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Review

***Solanum paniculatum* Linn: A potential antimicrobial agent against oral microorganisms**

Maria Regina Macêdo-Costa^{1*}, Pedro Henrique Sette-de-Souza², Shenia Eliane do Rego Carneiro³, Julia Moraes Fernandes³, Silvana Maria Zucolotto Langassner³, Maria do Socorro Vieira Pereira⁴ and Kenio Costa Lima¹

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In Brazil, several species of potentially medicinal native plants exist, including *Solanum paniculatum*. This species is commonly known as “*jurubeba*” and belongs to the *Solanaceae* family, which is found in several regions in Brazil. It is widely used as a remedy for bronchitis, coughs, arthritis, jaundice, hepatitis, fever and stomach problems. The plant is believed to possess anti-viral, anti-cancer, anti-inflammatory, antioxidant, diuretic, hepatoprotective and antimicrobial properties. The chemical constitution of the species contains flavonoids, amides, steroids, lignans, steroidal saponins and steroidal alkaloids. This species is listed in the Brazilian Pharmacopoeia and the “National Report on Medicinal Plants of Interest to the Single Health System (Renisus), due to its potential for use in products of interest to the Brazilian Ministry of Health. Therefore, the value of the present review manuscript lies in its aim to discuss the antimicrobial, antiadherent, bactericidal, fungicidal and anti-inflammatory action of *S. paniculatum* Linn (*jurubeba*). Furthermore, it is extremely important to characterize their chemical profile and cytotoxicity, thereby favoring the safe application of natural bioactive substances. A phytotherapeutic agent causes less damage to the body, is inexpensive and is more accessible to the general public. The present review is important to provide the concrete possibility of using phytotherapeutic and medicinal plants as a therapeutic resource in Basic Healthcare Units operated by the *Sistema Único de Saúde* (SUS), particularly those represented by the Family Health Strategy.

Key words: *Solanum paniculatum*, microbiology, phytotherapy, toxicity, chromatography.

INTRODUCTION

Over the centuries, humanity has benefited from the natural medicine to treat or prevent a wide range of

diseases. Secondary metabolites of plants and microbial and marine products have been considered a valuable

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source of novel molecules with potential for drug development (Newman, 1860). Several conditions affecting oral health can be prevented, controlled and/or treated with the use of natural resources - drugs or formulations. There is a long list-based and derived from natural products drugs, mouthwashes, toothpastes, among others, that are available for consumption or are administered as prescription (Cragg, 2014). However, the reasons for seeking new treatment modalities did not cease: microbial resistance, short and long-term toxicity, adverse and side effects and high costs. Thus, it seems accepted that there is a continuing need to obtain more potent, effective formulations for oral hygiene and low cost, with microbiota being safe and well tolerated (Rosalen, 2016). Accordingly, the use of plants in the prevention and treatment of oral infectious diseases, and as antibiofilm agent continues to be valued in many parts of the world, it is recommended by World Health Organization (WHO) (1987) and in our country. Many studies have been developed to assess the popular use of plants in dentistry, making it possible to identify plant species with potential biological activity (Ocheng et al, 2014; Mogosanu et al, 2015).

In Brazil, there are several potentially medicinal plants such as *Solanum paniculatum* Linn. This species is listed in the Brazilian Pharmacopoeia (Brasil, 1959) and belongs to the "National List of Medicinal Plants of Interest to the Unified Health System (Renisus)" (Brasil, 2009) to present potential to generate products of interest to the Ministry of Health. *S. paniculatum* Linn it is a species, commonly known as Jurubeba whose main pharmacogens are leaves, roots and fruits that are widely used in traditional medicine as a tonic, antipyretic in the treatment of gastric disorders, bronchitis, anemia, arthritis, jaundice and hepatitis. Jurubeba root is considered the most active part of the plant (Mesia-Vela et al., 2002; Botion et al., 2005). As regards, the scientific aspects are ascertained antibacterial, antifungal, antiviral, molluscicide, anticancer, anti-inflammatory, anti-oxidant, diuretic, antidiarrheal, hepatoprotective, gastroprotective and antiulcer.

However, *S. paniculatum* L. can determine signs of toxicity, diarrhea, nausea, vomiting, gastritis and erosive duodenitis, elevated liver enzymes and possibly neurological symptoms (Mesia-Vela et al., 2002; Oliveira et al, 2006; Carvalho et al., 2007; Valadares et al, 2009; Lôbo et al., 2010; Vieira et al., 2013; Silva et al., 2013; Macêdo-Costa et al., 2014; Vieira-Júnior et al., 2015; Gregoris et al., 2013; Stehmann et al., 2015; Martins et al., 2015; Macêdo-Costa, 2016; Clementino-Neto et al., 2016). Due to the biological potential of *S. paniculatum* L., the aim of this study was to review the literature on the antimicrobial activity of this plant.

GENERAL OVERVIEW OF *S. paniculatum* Linn

The genus *Solanum* is considered one of the largest and

complex among angiosperms, having 1500 species (Hameed; Hussain, 2015). Native to Brazil, belongs to the *Solanaceae* family, common in several states, extends from the limits of the Guianas to São Paulo and Minas Gerais. It is also commonly found in Paraguay, Bolivia and Argentina. This plant is already component of several pharmaceutical formulations including syrups, infusions and decoctions, extracts, tinctures and elixirs. Infused flowers are indicated for bronchitis and cough, while the macerated roots are recommended for arthritis and fruit for anemia. The decoction of leaves is used to treat intestinal parasites, but is also suitable for stomach disorders (Mesia-Vela et al., 2002; Botion et al., 2005).

There is a plant protection in Brazil, which has in its formulation fluid extract of leaves of *S. paniculatum*, the Lerobina® (Belfar Laboratory, Belo Horizonte, Brazil). The product, marketed for decades, also has extracts *Remijia ferruginea*, Jacaranda carob, and exotic *Erythraea centarium*. With the exception of the latter, these plants occur in Brazil and are popularly employed for treating different diseases, among which is dyspepsia (280 mg/kg/day). However, more studies are needed to identify the compounds responsible for antidiarrheal action Lerobina® (Botion et al., 2005; Tagliati et al., 2008).

There are other products on the market that have *S. paniculatum* in its composition, such as Jurubeba Composed Elixir® (Infabra Ind. Farm. Bras. Ltda, Rio de Janeiro, Brazil) and the Watchtower Jurubeba® (Farmabraz, Rio de Janeiro, Brazil). Both have jurubeba and boldo extracts in their composition, and are indicated as choleric / bile duct, in dyspepsia, flatulence and gastrointestinal discomfort. Classic drinks like spirits Jurubeba Lion Norte® (North Ltda Lion, Bahia, Brazil), Jurubeba Nordestina® (Pernambuco, Brazil) and Coleguinha Jurubeba® (Colonial, Ceará, Brazil) represent a combination of macerated fruit jurubeba, extracts alcoholics herbs, decoctions of bitter plants, cane sugar syrup and ethanol. Users highlight the medicinal properties of the plant and its qualities liver, digestive, tonic and aphrodisiac.

Justifying the numerous medicinal properties, some of the chemical constituents of jurubeba used are the alkaloids (jurubebina, jubebina, isojurupidina); the solaninas (solamina, solanidina, solasodine); resins (jupebina and jupebenina); saponins (isojurubidina, isopaniculidina, and isojurupidina jurubidina); steroidal nitrogenous compounds (paniculina, jurubina); aglycone; fatty acids; organic acids; glycosides (paniculoninas A and B), and mucilages, and bitter principles (Ripperger 1966; Ripperger et al., 1967; Blankemeyer et al., 1998; Mesia-Vela et al., 2002). Phytochemical analysis performed with the ethanol extract of root jurubeba also indicate the existence of flobabênicos tannins (condensed or catechin tannins), flavonols, flavanones, free pentacyclic triterpenoid and saponins (Cordeiro, 2008). In various phytochemical studies of the genus

Solanum species, many alkaloids have been isolated as described earlier, as well as a large variety of steroids, saponins, glycoalkaloids and flavonoids which are important in the natural defense of plants and have various biological activities (Silva et al., 2005; Cheng et al., 2008; Li et al., 2014; Mannanase et al., 2012; Miranda et al., 2013; Pinto et al., 2013; Zhang et al., 2013). A study by Lôbo (2009) detected an average tannin 4.6% (46g TC/kg of DM) of *S. paniculatum* root, using the Stiasny method (Guangcheng et al., 1991), adapted by Paes et al. (2006).

By fractionation of the ethanol extracts (70%) of aerial parts (leaves and branches) of *S. paniculatum*, Vieira Junior et al. (2014) isolated new saponins, (22R, 23S, 25R) 3b, 6a, 23-trihydroxy-5a -spirostane 6-OB-D-xylopyranosyl-O- [bD-quinovopyranosyl - O- [al-rhamnopyranosyl - OBD-quinovopyranoside (1) and diosgenin 3-OB-D-glucopyranosyl-ObD-glucopyranoside (2) as well as four known components: caffeic acid (3), diosgenin bD-glucopyranoside (4), rutin (5) and quercetin 3-OaL -rhamnopyranosil-ObD-glucopyranoside (6).

Macedo-Costa et al. (2014) and Macedo-Costa (2016) analyzed the extract from the root of *S. paniculatum* by preliminary phytochemical screening and fingerprints Thin-Layer chromatography (TLC) with different developers in order to identify the classes of secondary metabolites. The pharmacological results confirm previous studies, and revealed the presence of phenols (among which strokes pyrogallol tannins and flavonoids), gums, lactones and alkaloids (in the presence of reactive Dragendorff and Bertrand), and saponins. Qualitative analysis of phenols in relation to flavonoids and tannins, met suggestive spot isovitexin and tannic acid, respectively.

ANTIMICROBIAL POTENTIAL

In view of the potential antimicrobial activity of these compounds was evaluated *in vitro* action on planktonic *S. paniculatum* oral microorganisms and organized in biofilms. Macedo-Costa et al. (2014) evaluated the antibacterial action of *S. paniculatum* root extract on endogenous oral bacteria in planktonic form: *Streptococcus mitis*, *Streptococcus mutans*, *Streptococcus sanguinis*, *Streptococcus oralis*, *Streptococcus salivarius* and *Lactobacillus casei*. The extract showed minimum inhibitory concentration (MIC) of 7.81 mg / mL, minimum inhibitory concentration of adherence (MICA) of 62.5 mg / mL and bactericidal in the concentration of 500 mg / ml in 2 h of contact with *S. mutans* and MIC for 4 h. This study also evaluated the action of crude extract and diluted MIC of *S.paniculatum* on microorganisms in mixed culture in planktonic form and organized in biofilms, from human saliva. It was observed that the crude extract of jurubeba presented antimicrobial activity on the microorganisms in mixed

culture in planktonic form and organized in biofilms, however, considering its MIC did not present such action.

Macedo-Costa (2016) also evaluated the antimicrobial activity of *S. paniculatum* on superinfecting microorganisms of the oral environment: *Enterococcus faecalis* and *Candida albicans* ATCC (American Type Culture Collection) and clinical isolates. *S. paniculatum* presented bacteriostatic and fungistatic action, and there was a statistically significant difference between the extract and the positive control (chlorhexidine digluconate 0.12% and Nystatin 100,000 IU) to dilutions/concentrations: 1: 64/7.81 mg/mL (*E. faecalis* ATCC), 1:32/15.65 mg/mL (*E. faecalis* isolated from the oral environment - AB) 1:128/3.90 mg/mL (*C. albicans* AB) and 1:64/3.90 mg/mL (*C. albicans* ATCC). Non-stick action (1:512/0.97 mg/mL) was seen to be higher than controls and bactericidal.

Lôbo et al. (2010) and Pereira et al. (2010) found the antibacterial action of roots of *S. paniculatum* of *S. aureus* (ATCC, and bovine), *Escherichia coli* and *Pseudomonas aeruginosa*. Valadares et al. (2009) evaluated *in vitro* antiviral activity of leaf extract of *S. paniculatum* Herpes virus type I (HSV-1), murine encephalomyocarditis virus (EMCV), and vaccinia virus. *S. paniculatum* inhibited the replication of HSV-1 (50% effective concentration antiviral = 298.0 ± 11.2) g/mL and showed no effect on the EMCV and VACV.

Different doses (31.25 to 500 mg/kg) of ethanolic extract of leaves of *S. paniculatum* were evaluated against induced gastric ulcer in rats. The lowest dose of the extract was able to promote anti-ulcer effect which was 125 mg/kg. Treatment with *S. paniculatum* orally was able to decrease the area of gastric lesion, and also reduce levels of myeloperoxidase (MPO) in the gastric mucosa (Vieira-Júnior et al., 2014). Endringer et al. (2010) found Brazilian plants with anti-inflammatory activity, among which *S. paniculatum* are promising sources of chemopreventive agents of cancer. However, studies are needed to identify the active principles that are related to this pharmacological action.

With respect to toxicity, Vieira et al. (2010) evaluated the cytotoxic and mutagenic activities of ethanolic extracts of leaves and fruits of *S. paniculatum*, using the micronucleus test in bone marrow of mice. The results indicated that the ethanol extracts, both the leaves as the fruits of *S. paniculatum* showed no mutagenic action in bone marrow of mice, but at higher doses, both extracts showed cytotoxic activity. However, this study did not show an increase in cytotoxicity dose-response of 200 and 300 mg/kg.

The acute toxicity of root *S. paniculatum* in mice by determining the lethal dose (LD50) and the cytotoxic potential of human erythrocytes were assessed according to the study of Macedo-Costa et al. (2014). The extract did not cause mortality in any of the tested concentrations (500 mg / ml-0.97 mg / ml) after 24, 72 h and 15 days. As the major behavioral changes observed in mice treated

with the extract at times 30, 60, 90 and 240 min, and piloerection were observed only intense movements vibrissae until the first 60 min until the concentration of 31.25 mg / ml (dilution 1:16).

There were no serious side effects, and the animals showed only minor behavioral changes suggestive of CNS stimulation. In cytotoxicity assessment, it was observed in this study that *S. paniculatum* induced a low hemolytic activity (less than 50%) compared to 158 of human erythrocytes types A, B, O and AB at a concentration of 7.81 mg/mL (MIC), however showed cytotoxicity at a concentration of 250 mg/mL (dilution 1:2).

CONCLUSION

Several studies have shown that *S. paniculatum* Linn presents bacteriostatic activity, fungistatic, nonstick, bactericidal, fungicidal and antiviral *in vitro* on microbial suspension monoculture, mixed culture planktonic and biofilm on multispecies justified by pharmacological findings. Virtually no deleterious effects in preclinical toxicological, enabling the completion of a randomized controlled trial and stimulating research of bioactive natural substances for the treatment of oral infections associated with endogenous and superinfecting microorganisms was shown. Clearly, there is great potential for the use of therapeutically relevant compounds from nature. These should be the result of interdisciplinary collaboration based on empirical knowledge from the "healers", "bushmen", "mourners", herbalists, healers, shamans and others; combined with ethnopharmacology, botany, chemistry of natural compounds, microbiology and pharmacology to minimize the gap between *in vitro* and *in vivo*, providing clinical efficacy and safety in humans. Until then some progress has been achieved, but there is still a long way to overcome.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Genotyping of high-risk human papilloma virus (HR-HPV) and its role in cervical cancer among suspected women at reproductive age

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One of the important causes of uterine and cervical malignancy and premalignancy lesions in human is the infection with papilloma virus, especially genotypes that belong to high-risk human papilloma virus (HR-HPV) group. The involvement of HPV DNA testing in an adjacent to cytological screening will be of positive impact in early discovery of cervical neoplasia among women at risk and will significantly minimize the mortality rate. The study subjects were screened for cervical neoplasia which may be caused by different genotypes of HR-HPV among Saudi females. Two hundred and thirty eight (n=238) cervical scrapings were collected from women who attended Maternity and Children Hospital (MCH) at Al-Madinah Al Munawarah between August 2015 to January 2017, and thereafter processed and examined cytologically using liquid based cytology (LBC). Simultaneously, one-step Polymerase chain reaction (PCR) was performed to examine the presence of HR-HPV different genotypes. Low incidence of precancerous epithelial lesions was observed among enrolled patients 13 (5.5%) with the following distribution: high-grade squamous intraepithelial lesion in 5 cases (38.5%), low-grade squamous intraepithelial lesion in 4 (30.8%) and four cases (30.8%) as a typical squamous cells of undetermined significance. Nevertheless, HR-HPV was detected only in 6 (2.5%) cases and overall prevalence of HR-HPV in abnormal Pap smears was 15.4% (2/13). On the other hand, HPV were also seen in 1.9% (4/238) among smears that were registered free from any type of malignancy (NILM). The study concluded very low prevalence of HR-HPV in routine cervical screening samples among suspected Saudi women. Additionally, weak correlation between HPV and the incidence of cervical neoplasia was also observed. This may direct the clinicians and researchers to look for other suspected HPV genotypes in this regards.

Key words: Cervical cancer, high-risk human papilloma virus (HR-HPV), low-risk human papilloma virus (HR-HPV), Saudi women, Al-Madinah Al Munawarah, Saudi Arabia.

INTRODUCTION

Cervical cancer remains a major public health concern, affecting communities with low socio economic status

(Sankaranarayan et al., 2009). It is a type of fatal neoplasia with mortality that exceeds 275,000 annually;

the majority of which are in the third world countries (WHO/ICO, 2011). One of the major causes of his type is the high risk human papilloma virus, especially types 16 and 18 (Lowy and Schiller, 2012).

Whilst Saudi Arabia possess a population of more than nine millions females within the reproductive age (>15 years old), the risk of having cervical cancer will be more likely. Recent studies announced that the mortality rate was 84 women among 241 confirmed cases of -cervical malignancy. This type of cancer is accounted to be the 8th most frequent cancer among Saudi females within ages of 15 to 44 years old. Concerning burden of HPV in KSA, there is no available data. However, in other parts of Western Asia, different studies estimated 2.3% prevalence of HPV among suspected females, with most of the infections attributed to 16 or 18 genotypes (Information Centre on HPV and Cancer - ICO, 2010).

Scientific studies over the past few decades revealed definitive evidence that cervical cancer is a sexually transmitted disease caused by a certain type of high-risk, oncogenic HPV infection (Parkin, 2002; McLaughlin-Drubin and Munger, 2008). The prevalence of HPV in cervical neoplasia was suggested to be within the range of 85 to 99% globally (Walboomers et al., 1999; de Sanjose, 2010). In recent studies, several factors were proved to be correlated with HPV persistence; the most important are HIV/AIDS, cervicovaginal dysbiosis, contraceptives and smoking (Mitra et al., 2016).

For instance, there are two genotypes of genital HPV, low oncogenic risk including genotypes 6 and 11, while genotypes 16 and 18 are of high oncogenic risk belonging to the second genotype. Thus, developing to precancerous lesions by these viruses depend mainly on the infecting genotype. At the present time, genotypes 18 and 16 are carrying the responsibility for about 70% of all global infections (Koshiol et al., 2000; Stanley, 2009). Nevertheless, progression to cervical neoplasia caused by LR genotypes only (without the involvement of HR genotypes) was not detected among different forms of the diseases (Ostor, 1993). Moreover, the prevalence of HPV associated cervical cancer could be better assessed by self sampling. Screening of human papilloma virus has shown a recent increase when self-sampling- which means the participants do not need to attend the clinics for sampling- was applied, (Enerly et al, 2016; Sultana et al, 2016). It showed higher detection rates for \geq CIN2 than routine cytology-based screening, and similar detection rates as HPV and cytology co-testing, revealing its importance (Lam et al., 2018).

For Saudi Arabia, we received limited published data on the prevalence of HPV infection (Al-Badawi et al., 2011; Alsbeih et al., 2011). The involvement of cytological screening with the aid of molecular techniques

will greatly help in the detection of suspected cases. Therefore, rapid detection and identification will greatly reduce the mortality and morbidity among risky groups. Moreover, identification of HPV genotypes in KSA may help in the development of vaccination for HPV types.

PATIENTS AND METHODS

Study type, duration and clinical specimens

Two hundred and thirty-eight women (n=238) attended Maternity and Children Hospital (MCH) in Al-Madinah Al Munawarah with different ages (15-80 years) and gynecological symptoms (menorrhagia, urine incontinence, vaginitis and other vaginal diseases) during the period from August 2015 to January 2017 were included in this study after being given their informed consent. They were investigated for cytological appearance and the presence of HPV as well. Vaginal fluid was collected by using modified cyto-brush and rinsed into a labeled vial containing 15 ml of PreservCyt® transport medium, then transported to the laboratory for investigation. The samples were then processed using (Beckton, Dickson; PrepStain Slide Processor/TriPath Imaging Inc, 2005). The remaining specimens were investigated for high risk HPV DNA.

DNA extraction, PCR amplification and gel electrophoresis

DNA was extracted by DNA extraction kit according to the manufactures' instructions (Sacace Biotechnologies- Italy). Purity of the harvested DNA was adopted by NanoDrop (Thermo SCIENTIFIC, US).

In the amplification protocol, three sets of PCR premix-1 were used to check for the presence or absence of 12 possible genotypes (PCR mix-1 "16-35", PCR mix-1 "18-59", and PCR mix-1 "52-66"). Polymerase chain reaction (PCR) was adopted in 25 μ l according to (Sacace Biotechnologies), in a PCR tube containing 5 μ l of PCR-mix-1, 10 μ l of 2.5 PCR buffer, 0.5 TaqF polymerase and 10 μ l of template DNA were mixed. Also, control tubes were prepared to be included in the reaction. Amplification of the target sequences was conducted using PCR machine (SYNGENE, UK). PCR protocol was carried out according to Sambrook et al. (2001). Finally, the target amplicons were monitored under UV light on 3% agarose gel, with reference to Table 1 to compare the products sizes.

RESULTS

Two hundred and thirty-eight (n=238) cervical smears were examined cytologically and assessed for the presence of HPV. The ages of the study population were from 15 to 80 years, with a mean of (39.7 \pm 1.1 years); additionally, age groups 31-40 and 41-50 were found to be the most frequent in the study (Table 2).

The cytological investigation revealed that the incidence of abnormal epithelial cells is low constituting 13 (5.5%) cases which are distributed as follows; ASC-US in 4 (1.7%) cases, LSIL in 4 (1.7%), HSIL in 5 (2.1%)

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Table 1. Different lengths of specific amplified DNA fragments of suspected HPV.

Mix-1 (52 – 66)		Mix-1 (18 – 59)		Mix-1 (16 – 35)	
HPV 66	304 bp	HPV 59	395 bp	HPV 35	280 bp
HPV 65	325 bp	HPV 45	475 bp	HPV 33	227 bp
HPV 58	240 bp	HPV 39	340 bp	HPV 31	520 bp
HPV 52	360 bp	HPV 18	425 bp	HPV 16	325 bp

Table 2. Cytological results and age groups among enrolled subjects.

Age group	Cytological +HPV results										Total
	Normal	Normal +HPV	Negative for intraepithelial lesion or malignancy (NILM)				Epithelial cells abnormalities				
			Inflammation	Inflammation +HPV	Hormonal effect	Candida/Trichomonas	ASC-US	LSIL	HSIL	HSIL+ HPV	
Less than 20	2	0	0	0	2	0	0	0	0	0	4
30-20	26	1	15	1	10	2	2	0	0	1	54
31-40	34	0	28	1	6	2	0	1	0	0	70
41-50	30	0	33	0	6	3	1	2	1	1	75
51-60	15	0	5	1	0	0	1	1	1	0	24
More than 60	6	0	4	0	0	0	0	0	1	0	11
Total	105	1	85	3	24	7	4	4	3	2	238

ASC-US=Atypical squamous cells of undetermined significance; LSIL=low-grade squamous intraepithelial lesion; NILM=Negative for intraepithelial lesion or malignancy; HSIL= high-grade squamous intraepithelial lesion.

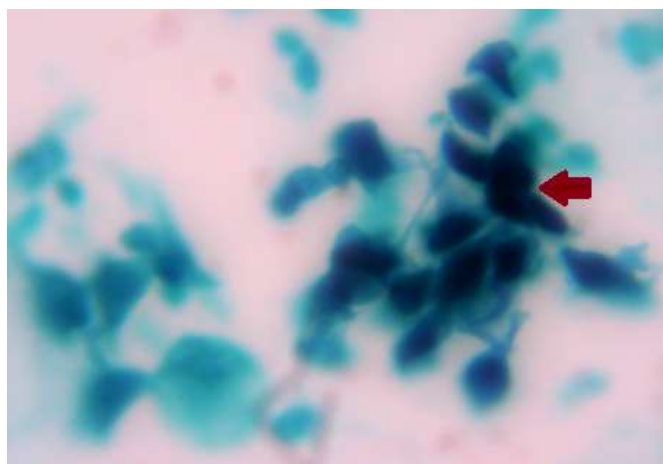


Figure 1. Cervical smear of 48 years old patient with HSIL, (HPV genotype 16), syncytial cluster of hyperchromatic cells with increased nuclear/cytoplasmic ratio and irregular nuclear membrane (liquid-based preparation, Thin Prep, X40).

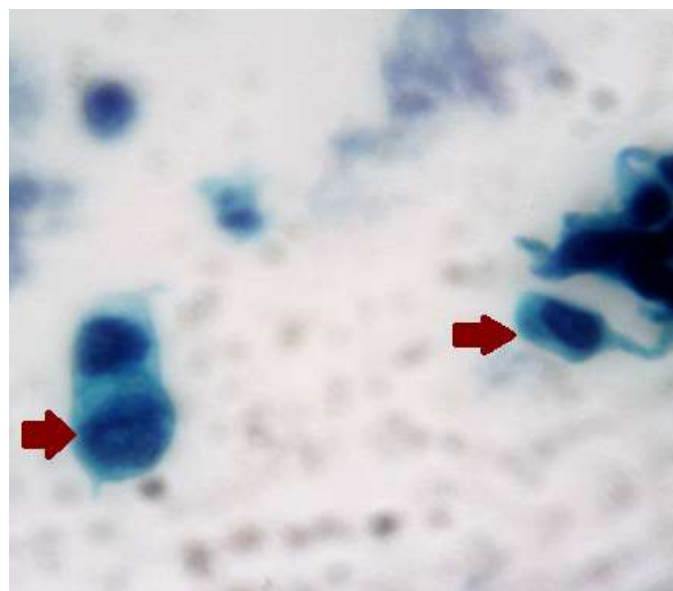


Figure 2. Cervical smear of 30 years old patients with HSIL, (HPV genotype 16), enlarged cells with increased nuclear/cytoplasmic ratio with granular chromatin and slightly irregular nuclear membrane (liquid-based preparation, Thin Prep, X40).

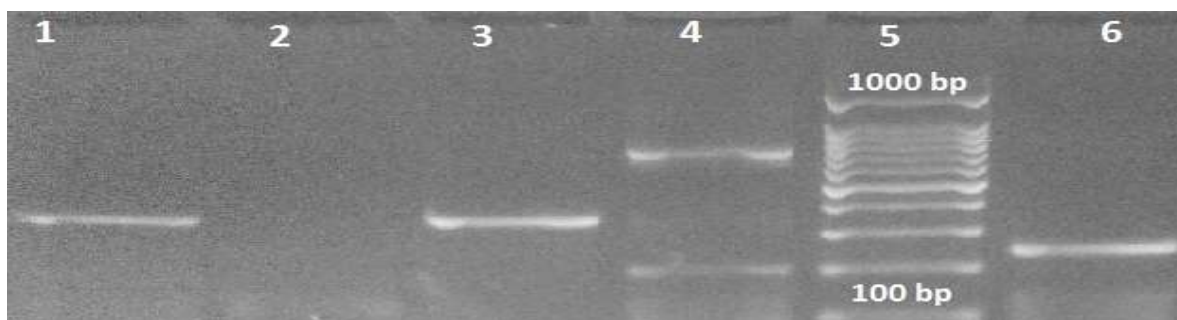
cases and the majority were in the age group (41-50) and (51-60). Among the enrolled subjects, 225/238, (94.5%) were reported free from any type of cervical cancer cells, among which 88/238 (37%) were cells with inflammations, 13/238 (5.5%) cells with Estrogen effect, 11/238 (4.6%) cells with Progesterone effect, 7/238 *vaginalis* and the remaining 106 (44.5%) were normal cells (Table 2 and Figures 1 and 2).

Detection of HPV

HPV was detected in only six cases (2.5%), with two of them present in patients with epithelial change (2/13

Table 3. Distribution of HPV genotypes among enrolled subjects.

HPV genotype	Cytological diagnosis			Total
	Normal	Inflammation	HSIL	
HPV 16	0	0	2	2
HPV 33	0	1	0	1
HPV 52	0	2	0	2
HPV 58	1	0	0	1
Total	1	3	0	6

**Figure 3.** 3% agarose gel electrophoresis of PCR amplicons of HPV from cervical smears. Lanes: L= 100 bp ladder; C1= positive control for HPV33 (325 bp); C2= positive control for HPV 16 (227bp); N= negative control; 1, 5 negative samples; 2,4 = HPV33 positive samples; 3= HPV33 positive sample.**Figure 4.** 3% agarose gel electrophoresis of PCR amplicons of HPV from cervical smears. Lanes: 5= 100 bp ladder; 1= positive control for HPV 52 (360bp); 2=negative control; 3 = HPV52 positive sample; 4= internal control (800-200bp); 6= HPV58 positive sample (240 bp).

hand, the remaining four genotypes were detected within cells that appeared free from any types of neoplasia as follows; HPV genotype 52 was identified in two patients with inflammatory condition and genotype 33 was present in one case with inflammation. However, HPV-58 was confirmed in women with normal smears (Figures 3 and 4 and Tables 2 and 3).

DISCUSSION

In this study, the incidence of abnormal epithelial cells is low, constituting 6.3% (12/190) of screened cases. This result is not far from that of Altaf (2008), who studied

cervical neoplasia among Saudi women in the period from 1990 to 2004. Here, the author found that the incidence of abnormal pap smears was 4.7% of study group (5132 patients) and also reported the classification of abnormal pap smears in the study as follows; atypical squamous cell (2.4%), atypical glandular cells (1.1%), low scale squamous intraepithelial (0.6%) and high scale squamous intraepithelial (0.4%). Different malignant groups were observed which include: adenocarcinoma of the cervix, neuroendocrine carcinoma and squamous cell carcinoma.

This study showed that the most of precancerous lesions were distributed in the age group (41-50) followed by (51-60) years. This result totally disagree with Sawaya

et al. (2000) who studied the percentage of cervical changes in a three years longitudinal study, their results indicated that the target cervical abnormalities were found to be predominant among women under 30 years old followed by age group of 30-49 and 50-64 years old respectively, while women over 65 years old showed the lowest frequency. Nevertheless, Saudi Cancer Report (SCR, 2011) showed evidences that cervical neoplasia among Saudi females were mostly found among age group of 15-44 years old. Our findings also suggested that HPV-16 was detected among women of the age group 21-30 and 41-50 while HPV-33 was isolated at age group 51-60 years old. These results are not far from that of Bruni et al. (2010), who recorded cervical cancer among Saudi females to be most likely concentrated among the age group (41–45 years) followed by the age group (56–60 years) as has been observed in other communities.

One of the major concerns of this project is the estimation of the prevalence of HPV among suspected women. Different studies estimated that the prevalence of HPV among suspected females may exceed 99% worldwide (Walboomers et al., 1999; de Sanjose et al., 2010), whereas in KSA and other similar regions the ratio between HPV and cervical cancer is scanty (Hussain et al., 2012; Khorasanizadeh et al., 2012; Hammouda et al., 2011; Alsbeih et al., 2011; Al-Badawi et al., 2011).

On the other hand, many researchers noted that the situation of cervical neoplasia among Saudi women is comparable and the participation of HPV in this phenomenon is also within the normal range worldwide (Ghazi, 2014). The majority of these studies suggested that the most frequent genotypes were HPV 16 and 18 respectively (Alsbeih et al., 2011; Al-Badawi et al., 2011). Furthermore, multiple infections caused by more than one genotype were recorded among Saudi females (Ghazi et al., 2013; de Sanjose et al., 2010; Walboomers et al., 1999). One important finding is the presence of epithelial cell changes among 11 (84.6%) cases with the absence of any HPV genotypes, a result that was previously suggested by many researchers worldwide. Farnsworth (2011) observed significant prevalence (8%) of squamous carcinoma among Australian females identified negative for HPV. Similarly, Poljak et al. (2009) with the agreement of Tjalma et al. (2013) proved the occurrence of 12.6% of cervical cancer cases without any evidence of the existence of HPV as causal organism. This may provide evidences to adjust and standardize all the available diagnostic kits to be more reliable for a wide spectrum check of HPV genotypes (Sin et al., 2014). One more additional point, is the presence of HPV among 4 (30.8%) cases with normal cytological smears, which may be an inquiry to search for the any suspected participation of HPV in the initiation of future abnormalities among Saudi females, especially if we consider the appearance of these non-classical genotypes; 33 and 52, among cells with inflations. Similar

findings which prove the role of these new genotypes were reported in this regard in the Middle East communities (Darnel et al., 2010) and elsewhere (Bruni et al., 2010; Sawaya et al., 2000).

Based on the previously mentioned studies, this research finding totally agrees with the fact that the occurrence of cervical neoplasia among Saudi female is scanty compared to different part of the world. On the other hand, this study proved the existence of new genotypes beside HPV 16 (HPV 33 (33.3%), HPV 52 (16.7) and HPV 58 (16.7%)) which were not previously observed in this region. However, Darnel et al., 2010, suggested that HPV 33 could be the most dominant genotype responsible for this condition in the Middle East followed by genotypes 18 and 16 respectively (Darnel et al., 2010). These findings prove that there are genotypes other than 16 and 18 in charge of causing cervical cancer among Saudi women, a point that should be considered by researchers and specialists in the medical field, putting into consideration the impressive scanty distribution of HPV in this region.

Conclusion

Our results suggested decreasing prevalence of precancerous epithelial lesions in routine cervical screening samples (5.4%), with scanty occurrence of HR-HPV compared with findings elsewhere. Thus, future research may concentrate on other HR-HPV and/or LR-HPV genotypes, which are currently not proved for this region. However, molecular tools provide an accurate and reliable tool for early detection in the population at risk and are recommended for routine screening.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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The background of the entire page is a microscopic view of green bacteria, likely Bacillus subtilis, showing various stages of growth and spore formation. The bacteria are illuminated from the side, creating a strong contrast and highlighting their cellular structures.

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