

Research Article

HPV Prevalence and Detection of Rare HPV Genotypes in Hong Kong Women from Southern China with Cytological Abnormalities

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Received 30 October 2013; Accepted 19 November 2013

Academic Editors: M. Ciotti and S.-L. You

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Human papillomavirus (HPV) has been identified as the primary cause of cervical squamous intraepithelial lesion and invasive cervical cancer. The emergence of various commercial HPV genotyping kits with different characteristics facilitates the detection of most high-risk and low-risk HPV genotypes, but the rare HPV types are usually underdiagnosed. In the present study, HPV detection was performed using the GenoFlow HPV Array Test kit (DiagCor Bioscience), which can identify 33 HPV subtypes by specific probes. Besides, a HPV consensus probe (universal probe) was designed to capture not only the 33 genotypes but also rare subtypes. Of the 1643 Southern Chinese women tested between 2012 and 2013, the HPV prevalence was 42.3%, with HPV 52 (139/1643, 8.5%), HPV 81 (89/1643, 5.4%), and HPV 16 (63/1643, 3.8%) being the most frequent subtypes detected. Among all 695 HPV-positive cases, 56 (8.1%) cases were only detected by the universal probe, in which 5 were either ASCUS or LSIL cases. Sequencing results confirmed HPV types 30, 91, and 74, and the intratypic variants of HPV 72 and 82 were present in the 5 cases. The result suggests that some rare HPV subtypes might be involved in cervical lesions.

1. Introduction

Human papillomavirus (HPV) infection is the most common sexually transmitted infection worldwide. To date, 170 HPV types have been completely sequenced [1]. Phylogenetic analysis based on the L1 ORF sequences identified five evolutionary groups with different epithelial tropisms and disease associations: Alpha-, Beta-, Gamma-, Nu-, and Mu-papillomavirus (PV). The Alpha PVs include the low-risk cutaneous types that typically cause skin warts, the low-risk mucosal types that are associated with benign lesions and genital warts, and the high-risk mucosal types that can cause cervical cancer [2]. Persistent infection with high-risk Alpha PVs is a prerequisite for the development of cervical cancer and its histological precursor, cervical intraepithelial lesions. Twelve HPVs (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) are classified by the International Agency for Research on Cancer as being carcinogenic to humans, with HPV 68 being recognized as probably carcinogenic. Several

other HPV types (26, 30, 34, 53, 66, 67, 69, 70, 73, 82, 85, and 97) are possibly carcinogenic based on evolutionary similarity to the known cancer-causing types [3]. HPV 16 and HPV 18 are generally recognized as the most important oncogenic viruses, which account for >70% of all cervical cancer diagnosed worldwide [4]. However, several studies have shown the involvement of rare HPV types in cervical malignancy. HPV 61 and HPV 62 were detected in 2.3% of LSILs and 3.5% of HSILs [5]. A retrospective cross-sectional study also identified rare HPV types (include 42, 61, 74, and 91) in 1% of 8977 cases of invasive cervical cancer [6]. A recent study using PCR with MY09/11 degenerate primers followed by direct sequencing that showed 9.47% prevalence of rare HPV types in cervical lesions. In particular, HPV 54 and HPV 81 were detected in samples with histopathological findings of CIN I, HPV 61 in samples with CIN II, and HPV 81 in samples with CIN III [7]. Emerging studies suggested the potential roles of some rare HPV types in cervical lesions and cervical cancer.

Currently, HPV genotyping is important in four main clinical applications: primary cervical screening, HPV triage of low-grade cytological abnormalities, test of cure following treatment, and resolution of uncertainties [8, 9]. Molecular methods for HPV DNA detection are broadly divided into signal and target amplification assays. The Hybrid Capture 2 (HC2) system (Digene Corporation, Gaithersburg, MD, USA) and Cervista HPV HR test (Hologic, WI, USA), the FDA-approved HPV DNA tests, which detect 13 and 14 high-risk HPV types, respectively, by signal amplification methods. The Linear Array HPV Genotyping (Roche Molecular Diagnostics, Branchburg, NJ, USA) and the InnoLiPA HPV Genotyping (Innogenetics, Belgium) are based on the amplification of HPV L1 gene followed by reverse line-blot hybridization to detect 37 and 28 HPV types, respectively. All these assays are designed to detect only the most frequent and defined high-risk and low-risk HPV subtypes. Other subtypes beyond their defined scopes are unlikely to be detectable. In order to detect more rare HPV subtypes, GeneFlow HPV array Test kit utilized a set of degenerated PGMY primers to amplify the L1 gene of HPV followed by proprietary flow-through hybridization and captured by using a universal probe with consensus sequence of HPV. The universal probe was designed to detect not only the defined panel of 33 high-risk and low-risk HPV subtypes but also out-panel rare subtypes and the variant forms of specific HPV types. In this report, we used the GenoFlow HPV assays to evaluate HPV prevalence and genotype distribution from Hong Kong women, Southern China. We also investigated whether the out-panel HPV subtypes identified were associated with their cytology results.

2. Materials and Methods

2.1. Study Population. Liquid-based cytology specimens from a total of 1648 Southern Chinese women were tested when they attended private sector healthcare clinics from October 2012 to September 2013, with patient consent. All specimens were screened with conventional cytology and HPV testing was undertaken using GenoFlow HPV Array Test kit (DiagCor Bioscience, Hong Kong, SAR). Five cases were excluded from the study due to the missing cytology or age information.

2.2. HPV Genotyping. GenoFlow Human Papillomavirus Array Test Kit was used for HPV genotyping. This kit has been designed for the identification of the 33 most common genotypes of HPV. The assay covers 18 currently known high-risk HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82) and 15 low-risk HPV genotypes (6, 11, 26, 40, 42, 43, 44, 54, 55, 57, 61, 70, 71, 72, 81, and 84). A universal probe (U) is also included to detect rare HPV subtypes that are outside the 33-genotype panel. Cervical samples preserved in liquid-based cytology medium (Thin-Prep; Cytoc Corp., Marlborough, MA, USA, or Surepath; BD Diagnostics-Tripath BD Biosciences, Oxford, UK) were washed once with phosphate buffered saline and subjected to DNA extraction by using the QIAamp DNA Blood

TABLE 1: Patient characteristics.

Characteristic	Hong Kong Chinese women ($n = 1643$)	
	No. of patients	%
Age (years)		
Mean	36.9	—
Median	29	—
(range)	(15–83)	—
Cytological results		
Normal	1505	91.6
ASCUS	91	5.5
LSIL	41	2.5
HSIL	6	0.4

ASCUS: atypical squamous cells of undetermined significance; LSIL: low-grade squamous intraepithelial lesions; HSIL: high-grade squamous intraepithelial lesions.

Mini Kit (Qiagen) in accordance with the manufacturer's instructions. The extracted DNA was mixed with the biotin-labeled primer mix and DNA *Taq* polymerase provided with the GenoFlow test kit; followed by the PCR amplification step using the stated thermocycling condition. The amplified products were then genotyped using the GenoFlow HPV array system. Briefly, the amplicons were denatured and hybridized to specific HPV DNA capturing probes via Flow-through hybridization. The hybridized DNA was subjected to a stringent wash and detected by colorimetric development. A result is valid only when there are signals at the hybridization control (HC) and the amplification control (AC) [10].

2.3. Confirmation of Out-Panel HPV Genotype. For samples with positive universal probe signal but negative for HPV specific types (out-panel HPV samples), the sample extracted DNA was amplified with MY09/11 and GP5+/6+ primer systems using standard reference protocol [11, 12]. The target amplicons were purified and sequenced using corresponding primer sets. The sequencing results were then searched through BLAST program to confirm the HPV genotype.

3. Results and Discussions

Among the 1643 eligible Chinese women, the age was ranged from 15 to 83 years and the median age was 29 years. Patient characteristics were shown in Table 1. Based on the cytological results, 1505 (91.6%, 1505/1643) samples were normal with no intraepithelial lesion or malignancy, 91 (5.5%) were atypical squamous cells of undetermined significance (ASCUS), 41 (2.5%) were low-grade squamous intraepithelial lesions (LSIL), and 6 (0.36%) were high-grade squamous intraepithelial lesions (HSIL). Using the GenoFlow HPV Array Test, genotyping test revealed that 695 cases were positive for HPV DNA, and the overall prevalence of HPV was 42.3% (695/1643). The three most prevalent types were HPV 52 (139/1643, 8.5%), HPV 81 (89/1643, 5.4%), and HPV 16 (63/1643, 3.8%) regardless of their cervical histological results (Figure 1). Our results were in-line with

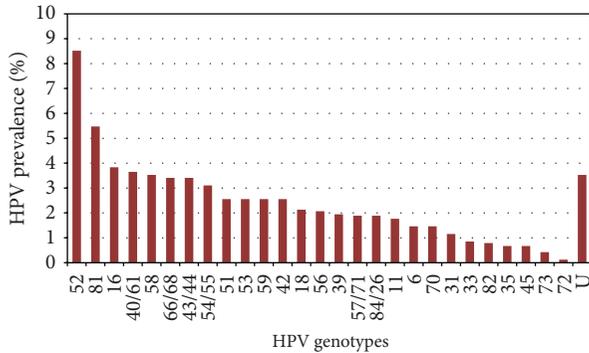


FIGURE 1: Distribution of genotypes in 1643 Hong Kong women. HPV 40 and 61, HPV 66 and 68, HPV 43 and 44, HPV 54 and 55, HPV 57 and 71, and HPV 84 and 26 share probe spots. U, cases showed positive for HPV DNA at the “universal probe” detection only, but not in-panel HPV specific types.

the reported meta-analysis studies showing that the most commonly detected HPV type in South China was HPV 52 [13–15]. Liu et al. reported that HPV 52 was the most frequently found genotype in the Guangzhou region of China while its prevalence was comparable to that of HPV 16 in Hong Kong [16]. Li et al. and Ye et al. also showed HPV 52 had the highest prevalence in Guangdong and Zhejiang of China, respectively [14, 17]. In this study, HPV 16 ranked the third and the prevalence (3.8%) was comparable with that worldwide. The population-based type-specific prevalence of HPV 16 is ranging from around 2% to 4% in all regions [6]; however, HPV 16 infection is strongly associated with risk of cervical neoplasia and causes over 50% of cases of cervical cancer [4]. HPV 81 (equivalent to CP8304) is also reported to be associated with precancerous or cancerous lesions [18, 19]. The prevalence of HPV 81 in worldwide population studies is not very high but HPV 81 is one of the most frequently observed HPV types in the HIV-positive patients [20, 21]. The finding that HPV 81 prevalence was more frequent than HPV 16 in our study may be due to the geographical and biological variations in the distribution of HPV types and the sensitivities of different genotyping methods on specific HPV types.

The GenoFlow test utilized a novel universal probe to detect HPV genotypes that were outside the 33 genotype panel, which can broaden the genotype detection coverage. In the present study, 56 cases were positive for HPV DNA at the “universal probe” detection only but not in-panel HPV specific types. It suggested that one or more out panel HPV genotypes were present in the samples and the prevalence was 3.4% (56/1643), which was only slightly lower than that of HPV 16 (Figure 1). Of the 56 cases positive for unknown HPV types, 91.1% (51/56) samples presented normal cytological results in the Pap test, 3.6% (2/56) samples were diagnosed as ASCUS, and 5.4% (3/56) samples were LSIL. These five cases with cell abnormality were sequenced to confirm the HPV types. The genotypes were successfully identified and listed in Table 2. Rare HPV types 30, 91, and 74 as well as the variant forms of HPV 72 and 82 were detected in women with ASCUS or LSIL.

TABLE 2: Genotype distribution of HPV in the 5 cases with cell abnormalities.

Sample no.	Cell abnormality	HPV genotype
16	ASCUS	HPV 72 variant
21	LSIL	HPV 30
23	LSIL	HPV 91
36	ASCUS	HPV 74
44	LSIL	HPV 82 variant

HPV 30, part of the alpha 6 species group along with HPV types 53, 56, and 66, is classified as possibly-carcinogenic because of its close phylogenetic relationship with HPV 56 which is a well-known cancer causing HPV type [3]. HPV 30 is not commonly detected in the genital tract, so it is usually excluded from the commercial HPV genotyping kits. However, by DNA microarray method, Rahman et al. showed that the prevalence of HPV 30 in 342 HIV-negative Japanese women was 3.8% and was detected in 1 case of ASCUA, 7 cases of LSIL and 1 case of HSIL [22].

HPV 91 and HPV 74 belong to alpha 8 and alpha 10 species, respectively, and are both classified as noncarcinogenic [3]. Only limited studies were carried out to examine their prevalence. HPV 74 was first identified from persisting vaginal lesions of low-grade intraepithelial neoplasia of an immunosuppressed woman [23]. Choi et al. performed DNA sequencing on 209 HPV-other samples (positive on HPV-PCR but negative when using specific HPV hybridization probes) to determine the HPV type. A case of HPV 74 infection was identified in LSIL patient [19]. In a cohort of 310 sexually active women in Trinidad, HPV 91 was found in 1 case by DNA sequencing but its role in cancer development is still not clear [24].

HPV intratypic variants are extensively reported, particularly in HPV 16. The oncogenic E6 and E7 proteins are commonly mutated, whereas synonymous and nonsynonymous mutations are also reported in the L1 gene in HPV 16 [25–27]. Several studies investigated the genetic variability of L1, E6, and E7 genes in HPV 18, 38, 52, and 53 and showed that the intratypic variants were frequently found [28–31]. Some of these mutations increased the oncogenic potential and the infection efficiency of the viruses. Unsurprisingly, if the mutations are situated at the position of the detection probes, the genotyping will give a false-negative result. In this study, although HPV 72 and HPV 82 specific probes were included in the kit, negative results were shown. However, the positivity of HPV infection was detected by the universal probe, and the identification of variant forms of HPV 72 and 82 was confirmed by DNA sequencing.

Nowadays, most of the HPV genotyping assays can only detect the most common high-risk and low-risk types but not the unknown-risk types, novel types, or even the intratypic variants. In order to improve the quality of HPV genotyping, next generation sequencing has been developed to sequence cervical DNA. Meiring et al. identified a total of 16 HPV genotypes from 109 cervical specimens from South African HIV-positive women using next generation sequencing, in which four genotypes (HPV-30, 74, 86, and 90) are not included in

the commercial kit [32]. Nevertheless, this platform is relative expensive, time-consuming, and requires costly equipment. For the GenoFlow test utilized in the present study, the use of PCR with modified PGMV primers targeting the L1 region of HPV followed by hybridization with a universal probe allow rare HPV species or variants outside the genotyping panel to be detected. The Flow-through hybridization system also allows the HPV genotyping to be completed within 3 hours. This rapid test with maximum HPV coverage will be a good alternative in HPV clinical applications.

4. Conclusions

In the present study, rare HPV types 30, 91, and 74 and the variant forms of HPV 72 and 82 were successfully detected in Chinese women with low-grade cytological abnormalities by the universal probe available in the GenoFlow HPV Array test. Data on the prevalence of rare HPV types and HPV intratypic variants will assist in clarifying the role of these viruses in cervical intraepithelial lesions and invasive cervical cancer.

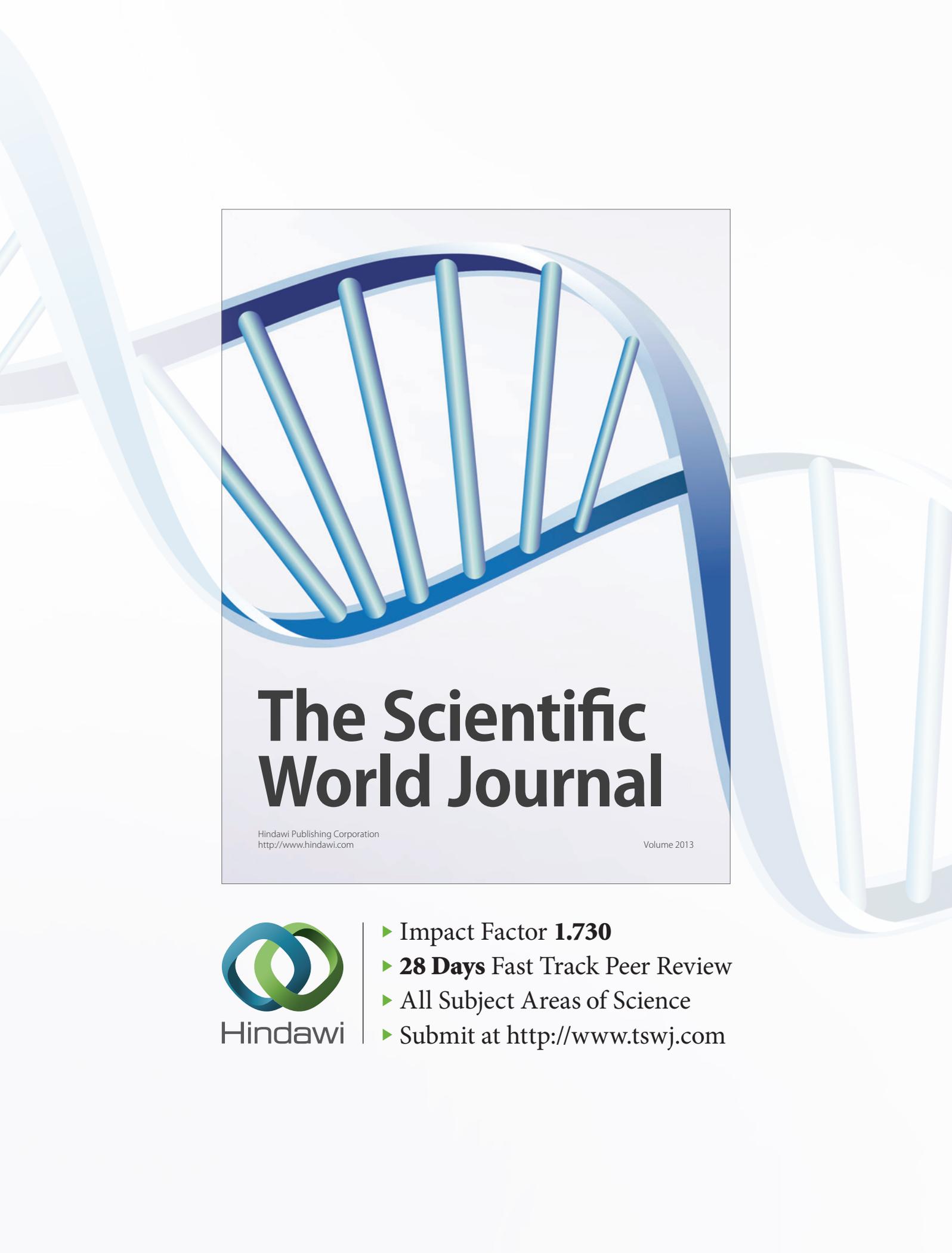
Conflict of Interests

Ngai Na Chloe Co, Lai-On Chu, Joseph K. F. Chow, and Joseph W. O. Tam are the staff of DiagCor Bioscience Inc. Ltd. Enders K. O. Ng is holding an Honorary Assistant Professorship in the University of Hong Kong and is also Senior Scientist at DiagCor Bioscience Inc. Ltd.

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