ISSN 2007-9737

Association of Endogenous PCR Final Product in HPV Human Samples with Ebis Measurements

Alejandro Corzo-Cruz[1,2], Virginia Sánchez-Monroy[1], Jacobo E. Munguía-Cervantes[3], César A. González-Díaz[1,*]

[1] Instituto Politécnico Nacional,
Escuela Superior de Medicina,
Mexico

[2] Secretaria de Defensa Nacional,
Mexico

[3] Instituto Politécnico Nacional,
Center for Nanosciences and Micro Nanotechnology,
Mexico

cgonzalez@ipn.mx

Abstract. Genomic medicine promises valuable support for the clinician in predicting pathological conditions. The Polymerase Chain Reaction (PCR) remains the gold standard for gene detection. Electrical Bioimpedance Spectroscopy (EBiS) is a non-invasive, low-cost technique that allows examining the electrical properties of biological materials. Human papillomavirus (HPV) is associated with cervical cancer and has recently been studied as a potential risk factor for prostate cancer (PCa). An EBiS-based genosensor has been proposed to detect fragments of the HPV L1 gene. As an initial approach, this study evaluates the feasibility of detecting a human endogenous gene present in PCa samples through EBiS measurements of the final PCR product. Thirty samples from patients diagnosed with PCa were examined, PCR amplification of the edogenous gene OGG1 was performed and the gene amplification, evidenced by electrophoresis, was estimated semiquantitatively based on the intensity of bands in agarose gel, which was correlated with EBiS measurements. The results indicate a significant association between the band intensity and the bioimpedance phase angle at low frequencies, particularly at 144 Hz ($R=0.52$, $p<0.05$). The detection of an endogenous gene in PCa samples is feasible using EBiS and opens the possibility of new studies to explore the feasibility of the same technique allowing the detection of PCa-specific genes, such as the GP 5+/6+ fragment of the HPV L1 gene.

Keywords. Bioimpedance, HPV, prostate cancer.

1 Introduction

The primary goal of a clinician is to reach an accurate diagnosis to develop effective strategies for establishing appropriate treatment for the patient [1]. Today, physicians have access to various devices and diagnostic tests with adequate sensitivity and specificity for pathogen detection [2].

Over the past three decades, molecular techniques have opened a new field of research in biomedical sciences by extensively studying Deoxyribonucleic Acid (DNA), known as “the molecule of life,” due to its characteristic information code of different living organism. Genomic medicine emerged from the interest in studying DNA fragments using the Polymerase Chain Reaction (PCR) a gold-standard technique, and gel electrophoresis to observe the amplifications of specific sequences [3].

DNA is a molecule with electrical properties that can be measured and characterized for developing devices that allow its detection [4].

In recent years, EBiS has become a promising field in the research of devices that use electrical principles for diagnostic tests. These devices provide clinicians with portable systems for

pathogen detection in the office, offering the advantages of being low-cost, non-invasive, easy to operate an analyze, without the need for laboratory studies requiring highly specialized and expensive personnel and equipment [4–6].

Juhun Lim et al. (2021) developed a device for real-time DNA detection using label-free PCR through EBiS, performing measurements per amplification cycle over a frequency range (100 Hz to 1 MHz) and implementing a performance index to monitor the optimal frequencies for detecting the amplification of the gene of interest, determining that the optimal frequencies were 3.984 kHz and 20.02 kHz for the real and imaginary parts of the impedance respectively, in concentrations of 10-4 ng/µL [7].

Chiticaru et al. (2023) developed a platform to detect hybrid DNA based on the fabrication of screen-printed carbon electrodes modified with oxidized graphene and gold nanoparticles.

This was followed by a preparation for the detection of hybrid DNA, involving the reduction of graphene oxide within the screen-printed carbon electrode, followed by the reduction of the gold nanoparticle to subsequently bind the hybrid DNA strand through physical adsorption.

The used Cyclic Voltammetry and EBiS for DNA detection after inserting the complementary hybrid strand, achieving high sensitivity to detect concentrations as low as 1 nM of the analyte under study [8].

Ames-Lastra et al. (2023) evaluated the feasibility of a biosensor based on measurements EBiS and PCR products labeled with chemical fluoroscopy using SYBER Green®.

Researchers amplified sequences of a 200 bp fragment of Entamoeba histolytica DNA, then diluted the amplifications at 1:1, 1:2, 1:5, 1:10 and performed measurements throughout EBiS.

The differential spectrum analysis showed clear differences between the DNA concentration with the spectrum ranges of magnitude an phase angle, observing that frequency ranges of 500-700 kHz and 700-900 kHz for magnitude ratio and phase, respectively, correlated with the different concentrations, demonstrating that EBiS was a feasible alternative technique to detect PCR product amplicons [9].

1.1 HPV as a Potential Risk Factor to PCa

Human Papillomavirus (HPV) is a pathogen that is classified among sexually transmitted diseases, belonging to the Papillomaviridae family, where around 200 different virus genotypes have been described, most of which cause benign genital warts, also known as low-risk genotype viruses. It is transmitted sexually and is the second most common pathogen worldwide that causes cancer after Helicobacter pylori.

Some HPV genotypes (16, 18, 33, 35, 45, 51, 56, 58, 59, 68, 73, 82) cause changes in the cellular morphology of the cervical epithelium, transforming them into cancerous cells, also known as high-risk genotypes. Cervical cancer is the second most common cancer in women worldwide, with genotypes 16 and 18 being the most prevalent [10, 11].

HPV has an icosahedral capsid composed of two types of proteins, L1 and L2, which protect a double-stranded DNA molecule of approximately 7 to 8 kbp and seven early coding regions that regulate transcriptions, replication, and overexpression of the gene during cellular infection [11, 12].

The L1 gene of HPV encodes the protein of the virus's icosahedral capsid, making it the most conserved in mutation rate and the one used to determine infection by the virus. It contains conserved regions in most species, with the MY11 fragment at 450 bp, L1C1 at 244 bp, and the GP 5+/6+ gene at 150 bp being the targets for HPV study [13].

In recent years, researchers have observed that genotypes such as 56 and 58 have been found in men with Benign Prostatic Hyperplasia (BPH) and in patients diagnosed with Prostate Cancer (PCa) [12, 14–16].

The aim of this study is to detect OGG1 an endogenous gene in PCa samples using the EBiS technique, which opens the door to future studies to investigate whether this methodology could also be applied to the detection of HPV-specific genes, such as the GP 5+/6+ fragment of the L1.

2 Materials and Methods

Thirty paraffin-embedded prostate tissue samples from patients diagnosed with PCa were obtained

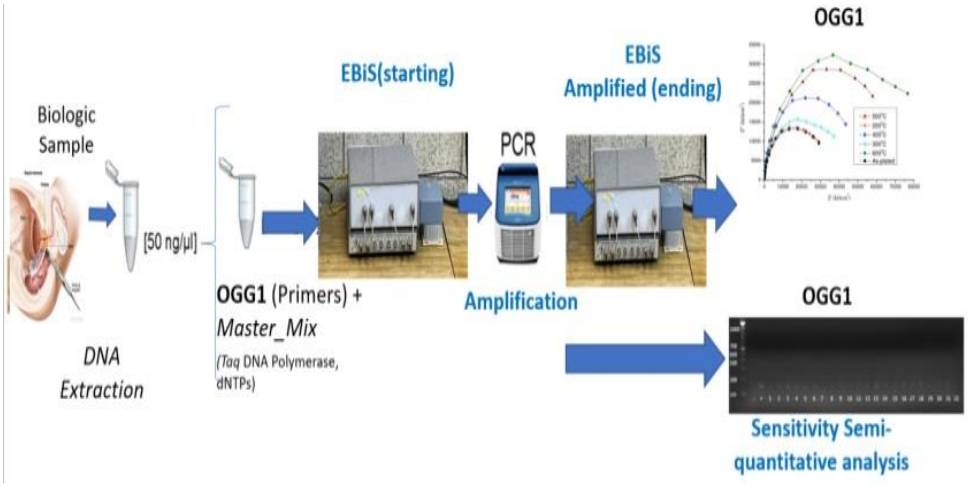

Fig. 1. Diagram of the experimental design. Sequence of steps for EBiS measurement and electrophoresis of samples

Table 1. Primers OGG1

Sense	Sequence
Forward	5'TTCCACCTCCCAACACTGTCA3'
Reverse	5'TGCCTGGCCTTTGAGGTAGT3'

from the physical pathology archive of the Central Military Hospital of Mexico.

2.1 Sample Preparation

The xylene technique was used for deparaffinization. Subsequently, the phenol-chloroform-isoamyl alcohol technique was used for DNA extraction.

The samples prepared for the amplification of the endogenous gene OGG1 with specific primers (Table 1) prepared 50 µL with master mix using the Platinum Hot Start PCR 2X Master Mix kit from Invitrogen® according to the manufacturer's instructions.

2.2 EBiS Measurement

The EBiS measurement were performed using the ISX-3 bioimpedance analyzer (Sciospec Scientific®, Leipziger, Bennewitz, Germany), serial number 01-000B-002F-0406 with a maximum voltage of 100 mV., coupled with gold film interdigitated microelectrodes embedded in a glass surface, with 10x10 mm features. The programming and storage for Sciospec Scientific® were carried out on a personal computer.

The procedure involved measurements in air, Milli-Q® water, and saline solution to establish impedance reference parameters. Subsequently, measurement was taken with 2 µL of the samples prepared before and after gene amplification according to the Figure 1. Following these steps:

1) The microelectrode is cleaned with 5 µL of Milli-Q® water.
2) The microelectrode is dried with specialized cleaning wipes (Kimwipes®, Kimtech Science™ brand S-8115 kimberly-Clark® Mexico).
3) 2 µL of the sample are placed on the microelectrode for EBiS measurement.
4) After the measurement, the sample is removed from the microelectrode with wipes, and 5 µL of Milli-Q® water is applied and dried.
5) Amplification is performed.

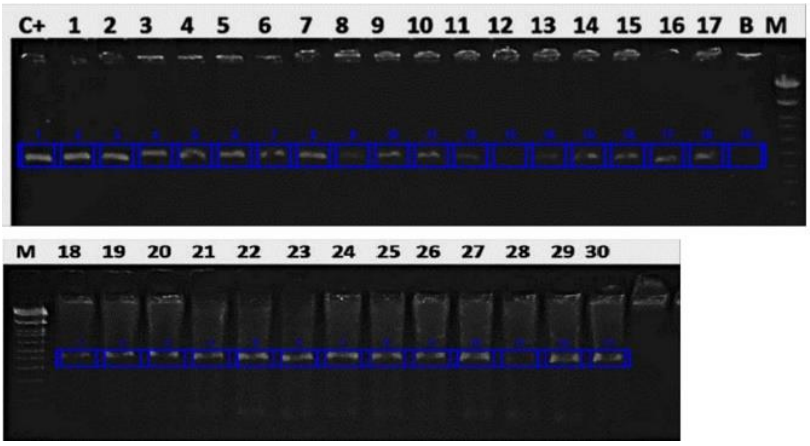

Fig. 2. OGG1 amplification on agarose gel. Semiquantification of band intensity using Image Lab. 6.1 software, BioRad®

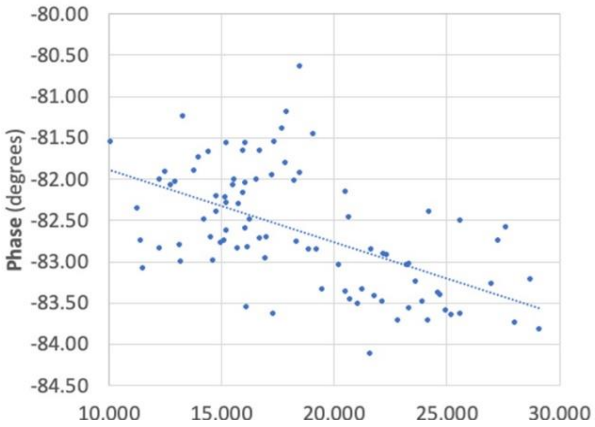

Fig. 3. Scatter plot intensity of bands on agarose gel by Image J software vs Bioimpedance phase angle at 144 Hz. A Pearson test shows statistically significative correlation (R=0.52, p<0.05)

6) After amplification, the steps mentioned above are repeated with amplification sample.

2.3 Electrophoresis and Semiquantification

The amplicons were evaluated by 1.5% agarose gel electrophoresis according to Figure 1. Subsequently, a semiquantification was performed based on the intensity of the amplicon band from electrophoresis gel of the thirty patients with PCa, using Image Lab 6.1 software, Bio-Rad®, as shown in Figure 2.

2.4 Data Processing

The data processing was carried out by elaborating a matrix in Excel® tables and using SPSS® Statistics software (version 21.0).

2.5 Ethical Conditions

This study follows the ethical conditions and research principles of international standards endorsed an approved by the Ethics and Research Committees of the Central Military Hospital of Mexico with registration numbers CEI-11/2022 and 062/2022 respectively.

3 Results

The endogenous OGG1 gene was amplified in all thirty patients, confirmed by the electrophoresis gel. The band intensity was semiquantified with software Image Lab 6.1 Bio-Rad®. The magnitude and phase angle results were obtained for the thirty patients. An association between band intensity and phase angle at low frequencies, particularly at 144 Hz ($R=0.52$, $p<0.05$), was observed as shown in Fig 3.

4 Discussion

A correlation was found between the results of the band intensity of the semiquantitative measurement of amplification of OGG1 and the EBiS measurement at low frequencies, compared to the results obtained by Ames-Lastra et al. [9] Unlike that work, the amplification was performed label free, opening the possibility of improving detection and lowering the cost of the label.

The amplification detection in this study at 144 Hz frequency was even lower than the one obtained by Juhum et al. [7] and we could look for a way to make a performance index as proposed in his work.

Additionally, unlike the approach by Chiticaru et al. [8], the electrodes were not functionalized, which simplifies the operation of the device and opens the potential for designing portable devices in the future.

5 Conclusions

The detection of the endogenous OGG1 gene in PCa samples is feasible through EBiS, opening the possibility of exploring new studies using the same technique for detecting the L1 gene fragment, specifically GP 5+/6+ of HPV in patients with PCa.

Acknowledgments

ACC extends thanks to the “Consejo Nacional de Humanidades, Ciencia y Tecnología- CONAHCyT, Mexico” for their support in the development of this work through a graduate student scholarship. Furthermore, this research was funded by the “Instituto Politécnico Nacional, Mexico” through the projects “SIP-20241622 and Innovación-20241041”.

Special thanks to the Military School of Health Graduates and the Department of Pathology at the Central Military Hospital of Mexico.

References

1. **Leeflang, M.M.G., Allerberger, F. (2019).** How to: Evaluate a diagnostic test. Clinical Microbiology and Infection. Vol. 25, No. 1. pp. 54–59. DOI: 10.1016/j.cmi.2018.06.011.
2. **Yang, B., Olsen, M., Vali, Y., Langendam, M.W., Takwoingi, Y., Hyde, C.J., et al. (2021).** Study Designs for Comparative Diagnostic Test Accuracy: A Methodological Review and Classification Scheme. Journal of Clinical Epidemiology. Vol. 138, No. 1. pp. 128–138. DOI:10.1016/j.jclinepi.2021.04.013.
3. **McCarthy, J.J., McLeod, H.L., Ginsburg, G.S. (2013)**. Genomic Medicine: a Decade of Successes, Challenges, and Opportunities. Sci Transl. Med., Vol. 12, No. 5. pp. 189. DOI: 10.1126/scitranslmed.3005785.
4. **Zhang, L., Su, W., Liu, S., Huang, C., Ghalandari, B., Divsalar, A., et al. (2022).** Recent Progresses in Electrochemical DNA Biosensors for MicroRNA Detection. Phenomics. Vol. 2, No. 1, pp. 18–32. DOI: 10.1007/s43657-021-00032-z.
5. **Bhalla, N., Jolly, P., Formisano, N., Estrela, P. (2016).** Introduction to Biosensors. Essays Biochem, Vol. 60, No. 1. pp. 1–8. DOI: 10.1042/EBC20150001.
6. **Magar, H.S., Hassan, R.Y.A., Mulchandani, A. (2021).** Electrochemical Impedance

Spectroscopy (EIS): Principles, Construction, and Biosensing Applications. Sensors. Vol. 21, No. 19, pp. 21. DOI: 10.3390/ s21196578.

7. **Lim, J., Kim, J., Lee, E., Park, H., Lee, J.H. (2021).** An analysis method to detect the presence of DNA using electrochemical impedance spectroscopy (EIS) for real-time PCR. Microsystem Technologies, Vol. 27, No. 3, pp. 1–7. DOI: 10.1007/s00542-020-05074-3.
8. **Chiticaru, E.A., Damian, C.M., Pilan, L., Ioniță, M. (2023).** Label-Free DNA Biosensor Based on Reduced Graphene Oxide and Gold Nanoparticles. Biosensors, Vol. 13, No. 8, pp. 14. DOI: 10.3390/bios13080797.
9. **Ames-Lastra, G., Sánchez, V., Sacristán-Rock, E., Gómez-López, M., Pérez-Vielma, N., Castillo-Salazar, I.A., et al. (2021).** Bioimpedance Spectroscopy as a Potential Technique to Detect Label-Free PCR Products. Journal of Physics: Conference Series. Vol. 17, pp. 7. DOI: 10.1088/1742-6596/2008/1/012016.
10. **Szymonowicz, K.A., Chen, J. (2020).** Biological and Clinical Aspects of HPV-Related Cancers. Cancer Biology and Medicine. Vol. 17, No. 4, pp. 864–878. DOI: 10.20892/j.issn.2095-3941.2020.0370.
11. **Tsydenova, I.A., Ibragimova, M.K., Tsyganov, M.M., Litviakov, N.V. (2023).** Human Papillomavirus and Prostate Cancer: Systematic Review and Meta-Analysis. Sci. Rep., Vol. 13, No. 1, pp. 8. DOI: 10.1038/s41 598-023-43767-7.
12. **Silva LL da Teles A.M., Santos, J.M.O., Souza de Andrade, M., Medeiros, R., Faustino-Rocha, AI., et al. (2023).** Malignancy Associated with Low-Risk HPV6 and HPV11: A Systematic Review and Implications for Cancer Prevention. Cancers (Basel), Vol. 15, No. 16, pp. 13. DOI: 10.3390/ cancers15164068.
13. **Bansal, A., Singh, M.P., Rai, B. (2016).** Human Papillomavirus-Associated Cancers: A Growing Global Problem. Int. J. Appl. Basic Med. Res., Vol. 6, No. 2. pp. 84–89. DOI: 10.4103/2229-516X.179027.
14. **Chang, H.J., Pong, Y.H., Chiang, C.Y., Huang, P.C., Wang, M.H., Chan, Y.J., et al. (2023)**. A Matched Case-Control Study in Taiwan to Evaluate Potential Risk Factors for Prostate Cancer. Sci. Rep., Vol. 10, No. 13, pp. 10. DOI: 10.1038/s41598-023-31434-w.
15. **Elvia, P., Fern, E., Oros-Pantoja, R., Medel-Flores, O., Miranda-Covarrubias, J.C., Virginia, S. (2021).** Proinflammatory and Oxidative Stress States Induced by Human Papillomavirus and Chlamydia Trachomatis Coinfection Affect Sperm Quality in Asymptomatic Infertile Men. Medicina (Kaunas), Vol. 57, No. 9, pp. 9. DOI: 10.3390/ medicina57090862.
16. **Medel-Flores, O., Valenzuela-Rodríguez, V.A., Ocadiz-Delgado, R., Josué, L., Hernández-Leyva, S., Lara-Hernández, G., et al. (2018).** Association Between HPV Infection and Prostate Cancer in a Mexican Population. Genet Mol. Biol., Vol. 41, No. 4. pp. 781–789. DOI: 10.1590/1678-4685-GMB-2017-0331**.**

Article received on 15/01/2025; accepted on 20/03/2025.
**Corresponding author is César A. González-Díaz.*