Pascual-Mathey LI, et al., J Reprod Med Gynecol Obstet 2023, 8: 153
DOI: 10.24966/RMGO-2574/100153

HSOA Journal of Reproductive Medicine, Gynecology & Obstetrics

Research Article

Determination of HPV Serotypes in Endocervical samples from Patients with Normal Cytology from a Mexican Population by PCR-RFLP

Pascual-Mathey LI[1]*, Díaz-Vallejo JJ[1], Córdoba-Colorado D[1], Pérez-Vásquez[1] and Lagunes-Báez E[2]

[1]*Facultad de Química Farmacéutica Biológica, Universidad Veracruzana, Xalapa, Veracruz, Mexico*

[2]*Unidad de Medicina Familiar No. 66, Instituto Mexicano del Seguro Social, Xalapa, Veracruz, Mexico*

Abstract

Human Papillomavirus (HPV) is the main infectious agent associated with cervical cancer. Genotypes 16, 18, 31 and 33 are the most prevalent and are considered high-risk (HR) factors for cancer. Currently, the Papanicolaou technique (Pap) is the routine method for the identification of morphological cell changes in cervical cancer screening. However, HPV infection may be present in normal Pap smears. This study aimed to estimate the prevalence of HPV and its genotypes in patients with normal cytology using universal oligonucleotides. HPV prevalence and genotyping were evaluated in 141 women with normal cytology. DNA was extracted from a cervical swab by the proteinase K method. The viral genome was screened by PCR, using MY09/11 and L1C1/L1C2 oligonucleotides and the genotypes by RFLP.

The estimated HPV prevalence increased to 16.3% (95%CI 10.1-22.5) when the combined oligonucleotides were used. Genotypes 11, 13, 33 and 59 were detected by RFLP; 33 and 59 are HR-VPH oncogenic risk factors for cervical cancer. The number of sexual partners was an associated factor for HPV infection, OR 5.2 (1.60-17.09) P=0.006. Although HPV 16 and 18 are the most reported genotypes, the increase in the prevalence of regional HR variations should be considered in cervical cancer prevention and treatment.

Keywords: High-risk HPV; Human Papillomavirus (HPV); Normal cytology; PCR-RFLP

***Corresponding author:** Luz Irene Pascual Mathey, Facultad de Química Farmacéutica Biológica, Universidad Veracruzana, Xalapa, Veracruz, 91190, Mexico,Tel: +52 2281251392, E-mail: lupascual@uv.mx.com

Citation: Pascual-Mathey LI, Díaz-Vallejo JJ, Córdoba-Colorado D, Pérez-Vásquez and Lagunes-Báez E (2023) Determination of HPV Serotypes in Endocervical samples from Patients with Normal Cytology from a Mexican Population by PCR-RFLP. J Reprod Med Gynecol Obstet 8: 153.

Received: November 20, 2023; **Accepted:** December 05, 2023; **Published:** December 12, 2023

Introduction

Cervical Cancer (CeCa) is the second highest prevalence for cancer in women aged 25-59 years after breast cancer [1]. Several environmental, genetic and carcinogenic factors play a role in the development of CeCa. The Human Papillomavirus (HPV) is the main pathogenic agent for anogenital sexually transmitted infections [2,3]. According to the World Health Organization, 604,000 new cases were estimated. Most of them occur in low- and middle-income countries, with an estimated increased prevalence of HPV in Mexico [4].

More than 200 types of HPV have been identified and subdivided into high- and low-oncogenic risk [5]. However, around 40 HPV infects the anogenital region; variants 16, 18, 31 and 45 are associated with persistent infections that can lead to cancer, presented in more than 70% of high-risk squamous intraepithelial lesions and cervical cancer [3,6,7]. Genotypes 31, 33, 35, 45, 52 and 58 are responsible for 20% of additional cases of CeCa worldwide [8], while types 6, 11, 42, 43 and 44 (known as low risk), are found in condyloma acuminatum and intraepithelial neoplasms of low risk and low malignant progression [5,9]. More than 80% of sexually active men and women will have an HPV infection in their lifetime [2,10,11]. Although most infections (with or without cytologic abnormalities) will be cleared spontaneously within 18 to 24 months, approximately 10% of cases will progress to a precancerous state within 10 to 20 years. However, some lesions progress to cancer within 1 to 2 years, depending on the genotype and the associated factors [5].

The Papanicolaou (Pap) is the traditional clinical test for the detection of vaginal mucosal lesions, whose histological observation allows the visualization of cells with aberrant phenotypes, being an effective test for the screening of malignant lesions (precancerous and cancerous) [12]. However, due to its low reproducibility and sensitivity, it does not allow the identification of about 50% of infections associated with cervical dysplasia or neoplasia related to HPV types of high oncological risk [13]. The combined use of high-sensitivity and specificity methods to identify and determine the HPV genotype, suggested by the American Cancer Society and the American Association for Colposcopy and Cervical Pathology, is indispensable [14]. The PCR-RFLP (Restriction Fragment Length Polymorphic Polymerase Chain Reaction) technique is an accessible tool in low- and middle-income countries that allows simultaneous identification and typing of HPV variants [15-17]. This study aimed to identify HPV genotypes in DNA samples from women with normal cytology using the PCR-RFLP technique as a primary screening test.

Materials and methods

Study Population

A cross-sectional study was conducted. Samples were randomly collected from healthy women with a normal cervical Pap smear examination during February - March 2021 in a family medicine unit No. 66 of the Mexican Social Security Institute in the city of Xalapa, Veracruz. The eligibility criteria were residing in Xalapa, Veracruz, during the last year and having a sexually active life. Exclusion criteria were presenting an abnormal cytology and being pregnant. All participants signed an informed consent form. A total of 141 endocervical samples from sexually active women were included. The present study had the approval of the IMSS National Committee for Scientific Research (registration number R-2021-785-015) and the informed consent. In addition, a questionnaire to evaluate the associated clinical and epidemiological factors was applied. All procedures were based on the Declaration of Helsinki, as well as the Mexican Official Standard NOM-012-SSA3-2012, which establishes the criteria for the execution of research projects for health in humans.

DNA Extraction

Endocervical samples were obtained by cytobrush and preserved in Phosphate-Buffered Saline (PBS) at 4°C. DNA extraction was performed by the proteinase K method [18]. DNA yield and purity were analyzed with a UV spectrometer at 260/280 nm. The integrity was visualized in 1.5% agarose gel electrophoresis with ethidium bromide.

PCR Assay

HPV was identified by the amplification of a fragment of the L1 sequence of the viral genome by two screening methods. The first amplified a 450 bp fragment by using the oligonucleotides MY09: 5'CGTCCAAAAGGAAACTGATC3' and MY11: 5'GCACAGGGACATAACAATGG3' [19]. Amplification was carried out in a final volume of 20 µL containing a reaction mixture of 1X $MgCl_2$-free buffer, 1.5 mM-$MgCl_2$, 200 µM-dNTP, 0.8 µM primers and 1.25U Taq Polymerase. The samples were subjected to amplification using a BIORAD T-100 Thermal Cycler, with the following program: 12 min at 95ºC, 40 cycles of 1 min at 95º C, 1 min at 55º C and 1 min at 72º C, with a final extension step at 72º C for 5 min. The 450 bp fragments were verified by electrophoresis in a 2% agarose gel. Negative samples were subjected to a second screening that amplified a 240-250 bp fragment using the oligonucleotides L1C1: 5'CGTAAACGTTTTTTCCCTATTTTTTTT3' and L1C2: 5'TACCCTAAATACCCTATATTATTG 3' [15]. The reaction mixture consisted of 1X $MgCl_2$-free buffer, 1.5 mM-$MgCl_2$, 200 µM-dNTP, 1.0 µM primers and 2.0 U Taq Polymerase. The thermal cycling program followed the following steps: 5 min at 95ºC, 40 cycles of 1 min at 95º C, 1.5 min at 55º C and 2 min at 70º C, with a final extension step at 72º C for 5 min. The ß globin gene was used as internal control [19].

Restriction Fragment Length Polymorphism (RFLP)

Positive samples obtained by the amplification methods with MY09/11 or LC1/LC2 oligos were typed by RFLP using the restriction enzymes BamHI, RsaI, PstI, HaeIII and DdeI (Promega, USA) [15,20]. Each restriction reaction was performed separately. The final volume was 20 µL; 2 µL of PCR product, restriction buffer 1X and 10 U restriction enzyme. The reaction was incubated in a water bath at 37°C for 3 hours. Inactivation was performed at 4°C and samples were visualized on 3% agarose gel stained with ethidium bromide. The results were verified on a trans illuminator by digitizing the gels using the Gene Snap software. The restriction sites reported by Bernard *et al*. (1994) were used for serotypes identification [21].

Statistical Analysis

A descriptive statistic was performed for all variables with a normal distribution. The Mann-Whitney U test was used to compare the sociodemographic factors (age, number of sexual partners and initial sexual age). A bivariate analysis of the main risk factors for HPV was performed. Odds ratios (OR) and 95% Confidence Intervals (CI) were obtained from multiple logistic regression models to assess the association between HPV infections and risk factors. The level of significance was set as a p-value of less than 0.05. All statistical analyses were performed with Statistical Package for Social Sciences version 25.0 (SPSS-IBM) (Armonk, NY: IBM Corp).

Results

Associated Risk Factors to HPV Infection

One hundred forty-one women participated in the study, with a mean age of 46.1±10.3 years; the range was 22-64 years. The age group with the highest proportion was 50-54 years (22%). Table 1 shows the frequency of contraceptive methods used, with BTO being the most used; 22.7% did not use a contraceptive method. Regarding clinical characteristics, all patients presented a Negative Cytological Result for Intraepithelial Lesion or Malignancy (NILM), of which 3.3% presented infection by *Trichomonas vaginalis, Candida albicans, or Gardnerella vaginalis.*

Contraceptives	Frequency n=141	Percentage (%)
Bilateral tubal occlusion	74	52.5
Condom	17	12.0
Use of copper IUD	8	5.7
Oral	7	5.0
Partner with vasectomy	3	2.1
None	32	22.7

Table 1: Frequency of contraceptive methods used. IUD: intrauterine device.

The highest prevalence was found in the 36-45 and 46-55 age ranges, with 34.8% and 34.8%, respectively. The number of sexual partners (more than two) (OR 5.2, 1.60-17.09; P=0.006) is a significant risk factor for HPV infection. Other factors like the use of a condom or the age at first intercourse, were not associated factors in our population. Table 2 shows the factors associated with HPV infection.

Characteristics	HPV+ n=23	HPV- n=118	OR (95%)	P-value
Age (range)				
20-35	1 (4.3)	23 (19.5)	1	
36-45	8 (34.8)	31 (26.3)	8.8 (0.85-90.1)	0.068
46-55	8 (34.8)	42 (35.6)	1.0 (0.26-3.66)	0.980
56-65	6 (26.1)	22 (18.6)	1.5 (0.42-5.04)	0.559
Number of sexual partners				
1	7 (30.4)	62 (51.7)	1.0	

2	7 (30.4)	30 (25.4)	2.1 (0.61-7.08)	0.238
≥3	9 (39.1)	26 (22.0)	5.2 (1.60-17.09)	0.006*
Age at first intercourse				
≥ 18	17 (73.9)	78 (66.1)	1.0	
< 18	6 (26.2)	40 (33.9)	0.60 (0.19-1.93)	0.608
Condom				
Yes	1 (4.3)	15 (12.7)	1.0	
No	22 (95.7)	103 (87.3)	2.9 (0.34-25.8)	0.320

Table 2: Risk factors for human papillomavirus infection. OR: odds ratio,*P value: <0.05.

Amplification and Typification of HPV Using Consensus Primers and Restriction Enzymes

The DNA samples extracted from endocervical smears were tested with MY09/11 oligonucleotides, obtaining six positive results (4.3%) (Figure 1). The negative samples were amplified with the L1C1/L2C2 oligonucleotides, with 17 additional positive results. Genotyping showed high and low oncogenic HPV serotypes, with an estimated prevalence of 16.3% (95%CI, 10.1-22.5). The genotypes detected were 11 (42.8%), 13 (28.6%), 33 (14.3%) and 59 (14.3%); 33 and 59 are considered high oncogenic risk factors (Figure 2).

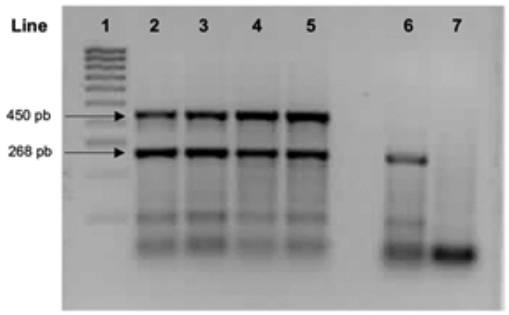

Figure 1: The MY09/MY11 oligonucleotides were used to identify HPV. Line 1) molecular weight marker, 100-1013 bp in 100-bp increments (Hyper Labdder, Bioline, AUS); lines 2 to 5, identification of HPV (450 pb) and the human ß-globin gene (268 pb); line 6 is negative to HPV; line 7 is the negative control.

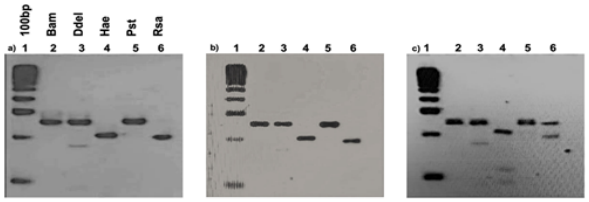

Figure 2: Restriction mapping of LC-PCR product. Twenty-three cloned HPV types were subjected to RFLP with the restriction enzymes BamHI, DdeI, HaeIII, PstI and RsaI. After electrophoresis, gels were visualized and photographed. From left to right, positive HPV samples: a) 118, b) 75, c) 26. Lines corresponded to: 1) molecular weight marker, 100-1013 bp; 2) BamHI; 3) DdeI; 4) HaelII; 5) PstI and 6) RsaI.

Discussion

Cervical cancer is a public health problem in low- and middle-income countries such as Mexico due to the high morbidity and mortality rates. Several studies have established a prevalence of HPV in women with normal cytology ranging from 9.1% to 40.9% [22]. However, this prevalence is differentiated according to the geographic area [23]. In our study, we estimated a prevalence of 16.3%, which is related to that reported by Navarro-Vidal *et al*. [24], with percentages of 15.83% in women with normal cytology and slightly lower than that reported in the region of Central America and Mexico (20.4%), with a prevalence of 23.7% in southern Mexico [25,26].

Worldwide, the genotypes most frequently reported are 16, 18, 31, 58 and 52; genotypes 16, 18, 31, 33 and 59 have been estimated in Mexico [27]. In this sense, in our population, two genotypes considered high-risk for cancer (33 and 59) were detected in women with normal cytology by Pap, which agrees with other investigations that have reported the presence of HPV serotypes 16, 18 and 33 in healthy female carriers [28]. Interestingly, the genotype detected with the highest prevalence was HPV 11, the most prevalent in men in the Latin American and Caribbean regions [10].

The age range of 36 - 45 and 46 - 55 years (34.8%) had the highest prevalence, without association with HPV infection. Some studies have reported that prevalence increases significantly with age, suggesting that health campaigns aimed at HPV screening could include the age criteria [29].

Several studies have shown a strong association between early sexual input, the number of sexual partners and the lack of contraceptive methods (condoms) with the risk for HPV infection [30,31]. In our study, we only found an association between the number of sexual partners and HPV with an OR 5.2 (1.60-17.09), similar to that reported by Morales-Figueroa *et al*. [32] of 5.9 (2.2-16.1) in northern Mexico. However, it differs from that reported by Lazcano-Ponce *et al*. in the state of Morelos (OR 3.4, 2.0-7.0) [30].

The PCR-RFLP allowed the identification and genotyping of HPV; hence, this technique could be implemented as a primary test for the screening of HPV in women with normal cytology. The combination of primers increases the identification of HPV in 13% of negative samples. In this sense, PCR-RFLP is a sensitive and low-cost molecular method for HPV diagnosis.

Conclusion

In our study, we detected the presence of high-risk genotypes in women with normal cytology, so it is necessary to know the distribution of HPV serotypes in women with intraepithelial lesions but also women without cytology changes detected by PAP. The implementation of public policies that allow a timely diagnosis improves the access to prevention and treatment of cervical cancer, according to the regional HPV serotypes.

Funding

The research was developed with own resources.

Author's Contribution

LIPM designed the study. LIPM, JJDV, ELB, DCC and MOPV designed and performed the experiments. JDV verified and analyzed the data and LIPM and JJDV wrote the manuscript. JJDV and LIPM

edited the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

This study was approved by the National Scientific Research Committee of IMSS (R-2021-785-015). Informed consent was obtained from each participant.

Consent for Publication

All authors consent for publication.

Competing Interest

The authors declare no competing interests.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, et al. (2021) Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 71: 209-249.

2. Bruni L, Serrano B, Roura E, Alemany L, Cowan M, et al. (2022) Cervical cancer screening programmes and age-specific coverage estimates for 202 countries and territories worldwide: a review and synthetic analysis. Lancet Glob Health 10: e1115-e1127.

3. Szymonowicz KA, Chen J (2020) Biological and clinical aspects of HPV-related cancers. Cancer Biol Med 17: 864-878.

4. Hull R, Mbele M, Makhafola T, Hicks C, Wang SM, et al. (2020) Cervical cancer in low and middle income countries (Review). Oncol Lett 20: 2058-2074.

5. Wendkuuni Djigma F, Tiendrebeogo F, Traore L, Mahoukèdè ZT, Tozoula BA., et al. (2022) Cervical Cancer Induced by Human Papillomaviruses in the Context of Africa: Contribution of Genomics. In: Molecular Mechanisms in Cancer. IntechOpen.

6. Jordá GB, Ramos JM, Mosmann J, Lopez ML, Wegert A, et al. Prevalencia del virus papiloma humano y factores de riesgo asociados en mujeres afiliadas al seguro de salud estatal en Posadas, Misiones (Argentina). Revista chilena de infectología 37: 111-116.

7. Smith ER, George SH, Kobetz E, Xu X (2018) New biological research and understanding of Papanicolaou's test. Diagn Cytopathol 46: 507-515.

8. Clarisse OT, Djigma FW, Idani B, Zohoncon TM, Sorgho PA, et al. (2020) Impact of glutathione S-transferase genes polymorphisms on human papillomavirus infection and precancerous lesions in west African women. Int J Genetic and molecular Biol 12: 59-70.

9. Okunade KS (2020) Human papillomavirus and cervical cancer. J Obstet Gynaecol (Lahore) 40: 602-608.

10. Bruni L, Albero G, Rowley J, Alemany L, Arbyn M, et al. (2023) Global and regional estimates of genital human papillomavirus prevalence among men: a systematic review and meta-analysis. Lancet Glob Health 11: e1345-e1362.

11. Chesson HW, Dunne EF, Hariri S, Markowitz LE (2014) The Estimated Lifetime Probability of Acquiring Human Papillomavirus in the United States. Sex Transm Dis 41: 660-664.

12. Kitchen FL, Cox CM (2023) Papanicolaou Smear. In: StatPearls Content Is King. StatPearls.

13. Tsikouras P, Zervoudis S, Manav B, Tomara E, Iatrakis G, et al. (2016) Cervical cancer: screening, diagnosis and staging. J BUON 21: 320-325.

14. Saslow D, Solomon D, Lawson HW, Killackey M, Kulasingam SL, et al. (2012) American Cancer Society, American Society for Colposcopy and Cervical Pathology and American Society for Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer. CA Cancer J Clin 62: 147-172.

15. Yoshikawa H, Kawana T, Kitagawa K, Mizuno M, Yoshikura H, et al. (1991) Detection and Typing of Multiple Genital Human Papillomaviruses by DNA Amplification with Consensus Primers. Jpn J of Cancer Res 82: 524-531.

16. Gravitt PE, Peyton CL, Alessi TQ, Wheeler CM, Coutlée F, et al. (2000) Improved Amplification of Genital Human Papillomaviruses. J Clin Microbiol 38: 357-361.

17. Golfetto L, Alves EV, Martins TR, Sincero TMC, Castro JBS, et al. (2018) PCR-RFLP assay as an option for primary HPV test. Braz J Med Biol Res 51.

18. Aljanabi SM, Martinez I (1997) Universal and rapid salt-extraction of high quality genomic DNA for PCR- based techniques. Nucleic Acids Res 25: 4692-4693.

19. Resnick RM, Cornelissen MT, Wright DK, Eichinger GH, Fox HS, et al. (1990) Detection and Typing of Human Papillomavirus in Archival Cervical Cancer Specimens by DNA Amplification with Consensus Primers. J Natl Cancer Inst 82: 1477-1484.

20. Quintero VM, Cruz GJF, Bastidas M, Márquez L, Puig PJ (2008) Detección y tipificación de virus del papiloma humano (VPH) mediante PCR-RFLP. Rev Obstet Ginecol Venez 68: 25-31.

21. Bernard HU, Chan SY, Manos MM, Ong CK, Delius H, et al. (1994) Identification and Assessment of known and novel human papillomaviruses by polymerase chain reaction amplification, restriction fragment length polymorphisms, nucleotide sequence and phylogenetic algorithms. J Infect Dis 170:1077-1085.

22. Vázquez-Vega S (2021) Prevalencia del virus del papiloma humano oncogénico en pacientes con lesión cervical. Rev Med Inst Mex Seguro Soc 58: 243-249.

23. Orozco-Colín A, Carrillo-García A, Méndez-Tenorio A, Ponce-de-León S, Mohar A, et al. (2010) Geographical variation in human papillomavirus prevalence in Mexican women with normal cytology. Int J Infect Dis 14 :e1082-e1087.

24. Navarro-Vidal E, Hernandez-Rosas F, Rey M, Flores-Peredo L (2018) Prevalence of Human Papillomavirus Genotypes in Women from Cozumel, Mexico. Asian Pac J Cancer Prev 19: 2417-2422.

25. de Sanjosé S, Diaz M, Castellsagué X, Clifford G, Bruni L, et al. (2007) Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. Lancet Infect Dis 7: 453-459.

26. Alarcón-Romero LDC, Organista-Nava J, Gómez-Gómez Y, Ortiz-Ortiz J, Hernández-Soleto D, et al. (2022) Prevalence and Distribution of Human Papillomavirus Genotypes (1997–2019) and Their Association With Cervical Cancer and Precursor Lesions in Women From Southern Mexico. Cancer Control 29:10732748221103331.

27. López Rivera MG, Flores MO, Villalba Magdaleno JD, Sánchez Monroy V (2012) Prevalence of Human Papillomavirus in Women from Mexico City. Infect Dis Obstet Gynecol 1-4.

28. Campos RG, Malacara Rosas A, Gutiérrez Santillán E, Delgado Gutiérrez M, Torres Ororzco RE, et al. (2019) Unusual prevalence of high-risk genotypes of human papillomavirus in a group of women with neoplastic lesions and cervical cancer from Central Mexico. PLoS One 14 :e0215222.

29. Shen Y, Xia J, Li H, Xu Y, Xu S (2021) Human papillomavirus infection rate, distribution characteristics, risk of age in pre- and postmenopausal women. BMC Womens Health 21: 80.

30. Lazcano-Ponce E, Herrero R, Muñoz N, Cruz A, Shah KV, et al. (2001) Epidemiology of HPV infection among Mexican women with normal cervical cytology. Int J Cancer 91: 412-420.

31. Yang D, Zhang J, Cui X, Ma J, Wang C, et al. (2022) Risk Factors Associated With Human Papillomavirus Infection, Cervical Cancer and Precancerous Lesions in Large-Scale Population Screening. Front Microbiol 13: 914516.

32. Morales-Figueroa GG, Bravo-Parra M, Olivas-Matas KM, Esparza-Romero J, Valenzuela-Zamorano M, et al. (2022) Associated factors with human papillomavirus infection in adult women from northwest Mexico. Biotecnia 25: 133-139.

HERALD

Advances In Industrial Biotechnology | ISSN: 2639-5665

Advances In Microbiology Research | ISSN: 2689-694X

Archives Of Surgery And Surgical Education | ISSN: 2689-3126

Archives Of Urology

Archives Of Zoological Studies | ISSN: 2640-7779

Current Trends Medical And Biological Engineering

International Journal Of Case Reports And Therapeutic Studies | ISSN: 2689-310X

Journal Of Addiction & Addictive Disorders | ISSN: 2578-7276

Journal Of Agronomy & Agricultural Science | ISSN: 2689-8292

Journal Of AIDS Clinical Research & STDs | ISSN: 2572-7370

Journal Of Alcoholism Drug Abuse & Substance Dependence | ISSN: 2572-9594

Journal Of Allergy Disorders & Therapy | ISSN: 2470-749X

Journal Of Alternative Complementary & Integrative Medicine | ISSN: 2470-7562

Journal Of Alzheimers & Neurodegenerative Diseases | ISSN: 2572-9608

Journal Of Anesthesia & Clinical Care | ISSN: 2378-8879

Journal Of Angiology & Vascular Surgery | ISSN: 2572-7397

Journal Of Animal Research & Veterinary Science | ISSN: 2639-3751

Journal Of Aquaculture & Fisheries | ISSN: 2576-5523

Journal Of Atmospheric & Earth Sciences | ISSN: 2689-8780

Journal Of Biotech Research & Biochemistry

Journal Of Brain & Neuroscience Research

Journal Of Cancer Biology & Treatment | ISSN: 2470-7546

Journal Of Cardiology Study & Research | ISSN: 2640-768X

Journal Of Cell Biology & Cell Metabolism | ISSN: 2381-1943

Journal Of Clinical Dermatology & Therapy | ISSN: 2378-8771

Journal Of Clinical Immunology & Immunotherapy | ISSN: 2378-8844

Journal Of Clinical Studies & Medical Case Reports | ISSN: 2378-8801

Journal Of Community Medicine & Public Health Care | ISSN: 2381-1978

Journal Of Cytology & Tissue Biology | ISSN: 2378-9107

Journal Of Dairy Research & Technology | ISSN: 2688-9315

Journal Of Dentistry Oral Health & Cosmesis | ISSN: 2473-6783

Journal Of Diabetes & Metabolic Disorders | ISSN: 2381-201X

Journal Of Emergency Medicine Trauma & Surgical Care | ISSN: 2378-8798

Journal Of Environmental Science Current Research | ISSN: 2643-5020

Journal Of Food Science & Nutrition | ISSN: 2470-1076

Journal Of Forensic Legal & Investigative Sciences | ISSN: 2473-733X

Journal Of Gastroenterology & Hepatology Research | ISSN: 2574-2566

Journal Of Genetics & Genomic Sciences | ISSN: 2574-2485

Journal Of Gerontology & Geriatric Medicine | ISSN: 2381-8662

Journal Of Hematology Blood Transfusion & Disorders | ISSN: 2572-2999

Journal Of Hospice & Palliative Medical Care

Journal Of Human Endocrinology | ISSN: 2572-9640

Journal Of Infectious & Non Infectious Diseases | ISSN: 2381-8654

Journal Of Internal Medicine & Primary Healthcare | ISSN: 2574-2493

Journal Of Light & Laser Current Trends

Journal Of Medicine Study & Research | ISSN: 2639-5657

Journal Of Modern Chemical Sciences

Journal Of Nanotechnology Nanomedicine & Nanobiotechnology | ISSN: 2381-2044

Journal Of Neonatology & Clinical Pediatrics | ISSN: 2378-878X

Journal Of Nephrology & Renal Therapy | ISSN: 2473-7313

Journal Of Non Invasive Vascular Investigation | ISSN: 2572-7400

Journal Of Nuclear Medicine Radiology & Radiation Therapy | ISSN: 2572-7419

Journal Of Obesity & Weight Loss | ISSN: 2473-7372

Journal Of Ophthalmology & Clinical Research | ISSN: 2378-8887

Journal Of Orthopedic Research & Physiotherapy | ISSN: 2381-2052

Journal Of Otolaryngology Head & Neck Surgery | ISSN: 2573-010X

Journal Of Pathology Clinical & Medical Research

Journal Of Pharmacology Pharmaceutics & Pharmacovigilance | ISSN: 2639-5649

Journal Of Physical Medicine Rehabilitation & Disabilities | ISSN: 2381-8670

Journal Of Plant Science Current Research | ISSN: 2639-3743

Journal Of Practical & Professional Nursing | ISSN: 2639-5681

Journal Of Protein Research & Bioinformatics

Journal Of Psychiatry Depression & Anxiety | ISSN: 2573-0150

Journal Of Pulmonary Medicine & Respiratory Research | ISSN: 2573-0177

Journal Of Reproductive Medicine Gynaecology & Obstetrics | ISSN: 2574-2574

Journal Of Stem Cells Research Development & Therapy | ISSN: 2381-2060

Journal Of Surgery Current Trends & Innovations | ISSN: 2578-7284

Journal Of Toxicology Current Research | ISSN: 2639-3735

Journal Of Translational Science And Research

Journal Of Vaccines Research & Vaccination | ISSN: 2573-0193

Journal Of Virology & Antivirals

Sports Medicine And Injury Care Journal | ISSN: 2689-8829

Trends In Anatomy & Physiology | ISSN: 2640-7752