains and highlighting other risk factors that may play a prominent role or catalyze the carcinogenesis process. For instance, Ngelangel *et al*. (1998) in their hospital-based case control studying the Philippines detected HPV-DNA in 90.9% case subjects with adenocarcinoma, 93.8% in case subjects with squamous cell carcinoma as opposed to just 9.2% of control subjects (Ngelange *et al*., 1998). They observed the presence of 6 HPV types in adenocarcinoma, and 15 HPV types in squamous cell carcinoma while noting that, apart from HPV 16 and HPV 18, HPV45 had the strongest association with squamous cell carcinoma (Ngelange *et al*., 1998). The same year saw the publication of the results of the Morocco-based case control studies by Chaoki *et al*. (1998) which, among other observations, added to the mounting evidence associating high parity, oral contraceptive, multiple sexual partners and low socioeconomic status to the HPV-cervical cancer link.

On the same note, the results of a nested study in Sweden by Ylitalo *et al*. (2000) found a strong relationship between HPV viral load and cervical cancer when it concluded that females with high HPV 16 viral loads were at least 30 times the relative risk of HPV-16-negative females terms of developing cancer of the cervix. Franceschi *et al*. (2003), after reviewing their case control study results even suggested that a vaccine against HPV 16 and 18 may be effective in over 75% cases of invasive cervical carcinoma. The early 2000s witnessed the supplementation of facts and extension of dimensions of studies heralded by the emergence of results of longitudinal studies, which provides information on the dynamics of cumulative or persistent exposure to HPV infection, commenced mostly in the 1990s and the drive to intervention studies. One of such papers was the result of the Ludwig-McGill cohort study in Brazil conducted between November 1993 and March 1997 with follow-up until June 2000 with a total of 1611 women (Schlecht *et al*, 2001). The paper asserted that there was a strong relationship between persistent infection with HPV and the incidence of squamous intraepithelial lesion (SIL), especially for HPV 16 and 18 types (Schlecht *et al*., 2001).

Again, cross- sectional studies have repeatedly shown that asymptomatic HPV infections are common in younger age groups, but invasive cancer occurs at an older age, 30 years and above (Bosch *et al*., 2002). The cross-sectional prevalence of HPV-DNA diminishes to a background level of 2-8% in most populations in groups that are over 40 years of age (Bosch *et al*., 2002). In some populations, such as rural Costa Rica, a second mode of HPV-DNA prevalence has been observed for females older than 50 with uncertain relevance regarding the risk of cancer of the cervix (Herrero, 2000). Furthermore, over the years, triage studies have shown that HPV testing is more sensitive than repeated pap smear test (cytology) in identifying underlying high grade lesions in females with atypical squamous cells of undetermined significance (ASCUS) (Bosch *et al*. 2002). From different types of studies, it is clearly evident that HPV infection precedes cervical intraepithelial neoplasia and cervical cancer by some years. The result is that clinical use of HPV-DNA testing in screening has been validated by epidemiological studies over the years: cross-sectional design and large randomized trials (Cox, 2009). The association occurs in almost every case of cancer of the cervix all over the world including developed and developing countries (Bosch, 2008). In countries where the test has been widely used with good quality assurance, the number of new cases as well as the mortality rate has reduced by more than 50% (Kitchener *et al*., 2006). With so much evidence gathered over the years and the results of cohort studies reaffirming the already known, the scientific community and the pharmaceutical companies joined in the race to find a therapeutic intervention to the virus-cancer link. The early results of Randomized Controlled Trials (RCT) with vaccines targeted at the causative virus proved promising: Koutsky *et al*., in their double-blind RCT with 2392 women published in The New England Journal of Medicine in 2002, found that the incidence of persistent HPV-16 infection was 3.8% per 100 woman-years at risk in their placebo group compared to zero per 100 woman-years at risk in the vaccine group. This discovery, even though somewhat anticipated, was remarkable.

The results of other RCTs strengthened this position proving that therapy/prevention was possible. For instance, significant vaccine efficacy was observed against HPV-16 and HPV-18 according to the RCT results by Harper *et al*. (2006). Their study showed a vaccine efficacy of 100% (42.4-100) against CIN lesions associated with vaccine types. Similarly, in a randomized, placebo-controlled, double-blind trial with 5455 women in multiple centers over a three-year period using a quadrivalent vaccine against HPV type 6, 11, 16 and 18, vaccine efficacy was 100% for each of the coprimary end points (Garland *et al*., 2007). The advent of effective preventive vaccines prove the etiological role of persistent HPV infections in cancer of the cervix development (Bosch *et al*., 2002) and it has taken clinical, laboratory and several designs of the epidemiological study to arrive at this point.

Conclusion

The etiological role of HPV infection in cancer of the cervix has been greatly documented without any doubt. The association occurs in almost every case of cancer of the cervix all over the world including developed and developing countries. Research about the cause of cancer of the cervix has made a tremendous impact in the last two decades both scientifically and clinically. The epidemiological research was very crucial in establishing the relationship between the risk factors and cancer of the cervix and also to describe the type of the association. Cervical screening HPV testing has been very helpful in early detection of cervical intraepithelial neoplasia thereby reducing the incidence of full blown cancer of the cervix, but this screening test is still limited to developed countries and not readily available in most developing countries where disease prevalence is very high. HPV testing programmes should be organized worldwide so that there will be equal access. Other aspects that must be looked into are HPV type geographical variations and multiple HPV type

infections, so as to determine the types of screening and vaccine that would be most effective in each geographical location.

Contributions of Author

FFO conceived and designed the study, gathered and reviewed the articles and wrote the manuscript.

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