Houston study augurs possible shift in hrHPV genotypes

Anne Paxton

March 2013—First identified in an HIV patient in 1998, HPV 90 is a genotype of the human papillomavirus that, until now, has received little attention. It is not counted as one of the few well-defined high-risk genotypes, like HPV 16 and HPV 18, that are known to cause the majority of cervical cancer cases.

But after studying data from 808 mostly Latina women patients, pathologists at The Methodist Hospital in Houston recently made surprising discoveries that are likely to raise HPV 90's profile. HPV 90, these researchers found, may be not only more prevalent than previously thought but also a genotype associated with cervical intraepithelial lesions.

"Unexpected high prevalence of HPV 90 infection in an underserved population: Is it really a low-risk genotype?," newly published online in the *Archives of Pathology & Laboratory Medicine* (doi: 10.5858/arpa.2012-0640-OA), is the first study to characterize HPV 90 infection in an underserved North American population, says senior author Yimin Ge, MD, a surgical pathologist and cytopathologist at The Methodist Hospital. Very few previous studies included HPV 90, and those that did were conducted outside the U.S. "HPV 90 has never been looked at in a U.S. population." Nor, Dr. Ge adds, has anyone proved that HPV 90 is related to disease of any type.

Dr. Ge

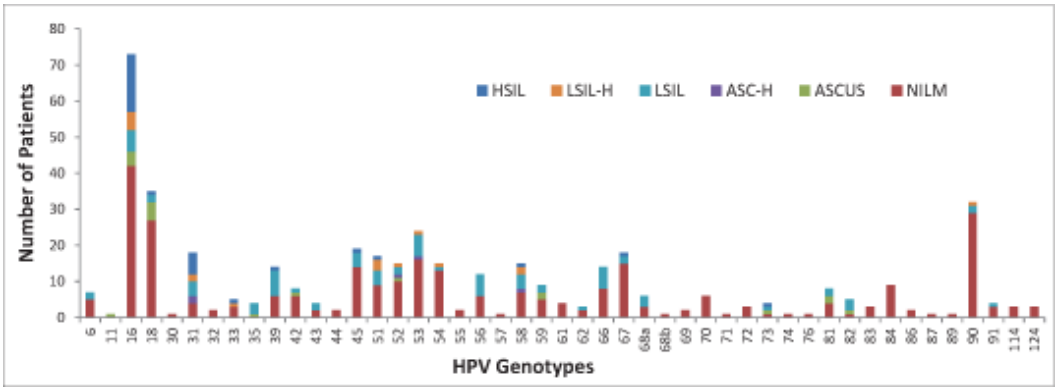
"Our original idea was we thought that the demographic change in the Houston area would change the prevalence and distribution of HPV genotypes in cervical disease," Dr. Ge explains. In the last 10 years, the Houston area has seen a 50 percent increase in its Hispanic residents, who now account for about 44 percent of the city's population. "Due to the significant demographic change, we thought we might find some differences in this particular Latina population compared to the U.S. general population." As it turned out, the differences went further than he and his colleagues hypothesized.

The women patients in the study were referred to The Methodist Hospital from 84 charity clinics in the greater Houston area for abnormal Pap test results between 2009 and 2011. After performing liquid-based Pap tests, the researchers extracted HPV DNA and amplified it with PCR, then hybridized the samples with an HPV DNA Genotyping Chip Kit, made by the Korean company GoodGene. This genotyping kit simultaneously detects 40 HPV genotypes—many more than the kits approved by the Food and Drug Administration, which can detect a maximum of 14. A GenePix 4000B Microarray Scanner, made by Molecular Devices, was used to visualize the signal.

Based on 2009 recommendations by an expert working group, the IARC (International Agency for Research on Cancer) classification system on cancer places known genotypes of HPV into four groups. The first, Group 1, includes 14 genotypes currently referred to as carcinogenic or high-risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68A/68B) because there is evidence they will cause cancer. Groups 2A and 2B are intermediate-risk classifications of "probably carcinogenic" and "possibly carcinogenic." Last is Group 3, "not classifiable," with genotypes considered low risk because there are no data. HPV 90 has been assigned to Group 3.

Dr. Ge and colleagues found that, overall, 93 percent of the 808 women in the study were infected with HPV, and most of those had high-risk or intermediate-risk genotypes. But HPV 90 was detected in 32 of the women—four

percent of the study population—with all but one of those women having no co-infections with other genotypes. Ten percent of the women infected with HPV 90 had cytologic abnormalities, including one with low-grade SIL with features suspicious for high-grade SIL.



Distribution of human papillomavirus genotypes in cytology diagnostic categories demonstrates an unexpectedly high prevalence of HPV 90 (4%). Approximately 9.4% of the women infected with HPV 90 had abnormal cytology in the same specimen. Abbreviations: ASC-H, atypical squamous cells, cannot exclude high-grade intraepithelial lesion; ASCUS, atypical squamous cells of uncertain significance; HSIL, high-grade intraepithelial lesion; LSIL, low-grade intraepithelial lesion; LSIL-H, low-grade intraepithelial lesion, cannot exclude high-grade intraepithelial lesion; NILM, negative for intraepithelial lesion. (Reproduced with permission, Archives of Pathology & Laboratory Medicine.)

"The hypothesis was that the most frequent HPV genotypes in this cohort would be different from the U.S. general population. We did a very extensive and comprehensive panel for HPV to include many rarely studied genotypes such as HPV 90," says study co-author Gabriela Quiroga-Garza, MD, a fourth-year pathology resident at The Methodist Hospital. "I looked at different studies and they don't usually screen for HPV 90."

A particularly unexpected finding of the study is that the four percent prevalence of HPV 90 in the study population was even higher than the prevalence of many of the well-studied genotypes such as HPV 11, 32, 33, 43, 44, and 68B. "We never expected HPV 90 to be that high, and we are not sure why HPV 90 is high in this cohort. But this is a very, very high-risk population from charity clinics with history of cervical disease and that could have contributed to this result," Dr. Ge says.

Dr. Schwartz

Study coauthor Mary R. Schwartz, MD, director of anatomic pathology at The Methodist Hospital, also says it's unclear why HPV 90 was found in this group. "We do know that there is regional variation on HPV types associated with cervical cancer. It varies from Africa to Asia to the U.S. What we don't know—because it hasn't been studied—is if the increased prevalence of HPV 90 will be found in other communities with Latina populations."

It's far too early, as well, to conclude that HPV 90 belongs in a higher-risk IARC classification. Dr. Ge cautions that many of the HPV genotypes that have been categorized have been intensively studied, and 808 patients, by comparison, is a very small number. "In order to be classified, a genotype has to have a multicenter study including a lot more patients."

But in addition to a high prevalence, the study found that 31 of the 32 patients who had HPV 90 infection had no co-infection with another genotype, which could mean that HPV 90 is the cause of their cervical disease. “This is a key point of this paper,” says study coauthor Haijun Zhou, MD, PhD, a second-year pathology resident who has spent the last 14 years studying cancer biology in China and the U.S. “These 31 patients don’t have co-infection with other genotypes, but several of them have concurrent dysplasia, so we think HPV 90 may be a causative mechanism.”

Dr. Zhou

However, much research remains to be done, he stresses, pointing out that for HPV 16 and HPV 18, there is not only epidemiological data but also biological data showing they cause cancer. “For HPV 90, this is the first study in a U.S. population. We can make people aware that HPV 90 is a possible cause for cervical disease, but we need more biological data to support this hypothesis.”

It’s possible that HPV 90 functioned as a “bystander” genotype in this group of women patients, Dr. Ge adds. “All of these women had cervical disease before, and there’s a reasonable argument that the cervical lesions with a concurrent HPV genotype might be a residual effect of previously high-risk HPV that has since cleared up, with HPV 90 acting only as a bystander—in other words, it showed up on tests but it’s not the real cause of the disease.”

However, the study notes, the absence of other HPV genotypes and presence of unequivocal viral cytopathic effect in concurrent cytologic and histologic specimens strongly argue against the bystander notion. “The only way to definitively prove that is to study the cancer tissues to see which genotypes are incorporated in the cancer cells,” Dr. Ge says.

For three of the patients with abnormal cytology and HPV 90 infection, the researchers also performed HPV-ISH (in situ hybridization tests) to look for evidence of residual high-risk HPV, and didn’t find any, Dr. Quiroga-Garza says. “That’s why we think, although we cannot predict with 100 percent certainty, that the HPV 90 was probably an independent infectious agent associated with cervical dysplasia.”

The HPV 90 associated with the three cases with cervical lesions might not be a “wild” type of HPV but rather a potent mutant that may act as powerfully as HPV 16 in tumorigenesis, Dr. Ge says. “We do know that high-risk HPVs cause cancer by degrading the tumor suppressor gene p53, and ‘wild’ type HPV 90 doesn’t have the ability.” However, it was demonstrated recently that the mutation of HPV 90 E6 enables it to fully degrade p53, he says. It is possible that the HPV 90 associated with the three cases of cervical disease might not be the wild type of HPV 90; rather, it could be a potent HPV 90 mutant that may act as powerfully as HPV 16 in tumorigenesis. “Unfortunately, we could not discriminate the wild type from a mutant HPV 90 in the current study, and further investigation is certainly warranted,” Dr. Ge says.

While the study authors urge that their results be interpreted with caution—for example, HPV 90 could be over-represented in this particular population—it’s clear that unexpected changes in the risk levels of various HPV genotypes could have implications for the HPV vaccine, which the Centers for Disease Control and Prevention considers a strong weapon of prevention.

The two current HPV vaccines (Gardasil and Cervarix) vaccinate against only two high-risk HPV types: HPV 16 and 18, Dr. Schwartz notes. (Gardasil also vaccinates against HPV 6 and 11, which are not high-risk for cervical cancer but are the most common causes of genital warts.) “Merck is working on a nine-valent HPV vaccine, but HPV 90 is not included; the vaccine is directed against HPV 16, 18, 6, 11, 31, 35, 45, 52, and 58. So these Latina women who

get one of the current HPV vaccines, or who will get the nine-valent vaccine if and when approved, will also not be protected against HPV 90."

With Houston's dramatic surge in Hispanic population, the city now has the third highest concentration of Hispanic residents in the country, Dr. Zhou notes. But Houston is not alone; Los Angeles, Miami, Chicago, New York, and other cities are also showing significant growth in the percentage of Hispanic population. "So for future vaccination programs, we need a broader strategy that will take the demographic change into consideration and monitor the dynamics of HPV composition and emerging genotypes."

As Dr. Ge points out, the origin of HPV 90 infection in these patients may be difficult to prove, but this virus is here to stay. "It will impact the future trend of HPV makeup of the U.S. population," Dr. Ge says.

In addition, "If the HPV vaccination program is successful, we don't know which genotypes will emerge as the dominant HPV genotypes in 10 years." The vaccination programs may be knocking down the current top high-risk genotypes, HPV 16 and HPV 18, but it's difficult to predict which genotypes will take their place, Dr. Ge says.

"The next one may not necessarily be HPV 31, even though by prevalence it is next in line, because of the complexity of host-viral interactions and competition among the HPV genotypes. But we think this study is kind of a wakeup call, because some of the previously unnoticeable genotypes may be emerging into important genotypes after the vaccination programs." All of this information will be important in defining future generations of vaccines against HPV, Dr. Ge adds.

The recently changed clinical guidelines for Pap test screening, which now recommend longer intervals for women in lower-risk age groups as well as co-testing for high-risk HPV, may need another look if, indeed, high-risk categories are more in flux than predicted. Says Dr. Schwartz: "The commercial tests for high-risk HPV testing—for example, Cervista HPV HR and Digene Hybrid Capture assay—do not test for HPV 90. So if you are a woman with an HPV 90 infection, your HPV infection will not be detected by current high-risk HPV assays, and you will not benefit from management pathways recommended for women found to have high-risk HPV infections."

The Methodist study is also likely to provide information for optimizing the future screening strategy for cervical cancer. The new guidelines say that for women ages 30 to 65, co-testing with cytology and HPV testing is the "preferred" approach, with cytology alone rated "acceptable." But "whether to screen for cervical cancer and its precursors using primary HPV screening is controversial," Dr. Schwartz says.

In Europe, in fact, the trend is to shift to HPV testing and leave cytology behind. "HPV testing has been traditionally used as a reflex test for patients with ASC-US on cytology," notes Dr. Ge, who has written a paper on HPV testing and cytology testing. But more clinicians are now likely to order both HPV and cytology as kind of a double screen. "We're looking into the data and we're finding if they order single testing, either HPV only or cytology only, they actually miss some high-grade lesions or cancer. We believe that cytology and HR-HPV testing are complementary, and the co-testing strategy will greatly increase the sensitivity for detecting squamous intraepithelial lesions, while retaining the ability to identify other significant lesions that are unrelated to HPV infection."

Since HPV testing currently tests for only 14 genotypes out of the 40-plus that are known, "We often get calls from clinicians to question the abnormal cytology diagnosis when the HPV test is negative. A negative HPV test often gives clinicians as well as patients a false assurance. A variety of factors may cause a negative HPV test. Either the viral titer is not high enough or the genotype is simply not included in the testing panel."

"All science is based on the accumulation of data, and our study is just one piece of information," Dr. Ge says. But the study is important, in his view, first, because HPV 90 has never been reported in a North American population, and, second, they proved an association with cervical disease. "And because this has never been studied before, we may need further study of this genotype, especially in the general U.S. population."

It is difficult to screen for all HPV genotypes, Dr. Quiroga-Garza notes. "But we're in a new era now, especially with vaccination." As further HPV studies proceed, she believes that particular attention must be paid to the nation's

changing demographics. “HPV genotypes are going to change, and genotypes that might be actually considered non-carcinogenic might emerge as important. And that might have prevention implications in the future.”

Anne Paxton is a writer in Seattle.