A review of dietary prevention of human papillomavirus-related infection of the cervix and cervical intraepithelial neoplasia

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ABSTRACT

The natural history of cervical cancer suggests that prevention can be achieved by modification of the host's immune system through a nutrient-mediated program. This study reviews the preventive role of dietary intake on cervical intraepithelial neoplasia (CIN) induced by human papillomavirus (HPV). Electronic databases were searched using relevant keywords such as, but not limited to, "human papillomavirus infection", "cervical intraepithelial neoplasia", "lifestyle factors", "nutrients intake" and "diet". High consumption of fruit and vegetables appears to be protective against CIN. The findings also highlight the possibility of consuming high levels of specific nutrients, vitamins and minerals, and retaining sufficient level of these elements in the body, especially those with high antioxidants and antiviral properties, to prevent progression of transient and persistent HPV infections to high grade CIN 2 and 3 (including *in situ* cervical cancer). The protective effect is not significant for high risk HPV persistent infections and invasive cervical cancer. While it appears that intake of specific nutrients, vitamins and minerals may be good in CIN prevention, there is lack of evidence from controlled trial to confirm this. Health professionals shall focus on implementation of a balanced-diet prevention strategy at an early stage for cervical cancer prevention.

INTRODUCTION

With a prevalence of 10%, cervical cancer is the second most common cancer (after breast cancer) affecting women around the world (1). While vaccination and safe sex education are effective in preventing the development of cervical intraepithelial neoplasia (CIN) to cervical cancer (2), women from many developing countries are not provided with such services. The morbidity rates of cervical cancer are therefore higher in these countries (3,4). It is predicted by 2020, if the risks and population growth remain constant as year 2002, there will be a 42% increase in cervical cancer cases (a total of 702,500 cases) if no novel preventive interventions are undertaken (3). The high incidence and cost to screen, treat and provide psychological support contribute to the large economic burden of the disease (5).

It is well established that deficiencies of dietary vitamins, minerals and nutrients can lead to DNA damage and immune-incompetence, permanent genetic alteration, and subsequently, a higher risk of cancer (6-11). New 'nutrient-mediated' prevention strategies are implemented to stimulate host's cell-mediated immune response against the viral oncogenes E6 and E7 (2,7). The constant expression and transforming properties of E6 and E7 genes and their interaction with the host cell proteins are indeed essential for the development of cervical cancer (12). The natural history of HPV infected high grade CIN to cervical cancer suggests that prevention can be stimulated via the immune system (12-15). The underlying principle of the 'nutrient-mediated' prevention strategy is the ability of a particular dietary constituent to interact with metabolising enzymes and proteins and regulate DNA methylation, synthesis and chromatin organization that prevent DNA damage (9,16,17).

Consuming foods, particularly plant-based foods, that support normal DNA methylation has the potential to suppress expression of viral oncogenes, promote proper signalling pathways, avoid cell transformation and reduce the risk of cancer in human (2,6,9). It has been documented that cruciferous vegetables have the ability to induce apoptosis through bioactive isothiocyanates and indoles (18-20). Vitamins such as vitamin A (retinoic acid), C (ascorbic acid), E (tocopherol) can inhibit proliferation of cancer cells (17,21), stabilise the p53 protein (9), prevent DNA damage and reduce immune-suppression (11,17,22), and support the receptor signal transduction pathways (22). The membrane-modifying effects of long chain fatty acids have also demonstrated positive effect in cancer prevention as docosahexaenoic acid induce mitochrondria-mediated apoptosis (23) and alter the receptor signal transduction pathway to activate lymphocyte proliferation (11). In addition, the active metabolite of vitamin D, 1,25-dihydroxyvitamin D3 was found to have the ability to avoid cell proliferation (10,24). The anti-viral property of minerals such as selenium and iron, while reliant on the activity of macrophages to produce nitrogen oxide, are also beneficial for cancer prevention (11).

Following HPV infection, it takes six years on average before progressing to high grade CIN including cervical cancer (5). Although the literature has suggested the role of diet in gene expression and cancer prevention, the relationship between a specific dietary constituent and the amount required to prevent cancer are not well established. Most of the studies were also based on animal models or *in vitro*. With the high disease burden of CIN, it is necessary to improve the understanding of dietary intake in the prevention of CINs and cervical cancer. The present review will examine recent evidence from epidemiological studies and randomized clinical trials with the aim to elucidate a better understanding about the role of diet in CINs prevention at an early stage.

MATERIAL AND METHODS

A search was conducted on the MEDLINE and Web of Knowledge databases in February 2012 to identify relevant articles. For the purpose of this review, the nomenclature used by the *Australian Modified Bethesda System 2004* is followed; low grade CIN includes HPV infection (both transient and persistent infections), high grade CIN includes CIN 2 and 3 while cervical cancer includes *in situ* carcinoma and invasive cervical cancer (25).

Keywords used for searching the electronic databases were "human papillomavirus infection OR cervical cancer OR carcinoma in situ OR cervical intraepithelial neoplasia OR cervix neoplasia OR cervix neoplasm" AND "lifestyle factors OR diet OR foods and nutrients intake OR nutrition OR dietary intakes OR food groups OR vitamins OR minerals OR antioxidants OR anticancer OR immunological prevention" AND "cohort study OR case-control study OR cross-sectional study OR trial". Only peer-reviewed, full-text articles written in English and published after the year 2000 were selected. The topic and abstract were initially screened to determine their eligibility for inclusion. Reference lists of the eligible articles were also manually searched for additional papers. The selection process adopted the preferred reporting items for systematic review and meta-analyses (PRISMA) approach (26).

RESULTS

Following the PRISMA approach, a total of 22 articles are selected (Figure 1). There are three cross-sectional studies, seven cohort studies, 11 case-control studies and one randomised controlled trial. The majority (95%) are observational studies, with about 48% conducted in the United States of America (USA), followed by 33% in Asian countries and

the rest from Europe and Brazil. All of the studies were conducted on women over the age of 18.

The results are organized and presented in terms of whole food intake (Table 1), nutrients, minerals and vitamins intake (Table 2) and circulating plasma or serum nutrients (Table 3), in relation to the risk of HPV infection, CIN 1, 2 and 3. In the reviewed publications, the adjusted odds ratio estimated the association between the exposure variable of interest and the development of HPV infection and CIN, while accounting for the effects of other plausible confounding factors.

DISCUSSION

As summarised in Table 1, the epidemiological evidence suggested that high intake of certain whole foods may be protective against HPV transient infections. The results highlighted the benefits of high consumption of whole fruits and vegetables, nuts and fish. Meanwhile, low consumption of whole vegetables (including dark green-, dark yellow-, dark orange-coloured vegetables and cruciferous vegetables), fruits (including fruit juices), yogurt, tofu, fish and meat, are not protective against HPV persistent infection, CIN 1, 2 and 3. Low intakes of fruits and vegetables are associated with three-fold increase in the risk of CIN 2 and 3in subjects with high HPV viral load (27). Therefore, high risk patients, especially those infected by high risk HPV types, should increase and maintain their fruits and vegetables intake at moderate levels. This recommendation is supported by findings presented in Table 2. Consumption of foods which provide rich sources of retinol and vitamin A, bioavailable calcium, antioxidants (including vitamin C, E, carotene, lutein and lycopene), as well as long chain polyunsaturated fatty acids, can significantly reduce the risk of *in situ* cervical cancer.

We believe that the antioxidant and/or antiviral activities from these whole foods contribute to the observed risk reductions.

Table 3 illustrates the relationship between circulating bioactive compounds (and their isomers) and risk of HPV-related diseases. The findings are consistent with the reported protective effects of nutrients, vitamins and minerals in Table 2. It appears that large amounts of whole foods should be consumed at an early stage to maximise their beneficial effect. For instance, circulating ascorbic acid (vitamin C) is not effective in preventing progression beyond HPV persistent infections. Similar results are observed between alpha tocopherol, gamma tocopherol, total carotene and the risk of CIN.

Low serum levels of lycopene, retinol, alpha tocopherol and gamma tocopherol tended to be positively associated with risk of CIN 3, while medium to high levels of serum alpha-carotene, beta-cryptoxanthin, lutein/zeaxanthin, gamma tocopherol and particularly serum lycopene could reduce the risk of high grade CIN (Table 3). It has been suggested that some antioxidants may be effective in preventing high grade CIN if it was initiated from the stage of cervical inflammation that promoted HPV infections, rather than directly infected by HPV (28). Their molecular structures can interact with the receptors and substrates within the host's cells and either regulate the activity of reactive oxygen species, which in turn inhibit transcription factor (activator protein, AP1) and expression of oncogenes E6 and E7, reduce immunosuppression, stabilise p53 protein, or prevent DNA damage (9,11,22,28). As a result, these antioxidants have different abilities to intervene the natural history of HPV-infected diseases.

Vitamin A (retinoid acid) is essential for the replication of basal and mucous cells and synthesis of protein blocks (11). Vitamin A deficiency may thus lead to a higher risk of squamous metaplasia and infection. Therefore, a low-to-medium level of serum retinol is only effective in reducing the risk of low grade CIN I (Table 3). Retinoids have the ability to inhibit cell proliferation as their isoform retinoic acid receptors (RARβ2) stimulate the transcription of RARβ2 tumor suppressor, diminish formation of AP1 that regulates expression of oncogenes and prevent cell proliferation and induce apoptosis while enhancing the expression of p53 proteins (19). Consequently, sufficient amount of retinoic acids in the blood can prevent progression of a low grade CIN to high grade CIN.

It is well known that folate plays an important role in DNA synthesis, repair, methylation and cell proliferation (29,30). Low folate levels are linked to weaker structure of DNA and thus more prone to DNA damage induced by virus and subsequent expression of the viral oncogene (17). As illustrated in Table 3, a low level of serum folate is associated with a higher risk of both low and high grade CIN, whereas medium levels of serum folate lead to a higher risk of low grade CIN but not high grade CIN. As folate can initiate DNA repair, methylation and expression of viral oncogenes, increasing the level of serum folate may provide an effective intervention in high grade CIN preventions.

Circulating antioxidants, at high levels, can enhance the clearance of high risk HPV infections and transient high risk HPV infections by up to 3 and 4 folds, respectively (Table 3). In this regard, lycopene has emerged as the most effective. In contrast, as HPV infections evolve from transient to persistent, there is no significant increment in clearance of persistent HPV infections with any levels of plasma circulating nutrients. This relationship stresses the importance of early intervention and prevention of HPV-related diseases. Nutrients, vitamins

and minerals are important in regulating viral integration and gene stability and preventing cancer development (17).

It appears that high consumption of whole foods has little benefit for the prevention of persistent HPV infections and beyond (Table 1), which is consistent with other studies (17,22,31). It should be noted that these studies investigated the effect of a particular food group but not the whole spectrum of food groups. The World Cancer Research Fund recommends the consumption of a wide variety of whole foods, as they naturally contain various nutrients, vitamins, minerals and bioactive compounds not found in a specific type of supplement (32). The synergistic effect of all food groups was not examined in these studies, which could have masked the protective effect of whole food intake on HPV infections and CIN. Dietary supplements should only be taken by those with a specific nutritional deficiency rather than for the purpose of cancer prevention (32-34). In addition, the problem of 'collective error' (33) should not be ignored. Moreover, explanation for the observed phenomenon cannot be elucidated without information on the history of the infection status of participants involved in the studies.

Despite the emerging evidence from these studies, several limitations should be taken into consideration. Only one prospective study had investigated the association between nutrients and cervical cancer. The majority of the observational studies are prone to biases. These include information bias with reliance on a 'standard serving' of fruit and vegetables, recall bias of past dietary history, and the inability of the cross-sectional studies to establish a cause-and-effect relationship. In addition, the controls in these epidemiological studies should ideally be women exposed to HPV infection to enable a fair comparison of the risk of HPV-related diseases (35). The effect of polyphenols as antioxidants and enzyme inhibitors on the

development of cervical cancer has also been overlooked (36). Well-designed controlled clinical trials are needed to confirm the observed associations between nutrients and cervical cancer. Until then, clinicians should encourage those at risk to take up healthy eating habits.

CONCLUSIONS

High consumption of certain nutrients, vitamins and minerals, particularly those with antioxidant and antiviral properties, appears to be effective in preventing HPV infections from progressing to high grade CINs. Further evidence from human clinical trials is required to confirm the relationship. Health professionals should continue to recommend consumption of a wide variety of whole foods rich in these nutrients, vitamins and minerals. Dietary supplements should only be taken when these micronutrients are deficit in the food system of the under-nourished populations.

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REFERENCES

1. Bosch, F.X., Burchell, A.N., Schiffman, M., Giuliano, A.R., de Sanjose, S. et al. Epidemiology and Natural History of Human Papillomavirus Infections and Type-Specific Implications in Cervical Neoplasia. *Vaccine* **26,** K1-K16, 2008.
2. zur Hausen, H. Papillomaviruses and cancer: from basic studies to clinical application. *Nat Rev Cancer* **2,** 342-350, 2002.
3. Parkin, D.M. & Bray, F. Chapter 2: The burden of HPV-related cancers. *Vaccine* **24,** S11-S25, 2006.
4. Seoud, M. Burden of Human Papillomavirus–Related Cervical Disease in the Extended Middle East and North Africa—A Comprehensive Literature Review. *J Lower Genital Tract Dis* **16,** 106-120, 2012.
5. Frazer, I.H., Cox, J.T., Mayeaux, E.J.J., Franco, E.L., Moscicki, A.-B. et al. Advances in Prevention of Cervical Cancer and Other Human Papillomavirus-Related Diseases. *The Pediatr Infect Dis J* **25,** S65-S81, 2006.
6. Gadducci, A., Barsotti, C., Cosio, S., Domenici, L. & Genazzani, A.R. Smoking habit, immune suppression, oral contraceptive use, and hormone replacement therapy use and cervical carcinogenesis: a review of the literature. *Gynecol Endocrinol* **27,** 597-604, 2011.
7. Moscicki, A.-B., Schiffman, M., Kjaer, S. & Villa, L.L. Chapter 5: Updating the natural history of HPV and anogenital cancer. *Vaccine* **24,** S42-S51, 2006.
8. Rampersaud, G.C., Bailey, L.B. & Kauwell, G.P.A. Relationship of Folate to Colorectal and Cervical Cancer: Review and Recommendations for Practitioners. *J Am DietAssoc* **102,** 1273-1282, 2002.

9. Reddy, L., Odhav, B. & Bhoola, K.D. Natural products for cancer prevention: a global perspective. *Pharmacol Ther* **99,** 1-13, 2003.

10. Ames, B.N. & Wakimoto, P. Are vitamin and mineral deficiencies a major cancer risk? *Nat Rev Cancer* **2,** 694-704, 2002.

11. Field, C.J., Johnson, I.R. & Schley, P.D. Nutrients and their role in host resistance to infection. *J Leukocyte Biol* **71,** 16-32, 2002.

12. Bosch, F.X., Lorincz, A., Muñoz, N., Meijer, C.J.L.M. & Shah, K.V. The causal relation between human papillomavirus and cervical cancer. *J Clin Pathol* **55,** 244-265, 2002.

13. Doorbar, J. Molecular biology of human papillomavirus infection and cervical cancer. *Clin Sci* **110,** 525-541, 2006.

14. Kadish, A.S., Timmins, P., Wang, Y., Ho, G.Y.F., Burk, R.D. et al. Regression of Cervical Intraepithelial Neoplasia and Loss of Human Papillomavirus (HPV) Infection Is Associated with Cell-mediated Immune Responses to an HPV Type 16 E7 Peptide. *Cancer Epidemiol Biomarkers Prev* **11,** 483-488, 2002.

15. Garcia-Hernandez, E., Gonzalez-Sanchez, J.L., Andrade-Manzano, A., Contreras, M.L., Padilla, S. et al. Regression of papilloma high-grade lesions (CIN 2 and CIN 3) is stimulated by therapeutic vaccination with MVA E2 recombinant vaccine. *Cancer Gene Ther* **13,** 592-597, 2006.

16. Rock, C.L., Lampe, J.W. & Patterson, R.E. Nutrition, Genetics, and Risks of Cancer. *Annu Rev Public Health* **21,** 47-64, 2000.

17. García-Closas, R., Castellsagué, X., Bosch, X. & González, C.A. The role of diet and nutrition in cervical carcinogenesis: A review of recent evidence. *Intl J Cancer* **117,** 629-637, 2005.

18. Higdon, J.V., Delage, B., Williams, D.E. & Dashwood, R.H. Cruciferous vegetables and human cancer risk: epidemiologic evidence and mechanistic basis. *Pharmacol Res* **55,** 224-236, 2007.

19. Gariglio, P., Gutiérrez, J., Cortés, E. & Vázquez, J. The Role of Retinoid Deficiency and Estrogens as Cofactors in Cervical Cancer. *Arch Med Res* **40,** 449-465, 2009.

20. Rieck, G. & Fiander, A. The effect of lifestyle factors on gynaecological cancer. *Best Pract Res Cl Ob* **20,** 227-251, 2006.

21. Borutinskaite, V.V., Navakauskiene, R. & Magnusson, K.-E. Retinoic Acid and Histone Deacetylase Inhibitor BML-210 Inhibit Proliferation of Human Cervical Cancer HeLa Cells. *Ann N Y Acad Sci* **1091,** 346-355, 2006.

22. Key, T.J., Allen, N.E., Spencer, E.A. & Travis, R.C. The effect of diet on risk of cancer. *The Lancet* **360,** 861-868, 2002.

23. Siddiqui, R.A., Harvey, K. & Stillwell, W. Anticancer properties of oxidation products of docosahexaenoic acid. *Chem Phys Lipids* **153,** 47-56, 2008.

24. Luong, K.v.q. & Nguyen, L.T.H. The beneficial role of vitamin D and its analogs in cancer treatment and prevention. *Cr Rev Oncol-Hem* **73,** 192-201, 2010.

25. Blomfield, P., Davy, M., Hammond, I. & Wain, G. The 2005 NHMRC guidelines for the management of abnormal pap smears in asymptomatic Australian women. Department of Health and Ageing, Australian Government, 2011.

26. Liberati, A., Altman, D.G., Tetzlaff, J., Mulrow, C., Gøtzsche, P.C. et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *J Clin Epidemiol* **62,** e1-e34, 2009.

27. Hwang, J., Lee, J., Kim, T. & Kim, M. The association between fruit and vegetable consumption and HPV viral load in high-risk HPV-positive women with cervical intraepithelial neoplasia. *Cancer Causes Control* **21,** 51-59, 2010.

28. Castle, P.E. & Giuliano, A.R. Chapter 4: Genital Tract Infections, Cervical Inflammation, and Antioxidant Nutrients—Assessing Their Roles as Human Papillomavirus Cofactors. *JNCI Mono* **2003,** 29-34, 2003.

29. Piyathilake, C.J., Henao, O.L., Macaluso, M., Cornwell, P.E., Meleth, S. et al. Folate is associated with the natural history of high-risk human papillomaviruses. *Cancer Res* **64,** 8788-8793, 2004.

30. Powers, H.J. Interaction among Folate, Riboflavin, Genotype, and Cancer, with Reference to Colorectal and Cervical Cancer. *J Nutr* **135,** 2960S-2966S, 2005.

31. Castellsagué, X., Bosch, F.X. & Muñoz, N. Environmental co-factors in HPV carcinogenesis. *Virus Research* **89,** 191-199, 2002.

32. World Cancer Research Fund/ American Institute for Cancer Research. Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective. Washington DC, AICR, 2007.

33. Bjelakovic, G. & Gluud, C. Vitamin and Mineral Supplement Use in Relation to All-Cause Mortality in the Iowa Women's Health StudyComment on “Dietary Supplements and Mortality Rate in Older Women”. *Arch Int Med* **171,** 1633-1634, 2011.

34. Redberg, R.F. Vitamin Supplements: More Cost Than ValueComment on “Dietary Supplements and Mortality Rate in Older Women”. *Arch Int Med* **171,** 1634-1635, 2011.

35. Piyathilake, C.J. Update on micronutrients and cervical dysplasia. *Ethnic Dis* **17,** 14-17, 2007.

36. Di Domenico, F., Foppoli, C., Coccia, R. & Perluigi, M. Antioxidants in cervical cancer: Chemopreventive and chemotherapeutic effects of polyphenols. *Biochim Biophys Acta* **1822,** 737-47, 2012.

37. Feng, C.-Y., Lin, M., Lakhaney, D., Sun, H.-K., Dai, X.-B. et al. The association between dietary intake and cervical intraepithelial neoplasia grade 2 or higher among women in a high-risk rural area of china. *Arch Gynecol Obstet* **284,** 973-980, 2011.

38. González, C.A., Travier, N., Luján-Barroso, L., Castellsagué, X., Bosch, F.X. et al. Dietary factors and in situ and invasive cervical cancer risk in the European prospective investigation into cancer and nutrition study. *Intl J Cancer* **129,** 449-459, 2011.

39. Giuliano, A.R., Siegel, E.M., Roe, D.J., Ferreira, S., Baggio, M.L. et al. Dietary intake and risk of persistent human papillomavirus (HPV) infection: The Ludwig-McGill HPV Natural History Study. *J Infect Dis* **188,** 1508-1516, 2003.

40. Tomita, L., Roteli-Martins, C., Villa, L., Franco, E., Cardoso, M. et al. Associations of dietary dark-green and deep-yellow vegetables and fruits with cervical intraepithelial neoplasia: modification by smoking. *Br J Nutr* **105,** 928-937, 2011.

41. Tomita, L.Y., Filho, A.L., Costa, M.C., Andreoli, M.A.V., Villa, L.L. et al. Diet and serum micronutrients in relation to cervical neoplasia and cancer among low-income Brazilian women. *Intl J Cancer* **126,** 703-714, 2010.

42. Hosono, S., Matsuo, K., Kajiyama, H., Hirose, K., Suzuki, T. et al. Association between dietary calcium and vitamin D intake and cervical carcinogenesis among Japanese women. *European J Clin Nutr* **64,** 400-409, 2010.

43. Shannon, J., Thomas, D.B., Ray, R.M., Kestin, M., Koetsawang, A. et al. Dietary risk factors for invasive and in-situ cervical carcinomas in Bangkok, Thailand. *Cancer Causes Control* **13,** 691-699, 2002.

44. Ghosh, C., Baker, J.A., Moysich, K.B., Rivera, R., Brasure, J.R. et al. Dietary Intakes of Selected Nutrients and Food Groups and Risk of Cervical Cancer. *Nutr Cancer* **60,** 331-341, 2008.

45. Lee, G.J., Chung, H.W., Lee, K.H. & Ahn, H.S. Antioxidant vitamins and lipid peroxidation in patients with cervical intraepithelial neoplasia. *J Korean Med Sci* **20,** 267-272, 2005.

46. Sedjo, R.L., Inserra, P., Abrahamsen, M., Harris, R.B., Roe, D.J. et al. Human Papillomavirus Persistence and Nutrients Involved in the Methylation Pathway among a Cohort of Young Women. *Cancer Epidemiol Biomarkers Prev* **11,** 353-359, 2002.

47. Sedjo, R.L., Roe, D.J., Abrahamsen, M., Harris, R.B., Craft, N. et al. Vitamin A, carotenoids, and risk of persistent oncogenic human papillomavirus infection. *Cancer Epidemiol Biomarkers Prev* **11,** 876-884, 2002.

48. Kim, J., Kim, M.K., Lee, J.K., Kim, J.-H., Son, S.K. et al. Intakes of Vitamin A, C, and E, and β-Carotene Are Associated With Risk of Cervical Cancer: A Case-Control Study in Korea. *Nutr Cancer* **62,** 181-189, 2010.

49. Castanon, A., Tristram, A., Mesher, D., Powell, N., Beer, H. et al. Effect of diindolylmethane supplementation on low-grade cervical cytological abnormalities: double-blind, randomised, controlled trial. *Br J Cancer* **106,** 45-52, 2012.

50. Palan, P.R., Woodall, A.L., Anderson, P.S. & Mikhail, M.S. α-Tocopherol and α-tocopheryl quinone levels in cervical intraepithelial neoplasia and cervical cancer. *Am J Obstet Gynecol* **190,** 1407-1410, 2004.

51. Sedjo, R.L., Fowler, B.M., Schneider, A., Henning, S.M., Hatch, K. et al. Folate, vitamin B12, and homocysteine status: Findings of no relation between human papillomavirus persistence and cervical dysplasia. *Nutrition* **19,** 497-502, 2003.

52. Sedjo, R.L., Papenfuss, M.R., Craft, N.E. & Giuliano, A.R. Effect of plasma micronutrients on clearance of oncogenic human papillomavirus (HPV) infection (United States). *Cancer Causes Control* **14,** 319-326, 2003.

53. Goodman, M.T., Shvetsov, Y.B., McDuffie, K., Wilkens, L.R., Zhu, X. et al. Hawaii Cohort Study of Serum Micronutrient Concentrations and Clearance of Incident Oncogenic Human Papillomavirus Infection of the Cervix. *Cancer Res* **67,** 5987-5996, 2007.

54. Kwanbunjan, K., Saengkar, P., Cheeramakara, C., Thanornsak, W., Benjachai, W. et al. Low folate status as a risk factor for cervical dysplasia in Thai women. *Nutr Res* **25,** 641-654, 2005.

55. Yeo, A.S.S., Schiff, M.A., Montoya, G., Masuk, M., van Asselt-King, L. et al. Serum Micronutrients and Cervical Dysplasia in Southwestern American Indian Women. *Nutr Cancer* **38,** 141-150, 2000.

56. Schiff, M.A., Patterson, R.E., Baumgartner, R.N., Masuk, M., van Asselt-King, L. et al. Serum Carotenoids and Risk of Cervical Intraepithelial Neoplasia in Southwestern American Indian Women. *Cancer Epidemiol Biomarkers Prev* **10,** 1219-1222, 2001.

57. Thompson, F.E., Patterson, B.H., Weinstein, S.J., McAdams, M., Spate, V.L. et al. Serum selenium and the risk of cervical cancer among women in the United States. *Cancer Causes Control* **13,** 517-526, 2002.

APPENDIX: ABBREVIATION LIST

HPV: human papillomarvirus

CIN: cervical intraepithelial neoplasia

DNA: deoxyribonucleic acid

PRISMA: preferred reporting items for systematic review and meta-analyses

USA: United States of America

AP1: activator protein 1

RARβ2: retinoic acid receptors β2

FIGURE CAPTIONS

FIGURE 1: Selection process of publications included in this review following the preferred reporting items for systematic review and meta-analyses (PRISMA) approach

TABLE 1: Association between whole food intake and cervical intraepithelial neoplasia (CIN) status

TABLE 2: Association between nutrients, minerals and vitamin intake and cervical intraepithelial neoplasia (CIN) status

TABLE 3: Association between circulating plasma or serum nutrients and cervical intraepithelial neoplasia (CIN) status

TABLE 1: Association between whole food intake and cervical intraepithelial neoplasia (CIN) status

Study design (Reference)	**Sample size**	**Food intake**	**Types of CIN**	**Adjusted odds ratio** (95% CI)
Cross-sectional study in Shanxi, China (37)	2338	Among all women,		
		≥ 15.95 servings/week of onion vegetables	high grade CIN	0.654 (0.437, 0.978)
		≥ 2.69 servings/week of legumes	high grade CIN	0.655 (0.439, 0.978)
		0.24-0.60 servings/week of nuts	high grade CIN	0.533 (0.359, 0.790)
		≥ 0.61 servings/week of nuts	high grade CIN	0.590 (0.394, 0.882)
		≥ 0.94 servings/week of meat	high grade CIN	0.651 (0.429, 0.987)
		Among HPV positive women,		
		≥16.34 servings/week of onion vegetables	high grade CIN	0.589 (0.387, 0.897)
		≥2.81 servings/week of legumes	high grade CIN	0.591 (0.392, 0.892)
		0.30-0.60 servings/week of nuts	high grade CIN	0.590 (0.395, 0.883)
		≥0.61 servings/week of nut	high grade CIN	0.635 (0.426, 0.946)
		≥1.02 servings/week of meat	high grade CIN	0.624 (0.406, 0.958)
Cohort study in Korea	328	Among subjects with HPV viral load ≥ 15.5,		

(27)		fruits (≤ 70 g/day) and vegetables (≤ 260 g/day)	CIN 2 CIN3	2.84 (1.26, 6.42) 2.93 (1.25, 6.87)
Cohort study in multi-European countries (38)	299,649	Total fruit	invasive cervical cancer	hazard ratio for risk: 0.83 (0.72, 0.98)
Nested Case-control study in Sao Paulo, Brazil (39)	185 cases, 248 controls	carrots (≥ 1 time/year - < 1 time/month)	HPV persistent infection	0.30 (0.10, 0.87)
		papaya (≥ 1 time/year - < 1 time/month)	HPV persistent infection	0.43 (0.19, 0.97)
		papaya (≥ 1 time/week)	HPV persistent infection	0.30 (0.14, 0.64)
Case-control study in Sao Paulo, Brazil (40)	231 CIN 3 cases, 453 controls	dark-green and deep-yellow vegetables/fruits (≤ 39 g/day)	CIN 3	1.71 (1.15, 2.52)
		total fruit and fruit juices (≤ 79 g/day)	CIN 3	1.51 (1.05, 2.17)
		total citrus fruit and citrus fruit juices (≤ 79 g/day)	CIN 3	1.44 (1.02, 2.03)
		total vegetables and fruits (≤ 319 g/day)	CIN 3	1.52 (1.06, 2.17)
Case-control study in Sao Paulo, Brazil (41)	99 CIN 1, 95 CIN 2, 185 CIN 3, 82	carrots (203-1321 g/day)	CIN 3	0.50 (0.27, 0.95)

	cervical cancer cases, 331 controls			
Case-control study in Nagoya, Japan (42)	72 CIN 3, 333 cervical cancer cases, 2025 controls	milk (> 5 times/ week)	cervical cancer	0.68 (0.48, 0.96)
		bone-edible small fish (> 5 times/ week)	cervical cancer	0.46 (0.24, 0.90)
Case-control study in Bangkok, Thailand (43)	184 cervical cancer cases, 509 controls	>3.01 servings/day of high retinol Thai foods	*in-situ* cervical cancer	0.03 (0.01, 0.14)
		>3.29 servings/day of high total vitamin A Thai food	*in-situ* cervical cancer	0.09 (0.02, 0.50)
Case-control study in New York, USA (44)	239 cervical cancer cases, 979 controls	vegetables > 80 g/day	cervical cancer	0.58 (0.38, 0.89)
		total fruits and vegetables (>139 g/day)	cervical cancer	0.52 (0.34, 0.77)

TABLE 2: Association between nutrients, minerals and vitamin intake and cervical intraepithelial neoplasia (CIN) status

Study design (Reference)	**Sample size**	**Nutrient, minerals and vitamins intake**	**Types of CIN**	**Adjusted odds ratio** (95% CI)
Young Women Cohort study in Arizona, USA (46)	201	all levels of folate, vitamin B12, vitamin B6, Methionine	HPV persistence	N.S.
Young Women Cohort study in Arizona, USA (47)	206	lutein (1042.4-2377.2 µg)	HPV infection	0.37 (0.13, 0.82)
Nested Case-control study in Sao Paulo, Brazil (39)	185 persistent HPV infection cases, 248 controls	beta-cryptoxanthin	HPV persistent infection	0.47 (0.26, 0.85)
		lutein plus zeaxanthin	HPV persistent infection	0.49 (0.27, 0.87)
		vitamin C	HPV persistent infection	0.50 (0.27, 0.92)
Case-control study in Nagoya, Japan (42)	72 CIN 3 cases, 333 cervical cancer cases, 2025 controls	total calcium (502.6-607.9 mg/day)	cervical cancer	0.50 (0.34, 0.73)
		total calcium ≥607.9 mg/day	cervical cancer	0.68 (0.48, 0.97)
		total vitamin D ≥291 IU/day	cervical cancer	0.64 (0.43, 0.94)
Case-control study in Korea	144 cervical	medium (798-999 RE/day) dietary	cervical cancer	0.52 (0.29, 0.92)

(48)	cancer cases, 288 controls	vitamin A		
		high (>999 RE/day) dietary vitamin A	cervical cancer	0.36 (0.19, 0.69)
		medium (4213-5476 µg/day) dietary beta-carotene	cervical cancer	0.51 (0.28, 0.93)
		high (>5476 µg/day) dietary beta-carotene	cervical cancer	0.48 (0.26, 0.88)
		>146 mg/day dietary vitamin C	cervical cancer	0.36 (0.18, 0.69)
		medium (869-1183 RE/day) total vitamin A	cervical cancer	0.51 (0.28, 0.92)
		high (>1183 RE/day) total vitamin A	cervical cancer	0.35 (0.19, 0.65).
		>174 mg/day total vitamin C	cervical cancer	0.35 (0.19, 0.66)
		>9.67 mg/day total vitamin E).	cervical cancer	0.53 (0.28, 0.99)
Case-control study in New York, USA (44)	239 cervical cancer cases, 979 controls	polyunsaturated fat >12 g/day	cervical cancer	0.57 (0.34, 0.97)
		dietary fibre >29 g/day	cervical cancer	0.59 (0.37, 0.94)
		vitamin C >224 mg/day	cervical cancer	0.52 (0.33, 0.80)
		medium vitamin E (5.8-8.9 mg/day)	cervical cancer	0.59 (0.40, 0.89)

		high vitamin E (>8.9 mg/day)	cervical cancer	0.44 (0.27, 0.72)
		medium vitamin A (7421-12786 I.U. /day)	cervical cancer	0.55 (0.37, 0.82)
		high vitamin A (>12786 I.U./day)	cervical cancer	0.47 (0.30, 0.73)
		medium (595-1393 µg/day) alpha carotene	cervical cancer	0.68 (0.47, 0.97)
		high alpha carotene (>1393 µg/day)	cervical cancer	0.41 (0.27, 0.63)
		medium beta carotene (3921-7512 µg/day)	cervical cancer	0.66 (0.46, 0.96)
		high beta carotene (>7512 µg/day)	cervical cancer	0.44 (0.29, 0.68)
		medium lutein (3596-6558 µg/day)	cervical cancer	0.61 (0.41, 0.89)
		high lutein (>6558 µg/day)	cervical cancer	0.51 (0.33, 0.79)
		high lycopene (>5837 µg/day)	cervical cancer	0.65 (0.44, 0.98)
		high folate (> 433.2 µg/day)	cervical cancer	0.55 (0.34, 0.88)
Double blind randomised controlled trial (6 months)	551	diindolylmethane supplementation	CIN2	Relative risk: 0.7 (0.4, 1.2)

in Wales, UK (49)			CIN3	Relative risk: 0.9 (0.4, 2.0)

N.S.: not significant

TABLE 3: Association between circulating plasma or serum nutrients and cervical intraepithelial neoplasia (CIN) status

Study design (Reference)	**Sample size**	**Circulating plasma or serum nutrients**	**Types of CIN**	**Adjusted odds ratio** (95% CI)
Cross-sectional study in Korea (45)	58 with CIN, 86 without CIN	vitamin C	CIN	Lower in CIN women (p = 0.008)
		malonaldehyde	CIN	Higher in CIN women (p = 0.002)
		total antioxidant capacity	CIN	Lower in CIN women (p = 0.011)
Cross-sectional study in USA (50)	37 with CIN, 14 with cervical cancer, 21 without CIN	alpha-tocopherol	CIN	Lower in CIN women (p = 0.012)
		alpha tocopheryl quinone	CIN and cervical cancer	Lower in CIN and cervical cancer women (p = 0.005)
Young Women Cohort study in Arizona, USA (46)	201	vitamin B12 of > 493.2pg/mL	HPV persistent infection	0.40 (0.17, 0.96)

Cohort study in Arizona, USA (51)	91	folate (5.47-8.79 ng/mL)	HPV persistent infection	1.29 (0.27, 6.20)
		folate (8.80-23.44 ng/mL)	HPV persistent infection	1.59 (0.33, 7.59)
		vitamin B12 (240.84-444.14 pg/mL)	HPV persistent infection	1.51 (0.30, 7.54)
		vitamin B12 (444.15-1322.58 pg/mL)	HPV persistent infection	0.82 (0.13, 5.11)
		homocysteine (8.61-11.35 nM/mL)	HPV persistent infection	1.22 (0.23, 6.38)
		homocysteine (11.36-25.28 nM/mL)	HPV persistent infection	0.66 (0.12, 3.61)
Young Women Cohort study in Arizona, USA (52)	331	Trans-lycopene (0.18-0.263 µg/mL)	oncogenic HPV clearance	Hazard ratio: 3.03 (1.20, 7.65)
		Trans-lycopene (> 0.263 µg/mL)	oncogenic HPV clearance	Hazard ratio: 2.79 (1.17, 6.66)
		Cis-lycopene (0.175-0.245 µg/mL)	oncogenic HPV clearance	Hazard ratio: 3.50 (1.51, 8.08)
		cis-lycopene (> 0.245 µg/mL)	oncogenic HPV clearance	Hazard ratio: 2.92 (1.28, 6.63)

Cohort study in Hawaii, USA (53)	122	total trans-lutein/zeaxanthin (303.93-905.29 ng/mL)	high-risk HPV infections clearance	Hazard ratio: 1.59 (1.01, 2.51)
		total cryptoxanthin (296.13-1164.6 ng/mL)	high-risk HPV infections clearance	Hazard ratio: 1.72 (1.02, 2.88)
		total lycopene (59.31-245.11 ng/mL)	high-risk HPV infections clearance	Hazard ratio: 2.06 (1.23, 3.44)
		alpha-carotene (68.82-388.36 ng/mL)	high-risk HPV infections clearance	Hazard ratio: 1.78 (1.03, 3.06)
		total trans beta-carotene (252.16-1405.5 ng/mL)	high-risk HPV infections clearance	Hazard ratio: 1.84 (1.13, 2.99)
		cis beta-carotene (23.96-97.36 ng/mL)	high-risk HPV infections clearance	Hazard ratio: 1.94 (1.20, 3.13)
		Total carotene (379.96-1724.4 ng/mL)	high-risk HPV infections clearance	Hazard ratio: 1.98 (1.21, 3.23)
		total carotenoids (1755.9-3979.0 ng/mL)	high-risk HPV infections clearance	Hazard ratio: 1.75 (1.03, 2.97)

		total trans-lutein/zeaxanthin (303.93-905.29 ng/mL)	high-risk HPV infections clearance	Hazard ratio: 3.15 (1.04, 9.53)
		beta-cryptoxanthin (242.60-1114.6 ng/mL)	high-risk HPV infections clearance	Hazard ratio: 4.65 (1.65, 13.09)
		total cryptoxanthin (296.13-1164.6 ng/mL)	high-risk HPV infections clearance	Hazard ratio: 3.47 (1.36, 8.87)
		total lycopene (59.31-245.11 ng/mL)	high-risk HPV infections clearance	Hazard ratio: 7.03 (1.61, 30.70)
		alpha-carotene (68.82-388.36 ng/mL)	high-risk HPV infections clearance	Hazard ratio: 3.02 (1.07, 8.53)
		total trans beta-carotene (252.16-1405.5 ng/mL)	high-risk HPV infections clearance	Hazard ratio: 3.52 (1.44, 8.62)
		cis beta-carotene (23.96-97.36 ng/mL)	high-risk HPV infections clearance	Hazard ratio: 3.43 (1.18, 9.93)
		total beta-carotene (281.16-1496.4 ng/mL)	high-risk HPV infections clearance	Hazard ratio: 3.79 (1.59, 9.00)

		Total carotene (379.96-1724.4 ng/mL)	high-risk HPV infections clearance	Hazard ratio: 3.72 (1.50, 9.23)
		alpha-tocopherol (12.76-36621 ng/mL)	high-risk HPV infections clearance	Hazard ratio: 3.48 (1.33, 9.11)
		total tocopherol (13605-37092 ng/mL)	high-risk HPV infections clearance	Hazard ratio: 3.25 (1.21, 8.71)
Case-control study in Bangkok and Choburi, Thailand (54)	44 low grade CIN cases, 70 high grade CIN cases, 95 controls	median serum folate level	low grade CIN high grade CIN	Lower in low grade CIN cases ($p<0.001$) and high grade CIN cases ($p<0.001$)
		median red blood cells folate level	high grade CIN	Lower in high grade CIN cases ($p=0.006$)
		folate level <19.82 nmol/L	low grade CIN	6.13 (1.80, 20.82)
		folate level <19.82 nmol/L	high grade CIN	5.57 (1.70, 18.20)
		folate level (314.89-468.19 nmol/L)	low grade CIN	0.25 (0.06, 0.98)
Case-control study in	190 low grade	retinol	Low grade CIN	Lower in low grade CIN cases

New Mexico, USA (55)	CIN cases, 112 high grade CIN cases, 326 controls			(p= 0.001)
		alpha-tocopherol	High grade CIN	Lower in high grade CIN cases (p = 0.03)
		retinol level (<0.3186 mg/L)	low grade CIN	2.3 (1.3, 4.1)
		retinol level (0.3186-0.384 mg/L)	low grade CIN	1.9 (1.1, 3.5)
Case-control study in New Mexico, USA (56)	81 high grade CIN cases, 160 controls	alpha-carotene level (18.1-87.3 µg/dL)	CIN	0.46 (0.21, 1.00)
		beta-cryptoxanthin level (10.4-30.3 µg/dL)	CIN	0.39 (0.17, 0.91)
		lutein/zeaxanthin (29-76 µg/dL)	CIN	0.40 (0.17, 0.95)
Case-control study in Sao Paulo, Brazil (40)	231 CIN 3 cases, 453 controls	lycopene (≤ 0.40 µmol/L)	CIN3	1.88 (1.14, 3.08)
		retinol (≤ 1.49 µmol/L)	CIN3	1.51 (1.00, 2.27)
		alpha tocopherol (≤ 2.49 µmol/mmol)	CIN3	2.87 (1.76, 4.68)

		gamma tocopherol (≤ 1.02 µmol/mmol)	CIN3	1.51 (1.01, 2.24)
Case-control study in Sao Paulo, Brazil (41)	99 CIN 1, 95 CIN 2, 185 CIN 3, 82 cervical cancer cases, 331 controls	gamma tocopherol (0.96-1.14 µmol/L)	CIN3	0.44 (0.20, 0.95)
		gamma tocopherol (1.14-1.82 µmol/L)	CIN3	0.45 (0.21, 0.97)
		lycopene (0.78-1.39 µmol/L)	CIN3	0.47 (0.22, 0.99)
		lycopene (1.39-6.30 µmol/L)	invasive cervical cancer	0.18 (0.06, 0.52)
Case-control study in Birmingham, Chicago, Denver, Miami, Philadephia, USA (57)	456 cervical cancer cases, 545 controls	selenium (<97.5 ng/mL)	cervical cancer	N.S.
		selenium (97.5-106.9 ng/mL)	cervical cancer	N.S.
		selenium (107-11[40]3.9 ng/mL)	cervical cancer	N.S.
		selenium (114-124 ng/mL)	cervical cancer	N.S.

N.S.: not significant