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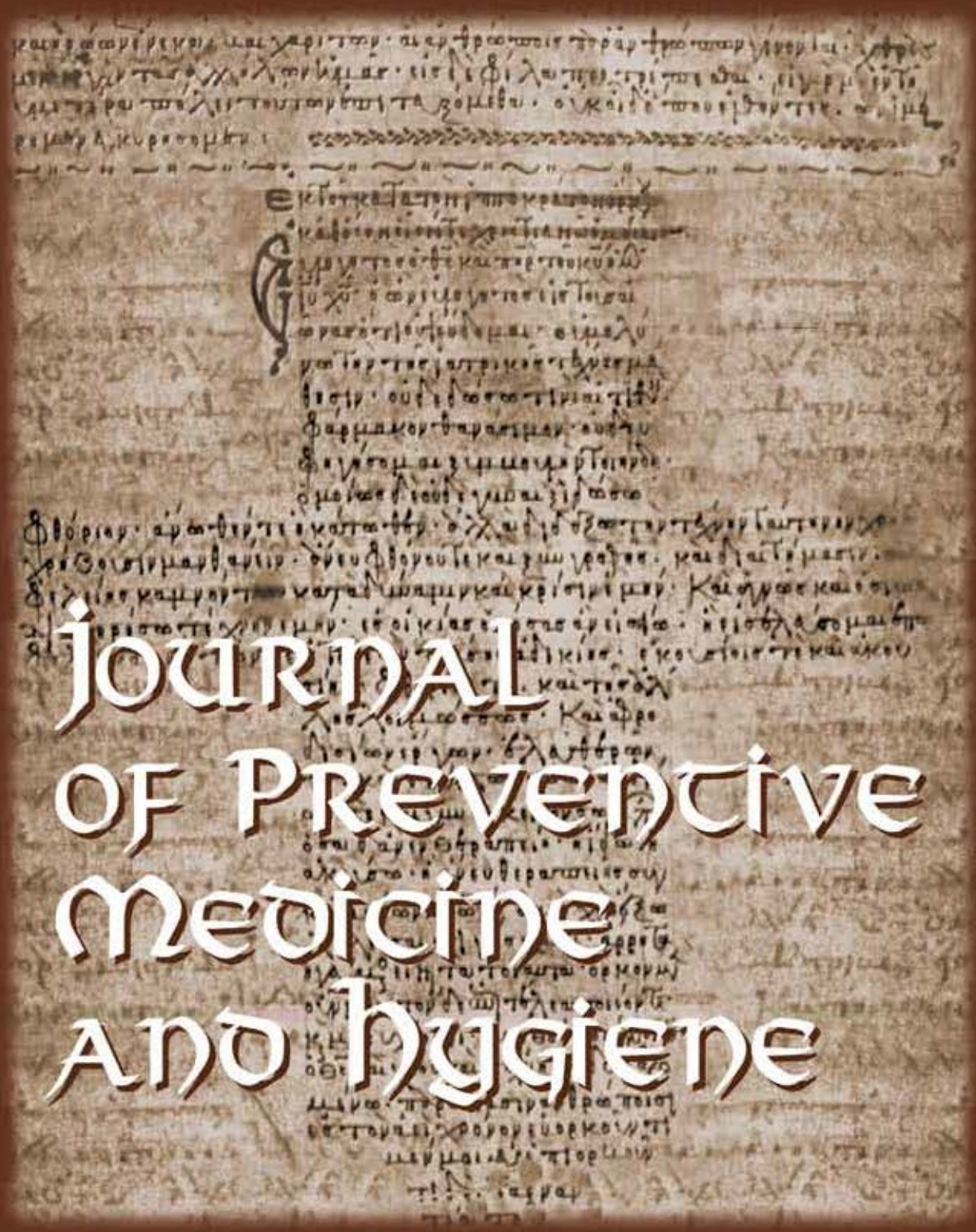

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REVIEW

HPV related diseases in males: a heavy vaccine-preventable burden

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Key words

HPV infections in males • HPV quadrivalent vaccine • HPV related cancers

Summary

Human Papillomavirus (HPV) has a significant impact in male's health, as cause of clinical manifestations ranging from genital warts to several cancers of the anogenital and aero-digestive tract. HPV types which most frequently affect men are 6,11,16 and 18, included in the HPV quadrivalent vaccine, recently approved for use in males by Food and Drug Administration (FDA) and European Medicines Agency (EMA). Although several data about the safety and efficacy of quadrivalent vaccine are available, the implementation of proper immunization plans dedicate to male's population cannot ignore the knowledge of the characteristics of the disease in men, which in some aspects should be clarify, in particular clearance of type-specific HPV infections and transmission dynamics. Purpose of this review is to summarise the main information about the burden and the natural history of the HPV related disease in males.

Introduction

The human papillomavirus also known as HPV is a ubiquitous virus causative agent of proliferative lesions of the skin and mucous membranes and represents one of the most frequent sexually transmitted infection in both men and women [1].
The demonstration of a causal link between some high-risk HPV sub-types and cervical cancer has focused the attention of scientific community on HPV infections in female leading to the development of highly effective prophylactic HPV vaccines [2-4].
The earlier studies on HPV in males were conducted on Man having sex with Man (MSM) and HIV+ to determine cancer risk associated to HPV infections [5, 6], subsequently the interest has shifted on HPV infections in heterosexual males, especially to clarify their role in the transmission of infection to woman [7, 8].
Several studies demonstrated the significant impact of HPV in males as cause of important disease ranging from genital warts to several cancers of anogenital and aerodigestive tracts [9].
The International Agency for Research on Cancer (IARC) in 2009 revised the carcinogenic role of HPV in human pathology reporting an association with penile and anal cancers and with cancers of oral cavity, oropharynx, larynx and esophagus [10].
Based on data derived from several studies of vaccine efficacy conducted on males, in 2009 Food and Drug Administration (FDA) licensed the use of the HPV quadrivalent vaccine for the prevention of genital warts in males aged 9-26 years and in 2010 the use of quadrivalent vaccine was also extended for the prevention of anal cancer and associated precancerous lesions for males and females aged 9-26 years [11, 12].
The use of quadrivalent vaccine against HPV has been extended for the prevention of genital warts in males 9 to 26 years also by the European Commission, which endorsed the opinion of the Committee for Medicine Products for Human Use (CHMP) European Medicines Agency (EMA) [13].
Despite the availability of the HPV vaccine for use in males, only few countries in the world have developed vaccine programs dedicated to this susceptible population, due to the lack of knowledge of some aspects of the natural history of the disease in men, in particular, the duration and the clearance of type-specific HPV infections together with transmission dynamics.
The explanation of such data is essentials for the implementation of extended screening programs and for the definition of appropriate vaccination plans directed to male's population.
The aim of this review is to summarize the latest knowledge about HPV epidemiology in males, which can provide a starting point for the implementation of cost effective preventive strategies.

Human papillomavirus

HPV, currently classified within papillomavirus family, is a naked icosahedral virus with a diameter varying between 45 and 55 nm containing a double-stranded circular DNA genome of 8000 bp [14].

HPV genome includes 8 "early genes" E1-E8, which encodes for proteins facilitating viral replication and stimulating cell proliferations, 2 " late genes" L1 and L2, encoding for structural proteins and an upstream regulatory region (URR) or long control region (LCR) including sequences for the control of transcription and the origin site for DNA replication.
Based on DNA sequence homology of the E6, E7 and L1 open reading frames (ORFs) more than 100 HPV types were identified, of which approximately 40 types are known to infect the genital epithelia.
Genital HPV types depending on their oncogenic propensity were classified at "low" risk, which usually give rise to a productive infection in permissive cells remaining episomial, and at "high/intermediate" risk, which in non-permissive cells integrates into genome inducing transformation.
A regulatory pathway involving E2, E6 and E7 genes has been shown to be at the origin of carcinogenesis.
In particular, the integration of HPV carcinogenetic type into cellular genome, lead to the inactivation of E2 viral protein which usually inhibits E6 and E7 genes expression [15-17].
E6 and E7 early genes play a key role in carcinogenesis acting as oncogenes by binding and deactivating p53 and p150rb tumor suppressor proteins respectively [18].
A set of about 40 types were frequently associated with sexually transmitted infections and with pathogenesis of the ano-genital tract cancer, among them HPV types 6, 11, 40, 42, 43, 44, 53, 54, 61, 68, 70, 72, 81, frequently associated to genital warts and low-grade squamous intraepithelial cervix lesions, are classified as low-risk, conversely, HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73 and 82 are referred to as high-risk and are related with malignant lesions of cervix, vulva, penis and anus [19].

Natural history of HPV infection in males

Little is known about HPV infection in men especially in terms of duration and transmission dynamics although such information are essential for the adoption of appropriate preventive interventions and are the subject of several investigations.
The first studies about HPV disease in males focused on small groups of men, especially homosexuals and HIV positive who were not representative of the general population. Therefore, data derived from such works have been of little use to the understanding of the natural history of the disease in male population.
Recently, the focus of research has shifted to heterosexual men, being heterosexual transmission the major route of spread of infection and the males seems to exert a key role acting as an asymptomatic reservoir of HPV [20].
Data derived from a cross-sectional study of HPV infection conducted in 463 men from 2003 to 2006 reported an overall prevalence of HPV infection of 65.4% with penile shaft, gland penis/coronal sulcus and scrotum sites presenting the highest positivity for HPV DNA among asymptomatic heterosexual men [21].
Results from a cohort study of HPV infections conducted on 240 heterosexually active male university student ages 18-20 years in United States showed a prevalence of any genital HPV type of 25.8% and a cumulative incidence of new infection of any genital HPV type of 62.4% (95% confidence interval [CI], 52.6%-72.2%). Cumulative incidence of high-risk HPV types was 47.9% (95% [CI], 38.6%–58.0%) [22].
The large continuous prospective HPV in Men study (HIM) whose aim was to study natural history of HPV disease in males aged 18-70 years in three countries (Brazil, Mexico and United States) reported an incidence of new genital HPV infection of 38,4/1000 months person for any HPV type. Concerning the incidence of HPV oncogenic type was estimated to be 22.2 / 1000 (95% [CI], 19.8%-24.9%) month's person, with types 16,51,52 and 59 showing the highest incidences.
The median time to clearance of HPV infection was 7.52 months for any HPV type, with the longest clearance observed in males aged 18-30 years, and 12.19 months for HPV oncogenic type 16, whose clearance was not age dependent. Moreover, clearance of oncogenic HPV types in males appeared inversely related to the number of lifetime female partners and has shown to be more rapid with increasing age. In this study also emerged a decreased clearance of oncogenic HPV types infection in men in Brazil and Mexico compared with the United States [23].
A recent study highlighted that prevalence of HPV in men varies according to different geographical areas and by race with the lowest prevalence among Asian/Pacific islanders (42.2%) compared to Blacks (66.2%) and Whites (71.5%). Further studies will be necessary to clarify the low prevalence of HPV in Asian populations, it has been suggested that sexual behavior and race-specific differences such as variations in the genes involved in immunomodulation can explain these data [24].
Giuliano et al. examined data about the incidence and clearance of HPV infection emerged from two studies conducted on women of 18-35 years and males 18-44 years, respectively. The comparison revealed similar period prevalences for any HPV infection (52.8% in men and 53.8% in women), and similar HPV incidence rates (29.4 per 1000 person-months in both men and women). Moreover males also showed a similar probability of acquiring infection with oncogenic and non-oncogenic types against a significantly higher probability of acquiring oncogenic types in females. The clearance of the infection was similar in males for all HPV types, while in females the clearance of oncogenic types was slowe [25].
About the transmission dynamics several studies of heterosexual partners have been performed to understand the role that partners may have in the spread of HPV infection.
Burchell et al. demonstrated a 64% concordance for at least one HPV type among partners of recently formed heterosexual couples [26].

The same authors in a more recent study have examined 179 newly formed heterosexual couples experiencing a transmission male-to-female rate of 3.5 per 100 person-months (95% [CI], 2.7-4.5) versus a female-to-male rate of 4.0 per 100 person-months (95% [CI], 3.0-5.5). Moreover, no change in the transmission rate across HPV genotypes and no changes in the rate in dependence on the lifetime number of partners reported by the initially uninfected partner were observed [27].
Nytray et al in examining 88 heterosexual couples demonstrated a 59% type-specific positive concordance and that the latter was associated with an increasing difference in partners' lifetime number of sex partners and inversely related to the increasing difference in age [28].
Several studies conducted on females partners of males with HPV lesions highlighted in these an increased risk of developing cervical carcinoma on the contrary, studies conducted on male partners of women suffering of cervical intraepithelial neoplasia (CIN I) showed HPV lesions detectable only in 50% of the cases, according to the hypothesis of a lesser receptivity to or a more effective clearance of HPV infection in men than in women [29, 30].
Martin-Ezquerra et al reported an overall 41% of HPV diagnosed infections among male partners of women with CIN II and III [31].
Recent evidence suggested that HPV infection in males, also with high-risk serotypes, is not always associated with the development of cervical lesions in female's sexual partners [30].
Centers for Disease Control and Prevention (CDCs) recommend the screening of male's sexual partners of women suffering from HPV infection in order to highlight and possibly treat any exophytic lesion [32] even if the treatment does not appear related to a reduction in the risks of development of precancerous cervical lesions.
Although sexual contact represents the major route of transmission, HPV can be transferred on genital organs indirectly due to its ubiquity and environmental resistance. In particular, skin contact would be the main route of indirect transmission (90% of cases); also transfer of infection by inanimate objects was demonstrated between sexual partners [33].

Clinical manifestation associated with HPV infections in males

HPV replicates in differentiated squamous epithelial cells of the skin and mucous membranes causing proliferative lesions ranging from asymptomatic infections to malignant lesions.
The healthy carrier state is the more common conditions for males so that several studies have focused on their role in the transmission of oncogenic HPV types to women [20].
The most frequent HPV clinical manifestations in males are condylomata acuminata (genital warts), wart-like lesions of oropharyngeal tract and less commonly cancers of the penis, anus and oral cavity cancer.

Genital warts

Genital warts, one of the most frequently diagnosed sexually transmitted infections, are benign genital lesions with symptoms such as local pain and bleeding without serious consequence, but frequently associated with psychosocial distress and significant medical costs [34-36].
HPV lesions are very infectious with a transmission rate of > 60% within sexual partnerships from an infected to a susceptible sexual partner. Most of genital warts develop 2-3 months after infection, with an incubation period ranging from 2-8 months [37].
Several studies reported a spontaneous regression rate up to 40% even if in some cases genital warts may recur [38-40].
The most common genotypes detected from condylomata acuminata are non-oncogenic 6 and 11 HPV types with a frequency of 70–100% of exophytic genital wart tissue containing one of these types, while one-third of lesions have multiple HPV types including co-infection with oncogenic types [41, 42].
It has been estimated that about 1% of sexually active men in the United States have genital warts at any one time with an incidence peak of 5.01 per 1000 person-years in the age group 25-29 years. [34, 43]
In Europe, data from two recent studies reported an incidence of genital warts in males ranging from 147.95 per 100,000 men-years in Germany (95% [CI], 144.48 to 151.48) to 168 per 100,000 men years in United Kingdom [44, 45].
Based on these studies Hartwig et al. estimated an expected number of new genital warts cases ranging from 335.301 to 380.961 [46].
Recent data about the trend in genital warts infections in UK based on Genitourinary Medicine (GUM) clinics attendances during the period 2006-2010 show a marked increase of diagnosed cases from 148.5 per 100,000 to 160.5 per 100,000 [47].
A study conducted to determine the incidence of genital warts before and after the introduction in female population of the quadrivalent human papillomavirus vaccine in Sweden, reported 1137 cases/100,000 among males aged > 24 years during 2006-2007; a further increase in incidence of genital warts among males together with a decrease in females was observed between 2008 and 2010 (Tab. I) [48].
The Italian Sentinel Surveillance of sexually transmitted infections (STIs) reported that between 1991 and 2007, the genital warts were the most common diagnosed sexually transmitted diseases with 73% of cases in males and a prevalence peak in the age group 14-25 years [49].
It has been assessed that in Italy, 89% of genital warts were diagnosed from heterosexual patients while 10,1% were from homosexual/bisexual. Among males, an association between number of sex partners (> 2) and concomitant STDs was demonstrated in 50% and 17.1% of genital warts cases, respectively. Moreover 6.9% of males presenting genital warts were also HIV+ [49].

Tab. I. Studies reporting incidence of HPV-related non-malignant diseases in different populations.

Country, year, Author	Disease	Population	Design	Incidence (95%CI)
United States, 2000 Insinga, 2003 [34]	Genital Warts	Privately insured populations	Retrospective (medical records)	5.01/ 1000 man-years
Germany, 2006 Kraut 2010 [44]	Genital Warts	General population (10-79 years)	Retrospective cohort	147.95/100,000 man-years (144.48-151.48)
United Kingdom, 2009 Desai, 2011 [45]	Genital Warts	General practice + GUM clinic attendees	Retrospective cohort	166/100,000 man-years
United Kingdom, 2006-2011 HPA, 2012 [47]	Genital Warts	GUM clinic attendees	Survey	148.5/100,000 man-years (2006) 160.5/100,000 man-years (2011)
Sweden, 2006-2007 Leval, 2012 [48]	Genital Warts	General populations (10-44 years)	Retrospective (medical records)	1137/100,000 man-years
United States, 1993-1994 Derkay, 1995 [51]	Recurrent Respiratory Papillomatosis	Children and Adults both sexes	Survey	4.3/100,000 children-years 1.8/100,000 adults-years
Denmark, 1965-1984 Denmark, 1974-1999 Hartwig, 2012 [46]	Recurrent Respiratory Papillomatosis	Children and Adults both sexes	Review	0,35-0,38/100,000 person-years

Recurrent respiratory papillomatosis

Recurrent Respiratory Papillomatosis (RRP) is a rare and highly morbid pathological condition caused by HPV types 6 and 11 and characterized by the recurrent appearance of warts like lesions in the respiratory tract, particularly at the larynx and vocal cords [50].

The disease is particularly severe among children who require frequent surgeries to remove recurrent obstructive lesions.

RRP is most commonly diagnosed among children without distinction between genders while it is rarely diagnosed in adults in which affects more males between ages 20 and 30 years [51].

Data from a national survey of practicing otorynolaryngologists in the United States in 1993-1994 provided an incidence of 4.3 per 100,000 in children and of 1.8 per 100,000 in adults [51].

Data derived from two European studies reported an RRP incidence of 0.35-0.38 per 100,000 person-years with a sex ratio for juvenile onset of 1:1; a different distribution of the disease were observed in adults, with a higher frequency in males (Tab. I) [46].

About transmission dynamics several studies demonstrated a vertical HPV transmission from mother to child during birth, while RRP cases among adults may develop as the results of oro-genital sexually transmitted HPV infections [52].

Anal cancer

The incidence of anal cancer is increasing worldwide in men and women with 99.000 new cases estimated in 2002, 40% of cases in men and 60% in women [53].

HPV infections are well known as the main cause of squamous cell carcinoma of the anus (anal SCC) and has been identified in more than 80% of anal canal cancer cases with HPV 16 and 18 oncogenic types responsible of 87% and 9% of intraepithelial lesions, respectively. HPV DNA detection in anal intraepithelial neoplasia (AIN) correlates with their cytological and histological severity: 75% in AIN 1 (mild), 86% in AIN 2 (moderate), and 94% in AIN 3 (severe) [54].

In Europe anal cancer cases among men generally first occurs in the age group 30-35 years, with an estimated value of 1821 new yearly-diagnosed cases due to HPV of which 1699 were attributable to HPV 16 and 18 types (Tab. II) [46].

A twofold increase in the anal cancer incidence was reported in United States after the advent of the HIV period during which was reached an estimated value of 35 per 100 000 among MSM (Tab. II) [5, 20].

Relative risk of developing anal cancer was highest among HIV infected man and among man who were HIV infected through homosexual contact with reported values of 37.9% (95% CI = 33.0-43.4) and 59.5% (95% CI = 51.5-68.4) respectively; consequently the majority of studies on prevalence of anal HPV infection has focused on these susceptible populations [55].

In a study about the incidence and the risk factors of anal cancer between 1992 and 2004 based on the French Hospital Database on HIV, Piketty et al reported that the majority of cases occurred among males (94%) of which 75% was homosexual. Moreover the incidence of anal cancers was higher in the years 1999-2004 (40/100,000; 95% [CI], 32 to 47) after the introduction of the highly effective antiretroviral Highly Active Antiretroviral Therapy (HAART) therapy [56].

Data derived from a study conducted in United States on 4.506 HIV infected males showed a continuous increase

in cancer rates reaching a value of 128/100.000 (95% [CI], 16 to 1042.2) in 2006-2008. In particular, cancer risk was higher among the patients who had acquired infection by more than 15 years and HAART therapy length did not seem to confer protection against anal cancer (Tab. II) [57].
This observation was confirmed in a recent work conducted to evaluate the trends in the incidence of anal cancers between HIV infected patients receiving long-term combined antiretroviral treatment (cART) between 1992 and 2008. In this study, standardized incidence ratios (SIRs) of anal cancer in HIV-infected patients proved to be high among MSM 109.8 (95% [CI], 84.6 to 140.3) and others HIV infected males 49.2 (95% [CI], 33.2 to 70.3) and no differences were observed between pre and post cART periods, consequently combined antiretroviral treatment appears to have no preventive effect on anal cancer [58].
Although little is known about immunomodulation mechanisms versus HPV infections, it is reasonable that immunosuppressive state could favor HPV infection at the early stage of transformation of precancerous lesions while the subsequent progression of the persistent lesions to invasive cancer could be induced by genetic mutations accumulated over time. The increased life expectancy of HIV infected patients favored by combined antiretroviral treatment may extend time to progression of lesions to cancer.
Specimens from anal cancers in MSM are almost always HPV DNA positive; suggesting that epithelial site of infection and frequency of exposure are factors that may affect the risk of infection [59].
It was postulated that the increased risk of developing anal cancer in MSM could be attributed to the presence of a HPV, susceptible transformation zone similar to that of the cervix [60].
Recent studies focused on HPV infections in traditionally non-high-risk groups, i.e. heterosexual men and HIV negative subjects, reported that anal and perianal HPV infections are very common.
Nyitray et al in a cross-sectional study conducted in United States to assess the prevalence of and risk factors for anal HPV infection in asymptomatic heterosexual men stated an overall prevalence of anal HPV infection of 24.8% in 222 men among which 33.3% had an oncogenic high-risk HPV type [61].
Data derived from the recent HIM study conducted on men residing in Brazil, Mexico, and the United States reported that the prevalence of anal canal HPV was 12,2% and 47,2% in both HIV negative heterosexual and homosexual men respectively [62].
The lifetime number of sexual partners is the main risk factor associated with anal HPV infections as emerged in both the cited studies.
Other reported risk factors for anal cancer common in men and women are history of genital warts, anal intercourse, and cigarette smoking [63].
Of particular interest are the findings from a study about the persistence and clearance of anal infection with HPV oncogenic types among men, showing a 6 month anal persistence of HPV 16 in 5.1% of MSM while no persistence was observed in heterosexual men [64].
The more rapid clearance of infection may partially explain the lower incidence of anal cancer than that of cervical, whereas the persistence of lesions could lead to neoplastic transformation and to the development of invasive cancer.

Penile cancer

Penile cancer is a rare disease accounts for less than 0,5% of cancer cases among males worldwide with an estimated incidence of less than 1/100.000 in developed countries [65].
The age-adjusted incidence rates for penile cancer in the United States during 2004-2008 was about 1.3 per 100.000 among Hispanic men compared to 0.7 per 100.000 in non-Hispanic men; [66] these data confirm the trend observed in Latin American countries (Brazil, Peru, Colombia), where penile cancer incidence is higher and amounts to 1.5-3.7 per 100,000 inhabitants. Incidence of penile cancer is highest in developing countries with a rate of 2,8/100.000 in Uganda and of 1,7/100.000 in Thailand and India (Tab. II) [10].
On the contrary, incidence is particularly low in the Jewish population that commonly practice neonatal circumcision with an estimated rate of 0,04/100.000 [10,66].
In Europe penile cancer tends to increase by age, reaching a peak in the seventh decade of life. Each year were diagnosed 3178 penile cancer cases (95% [CI], 2,623 to 3,751), the half of which, considering an overall HPV prevalence of 46,7%, could be attributed to HPV [46].
Data from the Italian Association of Cancer Registries reported a standardized rate of penile cancer of 1 per 100,000 during the year 2007 (Tab. II) [67].
Detection of HPV DNA in penile cancer has ranged from 40% to 50%, with HPV 16, 18 and 33 identified as the most common types [68].
HPV DNA positivity varies by histological subtype and is higher in basaloid and warty types with an estimated frequency of 80%-100%, while is low (30-60%) in squamous cell carcinoma, which is the most common type of penile cancer accounting for 90% to 95% of cases [69, 70].
Interestingly, incidence of penile cancer remains much lower than that of the cervix; a possible explanation could be the lower susceptibility of the penile tissue to HPV oncogenic types [59].
Among the risk factors associated with penile cancer may be cited: history of anogenital warts, number of multiple female sexual partners, lack of neonatal circumcision, phimosis, early age of first intercourse, cigarette smoking [71, 72].

Head and neck cancers

HPV related head and neck cancers include squamous cell carcinoma of the oral cavity, oropharynx, hypopharynx, and larynx.

It was estimated that in 2002 405.000 new cases of head and neck cancers occurred worldwide, with a high frequency in South and Central Asia [73].
The expected annual HPV associated oropharyngeal cancer cases based on United States cancer registry data amounted to 9.356 among males [74].
The estimated number of new HPV related head and neck cancers cases among men in Europe accounted to 14,098 (95% [CI], 11,455 to 17,077), including cancers of the oral cavity, oropharyngeal cancers and laryngeal cancers [46].
The Italian Association of Cancer Registries reported an age-adjusted incidence rates in males of 2,5 per 100,000, 1,3 per 100,000 and 2,6 per 100,000 for oropharyngeal, hypo-pharyngeal and oral cancers from all causes respectively, in 2007 (Tab. II) [67].
Although the main risk factors for head and neck squamous cell carcinomas (HNSCCs) are represented by

Tab. II. Studies reporting incidence, prevalence and expected number of cases of HPV related cancers in different populations.

Country, year, Author	Cancer type	Population	Incidence (95%CI)	Prevalence of HPV by cancer site (%) (95%CI)	Expected number of new cases, irrespective of HPV status (bounds)	Expected number of new cases attributable to HPV (bounds)
World WHO, 2012 [53]	Anal	Worldwide populations (both sexes)	ND	ND	99,000 /year	ND
Germany Varnai, 2006 [54]	Anal	Samples from 87 patients with diagnosed AIN (both sexes)	ND	80.9	ND	ND
Europe Hartwig, 2012 [46]	Anal	European Populations (males)	ND	84.2 (81.5-86.9)	ND	1,821/year (1,403-2,277)
United States Chin-Hong, 2004 [5]	Anal	MSM	35/100,000 man-years	ND	ND	ND
France Piketty, 2008 [56]	Anal	HIV infected patients (both sexes)	40/100,000 person-years (32-47)	ND	ND	ND
United States Crum-Cianflone, 2010 [57]	Anal	HIV infected patients (both sexes)	128/100.000 man-years (16-1042.2)	ND	ND	ND
United States CDC, 2012 [66]	Penile	General populations	0.7-1.3/100.000	ND	ND	ND
Latin American countries IARC, 2007 [10]	Penile	General populations	1.5-3.7/100,000	ND	ND	ND
Uganda IARC, 2007 [10]	Penile	General populations	2.8/100,000	ND	ND	ND
Europe Hartwig, 2012 [46]	Penile	General populations	ND	46.7	3178/year (2,623-3,751)	1484/year (1,102-1,925)
Italy AIRTUM, 2007 [67]	Penile	General populations	1/100,000	ND	ND	ND
World Parkin, 2002 [73]	Head and Neck	General populations (both sexes)	ND	ND	405,000/year	ND
United States CDC, 2012 [74]	Head and Neck	General populations males	ND	ND	9,356/year	ND
Europe Hartwig, 2012 [46]	Oral cavity, oropharynx larynx	General populations males	ND	ND	ND	14,098/year (11,455-17,077)
Italy AIRTUM, 2007 [67]	oropharynx hypo-pharynx oral cavity	General populations males	2.5-1.3-2.6/100,000 man-years	ND	ND	ND
World Kreimer, 2005 [77]	Head and Neck	5,046 HNSCC cancer specimens (both sexes)	ND	25,9 (24.7-27.2)	ND	ND

tobacco smoking and alcohol consumption the role of HPV is important with up to 60% of biopsies positive for HPV DNA [75, 76].
Data from a systematic review reported an overall HPV DNA prevalence of 25,9% (95% [CI], 24.7-27.2) among HNSCCs biopsies collected worldwide, with a prevalence peak of 35,6% (95% [CI], 32.6-38.7) in oropharyngeal cancer. HPV 16 was identified in the larger majority of HPV-positive oropharyngeal SCCs (86.7%, 95% [CI], 82.6-90.1) and remains the predominant type (Tab. II). [77]
D'Souza G. et al. in a case-control study demonstrated a significant association between oropharyngeal cancer and oral HPV type 16 infections (odds ratio 14.6%; 95% [CI], 6.3 to 36.6) both revealing a relationship between oral HPV infection and oral intercourse [78].

HPV and male fertility

Some studies have shown how HPV DNA is present in sperm cells both in infected and healthy individuals and although no direct relationship between HPV infection and male fertility has been demonstrated, preliminary data indicate that the presence of HPV can affect sperm function.
In particular it has been shown that HPV can bind to sperm cells surface reducing their motility revealing a possible cause of infertility [79].
A study by Foresta et al conducted to evaluate a possible association between HPV sperm infection and reduction of sperm cell function in infertile patients showed a significantly higher percentage of HPV infection on sperm of infertile patients [80].
Recent evidence on animal models suggested that HPV infected spermatozoa are able to penetrate the oocyte transferring the viral DNA within it acting as vectors for HPV transfer.
HPV genome within the oocyte is activated and transcribed and may interfere with embryo development reducing its ability to survive [81].
A prospective study performed on 199 infertile couples undergoing assisted reproductive technologies (ARTs) showed a highly statistically significant correlation between pregnancy loss rate (66,7%) and positive HPV DNA testing in the male partner. In the light of these findings HPV could be a causal factor both of infertility in man and of ARTs failure [82].

Vaccination

Currently, two HPV vaccines are available: a bivalent vaccine (Cervarix) targeted the oncogenic HPV16 and HPV18 types, recommended for the prevention of cervical cancer in women aged 10-25 years, and a quadrivalent vaccine (Gardasil) directed against the HPV types 6,11,16 and 18, recommended for the prevention of precancerous lesions of cervix, vulva and vagina and of genital warts in women aged 10-45 years and in men aged 9-15 years [83, 84].
Many data are currently available regarding the efficacy of quadrivalent vaccine, the only HPV vaccine approved for use in men, in the prevention of genital and anal lesions among males population.
Giuliano et al. in a double-blind, randomized, placebo-controlled trial evaluated the efficacy of the quadrivalent vaccine (Gardasil) in young men (4065 subjects) between 16 and 26 years of age. The results at a median follow-up of 2.9 years showed an effectiveness of 84% in preventing external genital lesions caused by all HPV types (warts, penile intraepithelial neoplasia, perianal and perineal neoplasia) caused by all HPV types. Vaccine efficacy was 90% against genital lesions related to the types 6,11,16 and 18 included in the vaccine [85].
Palefsky et al. in a double-blind trial conducted on 602 healthy homosexual men aged 16-26 years have evaluated the efficacy of the quadrivalent vaccine for prevention of anal intraepithelial neoplasia. Data from this study demonstrated a high reduction of anal precancerous lesions in the per protocol population (HPV negative subjects from 1 day to 7 month after the first vaccine administration receiving three doses of vaccine) with an efficacy up to 90% against anal intraepithelial neoplasia (AIN and AIN2+) lesions which are direct precursor of anal cancer [86].
A recent cohort study conducted on 202 MSM patients with a history of previously treated high-grade anal intraepithelial neoplasia (HGAIN) showed a significant HGAIN recurrence reduction among patients vaccinated with HPV quadrivalent vaccine. These data suggest the possible use of the vaccine as an effective post-treatment adjuvant form of therapy in MSM patients with previous diagnosis of HGAIN [87].

Discussion

The HPV infection in males are associated with a significant epidemiologic burden causing disease ranging from non-malignant conditions to malignant disease such as anal, penile and head and neck cancers. HPV related disease in males also have an important economic impact, as demonstrated by a recent study conducted in Italy which shows that the economic burden attributable to HPV 6,11,16 and 18 related disease in males, amount to 38.8 % of the total direct costs of HPV related diseases [88].
Based on epidemiological consideration and on data about HPV vaccine efficacy in male population, FDA and EMA approved the extension of HPV vaccination to boys and men.
Some mathematical models highlighted how the extension of HPV vaccination coverage to males could reflect positively in terms of herd immunity, although suggest that the predicted efficacy of the vaccine strategies depends on the characteristics of the infection in males that remain unclear in some aspects [89].

The herd immunity effects elicited by quadrivalent HPV vaccine between sexual partners has been demonstrated in a study conducted in Australia; in this analysis a decrease of genital wart diagnosed cases among heterosexual men was observed after the introduction of the HPV vaccination program targeting women [90].
Certain economic models concludeed that vaccination of males could be cost effective only in presence of a low vaccination coverage rate among females [91, 92].
These models were supported by the consideration that reaching vaccination coverage up to 50% in women the herd immunity effect could protect heterosexual men, disregarding MSM populations in which HPV infection and associated diseases incidence were significantly higher.
The introduction of the vaccine in MSM could exert a more positive benefit in terms of reduction in the number of anal cancer cases that represent in this population an important cause of health care assistance request with an incidence equivalent to that of cervical cancer in women and even higher in HIV infected MSM.
On the other hand, vaccination of MSM, representing a small portion of the males population could be cost effective but not ethically justified.
Moreover, in many developed countries, it has been observed an upward trend in the burden of HPV-related diseases, especially anal cancer and oropharyngeal squamous cell carcinomas in all men regardless of sexual orientation. In the light of these data, ethical considerations and reasons of social fairness would advise against the choice of protecting males only through herd immunity, effect achievable only after decades.
Nevertheless, the implementation of vaccination programs in males is complicated by the reduced knowledge about several aspects of natural history of the disease with particular regard to the transmission dynamics between sexual partners, concordance data about which are often conflicting. Another point to clarify is the definition of the factors such as immunomodulation pathways and genetic background that could influence the different duration and clearance of the infection in males respect to females. Furthermore an important aspect to consider is the lack of a reference-screening test universally accepted for clinical diagnosis of HPV in males.
Several countries including the United States, Canada and Australia have introduced vaccination programs for boys and men since 2009. Currently, in United States CDC recommends HPV quadrivalent vaccine for all boys aged 11 or 12 years, and for males aged 13 through 21 years, who did not get any or all of the three recommended doses when they were younger. HPV quadrivalent vaccine is also recommended for gay and bisexual men and men with compromised immune systems (including HIV) through age 26.
Given the clinical implications as well as the high economic burden associated to HPV in males, implementation of HPV vaccination strategies dedicated to men requires depth assessment.

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■ **List of abbreviations and acronyms:**
HPV, human papillomavirus
MSM, Man having sex with Man
IARC, International Agency for Research on Cancer
FDA, Food and Drug Administration
CHMP, Committee for Medicine Products for Human Use
EMA, European Medicines Agency
ORFs, open reading frames
HIV, Human immunodeficiency virus
URR, upstream regulatory region
LCR, long control region
HIM, HPV in Men
CIN, cervical intraepithelial neoplasia
CDCs, Centers for Disease Control and Prevention
GUM, Genitourinary Medicine
STIs, sexually transmitted infections
STDs, sexually transmitted diseases
RRP, Recurrent Respiratory Papillomatosis
SCC, squamous cell carcinoma
AIN, anal intraepithelial neoplasia
HAART, Highly Active Antiretroviral Therapy
cART, combined antiretroviral treatment
SIRs, Standardized incidence ratios
HNSCCs, Head and neck squamous cell carcinomas
ARTs, assisted reproductive technologies
HGAIN, high-grade anal intraepithelial neoplasia

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REVIEW

Preventive capacity of allergen immunotherapy on the natural history of allergy

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Key words

Allergen immunotherapy • Preventive capacity • Mechanisms of action

Summary

Allergen immunotherapy (AIT) is the practice of administering gradually increasing doses of the specific causative allergen to reduce the clinical reactivity of allergic subjects. A bulk of literature demonstrates that AIT is an effective and safe treatment to reduce allergic symptoms and the use of drugs. The preventive capacity of AIT is less investigated. The studies thus far available showed that this treatment, in both forms of subcutaneous immunotherapy (SCIT) and sublingual immunotherapy (SLIT) is able to prevent the development of asthma in patients with allergic rhinitis and the occurrence of new sensitizations in patients monosensitized. Such outcomes demonstrate the ability of AIT to change the natural history of respiratory allergy. Of particular importance, SCIT with Hymenoptera venom has an invaluable role in preventing potentially fatal anaphylactic reactions to the culprit sting in venom-allergic patients. Ongoing studies are aimed at evaluating the possible capacity of AIT in primary prevention of allergy. All these capabilities are related to the mechanisms of action of AIT. In fact, both SCIT and SLIT are able to modify the allergen presentation by dendritic cells that in turn modify the phenotype of allergen-specific T cells, switching from the Th2-type response, typical of allergic inflammation, to a Th1-type response. An important role is played by allergen-specific T regulatory (Treg) cells, which produce suppressive cytokines such as IL-10 and TGF-beta.

Introduction

Allergen immunotherapy (AIT) is the practice of administering gradually increasing doses of the specific causative allergen to reduce the clinical reactivity of allergic subjects. As stated in the World Health Organization document dedicated to AIT, concerning the products to perform the treatment "The historical term allergen extract was changed to allergen vaccine to reflect the fact that allergen vaccines are used in medicine as immune modifiers similarly to vaccines for infectious diseases" [1]. AIT was introduced more than one century ago, but remained a merely empirical treatment until 1954, when the first controlled trial was published, and was later continuously developed to reach full scientific evidence demonstrated by a number of meta-analyses [2, 3]. The only method to administer AIT was the subcutaneous injection until the 1980s, when a series of fatal adverse reactions both in Europe [4] and in the USA [5] raised the important issue of treatment safety. The sublingual route, introduced by a trial based on very low doses [6] that now we know to be ineffective, has been subsequently the object of thorough investigation and has currently achieved full scientific evidence of efficacy [7-9].

Therefore, today two forms of AIT are available, subcutaneous immunotherapy (SCIT), which is the only treatment recommended in patients with allergic reactions to Hymenoptera stings, and sublingual immunotherapy (SLIT) which is a valid option to treat patients with allergic rhinitis and allergic asthma. A large literature is accessible evaluating the efficacy and safety of SCIT and SLIT, the aim of this article is to focus the preventive capacity of both treatments.

Prevention of allergic reactions to Hymenoptera stings

Systemic reactions to insects belonging to the order of Hymenoptera, that include honeybees (*Apis spp*), bumblebees (*Bombus spp*), yellow jackets (*Vespula spp*), wasps (*Polistes spp*), and hornets (*Vespa spp and Dolichovespula spp*), affect about 3% of the general population, with particular interest for anaphylaxis, that is associated to a risk of potentially fatal reactions [10]. SCIT with Hymenoptera venom, known as venom immunotherapy (VIT) is highly effective in preventing further systemic reactions [11] and is also very well tolerated, as showed by the absence (differently from SCIT with inhalant allergens) of fatal adverse reactions to VIT [12]. In particular, the capacity to prevent fatal reactions to stings is 100%, and the capacity to prevent any kind of systemic reactions is estimated in 90-95% [13]. Of note, the patients not completely protected from stings can achieve full protection by increasing the maintenance

dose over the recommended amount of 100 mcg, being possible to determine the protective dose in each individual [14]. A recent important advance was represented by the recognition that VIT is effective and safe also in patients suffering from mastocytosis, which is a known risk factor for developing particularly severe reactions to insect stings [15, 16]. All these observations make VIT an invaluable treatment to prevent anaphylaxis from insect stings.

Prevention of asthma in patients with allergic rhinitis

Allergic rhinitis and asthma are closely correlated and patients suffering only from rhinitis have a substantial risk to develop asthma [17]. A pivotal experience in demonstrating the ability of AIT to prevent such progression was the Preventive Allergy Treatment (PAT) study [18]. The PAT study evaluated a group of 183 children, aged 6-14 years, with grass and/or birch pollen allergy after a 3-year course of SCIT, and found that the significant improvement in rhinitis symptoms observed at SCIT stopping persisted at a 5-year follow-up. The AIT treated children had, compared with control subjects treated only with drugs, significantly less asthma after 5 years as evaluated by clinical symptoms. In addition, 147 children were followed-up 7 years after termination of AIT: the significant improvement in rhinoconjunctivitis persisted at the follow-up, and significantly less actively treated subjects had developed asthma at the follow-up as evaluated by clinical symptoms. Patients who developed asthma among controls were 24/53 while in the AIT group were 16/64 [19]. The longitudinal treatment effect when adjusted for bronchial hyperresponsiveness and asthma status at baseline including all observations at 3, 5 and 10 years time points was statistically significant (P = 0.0075). The odds ratio for no-asthma was 4.6 (95% Confidence Interval [CI] 1.5-13.7) in favour of AIT. The authors concluded that AIT has long-term clinical effects and the potential of preventing development of asthma in children with allergic rhinoconjunctivitis up to 7 years after treatment termination.
There are also studies on prevention of asthma by SLIT. Di Rienzo et al. conducted a prospective parallel group study on 60 children (mean age 8.5 years) allergic to dust mites and divided into two matched groups: 35 underwent a 4- to 5-year course of SLIT with standardized extract and 25 received only drug therapy [20]. The patients were evaluated at three time points (baseline, end of SLIT and 4 to 5 years after SLIT discontinuation) regarding presence of asthma and use of anti-asthma drugs. In the SLIT group significant differences were found for the presence of asthma (P </= 0.001) and the use of asthma medications (P </= 0.01), whereas no difference was observed in the control group. Madonini et al showed in a retrospective survey on 302 patients treated with SLIT that, during a 1-year follow-up after stopping the treatment only 1% of non-asthma patients reported an onset of respiratory symptoms. The clinical benefits were associated with the length of treatment: patients with long-lasting benefits were treated for a mean duration of 29.1 months, while patients showing a return to pre-SLIT condition were treated for a mean 13.3 months [21]. In a more recent study, 216 children with allergic rhinitis, with or without intermittent asthma, were evaluated and then randomized to receive drugs alone or drugs plus SLIT for 3 years; 144 children received SLIT and 72 received only drugs. Asthma was less frequent in SLIT patients (odds ratio, 0.04; 95% confidence interval, 0.01-0.17), and the number of children with a positive methacholine challenge result decreased significantly after 3 years only in the SLIT group [22].

Prevention of new sensitizazions

As reported above, the development of new sensitizations following the initial monosensitization is typical of the natural history of respiratory allergy. The issue of preventing new sensitizations by AIT was investigated in various studies. The first investigations concerned small populations of patients. Des Roches et al. studied 22 children monosensitized to dust mites receiving SCIT with standardized allergen extracts and 22 other age-matched controls. Children were followed-up for 3 years, and it was found that 10 of the 22 children monosensitized to dust mites treated with SIT did not have new sensitivities compared with 0 of 22 children in the control group, this difference being significant (p = 0.001) [23]. In another small study, 23 patients allergic to grass pollen – 13 treated with SCIT and 10 controls – were prospectively followed for 6 years during the grass pollen season. At the last time point, 61% of the initially pollen-monosensitized children had developed new sensitizations to perennial allergens compared to 100% in the control group [24]. The difference was significant (p < 0.05), but not so impressive as in the study by Des Roches. Of course, the modest number of patients make likely a stochastic distribution of data in such studies.
A more robust study evaluated 134 children (age range 5-8 years) with respiratory allergy due to monosensitization to mites, 75 treated with SCIT and 63 children treated with medication only, who were considered as controls. SCIT was administered for 3 years and all patients were followed-up for a total of 6 years. New sensitizations were assessed by skin prick test and serum-specific IgE every year during the follow-up. At the end of the study, 69 SCIT treated and 54 controls were available; of them, 52 (75.4%) in the SCIT group showed no new sensitization, compared to 18 (33.3%) in the control group (p < 0.0002). The allergens most common responsible for the new sensitizations were pollens of Parietaria, grasses and olive [25].
A retrospective study evaluated a very large number of monosensitized patients, including 7182 patients treated with SCIT for 4 years and followed for further 3 years, and 1214 patients treated only with drugs for the same period years. All patients underwent prick test and specific IgE measurement before and after the 4 years of

SCIT and again 3 years later. The results showed that polysensitized subjects were 23.7% in SCIT-treated and 68% in drug treated after 4 years ($P < 0.0001$) and 26.95% and 76.77%, respectively, after 7 years ($p < 0.0001$). Asthmatic subjects were more prone to develop polysensitization in comparison to subjects suffering only from rhinitis (32.14% instead of 27.29% after 4 years, 36.5% instead of 31.33% after 7 years; $P < 0.0001$) [26]. Concerning SLIT, in the previously cited study by Madonini et al. on 302 patients followed-up for one year after stopping SLIT in only 9.6% of patients were detected by skin tests new sensitizations [21]. In the study by Marogna et al. the rate of new sensitizations was even lower, corresponding to 3.1% of SLIT treated patients and to 34.8% of controls, with an odds ratio to develop new sensitization in controls equivalent to 16.85 [22]. In a further study, the same authors prospectively evaluated the long-term effect of SLIT given for 3, 4, or 5 years on 78 patients, 59 of whom completed the study, compared with 12 control subjects. The total duration of the follow-up was 15 years [27]. According to new sensitizations, all the control subjects over the 15 years period developed positive test to allergens previously negative, while this occurred in less than a quarter of the patients receiving SLIT (21% in treated for 3 years, 12%, in treated for 4 years, and 11% in treated for 5 years, respectively).

Is AIT suitable for primary prevention of respiratory allergy?

The possibility to prevent the sensitization to inhalant allergens is currently under evaluation by Holt, who planned a prospective study of administration to newborns at risk of allergy (because of allergic parents) of sublingual extracts containing a mix of the most commonly sensitizing allergens [28]. The study is ongoing but a long time will be needed to achieve reliable observations on the real capacity to prevent, and not simply to postpone, the onset of allergy. It seems likely that the new method of AIT by intralymphatic injection, based on a very low number of administrations [29, 30], may represent in a near future a more suitable candidate method for primary prevention of allergy.

The mechanisms underlying the preventive capacity of AIT

The first mechanism suggested to explain the effectiveness of AIT was the generation of IgG antibodies. As IgG induced by vaccination neutralize the infectious agents [31], IgG induced by AIT should block the contact between the specific allergen and the IgE on the surface of mast cells and basophils [32, 33]. However, concerning prevention of anaphylaxis from insect stings, that is the most suitable model for a blocking role of IgG, a firm relationship between the amount of venom-specific IgG and clinical protection was never achieved, especially in the long-term [34]. Thus, finer mechanisms are likely to be involved, such as the inhibition by IgG of effector cells activation through their FC-gamma receptors [35]. In any case, the role of IgG antibodies should currently be viewed in the big picture of the effects of AIT on the immunologic response to the administered allergen, that seem similar in SCIT and SLIT [36-39]. In particular, the traditional, subcutaneous route of administration was first demonstrated to modify the allergen presentation by dendritic cells (DCs) that in turn modify the phenotype of allergen-specific T cells, switching from the Th2-type response, typical of allergic inflammation and characterized by a cytokine pattern including IL-4, IL-5, IL-13, IL-17, and IL-32 to a Th1-type response. This immune deviation is related to an increased IFN-gamma and IL-2 production as well as to the anergy of Th2 or to tolerance, the latter being related to the generation of allergen-specific T regulatory (Treg) cells, which produce cytokines such as IL-10 and TGF-beta. Comparable immunologic changes, with a pivotal role for Dcs in the oral mucosa and IL-10 producing Tregs, were observed during SLIT with administration of high allergen doses [38].

Conclusions

AIT for respiratory allergy, in both forms of SCIT and SLIT, has showed, along with a clinical efficacy starting from a few months from initiation of treatment [1, 2, 7], a clear preventive capacity on the development of asthma in subjects with rhinitis and on appearance of new sensitizations. This capacity is likely to be improved by the introduction of new materials to perform AIT. Valenta, who pioneered the diagnosis and treatment of allergy by using the molecular components of the allergens, suggested that the availability of the structures of the most common allergen molecules allows currently to produce well-defined recombinant and synthetic allergy vaccines able to target more precisely the mechanisms of allergy, and offers new possible allergen-specific strategies for prevention of allergic diseases [40]. However, to the purpose of preventing allergy in a child at risk it is strongly needed to increase the awareness on AIT in both the medical community and the lay, as currently conceived in international initiatives [41].

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ORIGINAL ARTICLE

Improving environmental quality in an operating room: clinical outcomes and economic implications

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Key words

Operating room • Environmental monitoring • Outcomes of quality improvement

Summary

An experimental study was conducted in a hospital in Liguria (northern Italy) on two groups of patients with the same disease severity who were undergoing the same type of surgery (primary hemiarthroplasty). Our aim was to assessing the results of a quality-improvement scheme implemented in the operating room.
The quality-improvement protocol involved analyzing a set of parameters concerning the operating team's behavior and environmental conditions that could be attributed to the operating team itself. A program of training and sanitary education was carried to rectify any improper behavior of the operating staff.
Two hundred and six hip-joint replacement operations (primary hip hemiarthroplasty - ICD9-CM 81.51) all conducted in the same operating room were studied: 103 patients, i.e. operations performed before the quality-improvement scheme and 103 patients, i.e. operations performed after the quality improvement scheme; all were comparable in terms of type of surgery and severity. The scheme resulted in an improvement in both behavioral and environmental parameters and an 80% reduction in the level of microbial air contamination ($p < 0.001$). Patient outcomes improved in terms of average postoperative hospitalization time, the occurrence and duration of fever (>37.5°C) and microbiological contamination of surgical wounds.
From an economic point of view, facility efficiency increased by 28.57%, average hospitalization time decreased ($p<0.001$) and a theoretical increase of € 1,441,373.58 a year in revenues was achieved.

Introduction

Surgical site infections (SSIs) are a major cause of morbidity and place an economic burden on hospitals worldwide [1]. Identifying and implementing evidence-based strategies designed to minimize SSI is therefore an important clinical goal. The rate of surgical wound infections is strongly influenced by operating room quality. An operating room is an extraordinarily complex system in which numerous risk factors are present, including not only the features of the structure and its fixtures, but also the management and behavior of healthcare workers. A safe and salubrious operating room is an environment in which all sources of pollution and any micro-environmental alterations are kept strictly under control. This can be achieved only through careful planning, maintenance and periodic checks, as well as proper ongoing training for staff [2].
Knobben et al. [3] observed that the combination of systemic and behavioral measures in the operating theater, such as wearing proper attire and limiting needless activity, led to a reduction in the incidence of intra-operative bacterial contamination and, consequently, of prolonged wound discharge and superficial SSI. Moreover, after one-year follow-up, fewer deep periprosthetic infections were recorded. While it is difficult to determine the relative influence of each individual measure on the final result, the combination of all these parameters evidently creates the most effective weapon against infections.
Many studies have shown that various methods can be adopted in order to minimize postoperative infection; one of the most effective ways has proved to be the administration of prophylactic antibiotics within 1 hour of surgical incision and continuation of their use during the immediate postoperative period [4]. However, microorganisms that are resistant to antibiotic treatment have been increasingly involved in SSIs, which is one of the main reasons why patients still suffer adverse outcomes from postoperative infections. Indeed, the number of SSIs caused by methicillin-resistant *Staphylococcus* (*S.) aureus* (MRSA) has increased dramatically [5], and recent studies have shown that reduced susceptibility to vancomycin and other glycopeptides is emerging in various MRSA clones all over the world [6, 7].
In 2008, the World Health Organization (WHO) published guidelines recommending several practices to ensure the safety of surgical patients worldwide [8]. Haynes et al. subsequently found that introducing the WHO Surgical Safety Checklist into operating rooms in eight hospitals was associated with marked improvements in surgical outcomes; postoperative complication rates fell by 36% on average, and death rates were reduced to a similar degree [9].
A management philosophy which seeks continuous improvement in hospital processes, products and services

and, in this specific case, in the operating room, is required in order to guarantee healthy conditions both for in-patients undergoing surgery and for health care providers. At the same time, maximum efficiency and effectiveness of the resources utilized must be ensured. For these reasons, measures undertaken to improve quality concern the entire organization, staff and facilities of the hospital [10-13].

Hence, quality improvement schemes yield both social and economic benefits. Social benefits comprise lower rates of postoperative complications and improved comfort and safety both of patients undergoing surgery and of health care providers. Economic benefits stem from the cost reductions resulting from shorter hospitalization due to optimization of operating room and staff use. Moreover, a lower postoperative complication rate reduces the need for antibiotic therapy and also shortens hospitalization.

We conducted an experimental study in a hospital in Liguria (northern Italy) on a group of patients with the same severity of illness (same ASA score) for whom the same resources were utilized (same patient management categories). Our purpose was to assess the results of a quality improvement scheme involving the operating room. Here, we report the results of the scheme in terms of environmental microbiological features and clinical outcomes.

Materials and methods

The study was conducted in 2012.

Quality-improvement protocol

The protocol was implemented over an 8-month period and included the following: (a) analysis of a set of parameters concerning the operating team's behavior (Tab. I); (b) evaluation of the airborne microbial load attributable to the operating staff; evaluation of microbiological features of surfaces and air from inlet ports of the ventilation system, upon completion of sanitation; (c) training and sanitary education to rectify inappropriate behaviors of the operating staff and to optimize plant and equipment maintenance and management by the technical staff. Conditions and behaviors (Tab. II) expected to improve the quality of the operating room environment were illustrated to the staff; particular emphasis was placed on clothing, operating room management, patient preparation and the various procedures used, through continuing medical education (CME) and the application of techniques focused on specific issues [14, 15].

Room quality and ventilation

The design of the operating suite provides adequate space for reception, anesthesia, surgery, recovery, and observation of patients. The air-conditioning system in the operating suite is equipped with high-efficiency particulate air (HEPA) filters, provides 24 air exchanges per hour by means of turbulent flow and is checked regularly by the maintenance staff. The same type of filter was used throughout the study.

Tab. I. Analysis of a set of parameters concerning the operating team's behavior.

1. During operations
   How many people are in the operating room?
   How many people not belonging to the operating staff are in the operating room?
2. In the operating room
   How many people use gloves?
   How many people replace their surgical mask after every operation?
   How many people wear proper clothes?
   How many people use universal precautions?
   Opening of doors
3. Assessment of procedures and protocols for
   hand-washing,
   routine and terminal cleaning,
   aseptic techniques,
   sterilization,
   infectious patients,
   pre-operative and post-operative management of patients,
   waste management

Tab. II. Behavioral and organizational interventions undertaken in the operating room.

1. Surgical staff
   Opening of doors kept to minimum
   Restricting access to the operating room of people not belonging to the surgical team.
   Movement of people kept to minimum
   No changing of personnel during operation
   Proper wearing of body covering:
   - use of disposable non-woven fabric suits,
   - use of clothes covering the whole body.
   - surgical mask replaced after every operation
2. Implementation of protocols for:
   Routine and final cleaning.
   Pre-operative and post-operative management of patients.
   Routine control of air-conditioning system.

Airborne and surface bacterial load

Airborne and surface bacterial loads were determined both before and after implementation of the quality-improvement scheme.

Microbial measurements were taken under rigorously aseptic conditions by means of a portable Surface Air System (SAS) SUPER 100 (PBI International®) impactor equipped with RODAC plates (Ø = 55 mm) containing γ-irradiated TSA (Tryptone Soy Agar) culture medium (Biotest Italia s.r.l.). In order to sample the air in the center of the room, the instrument was positioned in the immediate vicinity of the operating table, at a height of 1.5 m. During each procedure, a 1000 L volume of air was aspirated by means of a multi-aspiration modality; the impactor was switched on by remote control just as the skin was incised, and was switched off on completion of suturing [16]. To

determine the bacterial load of the airflow from the inlet ports of the ventilation system in at-rest conditions, the sampler was positioned in proximity to the flow-regulators. The total volume of air sampled was 1000 L. In order to prevent contamination, each plate was sealed after sampling and carried to the quality control laboratory in a biocarrier [17]. Plates were incubated at 37°C for 48 h before the total aerobic bacterial count was measured. Microbiological results are expressed as Colony Forming Units (CFU)/m^3.
Microbial sampling of surfaces was conducted by means of RODAC contact plates (Ø = 55 mm) containing Columbia blood agar culture medium (Biotest Italia s.r.l.). Sampling was carried out after sanitation of the operating room, as indicated by ISPESL and by the French guidelines [16, 18]. Plates were incubated at 37°C for 48 h before the total aerobic bacterial count was measured. Microbiological results are expressed as CFU/plates.
Subsequently, a search for *S. aureus* was undertaken. An 18-24 h culture of the test organisms was then prepared by inoculating a tube of Brain Heart Infusion Broth with a loopful of growth taken from pure culture, and a coagulase tube assay (Coagulase Plasma EDTA – Biolife Italiana S.r.l.s) was performed. The specificity of the reaction was checked by means of a control test carried out on each positive sample (*S. aureus* ATCC 25923).
The colonies that proved positive on the coagulase test were subjected to an antibiogram in order to evaluate methicillin/oxacillin resistance, according to the Oxacillin Agar Screen Test of the National Committee for Clinical Laboratory Standards (NCCLS, 2002, 12th Informational Supplement).

Patients

In order to obtain homogeneous and thus comparable data, the study recruited patients with the same disease severity (same ASA score) who were undergoing the same type of surgery.
We monitored 206 hip-joint replacement operations (ICD-9-CM 81.51) performed in the same operating room. These were divided into two groups: group 1 comprised 103 patients who underwent surgery before the beginning of the quality-improvement scheme; group 2 comprised 103 patients who underwent surgery after completion of the scheme.
Each patient from group 1 was matched with one patient from group 2. Both groups displayed comparable features: i.e. no concurrent diseases, age, sex, etc.. All patients in both groups followed the same protocol of prophylactic antibiotics: a single administration of 200 mg of Vancomycin combined with 400 mg of Pefloxacine 1 hour before surgery. All patients underwent surgery within 24 hours of hospitalization.
No joint sepsis or abnormal pain in the joint was noted in any of the patients.
The following parameters were considered for each patient:

- postoperative pyrexia above 37.5°C;
- administration, if any, of additional antibiotics;
- days of hospitalization.

Statistical analysis

Statistical analysis was carried out by means STATA/SE9^{tm} software (StataCorp LP, College Station, TX, USA). The results were analyzed in terms of descriptive statistics, and the relationships between data were examined by means of a t-test and Pearson's chi-squared test. A value of P less than 5% was considered significant. The rho Spearman rank correlation was used to assess the degree of association between the duration of pyrexia (>37.5°C) and hospitalization time.

Ethics statement

We did not need ethics committee approval, as the study was carried out as part of routine control tests that we conduct in the operating rooms of the hospital. As is the case of all studies conducted in the hospital environment, the General Management of the hospital approved the study protocol. The General Management is responsible for ensuring the ethical aspects of all activities of the hospital. Furthermore, the entire study was organized in accordance with a protocol agreed upon with the operating room teams. On entering the hospital, all patients sign an informed consent form regarding treatments in the hospital and the conditions of those treatments. Finally, the research was carried out in full respect of the Italian law on privacy (Legislative Decreee n. 196 of 30 June, 2003,).

Results

The behavioral and organizational interventions undertaken in the operating room are presented in Table II. Table III reports the average values of the various parameters considered.
With regard to environmental monitoring, the median value of the airborne bacterial load detected during the first set of measurements was 15 CFU/m^3; this value decreased to 3 CFU/m^3 (80% drop) in the second set of measurements (t-test = -13.5060, $p < 0.001$). The microbial load of the airflow through the inlet ports proved to be < 1 CFU/m^3 both first and second set of measurements. The median surface bacterial load decreased from 2 CFU/plate to 0 CFU/plate.
Analysis of the surfaces sampled before the quality-improvement scheme revealed contamination by 3 CFU of *S. aureus*, one strain of which proved to be MRSA; after implementation of the quality-improvement scheme, no *S. aureus* was found.
With regard to complications of patients, a 31.91% reduction in pyrexia (above 37.5°C) was recorded postoperatively. The number of patients with fever fell from 94/103 (91.26%) before implementation of the quality-improvement scheme to 64/103 (62.14%) after implementation. This difference proved statistically significant ($X^2 = 24.4462$, $p = 0.0000$), with an Odds Ratio of 6.36 (95% Confidence Interval [CI]: 2.77-15.86). The

Tab. III. Average values of the various parameters considered and percentage change after implementation of the quality improvement scheme.

	A	B	C
Total microbial contamination — air (CFU/m³)			
operating room - median (Q1-Q3)§	15 (10-20)	3 (0-5)	-80
air-conditioning plant	< 1	< 1	
Total microbial contamination — surfaces (CFU/plate)			
operating room - median (Q1-Q3) §	2 (2-4)	0 (0-1)	-100
Staphylococcus aureus surface (n.)	3	0	-100
MRSA contamination — surface (n.)	1	0	-100
Pyrexia above 37.5°C §	91.26%	62.14%	-31.91
duration of pyrexia (days) above 37.5°C (mean+S.D)§	6.84±3.15	3.16±1.05	-48.54
addditional antibiotic therapy§	32.98%	17.19%	-47.88
days of hospitalization (mean + SD)§	7.99 ± 3.24	4.92 ± 0.96	-28.57
Greater revenue***(Euro)*** due to more operations		€ 1,441,373.58	

Legend
A = case (before environment improvement); B = control (after environment improvement); C = percentage variation
§ = $p \leq 0.001$
SD = Stadard Deviation

duration of pyrexia (> 37.5°C) was longer in the patients belonging to group 1 (6.84 ± 3.15 days) than in the subjects belonging to group 2 (3.16 ± 1.05 days) (t = 8.1251, p = 0.0000). Some of the patients with pyrexia received additional antibiotics: 31/94 (32.98%) of group 1 versus 11/64 (17.19%) of group 2. The difference was statistically significant ($X^2 = 4.8651$, p = 0.020) with an Odds Ratio of 2.37 (95% CI: 1.03-5.72).
The average duration of hospitalization significantly decreased from 7.99 ± 3.24 days (group 1) to 4.92 ± 0.96 days (group 2) (t-test = 9.1998, p = 0.0000); a significant correlation (rho = 0.7730) emerged between the duration of pyrexia (> 37.5°C) and the time of postoperative hospitalization (p < 0.0000).
Finally, considering that in 2012, 596 operations were performed in this operating room, which is used exclusively for hip-joint replacement operations (ICD-9-CM 81.51) and also that, according to Italian law [19], the reimbursement is made by means of Diagnosis-related group (DRG n. 544) and the unit cost of hip-joint replacement operations amounts to € 8,565, we examined the functional and economic implications of the changes that were brought about. Owing to the reduction (3.07 days) obtained in the average duration of hospitalization, 828 more days became available for hospitalization. If we consider the average duration of hospitalization among group-2 patients, i.e. 4.92 days, 168 more operations could potentially be performed in this operating room. Thus, the waiting list (currently more than 2,000 people) could also be shortened, thereby producing clear social advantages.

Discussion and conclusions

A high degree of variability in air microbial contamination has been observed in some studies among operating suites and operating rooms, suggesting that factors which strongly affect the quality of air (e.g. number of people in the operating room, their movements, door-opening rate) may not be well controlled [3, 20-23].
Whyte et al. showed [24] that the incidence of joint sepsis progressively declines as air contamination is reduced, and that this trend is more marked below the value of 10 CFU/m³. Specific regulations concerning this issue have already been enforced in many countries [25, 26].
At the beginning of our study, the air bacterial load was fairly satisfactory (median 15 CFU/m³). Nevertheless, though the technological and structural features of the operating room remained unchanged, measures to improve staff behavior yielded a net improvement in clinical outcomes. This improvement was paralleled by the very low levels of air bacterial load (3 CFU/m³) obtained. Another our study confirms this result. [27].
With regard to surface bacterial load, an improvement was obtained after the implementation of sanitation protocols that included the use of hydrogen peroxide. Indeed, it has been shown that proper sanitation with hydrogen peroxide, which, even at low doses, reduces the bacterial load on surfaces by 50% [28], can eliminate *S. aureus*. Hence, clinical outcomes also improved, as indicated by the shorter duration of pyrexia (48.54% reduction) and hospitalization (28.57% reduction).
The presence of *S. aureus,* particularly MRSA, in the environment of the operating room reveals the importance of reducing the microbial load during surgery at high risk of SSI and highlights the fact that antibiotic prophylaxis, which is obviously necessary, should always be accompanied by good practice on the part of the operating staff.
Improved performance is mainly achieved through greater efficiency, rather than through the allocation of greater resources. Therefore, in addition to lowering the unit cost of the resources employed (overheads, structures, staff etc.), the hospital also receives more funds. The estimated increase in the number of operations that can be performed by the hospital means that revenues could be increased by € 1,441,373.58 a year (€ 8,565

X 168 extra hip-joint replacements that could be performed).
Moreover, shortening the waiting list would result in fewer patients going to hospitals outside their region. The Regional Healthcare System pays 20% more for a DRG performed in an extra-regional hospital (100%) than for the same DRG carried out in a hospital within the Regional Healthcare System (80%).

These data show the economic advantages deriving from a quality improvement scheme. However, the social and human benefits obtained should also be taken into account: namely, less discomfort for patients due to a shorter period away from home, higher quality of hospitalization as a result of fewer complications, and less time spent waiting for an operation that will improve the patient's quality of life.

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ORIGINAL ARTICLE

Clinical risk factors and bronchoscopic features of invasive aspergillosis in Intensive Care Unit patients

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Key words

Risk factors • Bronchoscopy • Invasive aspergillosis • ICU

Summary

Introduction. *Invasive aspergillosis (IA) is an important cause of morbidity and mortality in immunocompromised patients. During recent years, a rising incidence of IA in Intensive Care Unit (ICU) patients has been reported. The patterns of IA related infection may differ according to the type of underlying disease. Unfortunately little is known about the characteristics of IA in ICU patients. In the present study we assessed IA related clinical and bronchoscopy findings in ICU patients.*
Materials and methods. *This study was performed at the ICU units in Sari and Babul, Mazandaran from August 2009 through September 2010. We analysed 43 ICU patients with underlying predisposing conditions for IA. Bronchoalveolar lavage (BAL) samples were collected by bronchoscope twice a weekly. The samples were analyzed by direct microscopic examination, culture and non-culture based diagnostic methods. Patients were assigned a probable or possible diagnosis of IA according to the consensus definition of the EORTC/MSG.*
Results. *Out of 43 suspected patients to IA, 13 (36.1%) cases showed IA. According to criteria presented by EORTC/MSG, they were categorized as: 4 cases (30.8%) of possible IA and 9 (69.2%) of probable IA. The observed mortality was 69.2%. The main underlying predisposing conditions were neutropenia, hematologic malignancy, and COPD. The macroscopic finding in bronchoscopy included of Prulent secretion (46.6%), Mucosal bleeding (30.7%), Mucosal erythema (23%), Trachobronchomalasia (15.3%).*
Conclusion. *The diagnosis of IA in patients with critical illness in ICU is even more difficult. The clinical diagnostic process is often dependent on indirect circumstantial data enhancing the probability of IA. Bronchoscopy with inspection of the tracheobronchial tree, sampling of deep airway secretions and BAL can be helpful.*

Introduction

Invasive aspergillosis (IA) is a major cause of morbidity and mortality in immunocompromised patients receiving intensive chemotherapy, neutropenia, allogeneic stem cell transplantation, and solid organ transplantation [1-3]. Also intensive care unit (ICU) patients at risk for IA, which the specific defects in host defense mechanisms that increase their risk for IA [4]. During recent years, a rising incidence of IA in ICU patients (ranges from 0.3% to as much as 5.8%) has been reported and it carries an overall mortality rate exceeding 80% [5-8] This high mortality is at least partially related to difficulties in timely diagnosis, caused by insensitive and non-specific clinical signs and lack of unequivocal diagnostic criteria [7]. Thus IA must be considered as an emerging and mortal infectious disease in ICU patients even in the absence of an apparent predisposing immunodeficiency. The patterns of IA related infection may differ according to the type of underlying disease [9]. Unfortunately little is known about the characteristics of IA in ICU patients. It is often made late in the course of the infection because of clinical manifestations are usually non-specific, mycological cultures are difficult to interpret, and invasive procedures require to obtain histological specimens [10]. In Iran, there is no report on invasive fungal infections in ICU patients, in the present study we assessed IA related clinical risk factors and bronchoscopy findings IA in ICU patients.

Materials and methods

This study was performed at medical ICU units in Sari and Babul city in Mazandaran province from August 2009 through September 2010 all patients admitted to our were reviewed for inclusion in this study. We analysed ICU patients with one of the following underlying predisposing conditions who were at risk for developing IA; neutropenic patients including after chemotherapy or hematologic malignancy and non neutropenic patients including chronic obstructive pulmonary disease (COPD), solid organ transplant recipient, recipient of any other immunosuppressive treatment, and ICU stay more than 21 days. Radiological patterns of IA were classified either airway-invasive forms or angio-invasive forms, based on previ-

Tab. I. Demographic and characteristics of all patients with probable, possible and non-IA.

Characteristics	Probable IA (n = 9)	Possible IA (n = 4)	Non IA (n = 30)	All (n=43)
Age, yr., mean	47.8	62	58.4	56.5
Sex, male, (%)	6 (66.6)	2 (50)	16 (53)	24(58.8)
ICU length of stay, days	7.3	9.75	25	19.6
Mechanical ventilation, days	6.7	6.25	22	18.2
No. of deaths (%)	7 (77.8)	2 (50)	8 (26.7)	16(37)

Definition of abbreviations: IA= invasive aspergillosis; ICU= intensive care unit.

ous studies [11, 12]. Airway IA was considered when the predominant CT findings revealed a tree-in-bud pattern or peribronchial consolidation. During hospitalization, bronchoalveolar lavage (BAL) samples were collected by bronchoscope (Olympus BF20D) twice a weekly. The bronchus of the lobe in which consolidation was imaged by chest radiograph or chest CT scan was wedged, and 50 mL of 0.9% sterile saline solution at room temperature was instilled with a syringe through the working channel of the bronchoscope. The total volume of saline solution instilled into the lung was typically 150 mL, and 50 to 100 mL of BAL fluid was recovered. The presence of any tracheal or bronchial lesions was recorded by the endoscopist. The BAL samples were analyzed by culture and non-culture based diagnostic methods. BAL galactomannan (GM) antigen levels were measured by ELISA (Platelia Aspergillus GM EIA) assays. An optical density ratio of 1.0 was considered positive for GM in BAL samples [13].

Patients were assigned a probable or possible diagnosis of IA according to the consensus definition of the EORTC/MSG [14], with the modification. Probable IA was diagnosed when culture or cytology analyses of BAL fluid tested for Aspergillus species, and when one major clinical criteria (such as halo sign, air-crescent sign, or cavity within an area of consolidation on CT scan) or 2 minor clinical criteria (such as symptoms of lower respiratory tract infection, pleural rub, or a new infiltrate without an alternative diagnosis) were evident. Possible IA was defined by the presence of a host factor and either a positive culture or 1 major (or 2 minor) criteria. In this present study we were not be able to define a proven IA case (histologic evidence of tissue invasion) because there was an explicit refusal of the family to doing biopsy or autopsy.

Results

During the study period, 43 patients fulfilling the inclusion criteria were enrolled. According to the EORTC/MSG criteria, cases were classified as 9 (69.2%) of probable IA and 4 cases (30.8%) of possible IA. The median patient age was 56.5 years and 58.8% patients were male. The observed mortality in IA patients was 69.2% and 4 (30.7%) had survived. The characteristics of patients are summarized in Table I.

We found that the incidence of fever and rate of respiratory failure requiring mechanical ventilation were significantly higher in neutropenic patients than in patients with Solid organ cancer and COPD ($P < 0.001$). The macroscopic finding in bronchoscopy included of Prulent secretion (46.6%), Mucosal bleeding (30.7%), Mucosal erythema (23%), Trachobronchomalasia (15.3%). As showed in Table II, mucosal bleeding and prulent secretion were more prevalent in patient with IA. Neutropenic patients were more likely to exhibit peribronchial consolidation, mucosal bleeding, and mass-like consolidation. Hence, the airway-invasive pattern was more commonly observed in neutropenic patients (85.7%) than in other patients with IA.

Conclusions

Although a few studies have focused on the IA in ICU patients [15, 16], evaluation incidence of IA in ICU patients is important. The diagnosis of IA in patients with critical illness in ICU is even more difficult, because of Clinical manifestations are often non-specific, and diagnostic criteria have been adapted from standardized guidelines developed for ICU patients [10]. In addition, critically ill patients with prolonged stays in the ICU often develop pulmonary infiltrates, atelectasis and/or acute respiratory distress syndrome, whereas patients with prior lung disease (e.g. COPD) may present with pre-existing cavities on conventional chest radiographs [10]. In our study clinical signs are often lacking in ICU patients with IA, as reported by a previous study [8, 10, 16]. The radiological patterns of IA are usually characterized as airway-invasive or angio-invasive [17]. Therefore, our finding

Tab. II. Macroscopic finding in bronchoscopy of all patients with IA and non-invasive aspergillosis

Bronchoscopic features	IA (n = 13)	Non IA (n = 30)	p-value
Mucosal bleeding (%)	4 (30.7)	3 (10)	0.0154
Prulent secretion (%)	6 (46.6)	1 (3.3)	0.0082
Mucosal erythema (%)	3 (23)	4 (13.3)	0.0399
Tracheobronchomalasia (%)	2 (15.3)	0	0.0952

that the airway- invasive pattern was more commonly observed in neutropenic patients with IA is in agreement with previous reports [9, 17, 18]. Fiberoptic bronchoscopy with inspection of the tracheobronchial tree, sampling of deep airway secretions and BAL can be helpful [16]. This technique is a useful first procedure for the evaluation of IA patients, but a negative result does not exclude aspergillosis. Our study had several limitations. Firstly, we had a relatively small sample size which may have limited our power to detect differences between the groups. Secondly, because the critical condition of many patients did not permit an invasive diagnostic procedure and autopsy after death from patients suspected IA, has not been reported cases of proven IA.

In conclusion, the clinical and radiological features of IA differed between patients with underlying disease in ICU unit. Its occurrence in ICU usually entails a poor prognosis despite major recent improvements in the diagnosis and treatment of IA in patients with haematological diseases. Multicenter studies are warranted to explore the exact incidence and to better delineate clinical risk factors and bronchoscopy findings IA in ICU patients.

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ORIGINAL ARTICLE

Rs12979860 and rs8099917 single nucleotide polymorphisms of interleukin-28B gene: simultaneous genotyping in Caucasian patients infected with hepatitis C virus

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Key words

Hepatitis C virus • IL28B • Single nucleotide polymorphism

Summary

Introduction. *Recent studies have demonstrated the role of the interleukin 28B (IL28B) polymorphisms in predicting treatment induced and spontaneous clearance from Hepatitis C virus (HCV) infection, suggesting the possibility of tailored therapy in HCV infected patients. Genome-wide association studies have shown that single nucleotide polymorphisms (SNPs) near IL 28B gene on chromosome 19 are strong predictors of sustained virologic response (SVR) to pegylated interferon and ribavirin. This study was aimed at analyzing the co-prevalence of two common and clinically significant SNPs in a cohort of Ligurian patients.*

Methods. *Two SNPs (rs12979860, rs8099917) were genotyped in the IL28B locus from 175 DNA samples collected from HCV-infected consecutive patients in a Laboratory of Liguria Region, northern Italy. A real-time polymerase chain reaction in a Corbett Research Termocycler (Rotor Gene 3000A) by fluorescent probes (Fast Set IL 28B©, Arrow Diagnostics) was used for the detection, according to the manufacturer's instructions.*

Results. *Carriers of rs12979860CT genotype predominated (87/175, 50%), homozygotes for allele C were 68/175 (39%) and the remaining were homozygotes for IFN-resistant allele T (11%). As for the rs8099917 SNP, genotypes were thus distributed: 96/175 (55%) carried the rs8099917 TT genotype, whereas 70/175 (40%) and 9/175 (5%), were heterozygotes or homozygotes for the G allele. The variants rs12979860CC and rs8099917TT were found in 39% and 54% of overall patients with HCV genotype 1, respectively. The combined assessment of examined SNPs resulted in three most prevalent genotypes (rs12979860CC/rs8099917TT, rs12979860CT/rs8099917TG and rs12979860CT/rs8099917TT) with a frequency of 35%, 31% and 18%, respectively.*

Discussion. *Recent findings demonstrated that in carriers of rs12979860CT the determination of additional genotype of rs8099917 SNP could significantly improve the prediction of SVR. In our study cohort carriers of rs12979860CT represented 50% of all patients, who could take advantage with respect to SVR prediction by further determination of the rs8099917 SNP. The simultaneous genotyping of two IL28B SNPs should thus be recommended in HCV infected patients prior to treatment initiation.*

Introduction

Hepatitis C virus (HCV) infection is a global health problem with 120–180 million carriers worldwide; about 30% of infected individuals develop severe liver disease, as it is the leading cause of hepatocellular carcinoma, cirrhosis and liver transplants in Europe and the USA [1-4].

Combination therapy of long-acting pegylated interferon-α 2a or 2b (PEG-IFN) and oral treatment with ribavirin (RBV) represents the main antiviral tool; however, it is unsuccessful in about 50% of cases [5-8], expensive and associated with significant side effects, such as hematologic abnormalities, flu-like syndrome and adverse neuropsychiatric events, sometimes requiring dose reduction or premature termination of therapy [9].

In order to reduce several side effects and to avoid the heavy medical cost of PEG-IFN/RBV, a clinical tool able to predict an individual response before the treatment would be quite useful [10].

Several studies have demonstrated the role of both viral factors (such as HCV genotype, baseline viremia) and host factors (i.e. age, sex, ethnicity, liver fibrosis, body mass index) in predicting the natural course of hepatitis C and response to therapy.

A number of genome-wide association studies (GWAS) have shown that single nucleotide polymorphisms (SNPs) near the interleukin 28B (IL28B) gene on chromosome 19 coding for IFN-3 (rs12979860, rs8099917, rs12980275, and rs8103142) [11-16] are strong predictors of sustained virologic response (SVR) to PEG-IFN/RBV as well as of spontaneous viral clearance in HCV infected individuals [17, 18]. Other SNPs of IL28B (rs

8105790, rs 11881222, rs 28416813, rs 4803219 and rs 7248668) have been studied in HCV genotype 1 infected patients [17, 19, 20].
The SNPs rs12979860 and rs8099917 were found to be the most prevalent in different populations and strongly associated with treatment outcome. The first one, identified by Ge et al., has been associated with an approximately 2-fold difference in SVR rates in type 1-infected patients of European, African-American or Hispanic ancestry, with its favorable variant rs12979860CC, representing the best predictor of SVR in these ethnic groups [12, 20, 21-24].
The C allele distribution presents a geographical pattern with a lower frequency among African individuals than European descents [11].
In other studies a non-coding SNP, rs8099917TT, sited 7.5 kb upstream from the IL28 start codon, has been identified by Tanaka et al. and Suppiah et al. [14, 15]. These GWAS demonstrated that both SNPs (rs12979860, rs8099917) are the most critical for therapy outcome, in particular the favorable variants, homozygosis for the SNPs rs12979860 (CC) and rs8099917 (TT), are significantly associated with SVR in HCV-genotype 1–infected patients treated with Peg-IFN/RBV.
In the present study we describe genotype and allele frequency of IL28B polymorphisms rs12979860 and rs8099917 SNPs in 175 DNA samples collected from HCV-infected consecutive patients in a Laboratory of Liguria Region, northern Italy.

Methods

Genomic DNA was isolated from whole blood samples collected from 175 HCV infected patients. In a subset of these the following clinical data were available: HCV genotype, co-infection with hepatitis B virus (HBV) or human immunodeficiency virus (HIV), biochemical profile and SVR to antiviral therapy, defined as undetectable HCV RNA levels at least 24 weeks after the end of the treatment.
All the DNA samples were genotyped for two sets of IL28B SNPs, rs12979860 and rs8099917, specifying by C or T and T or G allele, using a real-time polymerase chain reaction (PCR) in a Corbett Research Termocycler (Rotor Gene 3000A) by fluorescent probes (Fast Set IL 28B©, Arrow Diagnostics), according to the manufacturer's instructions. The assay discriminated the different genotypes: wild-type homozygote (C/C, T/T), heterozygote (C/T, T/G), replaced homozygote (T/T, C/C), for rs12979860 and rs8099917, respectively. Fluorescence data were measured at the end of every cycle by the following profile: denaturation time of 3 min at 95°C, 35 cycle at 95°C for 15 s and 61°C for 45s. The results were analyzed using Allelic Discrimination Analysis. The samples were tested by duplicate and the IL28B genotypes were assigned by analysis of the reference cycle numbers for each fluorescence curve.

Results

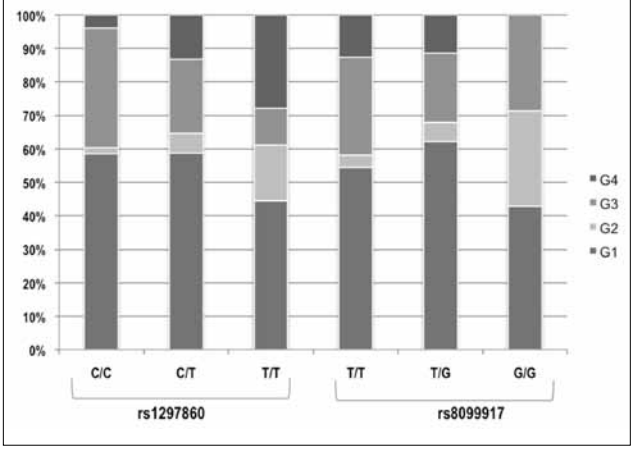
The characteristics of the study cohort are displayed in Table I. All patients in the present study were of Caucasian origin. They had a median age of 49 years (range 22-77 years) and included 121 (69%) men.
HCV genotype 1 was the most frequent (57%), followed by genotypes 3 (26%), 4 (12%) and 2 (6%).
Data on HIV and HBV co-infection were known in 97/104 (93%) and 28/102 (27%) patients, respectively. SVR to antiviral therapy was reached by 13/28 (46%) patients who received PEG-IFN/RBV.
Carriers of rs12979860CT genotype predominated (87/175, 50%), homozygotes for allele C were 68/175 (39%) and the remaining were homozygotes for IFN-resistant allele T (11%). As for the rs8099917 SNP, genotypes were thus distributed: 96/175 (55%) carried the rs8099917 TT genotype, whereas 70/175 (40%) and 9/175 (5%), were heterozygotes and homozygotes for the G allele.
The frequency of HCV genotypes according to IL 28B SNPs (rs1297860 and rs8099917) is depicted in 139 patients (Fig. 1). The preferred variants rs12979860CC and rs8099917TT were found in 39% and 54% of overall patients with HCV genotype 1, respectively. Patients infected with HCV genotype 2 were the minority in both SNPs (6%), whereas those infected with HCV genotype 3 were 36% and 20% carriers of rs1297860CC and non-CC, 29% and 22% of rs8099917TT and non-TT. The frequency of the IFN-resistant allele was 87% for rs1297860 and 37% for rs8099917 among all infected with HCV genotype 4.
The combined assessment of examined SNPs resulted in three most prevalent genotypes (rs12979860CC/rs8099917TT, rs12979860CT/rs8099917TG and

Tab. I. Baseline characteristics of study patients.

Variable	All patients (n = 175)
Age	49 (22 - 77)
Sex, male (%)	121 (69)
HCV genotype (%) (n=139)	
1	79 (57)
2	8 (6)
3	36 (26)
4	16 (12)
HIV Co-infected (%) (n = 104)	97 (93)
HBV Co-infected (%) (n = 102)	28 (27)
SVR (%) (n = 28)	13 (46)
Platelets (n = 58) (X10^9 L)	167x10^9 (16x10^9-406x10^9)
AST (IU/L)	56.5 (16-376)
ALT (IU/L)	78.5 (14-431)
WBC (/mmc)	5300 (2000-12700)
Haemoglobin (g/dl)	14.7 (10.2 - 17.5)

For categorical data, the number of patients is presented, whereas for continuous data median and range are shown. AST, alanine aminotransferase; AST, aspartate aminotransferase; SVR, sustained virological response; WBC, white blood cells.

Fig. 1. Frequency of HCV genotypes according to IL 28B SNPs (rs1297860 and rs8099917) in 139 patients.



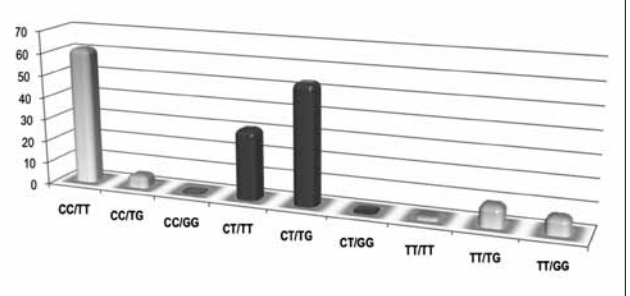
rs12979860CT/rs8099917TT) with a frequency of 35%, 31% and 18%, respectively (Fig. 2). The remaining genotypes for the combined two loci were less common and, in particular, the variant rs12979860CC/rs8099917GG was not observed.

Discussion

In the present study we investigated the prevalence of the haplotype and the combined genotyping of rs1297860 and rs8099917 SNPs in a set of samples of Caucasian descendent, by the simultaneous detection of the two IL28B loci. The homozygous variants rs1297860CC and rs8099917TT, the best predictors of SVR, were found in 39% and 55% of our cohort.

Several studies have demonstrated the role of IL28B polymorphisms in predicting treatment induced and spontaneous clearance from HCV infection, suggesting the possibility of tailored therapy and its relevant clinical and pharmacoeconomic implications. For example, carriers of rs1297860CC infected with HCV genotypes 1/4 may be haeded towards treatment with PEG-IFN/RBV, whereas carriers of the IFN-resistant allele T with HCV genotype 1 and no advance liver fibrosis

Fig. 2. Distribution (%) of combined genotyping of rs1297860 and rs8099917 in 175 HCV infected patients.



may defer therapy and wait for new direct-acting antiviral agents [25]. However, further cost-effectiveness analyses of response-guided therapy that include IL28B genotyping are needed for better understanding how current and future genotyping of other polymorphisms may help to evaluate the risk/benefit antiviral treatment profile.

The European Association for the Study of the Liver (EASL) Clinical Practice Guidelines for the management of HCV infection indicate the determination of IL28B variants as an available tool for diagnosis, useful to identify patient's likelihood of response to therapy, but with a low predictive value as other genetic variants may show correlation with disease progression in response to therapy [26]. In the scientific literature there is agreement about the need of integration of IL28B genotyping, which should not be *per se* considered a determining factor in deciding on a treatment strategy.

In this matter, a significant issue is which IL28B variant choose for diagnosis. A recent review by Soriano et al. summarized the main findings of GWAS conducted to assess the impact of different IL28B polymorphisms [25]: the authors underlined that, even though multiple SNPs around IL28B gene correlated to SVR, such as rs8099917, the current consensus considered rs12979860 as the strongest predictor [27]. The results of a 2013 meta-analysis confirmed that rs12979860CC and rs8099917TT genotypes could be used as independent predictors [28]; however, in consideration of the high frequency of rs12979860 in different populations and its relevant effect on treatment outcome, the determination of this SNP seemed sufficient for predicting response to therapy [29]. Halfon et al suggested that commercial tests for IL28B genotyping should include one simple polymorphism to give a simple message to physicians and, because of its highest predictive value to SVR, rs12979860 determination alone should be sufficient to predict treatment outcome [30]. In contrast with this opinion, Fisher et al. recently demonstrated that in carriers of rs12979860CT the additional genotyping of rs8099917 SNP could significantly improve the prediction of SVR [29]. In our study cohort, carriers of rs12979860CT represented 50% of all patients, who could take advantage with respect to SVR prediction by further determination of the rs8099917 SNP. This finding is line with the frequency observed by Fisher et al.

In conclusion, the simultaneous determination of two IL28B SNPs could be useful in some HCV infected patients to guide therapeutic decisions and improve treatment management and should therefore be included in the panel of pre-treatment evaluations.

Acknowledgements

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ORIGINAL ARTICLE

Clonal spread of vancomycin resistance *Enterococcus faecalis* in an Iranian referral pediatrics center

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Key words

Enterococcus faecalis • Vancomycin-resistant • Genotyping

Summary

Vancomycin-resistant enterococci (VRE) represent as an immediate threat to public health. Since few active compounds are available for VRE infections, rapid identification of these isolates are essential. In the absence of any report on the genetic relatedness of Enterococcus faecalis especially Vancomycin-resistant E. faecalis (VREF) isolates in Iran, we undertook this study to characterize these isolates using random amplification of polymorphic DNA (RAPD–PCR) genotyping method. In this study, E. faecalis strains isolated from various samples collected from different wards of Children Medical Hospital (Tehran, Iran). These isolates were identified by standard laboratory procedures and tested for antimicrobial resistance to vancomycin and teicoplanin. The genetic similarity of the strains was investigated by amplification of the RAPD–PCR.

In our study among 91 E. faecalis isolates, 15 (16%) were identified as VREF. The similarity pattern built for E. faecalis isolates by RAPD–PCR, demonstrated the presence of four distinct clusters (A, B, C, D). It is of interest to note that 100% of VREF isolates belonged to Clusters A, indicating that there may have occurred horizontal transmission of the same strain between patients. In conclusion, rapid spread of VREF from a clonal origin calls for implementation of careful isolation and infection control measures. Therefore, environmental control by routine disinfection of patient area as well as screening of high risk patients and isolation of colonized patients should be imposed in order to diminish risk of acquiring nosocomial VRE.

Introduction

Enterococci, an important cause of clinical infections, have emerged as an increasingly important cause of nosocomial infections in the last decades, being now the third to fourth-most prevalent nosocomial pathogen worldwide [1]. The emergence and spread of resistance to vancomycin as well as other glycopeptide agents like teicoplanin among *Enterococcus* species greatly reduces the number of treatment options. In addition, spread of vancomycin-resistant enterococci (VRE) represents an immediate threat to public health [2, 3].

Since few active compounds are available for VRE infections [4], rapid identification of these isolates are essential for implementation of control measures in order to restrict the emerging trouble of these strains.

A variety of typing methods have been used to examine clonal relatedness among human VRE isolates [5] but little is known about the epidemiology of vancomycin-resistant *Enterococcus faecalis* (VREF) [6]. Comparisons with respect to the epidemiological concordance and the overlap in the data for random amplification of polymorphic DNA (RAPD) versus pulsed field gel electrophoresis (PFGE) suggested that RAPD analysis is well-suited for epidemiological typing of enterococci [7]. In our previous study, high frequency of VREF as high as 16% was reported among hospitalized patients at Children's Medical Center from August 2009 to June 2010. In the absence of any report on the genetic relatedness of *E. faecalis* especially vancomycin resistant *E. faecalis* (VREF) isolates in Iran, we undertook this study to characterize these isolates using RAPD-PCR genotyping method.

Methods

The case definition for inclusion in this study was admission to the Children's Medical Center hospital (CMC) between August 2009 and June 2010, and a culture positive for *E. faecalis* at least 48 hours after hospital admission. No attempt was made to differentiate carriage, colonization or clinical infection.

These isolates were identified using standard microbiology methods [8] . The Kirby-Bauer disk diffusion method was used to determine the antimicrobial susceptibility of *E. faecalis* isolates to vancomycin and teicoplanin according to the Clinical and Laboratory Standards Institute. Isolates with potential vancomycin and teicoplanin resistance by this method were confirmed by E-test method (AB Biodisk, Solna, Sweden) according to the manufacturer's specification [9].

DNA Extraction

DNA was extracted from VREF isolates using QIAamp DNA Mini Kit (QIAGEN) according to the manufacturer's instruction.

Typing of *E. faecalis* isolates by RAPD-PCR

The genetic similarity of the strains was investigated by amplification of random amplified polymorphic DNA (RAPD). RAPD-PCR analysis was performed using primer (5'- ACG CGC CCT-3'). The reaction mixture (final volume 25 µl) consisted of 19 µl H_2O, 2.5 µl 10X reaction buffer, 0.75 µl $MgCl_2$ (100 mM), 0.6 µl dNTP mixtures (10 mM), 0.2 µl Taq-polymerase (5u/ µl), 1 µl primer (10mM), 1µl of the DNA. RAPD reactions were performed in a thermocycler by using the following temperature profile: initial denaturation at 94°C for 5 min followed by 36°C, 5 min; 72 °C, 5 min; repeat it 4 time then 94°C, 1 min; 36 °C, 1 min; 72°C, 2 min for 30 cycles [10]. Amplification products were analyzed by electrophoresis in 2% agarose gel with TBE buffer and stained with ethidium bromide 0.1%.

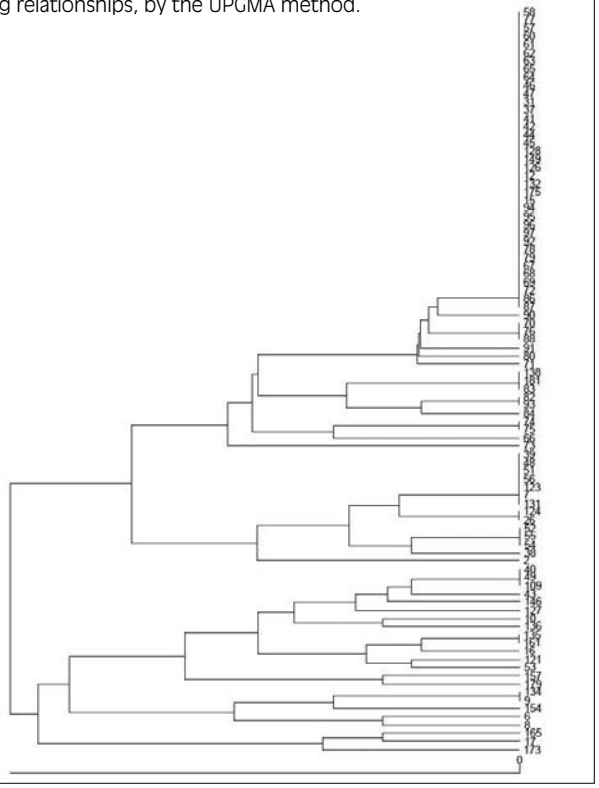
Similarity analysis

The electrophoretic profiles were scored according to the presence (1) or absence (0) of a particular band, Similarity of all pair-wise combinations of the numerical profiles was determined by Dice's coefficient and clustered by unweighted pair-group analysis using arithmetical averages (UPGMA) using the Freetree program (0.9.1.50 version). Phylogenetic trees were obtained and visualized using the TreeView program (1.30 version).

Results

During the study period, 91 children aged < 1 month to 12 years old were colonised or infected with *E. faecalis*.
Patients were hospitalized for a mean of 24 days (range 1-105 days). Twenty-one of the patients were hospitalized in urology ward, whereas the others were distributed in infectious ward (n = 14), surgical ward (n = 12), gastroenterology ward (n = 11), nephrology ward (n = 11), Neonatal Intensive Care Unit (NICU) (n = 7), Cardiovascular Intensive Care Unit (CICU) (n = 7), oncology (n = 4) and Pediatric Intensive Care Unit (PICU) (n = 4). Fifteen isolates (16%) were identified as VREF. The MICs to vancomycin and teicoplanin were ≥ 32 µg/ml in these isolates. Fingerprints of DNA fragments by RAPD-PCR were recorded. A single dendrogram of similarity was constructed for all isolates studied. Figure 1 demonstrated the similarity relationships between strains in 4 distinct clusters (A-D). Cluster A contained the majority of the isolates (n = 37, 41%), while cluster B contained 17 (19%), cluster C contained 14 (15%), and cluster D contained 23 (25%) of the isolates.
Surprisingly, all VREF isolates belonged to cluster A. Patients that were colonized/infected with VREF strains were identified in gastroenterology ward (n= 4), infectious ward (n = 3), urology ward (n = 3), CICU (n = 3) and NICU (n = 2).

Fig. 1. Phylogenetic tree among 91 *E. faecalis* isolates, constructed by Free Tree "software" and Distance Coefficient (DICE), showing relationships, by the UPGMA method.



Discussion

In the current study, we have investigated the genotypic characteristics of VREF isolates by RAPD method. Although the epidemiology of *E. faecium* is well described, little is known about the epidemiology of vancomycin-resistant isolates of *E. faecalis* [6]. Data on the prevalence of these strains are scarce in Iran, and to the best of our knowledge there is no published information concerning the genetic relatedness of VREF isolates.
The similarity phylogram built for theses strains, demonstrate the presence of four distinct clusters (A, B, C, D). In these clusters, some isolates were allocated in groups of higher or lower similarity and most strains were discriminated. Clusters A contained the majority of the isolates (n = 37, 41%) that were identical and forming one real clone. It is of interest to note that 100% of VREF isolates belongs to cluster A, indicating that there may have occurred horizontal transmission of the same strain between these patients. In our hospital, patients may be admitted briefly to a ward that does not match the medical care needed due to lack of bed on the appropriate wards. In addition, they might move to other wards when the level of care required changes. Therefore, patient movements may result in the dissemination of bacteria around the hospital, especially if proper infection control procedures are not instituted.
Predominance of one clone suggests frequent transfer of patients from one ward to another ward. In addition both person-to-person transmission and selective antibiotic

pressure can be probable mode of spread [10, 11]. Patients can remain colonized for prolonged period of time from months to years. In contrast to gram-negative bacteria, VRE broadly contaminates the environment and can remain for a long time; therefore, medical devices are commonly positive on wards with VRE patients [12] and patients might acquire nosocomial VRE from heavily contaminated environment [3].

In cluster B, 10 of 17 isolates had 100% similarity, forming four real clones. In cluster C, most of strains were similar, forming three real clones. Cluster D with 23 isolates had three real clones, which was formed by seven isolates and presented the biggest genetic distance among all. Due to the genetic distance observed especially in cluster B and D, it is probable that the non-VREF isolates in this study had diverse origins that may have been due to constant cross transmission of enterococal strains.

Our results are in agreement with those of studies of hospital-acquired VRE which indicated clonal dissemination as a major mechanism for the spread of isolates [6, 13, 14]. Typing of VREF isolates in the three studied hospitals in Spain by PFGE exhibited indistinguishable or closely related patterns [15].

Analysis of our results similar to other studies indicate *vanA* gene as common determinant for glycopeptide resistance in *Enterococcus* spp. [16-18] and clonal dissemination of VRE [19]. Identification of the source and route of dissemination of VRE is helpful for controlling outbreaks.

Clonal dissemination of *vanA* gene encoded VRE have been reported from other parts of world [19-22]. A study from Argentina have shown predominance of one epidemic clone carrying *vanA* gene [20]. Another molecular typing of VRE strains from UK revealed cross transmission of predominance VRE pulsotype with 92% containing *vanA* gene [22].

In conclusion, rapid spread of VREF from a clonal origin calls for implementation of careful isolation and infection control measures. Therefore, environmental control by routine disinfection of patient area as well as screening of high risk patients and isolation of colonized patients should be imposed in order to diminish risk of acquiring nosocomial VRE.

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ORIGINAL ARTICLE

Analysis of errors in histology by root cause analysis: a pilot study

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Key words

Root cause analysis • Anatomic Pathology • Laboratory errors

Summary

Introduction. *The study objective is to evaluate critical points in the process of pre-analytical histology in an Anatomic Pathology laboratory. Errors are an integral part of human systems, including the complex system of Anatomic Pathology. Previous studies focused on errors committed in diagnosis and did not consider the issues related to the histology preparation of routine processes.*
Methods. *Root Cause Analysis was applied to the process of histology preparation in order to identify the root cause of each previously identified problem. The analysis started by defining an 'a priori' list of errors that could occur in the histology preparation processes. During a three-month period, a trained technician tracked the errors encountered during the process and reported them on a form. 'Fishbone' diagram and 'Five whys' methods were then applied.*
Results. *8,346 histological cases were reviewed, for which 19,774 samples were made and from which 29,956 histologies were prepared. 132 errors were identified. Errors were detected in each phase: accessioning (6.5%), gross dissecting (28%), processing (1.5%), embedding (4.5%), tissue cutting and slide mounting (23%), coloring, (1.5%), labeling and releasing (35%).*
Discussion. *Root cause analysis is effective and easy to use in clinical risk management. It is an important step for the identification and prevention of errors, that are frequently due to multiple causes. Developing operators' awareness of their central role in the risk management process is possible by targeted training. Furthermore, by highlighting the most relevant points of interest, it is possible to improve both the methodology and the procedural safety.*

Introduction

The term 'clinical risk management' was introduced in the United States around 1970 to describe a tool that was used to check claims, lawsuits and requests of indemnification of patients who suffered adverse events caused by inefficient health care.
However, the consequences that may derive from the errors committed during clinical practice became of universal interest only in 1999, when a report published by the Institute for Medicine in the United States stated that a high number of deaths - between 44,000 and 98,000 every year - were caused by errors in clinical practice [1]. Since the publication of this report, the safety of patients has also become of interest to healthcare organisations and regulating bodies (such as the Joint Commission on Healthcare Organizations) that consider this topic as crucial for hospitals, in order to achieve accreditation and to meet the quality requirements of consumers' associations [2].
According to Khon [1], error is an integral part of human actions and the more complex a system is, the higher is the degree of risk. A healthcare system should be considered as a complex system with a number of variables (complexity of operations, specificity of individual patients, involvement of different professionals). Therefore, the risk of an error or incident is always present.
Anatomic Pathology can be taken as an example of a complex system, where errors could occur during different phases of the diagnostic process. The absence of a 'gold standard' for this discipline [3] makes the detection of errors complicated. However, they are easily and promptly identified when validated standard operating guidelines exist. There exists a small number of reports in medical literature concerning errors and their identification process in Anatomic Pathology. However, they mainly focus on inter-laboratory quality control and do not address the quality and the effective management of the specimens.
In the area of medical diagnostics, detecting such errors is heavily influenced by the subjectivity, education and attitude of the operator. The number of errors committed during the preparation of histology slides is also significant. A correct process is crucial for a correct and valid diagnosis, but this process is not automated and, therefore, it is dependent on the ability of the single operator [4, 5].
As data provided for laboratory medicine, and specifically by Anatomic Pathology, represent a central element of the whole diagnostic and therapeutic process, increasing attention is given to errors and potential 'pitfalls' of the diagnostic pathway in these areas that were of marginal interest in the past or were only considered

for clinical chemistry (numerical and quantitative) data [6]. However, to date, there are few papers concerning systemic analysis of errors within AP than papers concerning more specific clinical sectors.
This study attempted to widen the culture of procedural error prevention within Anatomic Pathology.
Assuming that education and training of personnel on the subject of error and the analysis of errors avoiding a punitive approach results in an increased level of attention in the workplace, and starting from the taxonomy defined by previous works [4], the study helped to increase the related knowledge and to detect deep causes of procedural errors that are committed within Anatomic Pathology.
This research did not focus on wrong diagnoses [7, 8], but on the problems related to the histology preparation of routine processes. Errors related to histology diagnosis, therefore, were not taken into consideration. The objective of the study was to evaluate the critical points in the process of pre-analytical histology in an Anatomic Pathology laboratory.
An increase in the perceived responsibility of each person involved in a process, results in the creation of a culture of prevention and risk analysis. Initially, therefore, errors found in the processes of accessioning, gross dissecting, processing, embedding, tissue cutting and slide mounting, coloring, and labeling and releasing, were collected and classified. These were then assessed, in order to identify contributing factors and causes of the most common and serious errors. Finally, the identification of solutions was undertaken, in order to reduce the errors and the consequent clinical risk.
Root cause analysis, which is one of the techniques used for detection of an error and identification of the cause, is of particular relevance. It is a structured investigation aimed at identifying the deep cause of a problem, and the necessary actions to eliminate the problem [9]. It is also a technique that, by analyzing the errors in a system, identifies the causes through an inductive method, i.e. through questions which explore the reason behind every action and the source of every possible deviation.
The main objective of this methodology is to understand in detail what happened, why it happened and what can be done to prevent it to happen again. It is, therefore, focused on the factors, the activities and the decisions that lead to an error [8].
To be effective, root cause analysis should be applied to all areas where an error may occur during the clinical process of patient care and involve both the therapeutic and the diagnostic activity in the laboratory.

Methods

Error detection

The study, that was performed during a trimester in a laboratory of a leading hospital in Lombardy, defined, on the basis of literature and of the experience gained by the staff involved, an 'a priori' list of errors that can occur in the processes (Tab. I) and then constructed a survey form to record: the date of the detected error; the type of error according to the classification by Reason [10] (based on rules, based on knowledge, slips, lapses), whether of omission or commission; the index of severity (Table II presents the severity rating scale adopted); the process in which the error was detected (accessioning, gross dissecting, processing, embedding, tissue cutting and slide mounting, coloring slides, labeling and releasing slides); and the operator who committed the error (piece selector, inclusor, cutter, secretary, freezer).
The survey form also included an area where the technician could suggest the possible causes of the error. The form was filled by trained technical staff, who had been previously identified as being responsible for the immediate recording of errors.
The activity of the technical staff was planned weekly where; two technicians were assigned to the procedures of accessioning and gross dissecting, two technicians to the procedures of processing and embedding, and four technicians to the procedures of tissue cutting and slide mounting, coloring, and labeling and releasing. The ability to detect and, at the same time, report the error instead of reporting it at the end of the process or even at the end of the working day prevented the loss of information. In addition, a person was assigned to fill the survey form prevented the collection of errors, thus reducing the risk of missing reports.

Analysis of errors and identification and analysis of the causes

In the first phase there was a preliminary assessment of all functional errors in the selection of cases to be submitted to root cause analysis, in particular, errors with gravity greater than 5 or high frequency of detection. The selected causes were then analyzed using two of the methods provided for in root cause analysis.
On the basis of assessments, that highlighted the absence of intermediate or higher levels of severity for the errors reported, it was decided to select, for the root cause analysis, errors with a higher frequency of detection; in particular, the causes of errors in the transcription of the number of identification from the generation of the code to the delivery phase of the prepared histology.
After the analysis of these causes, there was a further assessment to evaluate the potential risk; the incorrect transcription of the identification number may lead to an incorrect diagnosis and, also, to additional or unnecessary examinations or possible wrong therapy.
A multidisciplinary team of professionals trained to use root cause analysis was assembled. Team consisted of two technicians of the histology laboratory, the internal quality manager of the hospital and a medical doctor, trained in risk management, who works in a different hospital. The heterogeneity of the team allowed to highlight different points of view and to increase the robustness of results.
A 'fishbone' diagram [11] was built by the team, through a brainstorming activity that identified possible causes,

Tab. I. Errors that may occur in the processes

Accessioning	Gross dissecting	Processing	Embedding	Tissue cutting and slide mounting	Coloring slides	Labeling and releasing slides
The specimen was not in the container	Incorrect numbering of the slides containers	Unfinished program	Loss of specimen	Loss / exhaustion of specimen	Insufficient time for dewaxing	Error of number reported on the slide labels
Inconsistency between the specimen and request	Incorrect numbering of the containers of biological material	Instrumental error / error in temperature	Contamination	Number was reported incorrectly	Exhaustion of reactive	Exchange of slides
Request or supply not received	Error in checking of the request	Exchange of reagents	Exchange of specimen	Collection of incorrect section	Wrong coloring	Broken slide
Specimen wrongly accessioned	Incorrect choice of the containers	Mistaken choice of the program	Incorrect selection of paraffin	Contamination	Detachment of section from the slide	Slides were not delivered
Incomplete request	Loss of specimen	Excessive number of containers	The specimen was badly positioned	Thickness selection error	Breakage of slide	Specimen to be decalcified not reported
Allocation number error	Mistaken specimen	Loss of specimen		Damaged sample	Misuse of coloring solutions	Mistaken requests
Worksheet wrongly attached	Contaminated specimen			Error in identification of block to be cut	Error in assembling	
Registration error	The specimen was not decalcified			Error in coloring or lack of coloring	Error due to automatic coloring	
Incorrect type of fixative	The specimen was not loaded or Incorrectly loaded			Collection of slides in incorrect dyes	Error in the choice of the program	
					Incorrectly mounted slide	

Tab. II. Severity rate scale adopted in the study.

Index	Consequence
1	No effect
2	Repetition of a process
3	Repetition of multiple processes
4	Lengthening of response time
5	Re-sampling for sampling error
6	Partial loss of material - re-sampling
7	Loss of material: repeat sampling (non invasive)
8	Loss of material: repeat sampling (invasive)
9	Loss of material: it is not possible to repeat sampling
10	Incorrect diagnosis

contributing factors and, in addition, issues relating to the working environment, the system, staff and the external environment.

Starting from the reported errors, the 'fishbone' diagram was applied in order to identify potential causes and areas where the errors developed (Fig. 1).

In particular, this was undertaken to identify causes related to environmental factors (workplace noise), causes related to staff (operators tired due to workloads, different handwriting) as well as causes related to system factors (many overlapping activities related to the same operator, workloads to be conducted in a restricted time, routine and automatic work, long numerical codes of up to eight digits). In this analysis there were no causes linked to factors outside of histology.

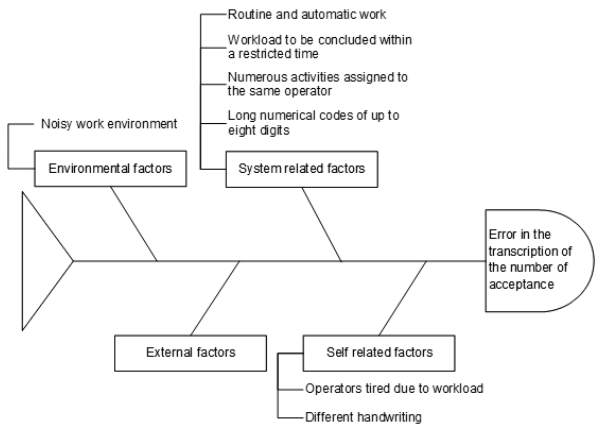
In the second phase, for each of the causes identified, the technique of 'Five whys?' [12] was applied in order to reach the root cause of each problem. However, it was not possible to answer the five "whys" in all the analyses carried out as the technique becomes exhausted when the root cause is identified. Finally, possible corrective measures were assessed in order to eliminate the identified causes.

Results

Errors highlighted in the individual processes

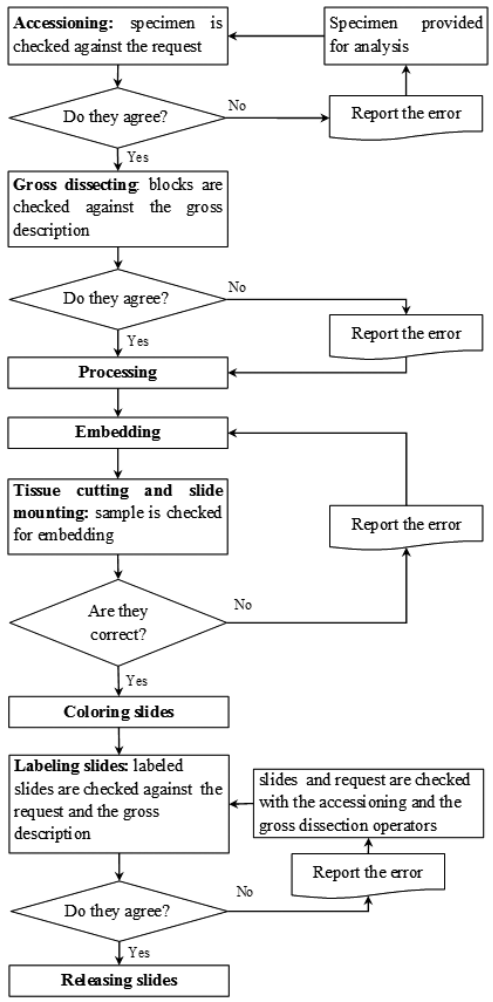
Over the whole period of the study 8,346 histological cases were reviewed, 19,774 samples were made and

Fig. 1. Incorrect transcription of the number of acceptance 'fish-bone diagram'.



29,956 histologies were prepared. The analytical process is reported as a flow-chart in Figure 2.
From February 1st 2010 to April 31st 2010, 132 errors were identified as reported in Table III.

Fig. 2. Flow chart of the histologies processing process.



Tab. III. Type and number of errors identified.

Type of error	Errors, No. (%)
accessioning	9 (6.5)
inconsistency between the specimen and request	3
requests or supply not received	3
allocation number error	1
worksheets wrongly attached to the request	2
gross dissecting	37 (28)
incorrect numbering of the slides container	26
errors in checking of the request	3
incorrect choice of the containers	3
specimen not decalcified	3
specimen incorrectly loaded	2
processing	2 (1.5)
exchange of reagents	1
instrumental error	1
embedding	6 (4.5)
specimen was badly positioned	5
contamination	1
tissue cutting and slide mounting	30 (23)
error in identification of block to be cut	7
the number was reported incorrectly	15
lack of coloring information	6
collection of incorrect section	1
thickness selection error	1
coloring slides	2 (1.5)
misuse of coloring solutions	1
detachment of the section from the slide	1
labeling and releasing slides	46 (35)
errors of number reported on the slide label	44
slides were not delivered	2
Total	132 (100)

During the different phases of processing there was no detection of the following errors: during the accessioning phase there were neither errors related to specimens which were not in the container, nor to specimens intended for another laboratory, incomplete requests, registration nor type of fixative used. During gross dissecting, there were no errors in the numbering of the containers of biological material, loss of specimen, specimen mismatching and sample contamination. During processing, there were no reported errors in the process temperature, the choice of the program, unfinished program, the quantity of loaded containers and in the loss of specimen. During embedding, there were no errors for incorrect selection of paraffin, mismatching or loss of specimen. During tissue cutting and slide mounting, there were no errors of loss, exhaustion or contamination of specimen, damage of samples on the slides and of the incorrect dyes, i.e. different from requested ones. During coloring, there were no errors related to insufficient time for dewaxing, to exhaustion of reagents, to wrong coloring, to breakage of slides, to wrong side mounting of the slide and, finally, to the use of automatic coloring. During labeling and releasing there were no errors in the mismatching of slides or of requests, either of specimen

to be decalcified not reported on the request and, finally, of breakage of slides.

Evaluation of Errors

The classification for each reported error took into account the following considerations:

- 98.5% of the errors were due to lack of attention and oversight and only 1.5% appeared to be due to a lack of knowledge of the process;
- 85% of the errors were of commission type, due to incorrect practical actions, while 15% of the errors were of an omission type or due to failure to perform an action;
- 10% of the errors had a severity index equal to 4; these errors resulted delayed report to physician;
- 2% had a severity index between 2 and 3, inducing the repetition of one or more processes;
- 88% had a severity index of 1 so they had no consequences.

It is important to consider that the majority of errors that were listed had no consequences for the patient (severity index not greater than 4) due to the ability of the working team in identifying and correcting them. However, as reported also in other works [13], these errors lead to a "near miss event" and required considerable time to be corrected.

- 73% of errors were detected only in the final process of labeling and releasing. In particular, all the errors related to copying and numbering committed during the gross dissecting process were recognized only at the time of delivery of the slides. Only 1.5% of errors was detected during the reading phase by the pathologist because of contamination and (or) material not consistent with that reported in the clinician's request.
- 85% of errors were detected during the processes of gross dissecting, tissue cutting and slide mounting, labeling and releasing. 80% of these errors appeared to be attributable to a number of incorrect transcriptions of containers identification, on slides and on labels applied to the slides at the time of delivery.

While considering it as a possible cause of error, the problem of different handwriting styles of each individual operator was not investigated.

Each proximate cause, identified in the 'fishbone' diagram, was investigated by the team using the question 'why?'.

Proximate cause 1: why is the work place noisy? Because safety cabinets are working and technician and pathologist need to talk. Why? Because safety cabinets are necessary to protect operators from dangerous substances. Pathologists describe specimens during gross dissection. Accessioning specimens are checked against request by two technicians: the first one reading the request the second one checking the specimen. Why? Because specimen description and identification number on blocks are handwritten by one technician during gross dissection. Why? Because automated blocks labeling system lacks in the laboratory.

Proximate cause 2: while considering it as possible cause of error it was not thought necessary to go deeper into the problem of different handwriting styles as an immutable feature of each individual operator. However the use of an automated specimen identification system would solve the problem.

Proximate cause 3: why are the operators tired? Because of personnel shortage.

Why? Handwriting of blocks, labels, slide and gross descriptions involves a number of technicians. Why? Automated specimen identification system is lacking in the laboratory.

Proximate cause 4: why is the work routine and automatic? Because the identification numbers on blocks slides and labels are handwritten. Why? Automated label maker for slides and blocks lacks in the laboratory. Why? Automated specimen identification system is lacking in the laboratory.

Proximate cause 5: why do activities overlap? Due to handwritten specimens and checking made during gross dissection. Why? Surgical pathology cases are accessioned in two large batches, one in the morning and one in the afternoon and the lack of automated label maker for blocks leads to overlapping. Why? Automated specimen identification system lacks in the laboratory. Surgical pathology cases are delivered from hospital twice a day. Why? Because histology and hospital are located in different buildings.

Proximate cause 6: numerical code of up to eight digits was not investigated because it is an unchangeable security system feature.

Proximate cause 7: why are workloads to be concluded in restricted times? Because large batches of accessioning specimens are checked in short time. Why? Because surgical pathology cases are delivered from hospital to histology twice a day, where inappropriate specimen or request have to be immediately returned to hospital. Why? Because histology and hospital are located in different buildings.

The answers identified were inserted into a chart with each answer below its parent, the result is a 'tree' where the roots are the deeper causes (Fig. 3).

In the path to find the root causes there was no disagreement; all the causes identified by the members of the team were considered and analyzed.

Discussion and conclusions

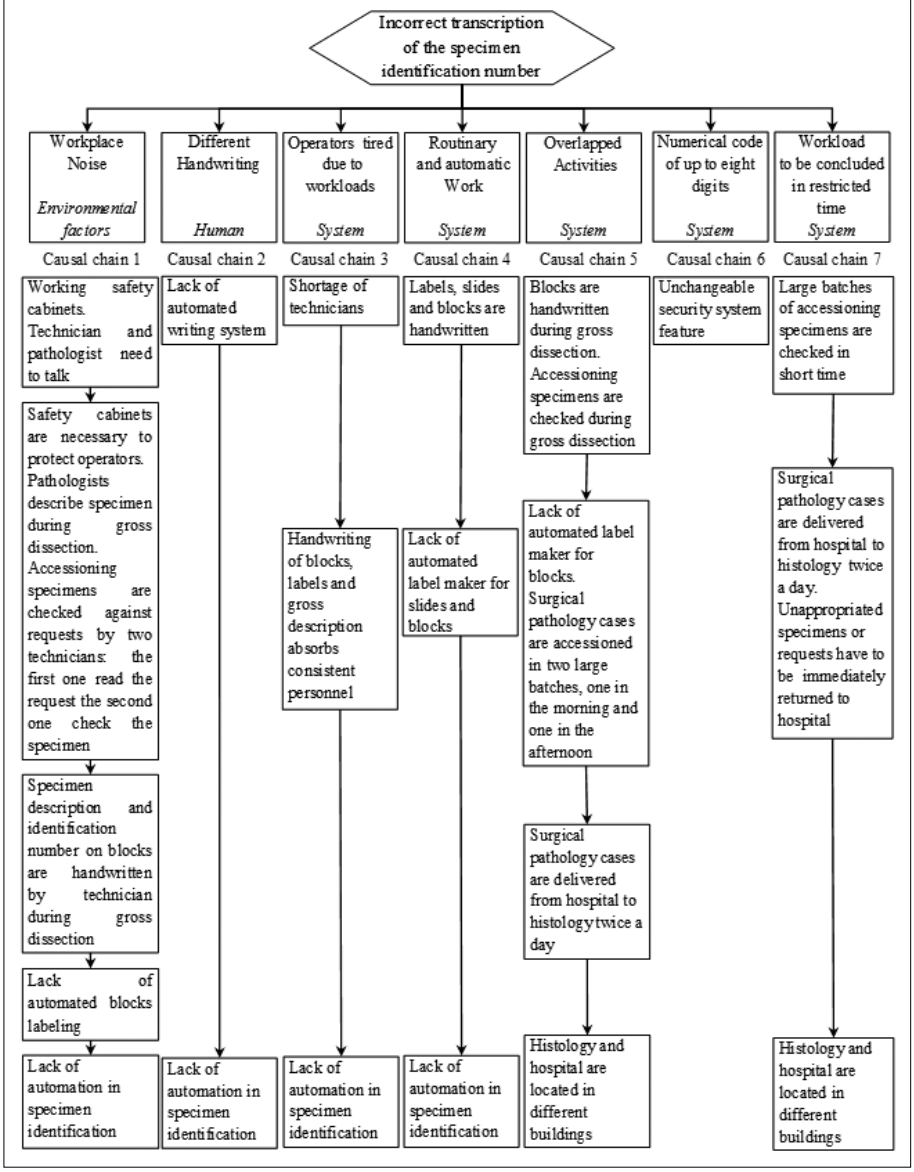
The root cause analysis carried out in the study revealed two causes which can be considered as the common root causes to most problems.

The first cause was the different locations of the histology laboratory and the hospital from where the test material came from. The material was delivered only twice a day, with the consequent need for accessioning in a short time, requiring more human resources, sometimes involved in other processes, in this unique operation. This root cause is to be referred to pre-analytic phase.

The second cause was the lack of automation in the numerical identification of containers, in the dictation and typing of the macroscopic description of the test material and in the numbering of the labels and the slides. This was not only the reason why the errors occurred, but also a waste of time for the personnel. The lack of automation is a root cause which influence both pre-analytic and analytic phases. As indicated by other authors[14] the introduction of technology and the adoption of automatic tracking system perfectly integrated with all the available technologies dramatically reduce errors in specimen processing.

In the study, the first cause, i.e. the problem of the location of histology, was not considered as it is beyond the possibilities of intervention. By contrast, the second cause allowed the possibility of some feasible solutions, such as the implementation of automatic systems for the management of the samples, e.g. a computerized system which includes the use of a unique bar code generated in the identification phase. Thus, by reading the bar code with an optical reader directly from the gross dissecting location, the pathologist can access the folder of the case, record in a voice recorder the description of the sample, and assign and print the number of the slides container.

Fig. 3. Incorrect transcription of the specimen identification number 'Five whys' technique results.



The focus of the study was the analysis of pre-analytic and analytic phases, therefore, root causes related to post analytic phase were not investigated.

The use of such systems would appear to be useful not only to obtain a decrease in the number of errors, but also to control the sample position during the processes.

It was found that root cause analysis is a technique that is useful and easy to use in clinical risk management. In particular, as shown in the study, it allows the possibility to highlight points of reliable importance for improving the methodology and assuring procedural safety.

The initiation of an error analysis program could become a stimulus for the detection of errors. Operators who already use a quality management system would be encouraged to report events. They would also be aware that this will be brought to the attention of the risk manager for enhancing process quality improvements. In this regard, it is useful that all staff involved is adequately informed about the objectives which must be achieved and about the possibility of changes resulting from reporting an incident and applying RCA.

In addition, the tools used for the analysis of cases, in order to investigate the factors involved, have proved effective and easy to use. The 'fishbone' diagram is particularly beneficial, also from a graphical point of view, helping to identify problems related to different types of factors involved (external factors, factors related to the system, factors related to staff and environmental factors) and to undertake an initial identification of causes. Furthermore, the 'Five whys?' method allows an in depth analysis of the causes, distinguishing all the steps, from the identification of the problem to the root cause.

Finally, root cause analysis can be applied within different contexts due to its focus on processes, and can there-

fore be successfully applied within hospitals accredited by National Health Service.
Thus, the proper identification of procedures to be submitted to this method, by carefully assessing the gravity and frequency of individual errors encountered in a routinely performed evaluation, is crucial.
The study identified errors, that are typical and specific of the laboratory where the analysis was performed, and it did not overlap with other studies, already published, relating to error collection databases. Most of the works concerning errors in surgical pathology are focused on misidentification or mislabeling cases or specimens [14, 15], it is therefore difficult to compare them with the results presented in this study.
In addition, the study framework, will help organisations and regulating bodies within the health care service to focus on two fundamental concepts:

1. the application of root cause analysis, to the processes that characterize a healthcare area, represents a useful tool to correct working habits that may generate errors and reduce safety;
2. the training of operators, to develop awareness of the fact that errors represent a central aspect of the risk management process.

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ORIGINAL ARTICLE

Tobacco smoking among students in an urban area in Northern Italy

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Key words

Smoking • Adolescents • Students • Social predictors • Smoking initiation

Summary

Introduction. *Tobacco smoking, which usually begins in teenage, is one of the most important lifestyle risk factors for chronic diseases and a major public health problem worldwide.*
The aims of the study were to determine the prevalence of tobacco smoking and the mean age of initiation among adolescents in Genoa (Italy) and to identify some socio-demographic predictors that could be associated with the onset of smoking.
Materials and methods. *2,301 randomly selected students (14-19 years old) in Genoa completed an ad hoc questionnaire. The Kaplan-Meier method was used to evaluate the instantaneous risk of experimenting with smoking. A multivariate logistic regression model was used to determine whether current or previous smoking status was associated with socio-demographic characteristics.*
Results. *59.5% of respondents had tried smoking, while 35.6% defined themselves as current smokers. No difference in current smoking prevalence emerged between males and females (35.2% and 35.9%, respectively, p = 0.83). The mean age on initiation was 13.5 years for males and 13.9 years for females. The instantaneous probability of trying smoking changed with age, reaching a maximum at 14 years. Subjects who tried smoking before this age were more inclined to continue smoking.*
The probability of being a current smoker was significantly higher among students from unmarried-parent families and those attending vocational and technical secondary schools.
Conclusions. *There is a great need for the activation of new health promotion interventions and enforcement of those already existing, in order to raise awareness of the damage caused by smoking among adolescents, especially those belonging to high-risk groups.*

Introduction

Cigarette smoking is one of the most important lifestyle risk factors for chronic diseases [1], which are responsible for a high number of deaths worldwide [2]. Throughout the world, more than 5 million deaths each year are due to tobacco smoking and, if the current rising trend persists, this figure is expected to reach a billion by the end of the 21st century [3]. Tobacco smoking has been classified by the International Agency for Research on Cancer (IARC) as having a Group I carcinogenic effect in humans, and about 70 carcinogens have been identified in tobacco smoke [4]. The leading smoking-attributable diseases are cancers and respiratory and cardiovascular diseases [5]. Gallus et al. reported 71,445 deaths (52,707 males and 18,738 females) attributable to smoking in 2010 in Italy (12.5% of total mortality). These deaths are due to lung cancer, other malignant neoplasms, cardiovascular disease and non-neoplastic respiratory diseases [6].
Tobacco smoking is a major public health problem worldwide. The majority of smokers start smoking in adolescence and approximately two thirds try smoking by the age of 15 [7]. The hazards of smoking depend on a variety of factors, such as age on initiation, the number of cigarettes smoked per day, degree of inhalation, nicotine and tar content, and filter type [5]. Cigarette smoking among the young is associated with numerous health problems during childhood/adolescence and with an increase in health problems in adulthood [1, 8].
The age at which an individual starts smoking determines the probability of addiction, the likelihood of stopping and the risk of adverse health outcomes [9]. In a recent survey conducted in the United Kingdom (UK), 53% of adolescents reported having smoked at least one whole cigarette by the age of 16 years [10]. The Health Behavior in School-aged Children (HBSC) study, carried out in Europe, reported the following weekly smoking prevalence rates among the young: 2% among 11-year-olds, 8% among 13-year-olds and 24% among 15-year-olds [11].
The process of becoming a smoker develops through phases of preparation, initiation, experimentation, regular smoking and addiction, with progression from the first to the fifth phase lasting on average 2-3 years [12].
Adolescent smoking may be associated with socio-demographic, environmental, behavioral and personal factors [13]. The individual's level of education may also

influence smoking initiation, the more highly educated being less likely to smoke [14]. The question of whether a lower socio-economic status of parents influences the onset of smoking in their children remains open. Several studies have documented an inverse relationship between adolescent smoking and parental socioeconomic variables, such as education and social class, in that a higher level of adolescent smoking has been observed in families with lower socioeconomic status [13, 15]. Other studies, however, have not found such a link [16 ,17].
In order to implement early health-promotion measures, especially among high-risk groups, it is essential to ascertain the prevalence of tobacco smoking among adolescents, the ages when smoking is first experienced and becomes regular, and related risk factors.
The aims of the present study were to determine the prevalence of smoking and the mean age on initiation among adolescents in Genoa (Italy) and to identify socio-demographic predictors that could be associated with the onset of smoking.

Materials and methods

Study design and population

This cross-sectional study was conducted in Genoa, Italy, between 2008 and 2010. About 30% of the city's secondary schools (high schools, technical schools and vocational schools) were randomly selected to participate in the study. Within each school, nearly 10% of the students were recruited by age-class (14-16 years and 17-19 years) by means of a randomized stratified sampling method. The boards of each school gave a permission for the testing the students.
As schooling is obligatory up to the age of 16 years in Italy, we recruited a higher number of subjects in the 14-16-year age-class (2:1) [18].

Smoker definition

We have adopted the World Health Organization (WHO) definition of smoker that defines a smoker as a person who smokes either daily or occasionally [19].

Questionnaire

We use an anonymous *ad hoc* questionnaire to record socio-demographic characteristics (gender, age, nationality, type of educational institution attended, family context, parents' education) and smoking habits (past and present smoking habits, age on first smoking experience). Questionnaires were self-administered and, in order to encourage openness and honesty in the answers, confidentiality and secrecy were ensured by avoiding any questions regarding the identity of the respondents. During administration of the questionnaire, physicians were available to explain the questions.
All questionnaires were checked on the basis of quality control.

Socio-demographic characteristics

Nationality, which was self-defined by the participants, was categorized as Italian or Foreign.
The type of family was subdivided into traditional and non-traditional. We considered married-parent families to be traditional, while any other family structure (e.g. single-parent family, remarried parent, etc) was defined as a non-traditional family. This distinction was prompted by the fact that several studies have reported different patterns of smoking among adolescents from families in which the parents are not married [20-22].
The educational level of the mother and father was classified as: high (high-school level or higher) or medium/low (middle-school level or lower).
The educational level of the participants was determined according to the type of educational institution attended. In Italy, the second phase of secondary education is broken down into 3 types of institution: high school, technical school and vocational school. High school is usually attended by students who want to continue their education at university; graduation from a technical school qualifies the individual to practice a technically skilled profession (e.g. chartered surveyor, accountant); vocational schools offer specialized training for specific occupations, such as electricians, designers etc. The data were dichotomized into two groups: (i) low educational level, which comprised vocational/technical schools and (ii) high educational level, which comprised high schools. This subdivision was based on the fact that, according to the Italian National Statistics Institute (ISTAT), more than 95% of high-school graduates continue their education at university, while the percentage of graduates from vocational (29%) and technical schools (53%) who try to enter university is significantly lower [23].

Statistical analysis

Quantitative variables are expressed as means and standard deviations (SD), and qualitative variables as frequencies and percentages with 95% Confidence Intervals (95% CI). Student's t test was used to estimate differences in age on first smoking experience between the different categories. The Kaplan-Meier product-limit method was used to evaluate the hazard rate of trying smoking at any age for males and females. The hazard rate is the instantaneous probability of smoking onset [9, 24]. In particular, age of the first cigarette was chosen as a dependent variable (for subjects declared to experiment with smoking at least once) while subjects who had never smoked were registered as censored observations. Fisher's exact test was performed to compare current smoking status among males and females and to ascertain whether there was a difference in smoking continuation between subjects who tried the first cigarette before or after the age of 14 years. Univariate logistic regression was first performed to determine whether smoking status (current and previous) was associated with gender, age-class, nationality, type of family, and parents' and participants' educational level. Multivariate logistic regression was then performed in order to

reduce confounding biases. The multivariate analysis considered significant variables obtained from the univariate analysis, in order to determine any association of each variable with current smoking status and previous smoking experience. Statistical analysis was performed by means of the SPSS 17.0 (SPSS, Chicago) software. A $p < 0.05$ was considered significant.

Results

A total of 2,500 students aged 14-19 years were invited to participate in the study, 61 of whom declined (response rate of 97.6% [95% CI: 96.9-98.1]). As few students refused to participate, the reasons for refusal were not investigated. The study therefore involved 2,439 students (926 males and 1,513 females). We excluded 138 questionnaires from the analysis on the basis of quality control. Thus, the pool of eligible participants consisted of 2,301 students (901 males [39.2%, 95% CI: 37.2-41.2] and 1,400 females [60.8%, 95% CI: 58.8-62.8]). The mean age of participants was 15.8 (SD 1.6) years (males: 15.6 [SD 1.6]; females: 16.0 [SD 1.6]). The socio-demographic characteristics of participants are reported in Table I.

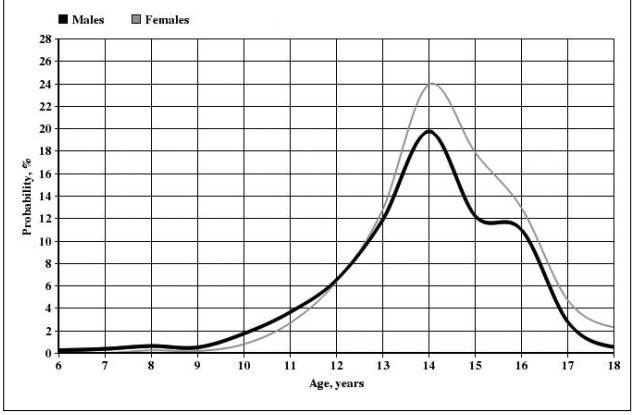
Of the students interviewed, 59.5% (95% CI: 57.4-61.6) stated having experimented with cigarette smoking at least once, and 35.6% (95% CI: 33.3-38.0) defined themselves as current smokers. The prevalence of smoking at the time of the study was similar among males and females (males: 35.2% [95% CI: 31.4-39.1]; females: 35.9% [95% CI: 32.9-38.9], p=0.83).

The mean age on first smoking experience was 13.5 (SD 1.7) years among current smokers and 13.8 (SD 1.8) years among subjects who had tried smoking at least once; this difference proved statistically significant ($p < 0.001$). Subjects who tried their first cigarette before 14 years of age were more inclined ($p<0.001$) to continue smoking than those who experimented with smoking at the age of 14 years or later (56.0% [95% CI: 51.5-60.5] vs. 41.6% [95% CI: 38.0-42.2]).

The mean age on first smoking experience differed significantly ($p < 0.001$) between males (13.5 years, SD 1.9) and females (13.9 years, SD 1.7). There was no difference in the mean age on first smoking experience between Italians and foreigners (13.8 and 13.9 years, respectively, $p = 0.72$), between students from traditional and non-traditional families (13.8 years for both), between low/medium and high educational level of the mother (13.8 years for both), between low/medium and high educational level of the father (13.9 and 13.8 years, respectively, $p = 0.38$), or between the educational levels of students: low educational level and high educational level (13.8 years for both).

The instantaneous probability of trying smoking changed substantially with age for both males and females (Fig. 1). Indeed, between the ages of 6 and 11 years, the risk of trying smoking was seen to increase slowly; after the

Fig. 1. Instantaneous probability of first smoking experience among participants.



Tab. I. Socio-demographic characteristics of participants.

Variable		Number (%)	95% CI
Nationality	Italian	2,174 (94.5)	93.5-95.3
	Foreign	81 (3.5)	2.8-4.3
	Not available	46 (2.0)	-
Age-class, years	14-16	1,536 (66.8)	64.8-68.6
	17-19	765 (33.2)	31.3-35.2
Family context	Traditional family	1,888 (82.1)	80.4-83.6
	Non-traditional family	311 (13.5)	12.2-15.0
	Not available	102 (4.4)	-
Type of secondary school	Vocational school	321 (14.0)	12.6-15.4
	Technical school	189 (8.2)	7.2-9.4
	High school	1,564 (68.0)	66.0-69.8
	Not available	227 (9.9)	-
Mother's level of education	High	1,631 (70.9)	69.0-72.7
	Medium/low	501 (21.8)	20.1-23.5
	Not available	169 (7.3)	-
Father's level of education	High	1,480 (64.3)	62.3-66.2
	Medium/low	590 (25.7)	23.9-27.5
	Not available	231 (10.0)	-

Tab. II. Univariate logistic regression for the main variables.

Current smoking status			
Variable	OR	95% CI	p value
Females vs. males	1.03	0.83-1.27	0.79
17-19 years vs. 14-16 years	1.97	1.59-2.43	<0.001
Foreigners vs. Italians	0.96	0.54-1.71	0.88
Non-traditional family vs. traditional family	1.62	1.22-2.15	0.001
Low/medium vs. high level of mother's education	1.44	1.12-1.84	<0.01
Low/medium vs. high level of father's education	1.31	1.04-1.65	<0.05
Low educational level vs. high educational level of participants	1.56	1.22-1.98	<0.001
Smoking experience			
Females vs. males	1.27	1.07-1.52	<0.01
17-19 years vs. 14-16 years	1.81	1.50-2.19	<0.001
Foreigners vs. Italians	1.02	0.63-1.65	0.94
Non-traditional vs. traditional family	2.21	1.67-2.94	<0.001
Low/medium vs. high level of mother's education	1.26	1.02-1.57	<0.05
Low/medium vs. high level of father's education	1.31	1.07-1.61	<0.01
Low educational level vs. high educational level of participants	1.88	1.50-2.34	<0.001

age of 12 years, there was a rapid increase, the maximum value (23.9% for females and 19.7% for males) being reached at 14 years. Subsequently, the risk diminished progressively, though this diminution was less marked between the ages of 15 and 16 years, especially among males.

Univariate logistic regression showed a significantly higher probability of being a smoker/experimenting smoking for higher age (17-19 years), non-traditional family context, low/medium education of parents, lower education of participants, and sex (for previous smoking experience only) (Tab. II). However, on multivariate logistic regression, only age, type of family and participants' educational level remained significant (Tab. III).

Discussion

Participation in the study was very high and the students showed considerable interest in smoking- related issues. The majority of interviewees stated that they had smoked at least once in their life. The prevalence of cigarette smoking among our respondents was higher than in some studies conducted in Italy in the same period as our research. Indeed, Gallus et al. reported a prevalence of 25.1% for 15-24-year-old males and 18.4% for 15-24-year-old females [6]. A survey conducted by the Italian Institute of Health (ISS) reported a prevalence of 21.9% among subjects aged 15-24 years [25] and Tramacere et al. found current-smoker prevalence rates among subjects aged 15-24 years of 29.6% and 23.3% in males and females, respectively [26].

The higher percentages in our study than in the three above-mentioned studies could be associated with the different definition of "current smoker" used in different studies. For instance, our "current smoker" definition was a person who smokes either daily or occasionally [19], while in the study conducted by Gallus et al., a current smoker was defined as someone who had smoked 100 or more cigarettes in his/her life [6]. The different forms of questionnaire administration may also influence the data collected. For instance, it has been shown that household surveys yield lower adolescent smoking rates than school-based surveys [27, 28].

Tab. III. Multivariate logistic regression for the main variables considered.

Current smoking status			
Variable	OR	95% CI	p value
17-19 years vs. 14-16 years	1.96	1.53-2.51	<0.001
Non-traditional vs. traditional family	1.44	1.02-2.04	<0.05
Low/medium vs. high level of mother's education	1.16	0.85-1.56	0.35
Low/medium vs. high level of father's education	1.06	0.81-1.40	0.67
Low educational level vs. high educational level of participants	1.39	1.06-1.81	<0.05
Smoking experience			
Females vs. males	1.11	0.90-1.35	0.33
17-19 years vs. 14-16 years	1.69	1.36-2.10	<0.001
Non-traditional vs. traditional family	2.22	1.57-3.14	<0.001
Low/medium vs. high level of mother's education	1.04	0.81-1.35	0.73
Low/medium vs. high level of father's education	1.11	0.87-1.40	0.40
Low educational level vs. high educational level of participants	1.75	1.37-2.40	<0.001

Our findings are in line with those of other studies. The Italian data from the HBSC study revealed prevalence rates of current smoking among 15-year-olds of 23% and 22% for females and males, respectively [11]; our study found a prevalence rate of current smoking among 15-year-olds of 20.7% (data not shown). A study conducted among 13-18-year-old students in Lombardy (Northern Italy) found prevalence rates of current smoking of 26.5% among 15-year-olds and 33.2% among 18-year-olds [29].

The mean age at which our respondents had first experienced smoking was 13.8 years, and the majority had smoked by the age of 16. This finding is in line with previous research in Italy and other Western European countries [11]. Moreover, in our study, males started smoking earlier than females. This finding is also in line with other research. Indeed, the European Tobacco Control Report revealed that boys started smoking earlier in almost all countries [11].

Subjects who experienced cigarette smoking before 14 years of age were more likely to carry on smoking than those who tried their first cigarette at the age of 14 years or later [9, 30].

Figure 1 shows that the risk trying smoking increases after the age of 11 years, and that this increase occurs after students move from elementary school to the first phase of secondary education; subsequently, the highest risk occurs after students transfer from the first phase to the second phase of secondary education (at the age of 14 years). Other researchers have also reported this trend [9].

On moving from childhood to adolescence, and in the first phase of adolescence, profound biological, social and relational changes take place. As personal identity forms, adolescents distance themselves from the family and forge closer bonds with peer groups, which become a new point of reference. Thus, adolescents feel the need to share emotions and experiences with their peers in order to feel part of the group. In terms of lifestyle, peer-group influence is a factor of primary importance and, according to previous research, the social exchange of cigarettes is one of the main sources of smoking among teenagers [31, 32]. In general, the influence of peer smoking is stronger than that of parental smoking, and this difference increases over time. However, the beneficial effect of having non-smoking friends seems to be greater than the deleterious effect of having friends who smoke [30]. Thus, the process of emulation and identification with the peer group could also be exploited as a strategy for the prevention of smoking [33].

After the age of 14 years, the risk of trying smoking decreases rapidly; interestingly, the decline in the risk of starting smoking appears to be less marked between the ages of 15 and 16 years, especially among males; this might be explained by the fact that 16 is the legal age limit for the purchase of tobacco products in Italy.

Our study highlights the importance of social and demographic factors that determine smoking habits. Regarding age, our study found a significantly higher prevalence of smoking among older students (17-19 years) than younger ones. Moreover, the highest percentage of smoking was seen among students attending vocational and technical secondary schools, a finding consistent with those of other studies [34, 35]. In addition, regular smoking proved to be statistically associated with a non-traditional type of family. Again, this is in line with previous research, which has suggested a higher risk of taking up smoking among adolescents living in single-parent families [21, 22] and step-parent families [20, 22]. Many authors have found that adolescents from less educated families are at greater risk of starting smoking [36]. This is very probably linked with the fact that parents with a low socio-economic status are more likely to be smokers [37], and parental smoking is a well-known predictor of smoking among offspring [38, 39]. In our study, a lower level of parental education was statistically associated with a higher prevalence of smoking among offspring in the univariate regression model; on multivariate regression, however, it did not prove significant.

In agreement with these observations and the WHO recommendations, curbing smoking should be an integral part of health policy objectives; these should include promoting healthy lifestyles and preventing the damage caused by smoking, through the adoption of multi-sector strategies and raising awareness among young people [40]. Moreover, in order to target adolescents at high risk of starting smoking, promotional and educational interventions should take into account differences in social, cultural, geographical and economic contexts. In this regard, the Italian Ministry of Health has activated a project "Gaining Health - Making healthy choices easier" (*"Guadagnare Salute - Rendere facili le scelte salutari"*) which provides multi-sector intervention to promote healthy lifestyles, not only by persuading smokers to give up smoking or to smoke less, but also by discouraging young people from starting [41]. The key points of this program are: the implementation of educational campaigns that target subjects by age, sex, family and work contexts; school-based programs; regulation of the sale of tobacco products; raising consumer awareness; the promotion of specific educational programs on smoking among doctors, healthcare workers and voluntary associations; strengthening anti-smoking centers and the development of initiatives for women (mothers and pregnant women). Indeed, our study reveals a great need for the prompt activation and enforcement of these interventions, the message to young people being that they can be successful and appreciated without smoking, and that smoking is harmful to the health of everyone.

The present study displays some limitations. First, our questionnaire did not contain items on the type and intensity of smoking (such as number of cigarettes smoked). Second, we did not investigate other social and environmental factors (such as parents' and friends' smoking) that can prompt young people to start smoking.

In conclusion the findings of our study highlight the fact that tobacco smoking is a major problem among adolescents and that the early onset of smoking is linked to a

high probability of continuing to smoke. Therefore, reducing smoking among adolescents is one of the most important challenges for public health authorities.
Finally, our study revealed great interest in smoking and smoking-related issues among adolescents. It would therefore be useful to introduce programs of health education into the secondary school curriculum, in order to promote a healthy lifestyle. Furthermore, a potentially effective approach may be the adoption of the peer education method, which has had great success in other programs of health promotion among young people.

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ORIGINAL ARTICLE

To start and quit smoking cigarettes: an evaluation of students in a Nigerian city

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Key words

Tobacco • Youths • University

Summary

Introduction. *Several factors have been shown to influence cigarette smoking and are important in creating measures for tobacco control. The aim of this study is to identify the factors responsible for making decisions to start or stop cigarette smoking among students.*
Methods. *This was a cross-sectional study which sampled 280 youths in tertiary institutions using multi-stage sampling technique. The data was collected using self- administered questionnaire that had been pretested and validated. Data analysis was done using SPSS version 16. Frequency tables and cross-tabulations were generated with a 95% confidence interval and predetermined p-value at less than 0.05.*
Results. *All the current smokers (100%) were males, most (73.2%) were within 21 to 25 years of age and 87.8% of them had a relative or friend who smoked. Some (29%) of the students who currently smoked were willing to quit smoking while 73.2% of them had ever attempted to quit smoking. Students who smoked to relieve stress were willing to stop smoking (100.0%), while 40% of those who smoked for pleasure/relaxation were willing to stop smoking. Students who had received lectures on smoking were significantly willing to quit (100.0%) compared with those who had not received such lectures (0.0%) (p=0.000).*
Discussion. *Understanding the role of the factors associated with smoking initiating and cessation is very crucial in planning appropriate intervention for the control of cigarette smoking among the youths and there is need for more youth oriented health education directed towards a proper attitude to tobacco control.*

Introduction

Cigarette smoking has been shown to adversely affect health and quality of life in humans of all age groups [1]. It also modifies and worsens several diseases like chronic obstructive airway disease (COPD) and has been implicated as a risk factor in the development of cancers, stroke, and ischaemic heart disease [2]. The cigarette smoking behavior mostly starts in adolescence and many smokers are initiated before the age of 16 [3, 4]. There are several preventive measures directed at reducing the prevalence of smoking and reducing the morbidity and mortality contributed by smoking- related diseases [5] but the prevalence of cigarette smoking is still high despite the measures in place for its control. According to the World Health Organization (WHO) report on Tobacco Use and Its Impact on Health [6], the global prevalence of cigarette smoking is 24% while the Center for Disease Control and prevention (CDC) stated that the United States of America has a tobacco use prevalence of 19.3% [7]. In England, the prevalence of tobacco smoking is 21% among adults but higher (27%) among those aged 20-24 [8] while the prevalence among adults aged 15 and above in South Africa is 27.1% [9]. In Nigeria, according to a study carried out in Ilorin by Fawibe and Shittu [3], 5.7% of university students were current smokers.

The attitude to smoking among young adults is vital to the tobacco control processes. Research has shown that up to 84% of current school adolescent smokers do not believe that cigarette smoking is harmful to health [10]. It is important to determine the factors responsible for initiating smoking so as to understand and create interventional measures that will be relevant for tobacco control. In previous studies, peer pressure, parental influence, stress, pleasure, lower self-esteem, absence of home-smoking restriction and exposure to cigarette adverts are very important sources of cigarette smoking initiation among the youths [11-13]. According to a study carried out among students in Kwara, Nigeria, 55.9% of smokers were introduced to cigarette smoking by their friends [14]. Also, alcohol intake and marijuana use have been shown to have an effect on cigarette smoking habits [15].
The desire and attempt to quit smoking have been studied over the years and certain factors have been associated with deciding to quit cigarette smoking. According to Arnsten et al. [16], 33.3% of the respondents intended to quit within the next six months and factors associated with their desire to quit include smoking related symptoms, social support for quitting and self efficacy (confidence in one's ability to quit). The effect of social support was also highlighted in another study [17] in which subjects were found to be quitting in groups. In this

study, cessation of smoking by a spouse reduced a person's chance of smoking by 67%, cessation by a friend reduced the chance by 36% and cessation by a sibling reduced the chance by 25%. Teater and Hammond [18] in their study found that 55.2% of respondents were planning to quit while 33.5% had made an attempt. They also discovered that people who smoked for 30 of the last 30 days before their research were least likely to desire to quit. Other factors that have been shown to impede readiness to quit smoking include depression and alcohol use [19].

Several factors have been shown to influence the onset and cessation of smoking of cigarette smoking among youths. Such factors directly or indirectly contribute to disease pattern, morbidity and mortality as well as social disruptions. These factors are important in further creating measures for tobacco control [20]. The aim of this study is to identify the factors responsible for making decisions to start or stop cigarette smoking among students in a tertiary institution in southwestern Nigeria in order to offer recommendations towards further research and policy making.

Materials and methods

This was a cross-sectional descriptive study of students in tertiary institutions carried out in 2012.

The sample size was calculated using Fischer's formula

$$N = \frac{Z^2pq}{d^2}$$

Where:

N= sample size

Z = Standard normal deviation (1.96)

p = Population in the target population estimated to have a particular characteristic (in this case, prevalence of smoking among youths= 0.21 [21])

q = 1.0 – p

d = degree of accuracy required (0.05)

$$N = \frac{1.96^2\ p\ (1\text{-}p)}{0.05^2}$$

$$= \frac{1.962 \times 0.21 \times 0.79}{0.052}$$

$$= \frac{0.9600}{0.0025}$$

$$= 254$$

The calculated sample size of approximately 254 was further increased to 280 to make up for cases of attrition. Multi stage sampling technique was used to select the students to whom the questionnaires were administered. There are two universities in Ekiti State: Afe Babalola University, Ado- Ekiti and University of Ado-Ekiti. One of the Universities was chosen by simple random sampling technique in the first stage, and five departments were selected in the second stage of sampling using simple random sampling technique by balloting.

In the third stage, systematic sampling was used to select the respondents. The total list of the students in the selected departments served as the sampling frame and the sampling interval was determined by dividing the sampling frame by the sample size. The respondents were identified by matriculation numbers, departments, levels of education and the number on the sampling frame. Students that were not around during the time of the study or those that were not willing to participate were excluded from the study. Such students were replaced by the next person in the sampling frame.

The research instrument was a questionnaire that was pre-tested among 30 students of College of Education, Ikere Ekiti and validated by a departmental panel. The questionnaire was self-administered after obtaining an informed consent from each respondent and designed to elicit information on socio-demographic characteristics, reasons for initiating smoking, cigarette smoking pattern and reasons for quitting (for those that have stopped smoking). Data generated were edited for errors and entered into the computer for analysis with SPSS version 16 using descriptive statistics while association was established with Chi square with 5% level of significance. Ethical clearance for the study was obtained from the Ethical Committee of the Federal Medical Centre, Ido-Ekiti.

Results

Out of the 280 students sampled, 41 were current smokers giving a smoking prevalence of 14.6%. Most of the current smokers (73.2%) were within 21 to 25 years of age (Tab. I), while the mean age was 21.78 ± 2.35 years. All the current smokers (100%) were males, and most (78.0%) were Christians. The monthly expenditure of the smokers were ≤ ₦5000 (31.25 US Dollars) (34.2%), ₦5000 – ₦10000 (31.25-62.50 US Dollars) (43.8%), and > ₦10000 (> 62.50 US Dollars) (22.0%). The mean monthly expenditure was ₦10,244 (64.03 US Dollars).

Tab. I. Socio-demographic characteristics of current smokers.

Variable	Frequency N = 41 (%)
Age Group (Years)	
18-20	11 (26.8)
21-25	30 (73.2)
Mean Age: 21.78±2.35 (Range: 18-30)	
Sex	
Male	41 (100)
Female	0 (0)
Religion	
Christianity	32 (78.0)
Islam	9 (22.0)
Monthly expenditure Naira (Dollars) *	
< 5000 (< 31.25 US Dollars)	14 (34.2)
5000-10000 (31.25-62.50 US Dollars)	18 (43.8)
> 10000 (> 62.50 US Dollars)	9 (22.0)
Mean monthly expenditure: 10,244 (64.03 US Dollars)	

(* 1 US Dollar = 160 Naira).

Tab. II. Determinants of cigarette smoking initiation and sustenance.

Factor	Frequency N = 41 (%)
Main location of smoking	
Parties/Clubs	19 (46.4)
Friend's house	11 (26.8)
In school	6 (14.6)
Hostel	5 (12.2)
Relative/friend who smoke	
Yes	36 (87.8)
No	5 (12.2)
Ever asked to quit	
Yes	35(85.4)
No	6 (14.6)
Marijuana use	
Yes	11 (26.8)
No	30 (73.2)
Ever been asked for proof of age when buying cigarette	
Yes	16 (39.0)
No	25 (61.0)
Smoking restrictions at home	
Yes	28 (68.3)
No	13 (31.7)

Majority of the smokers, 19 (46.4%) smoked in parties/clubs (Tab. II), while some of them, 11 (26.8%) smoked in friend's houses. Thirty six (87.8%) of the smokers had a relative or friend who smoked, and 35 (85.4%) of them had been ever asked to quit smoking. Eleven (26.8%) of the students who currently smoked also use marijuana. Only 16 (39%) of the smokers had been asked proof of age, while 28 (68.3%) had smoking restrictions at home.

Tab. III. Attitude to tobacco control.

Attitude		Frequency N = 41 (%)
Support smoking control		
	Yes	32 (78.0)
	No	9 (22.0)
Attempt to quit		
	Yes	30 (73.2)
	No	11 (26.8)
Support smoking ban		
	Yes	8 (19.5)
	No	24 (58.5)
	Abstain	9 (22)
Increase tax on cigarette		
	Yes	8 (19.5)
	No	24 (58.5)
	Abstain	9 (22.0)
Ban cigarette adverts		
	Yes	32 (78.0)
	No	0 (0.0)
	Abstain	9 (22.0)
Stop cigarette production		
	Yes	8 (19.5)
	No	24 (58.5)
	Abstain	9 (22.0)

Table III shows the attitude of current smokers to smoking, with 32 (78.0%) of them supported smoking control as well as banning of cigarette adverts, and 8 (19.5%) supported smoking ban, increase in tax on cigarette, and stopping cigarette production out rightly. While only 29% of the students who currently smoked were willing to quit smoking, 30 (73.2%) of them had ever attempted to quit smoking.

Students who smoked to relieve stress were more willing to stop smoking (Tab. IV), while those who smoked for pleasure/relaxation were not. This was statistically significant. There was more willingness to cease smoking among students who were introduced to smoking by their friends (outside school) than those who were introduced to smoking by their siblings or colleague at school, with statistical significance. Students who had received lectures on smoking were significantly willing to quit compared with those who had not received such lectures. Also, use of marijuana was found to be associated with less willingness to cease smoking. This was statistically significant.

Discussion

The prevalence of smoking from this study was 14.6%. Relatively higher prevalence rates of smoking were reported in other studies: 19.3% from a CDC study in the United States of America [7], 21% from a study in England [8], and 27.1% from a South African study [9] in which the prevalence of smoking among adults aged 15 and above was 27.1%. This may imply that there is still a high prevalence of cigarette smoking despite global efforts at curbing the menace. Most of the current smokers (73.2%) were within the age range 21 to 25 years of age. This is comparable to a study done in England where a higher prevalence (27%) of smoking was found among those aged 20-24 years [8]. Intervention measures would have to focus on these adolescents and young adults as they have been found to have a high smoking prevalence. The decision to smoke among students from our study is mostly influenced by having a relative of friend who smoked, as found out in 36 (87.8%) of the current smokers. Introduction to smoking by friends was also reported by a study in Kwara, Nigeria where 55.9% of smokers were introduced to smoking by their friends [14]. Considering that 28 (68.3%) of smokers had smoking restrictions at home, the commonest place where smoking is carried out is in parties/clubs (46.4%), while some, 11 (26.8%) smoked in friend's houses. Such venues are more conducive for decision to smoke as there are no restrictions.

Smoking among the students is also sustained by Marijuana use. It was found that eleven (26.8%) of the students who currently smoked also use marijuana. Use of Marijuana and alcohol intake has been identified by previous studies as having effect on smoking habits [15]. Though 35 (85.4%) of smokers in our study were ever asked to quit smoking, they had sustained the habit. It was also noted that only 16 (39%) of the smokers had been asked proof of age when buying

Tab. IV. Factors associated with willingness to smoke.

Characteristics of smokers	Willingness to quit (%)	
	No	Yes
Willing to quit	9 (21.9)	32 (78.1)
Reasons for smoking		
As a habit		5 (15.6)
For pleasure/relaxation	9 (100)	6 (18.8)
To reduce weight	0	3 (9.4)
To relieve stress	0	18 (56.3)
Total	9 (100)	32 (100)
Who introduced student to smoking		
Sibling	3 (33.3)	8 (25)
Colleague at school	6 (66.7)	8 (25)
Friends at home	0 (0)	16 (50)
Total	9 (100)	32 (100)
Received lecture on smoking		
Yes		32 (100)
No	9 (100)	0 (0)
Total	9 (100)	32 (100)
x2 = 84, p = 0.000		
Marijuana use		
Yes	3 (33.3)	8 (25)
No	6 (66.7)	24 (75)
Total	9 (100)	32 (100)
x2 = 1.31, p = 0.000		

cigarette, indicating the low level of societal contribution to smoking control. It was found out that many of the current smokers do not support strict intervention measures for tobacco control, including support for smoking ban (Yes, 19.5%), increase in tax on cigarette (Yes, 19.5%), and stopping cigarette production (Yes, 19.5%). They however support seemingly lighter smoking control measures (78.0%) and ban on cigarette adverts (78.0%).

While 32 (78.1%) of the students who currently smoked were willing to quit smoking, 30 (73.2%) of them had ever attempted to quit smoking. These values are different and more than those reported by Arnsten et al. [16] in a study in which 33.3% of the respondents intended to quit smoking within the next six months. They are also higher that what was observed by Teater and Hammond [18] who reported a 55.2% level of willingness to quit smoking in their study and recorded that 33.5% of smokers made an attempt to stop smoking. This disparity could be because many of the respondents (78.1%) in this study had received lectures on the risk of smoking, 85.4% had been asked to quit and up to 78.0% would support smoking control. This further shows the importance of organizing enlightenment lectures and programmes on the risks of tobacco smoking for young persons.

We found out that there was more willingness to stop smoking among students who smoked to relieve stress than among those who smoked for pleasure/relaxation. This could be due to the role of addiction in sustaining smoking habit. There was also more willingness to cease smoking among students who were introduced to smoking by their friends than those who were introduced to smoking by their siblings or colleague at school. This implies that smoking interventions should focus on peer groups for effectiveness in curbing smoking, as other studies have also identified the role of social support in smoking cessation showing that subjects quit smoking in groups [17]. Also, use of marijuana was found to be associated with less willingness to cease smoking. This compares to another study which shows that alcohol use impedes readiness to quit smoking [19]. In conclusion, understanding the role of these factors in smoking initiating and cessation is very crucial in planning appropriate intervention for the control of cigarette smoking among the youths. An important factor influencing willingness to quit smoking is being lectured on smoking. Students who had received lectures on smoking were significantly willing to quit compared with those who had not received such lectures. Thus, there is need for more health education towards a proper attitude to tobacco control and in the adolescents and young adults.

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ORIGINAL ARTICLE

Impact of anti-tobacco warning labels on behaviour of tobacco users in one of the cities of Gujarat, India

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Key words

Awareness • Impact • Tobacco users • Warning labels

Summary

Background. *Tobacco use continues to be the leading global cause of preventable deaths, killing nearly 6 million people worldwide each year. Tobacco control must be given the high priority by scaling up tobacco control measures. In India under Control of Tobacco Product Act, it is mandatory to keep the warning labels over all kind of tobacco products in order to minimise the use of tobacco.*
Objectives. *Review of the knowledge regarding warning labels printed on tobacco products among its users and to evaluate the impact of them on addicting behaviour.*
Methodology. *A Cross Sectional study was carried out among the group of people using tobacco in any form. Total 776 tobacco users were enrolled in the study.*
Results. *Mean age of tobacco user was 41.4 years. Out of total 776 tobacco users, 561(72.3 %) had ever noticed warning signals over the tobacco products. Among those who have noticed warning labels, 64.4 % became aware about health effects and 66% have thought to quit tobacco. Tobacco users of young age group (15-45) were more aware regarding warning labels. Females were less aware. As level of education increases number of tobacco users who tried to quit or reduced the daily quantity of tobacco intake were also increases.*
Conclusions. *Positive impact of warning labels has been seen among the tobacco users who have noticed them. Not all the tobacco users were aware regarding the presence of warning labels as per the findings of present study.*

Introduction

Tobacco is the prime and most perilous killer of humanity probably since its discovery. From all its care-takers in farms to stakeholders and mainly its users are suppose to suffer from multiple health hazards – the ultimate outcome of which is premature miserable death. Almost one million annual deaths from tobacco-related diseases occur in India, the world's second largest consumer of tobacco, where about one-third of adults use some form of tobacco [1-3]. According to the Million Death Study, Smoking alone causes 10% of all deaths. One in five of all adult male deaths and one in twenty of all adult female deaths in middle age are due to smoking.
In India, around 34.3% of youth are exposed to passive smoking at home. Among Daily tobacco users, 60.2% consumed tobacco within half an hour of waking up [4]. In India, beedi smoking is most popular form of tobacco smoking, followed by cigarette smoking. Paan with tobacco is the major chewing form of tobacco. Dry tobacco-areca nut preparations such as paan masala, gutka and mawa are also popular and highly addictive.
Indian Parliament passed the Cigarette and Other Tobacco Products (prohibition of Advertisement and Regulation of Trade and Commerce, production, supply and Distribution) Bill, 2003. One of the key provisions under this act is health warnings in both written and pictorial form. Health warnings on tobacco packages are among the most widespread policy initiatives implemented to raise awareness of the health risks of tobacco as well as to encourage consumers to quit. Communicating the health hazards of tobacco use is a primary goal for tobacco control Policy.
Despite such efforts to inform consumers about the dangers of tobacco products, little is known to them about the content of or even the existence of warning messages. Little is known about the impact of pictorial warnings or health messages on tobacco products on people's behavior. An analysis of the awareness of the presence of warning messages among tobacco users is important. Present study was conducted to know about the level of awareness about anti tobacco warning labels, among the current tobacco users residing in the urban slums of Ahmedabad City in Gujarat province of India.

Objectives

1. To study the pattern of use of tobacco among the tobacco users.
2. Review of the knowledge regarding warning labels printed on tobacco products among tobacco users.
3. To evaluate the impact of the existing textual and pictorial warnings.

Materials and methods

A Cross Sectional study was carried out among the group of people using tobacco in any form. A Questionnaire used for data collection was pre-designed. Questionnaire was designed so as to collect information regarding socio demographic profile of tobacco users, pattern of tobacco use and any kind of awareness about warning lables either in written form or in pictorial form. It was pre-tested by carrying out pilot study. Modifications were made in the questionnaire based on the findings of pilot study. Type of questionnaire was close ended, that require yes or no answer. Data we collected were of qualitative type.

Sample size was calculated by using following formulae [5]:

Sample size = $4pq/L^2$ (for 95% confidence)

where,

p is the prevalence of tobacco users (Which was kept as 34% as per the recent "Global Adult Tobacco Survey (GATS) India" report [1]

q = 1-p ,

L= Allowable error, which was kept as 10% of p for present study. So it was 10% of 34 i.e. 3.4.

So the calculated sample size was 776.4, to round about the figure it was taken as 776.

Thus, total 776 tobacco users residing in the field practice area covered under Urban Health Training Center of one of the teaching institutes in Ahmedabad city of Gujarat Province of India were selected. Personal interview technique was used for the data collection. Investigators visited each tobacco users and interviewed them personally. Informed verbal consent was taken prior to data collection. Those who refuse to give consent were excluded from the study. Study was carried out between March-May 2012. Data analysis was done by using SPSS software. Chi- square test was used as a test of significance considering the data as qualitative.

Results

Total 776 tobacco users have participated in the study. Maximum numbers of tobacco users (43.2%) were between 35 and 55 years. Mean age of tobacco user was 41.4 years with standard deviation of 14.6 years. 592 (76.3%) were males while 184 (23.7%) were females. Out of 776, 639 (82.3%) were married and 181(23.3%) were illiterate. 554 (71.4%) were currently employed and 11 (1.4%) were students yet (Tab. I). Out of 776, 523 (67.4%) were using smokeless form of tobacco, while 217 (28%) were consuming tobacco by smoking. 36 (4.6%) were consuming tobacco in both forms. Smokeless forms of tobacco included Gutkha, Masala and Snuff where as smoking included bidi and cigarette (Fig 1). 426 (54.9%) were using tobacco in any form since more than 10 years. 168 (21.6%) had started using tobacco since < 1 year (Fig 2). Out of total 776 tobacco users, 561 (72.3%) had ever noticed warning signals over the tobacco products. On doing analysis, it was found that tobacco users of young age group (15-45) were more aware regarding warning labels whereas tobacco users from extremes of age were less aware about the same. Gender difference regarding awareness about warning message was also statistically significant – females were less aware than males. Education wise comparison showed that literate tobacco users had more awareness towards the warning labels as compared to illiterate. Findings were significant statistically. Tobacco users who were consuming tobacco since less than 5 year were having more awareness regarding warning labels. Thus the level of awareness regarding warning

Tab. I. Socio demographic profile of study population (n = 776).

Socio Demographic Variables	Frequency	Percent
Age group		
< 15	5	0.6
15-24	99	12.8
25-34	154	19.8
35-44	170	21.9
45-55	165	21.3
56-64	129	16.6
> 65	54	7.0
Gender		
Female	184	23.7
Male	592	76.3
Marital Status		
Married	639	82.3
Un married	94	12.1
Widow/Widower	43	5.5
Education		
Illiterate	181	23.3
Primary	225	29.0
Secondary	238	30.7
Higher secondary	71	9.1
Graduate and Above	61	7.9
Employment		
Non working	211	27.2
Working	554	71.4
Students	11	1.4

Fig. 1. Distribution of the study population as per the use of tobacco products in different forms.

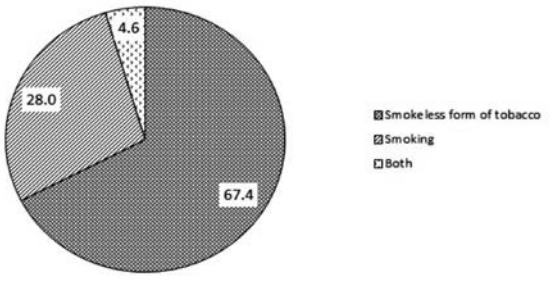
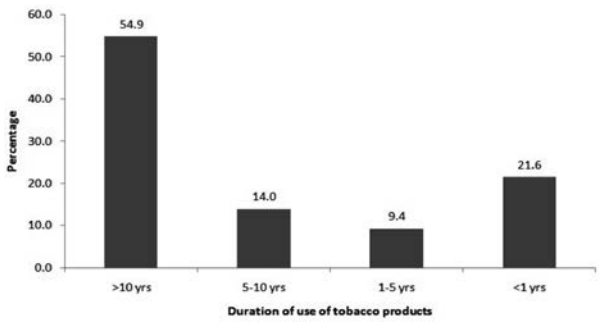


Fig. 2. Duration of use of tobacco products.



labels was significantly differs among tobacco users with different socio- demographic profile (Tab. II). The positive impact of education was seen in the perception of warning signals. As the level of education increases number of tobacco users who tried to quit the tobacco or reduced the daily quantity of tobacco intake were also increases. They become more aware about health hazards of tobacco (Tab. III). It was found statistically significant that the addictors using either smokeless form of tobacco alone or both smokeless and smoking form were more aware about health hazardous message or pictorial warnings than the addictors who use smoking form alone (Tab. IV).

Discussion

Out of 776, 523 (67%) were using smokeless form of tobacco including gutkha, masala and snuff, while 217 (28%) were using consuming tobacco by smoking. 36 (5%) were consuming tobacco in both forms. Our findings were similar to the findings of Global Adult Tobacco Survey [1] who found that the majority of tobacco users (60%) consume only smokeless tobacco. In present study, 426 (54.9%) were using tobacco in any form since more than 10 years. 168 (21.6%) had started using tobacco since < 1 year. Out of total 776 tobacco users, 561 (72.3%) had ever noticed warning signals over the tobacco products. Results were almost similar to that found in the study of Aravind Karinagannanavar et al. [6] who mentioned that 72.5% of total participants had seen the pictorial warnings.

Warning labels do have the impact on changing behavior of the tobacco users. In present study, among those who

Tab. II. Awareness about warning labels among tobacco users.

Variables	Warning signals not seen (%)	Warning signals seen (%)	Total	Chi-Square	p value
Age group					
< 15 (n = 5)	2 (40)	3 (60)	5		
15-24 (n = 99)	16 (16.2)	83 (83.8)	99		
25-34 (n = 154)	28 (18.2)	126 (81.8)	154		
35-44 (n = 170)	40 (23.5)	130 (76.5)	170	42.9	< 0.001
45-55 (n = 165)	49 (29.7)	116 (70.3)	165		
56-64 (n = 129)	51 (39.5)	78 (60.5)	129		
> 65 (n = 54)	29 (53.7)	25 (46.3)	54		
Gender					
Female (n = 184)	104 (56.5)	80 (43.4)	184	98.1	< 0.001
Male (n = 592)	111 (18.7)	481 (81.2)	592		
Education					
Illiterate (n = 181)	97 (53.6)	84 (46.4)	181		
Primary (n = 225)	64 (28.4)	161 (71.6)	225		
Secondary (n = 238)	45 (18.9)	193 (81.1)	238	98.5	< 0.001
Higher secondary (n = 71)	5 (7.0)	66 (93.0)	71		
Graduate and above (n = 61)	4 (6.6)	57 (93.4)	61		
Duration of tobacco use					
> 10 yrs (n = 426)	129 (30.3)	297 (69.7)	426		
5-10 yrs (n = 109)	17 (24.0)	92 (76.0)	109	17.4	< 0.01
< 5 yrs (n = 241)	44 (18.2)	197 (81.7)	241		
Total	215	561	776		

Tab. III. Education versus affirmative effect of warning signals on the tobacco users.

Education	Effect of warning signals			Total
	Tried to quit (%)	Reduced the quantity (%)	Aware about health hazards (%)	
Illiterate	48 (57.1)	57 (67.9)	46 (54.8)	84
Primary	85 (52.8)	120 (74.5)	85 (52.8)	161
Secondary	123 (63.7)	149 (77.2)	119 (61.7)	193
Higher secondary	41 (62.1)	52 (78.8)	42 (63.6)	66
Graduate and Above	44 (77.2)	47 (82.5)	42 (73.7)	57

Tab. IV. Impact of type of tobacco addiction on awareness regarding warning labels on tobacco products.

Type of tobacco used	Awareness regarding warning labels		Total
	Absent (%)	Present (%)	
Smokeless form	115 (23.0)	408 (78.0)	523
Smoking	92 (42.4)	125 (57.6)	217
Both	8 (22.2)	28 (77.8)	36
Total	215	561	776

Chi square – 32.4 P < 0.001

were aware about warning signals, 82.2% said that they had reduced the quantity after seeing the warning labels, whereas 64.4% became aware about health effects and 66% have thought to quit tobacco. In India, three in five current tobacco users (61.1%) noticed the health warning on tobacco packages and one in three current tobacco users (31.5%) thought of quitting tobacco because of the warning label [4]. Aravind Karinagannanavar et al. [6] mentioned that among the subjects who had seen the pictorial warning, 111 (25.5%) had interpreted correctly and 63(14.5%) had given a thought/ tried to reduce or quit tobacco consumption. As per the findings of European commission, one fifth of smokers reported that health warnings have been effective in getting them to smoke less and in helping them try to quit [7]. In countries with pictorial health warnings, such as Canada and Australia, impact of warning labels were high. More than 40% of Canadian smokers report that the pictorial warnings have motivated them to quit smoking. While among Australian smokers, 57% reported that the labels made them thing about quitting [8, 9].

In our study we found that tobacco users from age group of 15-45, Male, better educational status and who were consuming tobacco since less than 5 year all were more aware regarding warning signals. Results were significant statistically. Findings of present study were similar to those found in study of Aravind Karinagannanavar et al. [6]. They found that younger age group (< 25 years), better educational status, lesser duration (< 5 years) of tobacco usage was found to have significant association with awareness about pictorial warnings on tobacco products.

Impact of Education is there on the perception of warning signals. In present study 53.6% of illiterates were not ware about the warning labels where as in the study of G N Karibasappa [10] 40.6% of illiterates were not aware about the warning labels. As the level of education increases number of tobacco users with positive impact of warning labels increases. In present study, group of tobacco users who came in the category of graduate and above, 44 (77.2%) had tried to quit, 47 (82.5%) had reduced the quantity while 42 (73.3%) became aware of health hazards. GN Karibasappa in their study mentioned that awareness and impact of pictorial warnings was highest among graduates [10].

Conclusions

Though being stamped as one of the most common killer, tobacco is still being used passionately by the people especially young ones. Throughout the world almost all governments are making some form of legislations to increase the awareness about health hazards of tobacco products and to decrease the number of deaths directly or indirectly consequence of tobacco addiction in any form. It was accomplished from the findings of present study that pictorial message/photographic material can convey the relevant message to illiterates as well as enhance the impact for those who are literate. Although some of the addictors are so ardent for it that they are overlooking any kind of health warnings or messages but still there are many who have tried to quit or at least reduced the quantity after seeing the warning messages over the tobacco products. It was also concluded that those using only smoking form of tobacco were having more awareness regarding tobacco warning signals. Apparently it shows that tobacco users are presuming smokeless form of tobacco as less hazardous and were not keen to pay attention towards health warnings.

Recommendation

The education was found to be a mammoth savior to quit the tobacco or to reduce the daily quantity gradually. The same should be utilized for peer groups or for their cohabitants by means of educating them about health hazards of tobacco. From very initial years of study, i.e. possibly from primary school education, the health hazards of tobacco must be thrusted in the brains of young students that they can't think of addiction. Again the duration of addiction was also found posi-

tive factor. The recent users of any tobacco form must be taken care of by health workers and proper planning should be done for their counseling and rehabilitation by proper person. School and colleges should organize such screening camps frequently in good faith of their students – the would be growth engine of the country. In majority regions of India, it can be found that smokeless form of tobacco (i.e. mainly paan-masala, mawa and up to some extent gutkha) are being sold by local shopkeepers which is prepared instantaneously on demand and packed in hand made pouches which do not contains any kind of health warnings and messages. Strict enforcement of law in this regard should be enacted.

Limitation of the study

The personal interview technique was used in present study for data collection. One of the limitations for such kind of studies is that authors will have to rely on responses given by the study population.

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ORIGINAL ARTICLE

Risk perception of sexually transmitted diseases and teenage sexual behaviour: attitudes towards in a sample of Italian adolescents

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Key words

Sexually transmitted diseases • Youth • Knowledge

Summary

The aim of the study is to determine awareness about sexually transmitted diseases (STDs) and their prevention in people aged 14-19 of Ferrara and province.
The study was carried out using a self-administered standardised anonymous questionnaire in a sample of students attending to three upper secondary schools.
Total number of collected questionnaires was 2695, the average age of interviewed was 17.1. Only 52.3% of respondents correctly recognized STD definition. Over 95% of subjects identified acquired immune deficiency syndrome (AIDS), while properly classification of Hepatitis B increased with age and lowest degree of knowledge concerned herpes infection and Candidiasis. Sex without condom (95.97%) and needle exchange in drugs abusers (94.9%) are considered high risk behaviours. 80.3% of interviewed, without distinction of school attendance, sex, and age considered lack of information as a situation of high risk. Condoms are not used by 46.4% of the subjects in case of sex with a regular partner and by 9.5% with casual partner. Majority of students declared condoms very safe in preventing STDs but an important percentage indicated also contraception methods; correct answers were higher among females and increased with age. Main sources of information were TV (21.6%), school (21.1%) and friends (14.8%) and a few sought information from family doctor (7.4%) and web (4.8%).
The study suggests, as priority, to improve teenagers' awareness about risk behaviours and prevention of STDs. School can play an important role in reinforcement of sexual education programmes and directing young people to general practitioners and primary sexual health care services.

Introduction

Sexually Transmitted Diseases (STDs) are a large group of infectious diseases associated with different aetiologies (bacteria, chlamydia, mycoplasma, fungi, viruses, protozoa and ectoparasites), and characterized by their sexual transmission and genital location [1]. The risk of severe complications and their role in the increased risk of human immunodeficiency virus (HIV) transmission [2] make STDs a social problem worldwide. In fact, several studies demonstrated a strong association between both ulcerative and non-ulcerative STDs and HIV infection [3, 4], so that there is clear evidence that conventional STDs increase the likelihood of HIV transmission [5]. The World Health Organization (WHO) estimated 333 million/year worldwide the total of STDs cases, mostly localized in the south-east Asia, in Africa, and Latin America [6], where the higher prevalence is seen among sexually active young people [7]. The sex-related incidence of STDs is greater among males than in females (except during adolescence, when the ratio is reversed), yet the prevalence is greater among women and homosexual men [8, 9].
The current spread of STDs is related with a number of different factors involving not only their aetiology (emerging pathogens, and antibiotic resistance), but also inadequate health information [10], low income [11], and modified sexual lifestyles (frequent unprotected intercourse with different partners, an increased demand for prostitution, and the decreasing average age at which young men and women become sexually active) which have occurred in the general population in the last 50 years [12]. In regards to this last feature, adolescents and young adults (15-24 years old) represent only 25% of the sexually active population but almost 50% of all new acquired STDs [12] – this is caused by sexual ignorance, non-use of condoms, increased number of relationships between young subjects and older partners, use of psychoactive substances, and poor attitude towards consulting specialist health care services [13]. In a recent randomized controlled trial, it was demonstrated that the behavioural intervention promoted by Sexual Awareness For Everyone (SAFE) on teenagers (aged 14 to 18 years) significantly decreased high-risk sexual behaviours [14].
In Italy, even though adolescents have their first sexual experience at a very early age, and often without protection against STDs [15], a health education programme has been running for the last several years, with special attention given to the prevention of STDs [16]. In order to determine how information and awareness about STDs may modify sexual behaviours of Italian adolescents, a study was per-

formed on a sample of students of 14-19 years living in Ferrara and its province.

Materials and methods

Study design

A cross-sectional study of a sample of students population attending upper secondary school was developed to achieve the following objectives: a survey of the knowledge of STDs, the source of related information, knowledge level related risk and its influence in determining sexual lifestyles.

The questionnaire

A structured self-administered questionnaire containing 18 closed questions was used for data collection. The aim of this approach was to ensure that answers could be reliably aggregated and that comparisons could be made with confidence between subgroups or different survey periods. The design of the questionnaire was developed following the international guidelines [17]. The questions were close-ended, providing a set of responses or options from which respondents indicated their choice. (Close-ended questions are particularly useful where the study concerns factual issues, and can be narrowed down to a limited range of responses.)
As to the content of the questions, the items were formulated considering the following features:
1) basic knowledge of STDs and the risks involved;
2) perception of risk and measures to prevent it;
3) sources and quality of information;
4) sex and age.
To determine the best formulation and sequence of the questions, a pilot study in two phases with an increasing number of respondents was performed. In the first phase we asked each respondent of a small group the degree of understanding of each item in the questionnaire, the degree of self-awareness for each question asked, and the appropriateness of the terminology used. In the second phase the entire questionnaire was administered – based on the responses, it was corrected, supplemented, and amended. The distribution of questionnaires took place in the course of one day in all the considered school-classes. The completion time was 10 minutes.

The setting

The present study was performed in three different upper secondary schools located in the Emilia-Romagna Region (Northern-Italy).

The population

This investigation has considered students of both sexes, 15-19 years of age, attending upper secondary school. In order to verify the existence of different knowledge levels in students from different kinds of school, the questionnaire was distributed in three different high schools, one focussing more on humanities, one on sciences and one on technical studies. The sampling of the population was conducted among the students from the towns of Ferrara and Cento. In view of the sensitivity of the topics covered by the questionnaire, the under-age students needed written consent of their parents in order to take the test.

The data analysis

Statistical analysis was calculated by chi-squared test.

Results

This study was completed between December 2011 and February 2012. The sample consisted of 2800 students – 26.9% of the entire school population of the province of Ferrara. The questionnaire was completed by 2695 students, representing 96.2% of the considered population sample and 42.6% of the students attending upper secondary schools in the province of Ferrara. The average age of the respondents was 17.1 years (SD ± 1.25). The group of respondents consisted of 1145 males (42.5%) and 1550 females (57.5%). The division by year of attendance was the following: second year: 34.8% (mean age 15.9 years), third year: 22.8% (mean age 16.8 years), fourth year: 18.6% (mean age 17.8 years), fifth year: 23.8% (mean age 18.7 years).
The first area of investigation was the real knowledge of what an STD is (Tab. I). Five definitions were proposed to define this point. The 52.3% of respondents answered correctly, with a no significant difference among sexes ($p = 0.1352$). In contrast with the fact that the second-year students had many uncertainties [60.4% gave wrong answers], 65.5% of the fifth-year students were able to identify the correct definition ($p < 0.0001$). The kind of school attended by the students had no significant influence on their answering ($p = 0.1352$) .
To better determine the information level among the students, a list of 17 diseases was included (Tab. II). The results were fairly uniform only for AIDS, which has been correctly recognized by over 95% of subjects with no significant difference among sexes. Although the Hepatitis B had a considerable percentage of proper identification among all students (total: 74.6%, males: 70.9%, females: 77,3%), the level of knowledge increases with age, going from 73.0% in the 15-16 years old subgroup to 78.0% for students aged 18-19 ($p = 0.0294$). A slightly lower level of knowledge was demonstrated with regards to herpes infection (total: 69.1%) with a significant difference ($p < 0.0001$) between younger and older students of both sexes (62.0% and 74.2% respectively). Candidiasis was correctly recognized by 65.5% of subjects, with a significant gender-related difference. In fact, 77.1% of the female students answered correctly, compared to the much lower 48.9% of the male students ($p < 0.0001$). With regard to the no-sexually transmitted diseases, the respondents correctly identified botulism (88.4%), leptospirosis (62.2%), cholera (67.2%), measles (71.2%), tetanus (72.2%), chickenpox (73.9%), flu (74.1%), salmonellosis (75.8%), and rabies (81.1%). The most surprising finding was that the majority of respondents (72.0%) identifies Hepatitis A as a STD.
The second item considered the ability among the adolescents to recognize risk situations for STDs (Tab. III). In

Tab. I. Definition of what a sexually transmitted disease is. Expressed percentages according to gender and year of attendance.

A sexually transmitted disease is :	Total	Males	Females	Year of attendance	
				2nd	5th
• a disease transmitted by any form of physical contact (kissing, caressing, etc ...) between males and females	0,4	0,8	0,2	0,1	0,0
• a disease only transmitted by sexual intercourse	21,1	21,4	21,0	28,9	14,5
• a disease only transmitted by petting	25,5	22,6	27,6	30,9	18,7
• a disease only transmitted through males having sex with males	0,6	1,1	0,2	0,5	0,8
• a disease mostly transmitted by sex, but not only	52,3	54,1	51,0	39,6	65,5

Tab. II. Identification of a true or false sexually transmitted disease. Expressed percentages according to gender and year of attendance.

Disease	Total		Gender				Year of attendance			
			Males		Females		2nd		5th	
	yes	no	yes	no	yes	no	yes	no	yes	no
Rabies	18,9	81,1	22,4	77,6	16,3	83,7	19,0	81,0	18,8	81,2
Candidiasis	65,5	34,5	48,9	51,1	77,1	22,9	50,5	49,5	78,5	21,5
Pediculosis	16,8	83,2	20,9	79,1	13,7	86,3	19,8	80,2	12,5	87,5
Botulism	11,6	88,4	17,7	82,3	7,1	92,9	17,0	83,0	6,3	93,7
Flu	25,9	74,1	32,5	67,5	21,0	79,0	28,0	72,0	25,4	74,6
Tetanus	27,8	72,2	31,4	68,6	24,7	75,3	30,1	69,9	25,3	74,7
Leptospirosis	37,8	62,2	42,5	57,5	33,9	66,1	46,0	54,0	26,9	73,1
Cholera	32,8	67,2	37,7	62,3	29,0	71,0	38,8	61,2	29,3	70,7
AIDS	98,9	1,1	98,5	1,5	99,3	0,7	98,2	1,8	99,7	0,3
Herpes	69,1	31,0	67,1	32,9	70,4	29,6	62,0	38,0	74,2	25,8
Salmonellosis	24,2	75,8	29,3	70,7	20,4	79,6	28,0	72,0	18,6	81,4
Hepatitis A	72,0	28,1	71,6	28,4	72,0	28,0	73,2	26,8	70,1	29,9
Chickenpox	26,1	73,9	30,3	69,7	23,0	77,0	30,3	69,7	25,1	74,9
Hepatitis B	74,6	25,4	70,9	29,1	77,3	22,7	73,0	27,0	78,0	22,0
Parkinson's disease	4,0	96,0	5,5	94,5	2,9	97,1	4,8	95,2	2,4	97,6
Measles	28,8	71,2	33,2	66,8	25,5	74,5	31,8	68,2	28,6	71,4
Down's syndrome	8,1	91,9	10,0	90,0	6,6	93,4	11,3	88,7	5,1	94,9

Tab. III. Ability among adolescents to recognize risk situations. Expressed percentages according to gender and risk.

	Total			Males			Females		
	High risk	Low risk	No risk	High risk	Low risk	No risk	High risk	Low risk	No risk
Multiple partners	76,8	19,5	3,7	66,7	28,0	5,3	84,3	13,3	2,4
Sex without condom	96,0	3,4	0,6	94,1	4,7	1,2	97,5	2,3	0,2
Sex out of marriage	8,2	36,2	55,6	8,5	37,6	53,9	8,0	35,2	56,8
Sex before marriage	4,5	28,7	66,8	4,1	30,6	65,3	5,0	27,3	67,7
Needle exchange in drug abusers	94,9	2,5	2,6	92,9	3,1	4,0	96,3	2,0	1,7
Pregnancy	10,9	35,2	53,9	8,8	37,1	54,1	12,5	33,6	53,9
Blood transfusion	42,9	43,0	14,1	37,5	47,2	15,3	46,8	40,0	13,2
Vaginal sex	60,3	33,3	6,4	54,4	37,0	8,6	64,8	30,6	4,6
Anal sex	47,6	37,9	14,5	45,4	35,9	18,7	49,1	39,6	11,3
Oral sex	23,3	43,2	33,5	21,5	44,5	34,0	24,7	42,1	33,3
Kisses	2,2	24,7	73,1	1,9	23,6	74,5	2,5	25,6	71,9
Stings	6,1	31,9	62,0	6,8	34,9	58,3	5,5	29,4	65,1
Underwear exchange	4,1	31,2	64,7	3,2	25,1	71,7	4,8	35,9	59,3
Toothbrush exchange	9,6	35,9	54,5	7,9	39,6	52,5	10,7	33,4	55,9
Shaking hands	0,9	3,0	96,1	1,3	3,3	95,4	0,5	2,8	96,7
Hugging	0,6	2,2	97,2	0,7	3,0	96,3	0,5	1,7	97,8
Coughing and / or sneezing	3,4	36,2	60,4	3,6	38,9	57,4	3,3	34,3	62,4
Working in the same room	1,2	8,8	89,9	2,1	11,6	86,3	0,6	6,8	92,6
Sexual violence	92,6	6,0	1,4	89,5	8,3	2,2	94,9	4,3	0,8
Exchange of glasses and cutlery	6,1	38,5	55,4	5,3	40,7	54,0	6,7	36,7	56,6
Lack of knowledge of the STDs	80,3	16,0	3,7	76,9	18,6	4,5	82,7	14,2	3,1

Tab. IV. Prevalence among adolescents of attitudes to use condom in sex with casual or single partner. Expressed percentages according to gender and year of attendance.

	Total			Males			Females			2nd			5th		
	Never	Sometimes	Always	Never	Sometimes	Always	Never	Sometimes	Always	Never	Sometimes	Always	Never	Sometimes	Always
Single partner	10,0	36,4	53,6	12,4	42,8	44,8	8,2	31,7	60,2	5,8	38,5	55,7	18,0	36,1	45,9
Casual partner	3,9	5,6	90,5	4,7	7,4	87,9	3,3	4,3	92,4	5,6	5,5	88,9	3,8	6,7	89,5

this case, three options were provided: high risk, low risk, no risk. Among the students of both sexes perception of high risk was respectively attributed to the following features: sex without condom (95.97% of respondents) and the needle exchange among drug abusers (94.9% of respondents). When it comes to multiple sexual partners, however, a relevant sex-related difference ($p < 0.0001$) was observed (total: 76.8%, males: 66.7%, females: 84.3%). On the other hand, it is very comforting to note that the majority of respondents (80.3%), without distinction of school attendance, sex, and age considered the lack of information as a situation of high risk of contracting an STD.

As to sexual practices, a surprising absence of STDs-related risk was respectively attributed to oral sex by 33.5% of subjects of both sexes, to anal sex by 14.4% of subjects (males: 18.7%, females: 11.3%), and vaginal sex by 6.3% of the interviewed (males: 8.6%, females: 4.6%).

Table IV illustrates the findings as to the use of condoms within a relationship and with casual partners. 46.4% of the subjects do not use condoms regularly when in a relationship. This percentage falls at 9.5% in case of sex with casual partners (males: 12.1% , females: 7.6%). Surprising enough, age seems to have little influence over this behaviour (second-year students: 11.1%, fifth-year students: 10.5%) ($p = 0.7401$).

Questions about the means of prevention of STDs have been included within this area of information. Students were asked to establish whether these items were very safe, partly unsafe, or unsafe in preventing STDs (Tab. V). Even though the vast majority of students is correct in considering the use of condoms as very safe in preventing STDs, there is a surprisingly significant minority of students considering very safe the following items: contraceptive pills (total: 22.1%, males: 27.1%, females: 18.0%), spermicides (total:14.6%, males: 16.2%, females: 13.5%), intra uterine device (IUD) (total:18.2%, males: 20.6%, females: 16.6%). The morning-after pill (total: 9.8%, males: 13.6%, females: 6.9%) ($p < 0.0001$), and the *coitus interruptus* (total: 8.1%, males: 12.2%, females: 5.2%) ($p < 0.0002$) obtained lower percentages. In this area of questions, a gender difference that sees females give correct answers in much higher percentage than males was found. However, with older students these gender differences became less pronounced. Similarly, the percentages of those who identified as a "highly secure protection" contraceptive pills, spermicides, IUD, morning-after pills, and *coitus interruptus* appeared significantly reduced among fifth-year students ($p < 0.0001$).

The last part of the survey was aimed to investigate the role of information in STDs prevention. The first item concerned the role of the school to provide information about health and sexual education. 96.8% of the students declared that school should provide information, with the 68.7% of respondents thinking it should be given to them starting from their preteen years, while only 28.1% believed that they should receive this kind of information in high school only. With regard to the sources of information, television (total: 21.6%) and school

Tab. V. Knowledge about the means to prevent sexually transmitted diseases among adolescents. Expressed percentages according to gender and year of attendance.

	Total			Gender						Year of attendance					
				Males			Females			2nd			5th		
	Very safe	Partly safe	Unsafe	Very safe	Partly safe	Unsafe	Very safe	Partly safe	Unsafe	Very safe	Partly safe	Unsafe	Very safe	Partly safe	Unsafe
Contraceptive pills	22,1	23,5	54,4	27,1	26,2	46,7	18,0	21,7	60,3	27,5	29,9	42,5	13,3	18,7	68,0
Morning-after pills	9,8	30,0	60,2	13,6	35,6	50,8	6,9	25,9	67,2	12,0	39,8	48,2	7,3	18,2	74,5
Condom	89,0	10,0	1,0	88,6	10,0	1,4	89,2	10,1	0,7	88,0	11,3	0,6	91,2	7,1	1,7
Single partner	56,6	36,4	7,0	57,0	34,8	8,2	56,3	37,5	6,1	56,9	35,7	7,3	54,5	38,9	6,6
No pregnancy	13,7	31,4	54,9	16,0	33,2	50,8	11,7	30,1	58,2	20,4	34,6	45,0	6,2	25,3	68,5
Spermicides	14,6	46,9	38,4	16,2	49,1	4,7	13,5	45,4	41,0	19,0	55,0	26,0	6,0	36,9	57,1
Intra uterine device	18,2	39,1	42,7	20,6	43,9	35,5	16,6	35,5	47,9	25,7	45,0	29,3	10,4	30,8	58,8
Coitus interruptus	8,1	38,3	53,6	12,2	39,1	48,7	5,2	37,7	57,2	11,0	49,2	39,8	4,9	30,9	64,2

(total: 21.1%) were indicated as the most frequent sources, followed by friends (total: 14.0%), parents (total: 13.1%), and newspapers (total: 13.1%). The most surprising finding was that only a small percentage of subjects mentioned the family medical practitioner (total: 7.4%) and web search engines (total: 4.8%) as their source of information.

Discussion

This study was encouraged by the fact that it appeared advisable to investigate how the levels of knowledge about sex and STDs are able to influence young people's sexual behaviours. The use of a structured self-administered questionnaire seemed the easiest and cheapest way to investigate this aspect, because it is considered the only feasible technique to reach a number of responders large enough to allow a statistical analysis of the results [18]. The data analysis indicates several aspects deserving comment. The first important finding is the fact that, in spite of the questionnaire dealing with fairly sensible issues (sex and STDs), 96.2% of parents gave their consent. The first area of investigation was the real knowledge of what an STD is. Here just a little above half of the respondents answered correctly. In spite of this imperfect knowledge of the basics about STDs, students showed to have a good understanding of the risks of AIDS and hepatitis. Unfortunately, other STDs do not share this level of awareness. The lowest level of awareness regarded herpes infection and Candidiasis, with a strong significant gender-related difference ($p < 0.0004$), since females were shown to answer more correctly than males. This is most probably due to the greater awareness females have about gynaecological infections. The kind of school attended by the interviewed students showed to have no significant impact in the responses they gave.

The second area of investigation considered the young person's ability to recognize risk situations for STDs. Three quarters of the interviewed population were unanimous in considering the lack of information as a possible risk factor. However, this does not seem to significantly affect the sexual habits of a considerable percentage of teenagers who believe that oral, anal, and vaginal sex are at low risk of contracting an STD. So much so that they feel comfortable entrusting prevention of STDs to the use of condom, contraceptive pills, spermicides, and IUD. This result implies that a significant percentage of teenagers confuse STDs and pregnancy preventions. A general agreement was observed in the perception of risk related to sex with multiple partners, whereas a single partner was seen as a safety factor. However, this awareness was significantly higher among second-year female students, who also believe it is important to always use condoms [19] even with a single partner. The questionnaire shows that this particular belief is affected by the age of the interviewed student and that fifth-year female students are more likely to trust their partner (rather than rely on the use of condom). In spite of the increasing trust, the greater part of the respondents didn't see the partner as a sufficient source of information about sex and STDs. Indeed, the majority of males and females reported receiving sexual health information especially from television, school and friends. It is interesting to note that, contrary to what was observed in other studies [20, 21], internet as a source of information was considered less important than newspapers, and it was used only by a minority of respondents. The lack of knowledge about the appropriateness of using local health care services has been detected in the overall student population and at all ages, as noted by previous studies [22, 23]. The present study suggests that work is needed to improve teenagers' access to, and use of, primary sexual health care services. In particular, the identification of strategies that improve teenagers' awareness of services and general practitioners' approaches towards teenagers could be considered a priority.

A further key point to emphasize is the fact that no substantial differences were observed in the responses given by students of different kinds of schools. These findings lead to the conclusion that what the students know about sex and connected risk of STDs is uncertain and derives mostly from the media (especially TV) and their friends. So, the school has an important role in conveying basic information, but it does not help to create proper awareness of the risks associated with sex and fails in modifying sexual behaviours. Although the proportion of correct answers was observed to increase for fifth-year students, the fact that a high percentage of students confuses the prevention against STDs and pregnancy is an indication that a confusion of ideas thrives among our teenagers, despite the apparent easiness of their life conduct. Therefore, it is necessary to reinforce preventive measures in the younger age groups to design and implement sexual education programmes, and further studies are needed to better understand how our children think and act about their sexual life.

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ORIGINAL ARTICLE

Bacteriological study of raw and unexpired pasteurized cow's milk collected at the dairy farms and super markets in Sari city in 2011

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Key words

Raw milk • Pasteurized milk • Milk contamination

Summary

Introduction. *The quality of milk is influenced by different bacteria present in milk. This study was undertaken to investigate the bacterial contamination of raw and pasteurized milk in Sari Township, Iran, 2011.*
Methods. *In this investigation, 100 pasteurized milk samples were collected randomly from the super markets in the city and 100 raw milk samples from 4 dairy farms from suburb areas and evaluated for the presence of coliforms, Escherichia coli, Staphylococcus aureus and Listeria monocytogenes by culture methods and biochemical tests. Data analysis was performed by SPSS software using X^2 test and described in percentage.*
Results. *In the raw milk, contamination with E. coli, coliforms and Staphylococcus aureus was observed in 42 (42%), 36 (36%) and 22 (22%) of samples respectively, and the same for the pasteurized milk samples was 9 (9%), 2 (2%) and 2 (2%), respectively. Listeria monocytogenes was not detected in any sample. Presence of E. coli in the milk could be due to contamination with waste water and fecal materials.*
Conclusions. *Considering the contamination of raw and pasteurized milk with E. coli and coliforms, sanitary practice during collecting and transporting, particularly in the summer season is recommended.*

Introduction

Milk is an excellent high quality food providing major nutritional requirement to man at any age [1] and extremely susceptible to spoilage by microbes [2]. Unhygienic production of milk and milk products and improper storage, cause the early spoilage with microorganisms [3]. Bacteria present in the milk cause unpleasant effect on the taste and physical properties and disease. The bacterial contamination of milk not only reduces the nutritional quality but also consumption of such milk threatens health of the society [4].
Total number of organism in milk as disease causative agent in relation to its proper evaluation for consumption is important. The notable disease causing bacteria in milk are *Salmonella*, *Brucella*, *Staphylococcus (S.)*, *Listeria (L.)*, *E. coli* and *coliforms*. *Coliforms* and *E. coli* are normal inhabitants of the large intestine and their presence in milk could indicate fecal contamination.
Presence of organisms in the pasteurized milk is indicative of unhygienic for consumption. It has been shown that contamination of milk to *E. coli* in the milk distributing centers is increasing, which is indicative of the unhygienic conditions in preparing, distribution and transportation [5].

Considering the geological location of Mazandaran province and being a major milk and milk products center of Iran, and knowing that during processing, milk gets contamination with different types of microorganisms from a wide variety of sources, such as, infected cow's udder, feces, milk containers, dust in barns, workers' hand and lack of the workers' knowledge towards hygienic practice in keeping the quality of milk constant during storage and transportation. This study was aimed to evaluate the rate of contamination to different microorganisms of *E. coli*, *coliform*s, *S. aureus* and *L. monocytogenes* in the pasteurized and raw milk in Sari Township.

Materials and methods

In this descriptive study, 100 samples, each in 5 ml were collected during the spring, summer, fall and winter seasons from 4 dairy farms of Sari Township (only 4 dairy farms permited). Also 100 pasteurized milk samples were collected from the retail dealers (those dealers who had milk at the time of referring). The samples were preserved in ice container and transferred to the Sari Medical Collage laboratory.
In all, 200 pasteurized and raw milk samples were studied and cultured based on the standard methods for the iden-

Tab. I. Grade of raw milk contamination by referring to the National Standard of Iran, code number 2406.

Colony Forming Unit	$< 3\times10^4$	3×10^4 -1×10^5	1×10^5 -5×10^5	5×10^5 -1×10^6	$>1\times10^6$
Quality	Excellent	First grade	Second grade	Third grade	The unstandard raw milk
Number (%)	30 (30%)	33 (33%)	17 (17%)	12 (12%)	8%

tification of bacteria in the dairy products [6]. According to the recommended procedures (pour plate method) colony counting of the aerobic and mesoaerophilic bacteria was done. The Eosine Methylene Blue (EMB) and blood agar media were inoculated and kept at 37 °C for 24-48 hours. For identification of *coliforms*, and *E. coli*, the differential media, such as TSI, urea, and Simmon citrate were inoculated. For isolation and colony counting of *S. aureus* the medium blood agar and DNase media were inoculated and incubated at 35°C for 24 hours, the catalase and coagulase test, were performed too. For isolation of *L. monocytogenes* the samples were kept at 4°C for 7 days and identification was done according to the routine procedure. The colonies were counted using colony counter and the number was recorded as colony forming unit/ml (CFU). For testing, 5 dilutions of milk samples, 1, 0.1, 0.01, 0.001 and 0.0001 ml were used.

Aseptically, 1 ml of milk was added to the sterile test tube containing 9 ml of sterile distilled water, mixed properly by cyclometer, 15 ml of medium was poured in the plate containing 0.1 ml of sample and shaked to mix thoroughly and uniformly with the agar medium. The agar was allowed to be solidified and the petri-dishes were incubated at 37°C for 48 hours. A negative control was prepared using plate count agar only. The plates were placed on a colony counter and the number of bacterial colonies was recorded. The blood agar and DNase test media were inoculated and incubated at 37°C for 24 hours [5, 6].

On the pasteurized samples, the lactose broth with dilution of 1, 0.1, 0.01 was prepared and incubated at 37°C for 24 hours (National Standard of Iran 2002). For identification of *S. aureus*, on each blood agar and EMB media, 0.1 ml of milk sample was inoculated. In case of observing any colony, identification of *S. aureus* was intended. Also colony characteristics, catalase test and staining of the colony as well as manitol test were performed, the conformatory tests such as aerobic, anaerobic fermentation of glucose, sensitivity to lysostaphin and coagulase test were performed, too.

The obtained data were analyzed by SPSS software, using X^2 test, and recorded in percentage, mean and distribution rate.

Results

In this investigation, total of 200 samples (100 pasteurized and 100 raw milk) were studied. In the raw milk samples, contaminatation with *E. coli*, *coliforms* and *S. aureus* was observed in 42 (42%), 36 (36%) and 22 (22%), respectively. In the pasteurized samples, contamination with *E. coli*, *coliforms* and *S. aureus* was observed in 9 (9%), 2 (2%), and 2 (2%) samples, respectively.

By referring to the grading of raw milk contamination of national standard of Iran which is given in the Table I and considering the total bacterial count, the quality of raw milk was determined as follow: 30% had very good quality, 50% with first and second grades, that is, with good quality and the rest were of poor quality.

Table II shows the highest rate of isolates in the raw and pasteurized milk orderly as follow: *E. coli*, *coliforms* and *S. aureus*. From the statistical point of view, insignificant relationship was observed between the seasons of the year and the isolated organisms ($X^2 = 1.30$, df = 6 and P = 0.97). *Listeria* could not be isolated in any of the samples. The highest number of isolates were observed in the raw cow's milk. Isolated organisms from the pasteurized milk samples collected from the dairy companies indicate the higher rate of *E. coli* (Tab. III).

Table IV indicates the frequency of the isolates from the raw cows milk samples collected from the dairy farms under study , here also presence of *E. coli* and *coliforms* is significant, which is due to unhygienic production of milk.

The frequency distribution of the isolates from the pasteurized milk samples based on the seasons of the year is depicted in the Table V.

Discussion

In the present study, 200 (100 pasteurized and 100 raw) milk samples were collected and in the pasteurized milk *E. coli*, *coliforms* and *S. aureus* were observed in 9%, 2% and 2%, respectively and in the raw milk 42%, 36% and 22%, respectively. *L. monocytogenes* was not isolated in any of the samples under study. Data on 739

Tab. II. The frequency distribution of the organisms in 100 raw and 100 pasteurized milk samples in Sari Township in 2011.

Bacteria	Raw milk number (%)	Pasteurized milk number (%)
Escherichia coli	42 (42)	9 (9)
Coliforms	36 (36)	2 (2)
Staphylococcus aureus	22 (22)	2 (2)

Tab. III. The frequency distribution of the isolated organisms from 100 pasteurized milk samples, distributed from supermarkets of the Sari Township.

Dairy company	Samples number	Escherichia coli		Coliforms		Staphylococcus aureus		Total	
		No.	%	No.	%	No.	%	No.	%
Khaz shir	25	2	8	1	4	0	0	3	12
Maz shir	25	2	8	1	4	1	4	4	16
Kal shir	25	2	8	0	0	0	0	2	8
Pak shir	25	3	12	0	0	1	4	4	16

Tab. IV. Frequency distribution organisms from 100 raw cow's milk samples collected at 4 Township dairy farms.

Dairy farm	Sample number	Escherichia coli		Coliforms		Staphylococcus aureus	
		No.	%	No.	%	No.	%
1- Dimtron	20	6	30	6	30	8	40
2- Dinek	23	18	78.3	5	21.7	0	0
3- Magham	17	6	35.3	10	58.8	1	5.8
4- Daryek	40	12	30	15	37.5	13	32.5

Tab. V. Rate of contamination of the raw milk in different seasons of the year in 2011

Organisms	Spring number (%)	Summer number (%)	Fall number (%)	Winter number (%)	Total %
Escherichia coli	11 (26.2)	24 (57.1)	4 (9.5)	3 (7.2)	42
Coliforms	9 (25)	19 (52.8)	4 (11.1)	4 (11.1)	36
Staphylococcus aureus	6 (27.4)	10 (45.4)	3 (13.6)	3 (13.6)	22

pasteurized milk samples in Iran showed contamination of 8.68% with higher than the standard level, in a way that, in 15 samples, *coliforms* count was 20 to 40 per ml of sample [7].

A study in Iran, comparing the concurrent contamination of *E. coli* and *S. aureus* revealed that in milking 19.7%, transporting 49% and in milk selling centers 58.4% of samples were contaminated with 2 organisms [8]. Report on contamination of the raw milk samples in Malayer city of Iran was as follow: *E. coli* 75%, *Enterobacter* 42%, *Klebsiella* 36% and *S. aureus* 52% [9]. Also higher count of *E. coli* followed by *S. aureus* was reported too, which refers to the improper public health measurements, and poor cleaning, in addition to the primitive system of transportation. Report given by Asmahan in Khortoum indicated, 63% of raw cow's milk samples contaminated with *E. coli* [10].

Fulya revealed that 10% of the raw milk samples under study were contaminated with *E. coli* [11]. Crump et al., studied on 216 raw milk samples and found that 28 (13%) of them were contaminated with *E. coli* [12]. The reason could be due to the animals and their living environment. Another study showed that 1.46% of the milk samples in the milk storage tankers were contaminated with *E. coli* [13]. The reason was expressed as contamination of the milk with animal feces.

Zelalem and Bernard in Ethiopia found higher *Coliform* count in raw milk samples under study which could be due to the initial contamination of the milk samples from the cow's milk, the milkers, milk containers and the milking environment [14]. Chye et al., stated that the lower counts of bacteria may be due to good cleaning system and good handling from farms to the plant [15]. The higher percentage of *E. coli* could be due to the fact that *E. coli* may grow in raw milk and reaches higher number in tropical countries or in the absence of cooling system. The isolation of *Coliform* and other food pathogens from dairy products poses a serious threat to food safety [16].

High contamination of raw milk to *Escherichia* and *Coliform*, particularly in the summer was reported [17]. Presence of *S. aureus* in milk may originate from mastitic animals [18], or human sources, which is in agreement with our findings. *S. aureus* in milk and milk products is an indicator of the spoilage [19].

Ekici et al., in their study detected *S. aureus* in 75% of the raw cow's milk but no *E. coli* was isolated [20]. Findings showed that 38% of raw milk and 11% of pasteurized milk contaminated with *S. aureus* [21].

A study on 366 raw milk samples, revealed 25.3% contaminated with to *L. monocytogenes* and 9.2% to *Salmonella typhi* [22].

When the frequency distribution of the detected organisms in the raw and pasteurized milk samples are compared, it is noticed that the number of the isolates are higher in the raw cow's milk. It is because, during pasteurization process some organisms are killed, as is indicated from our findings which are given in the Table III and IV. When the number of isolates in the raw milk are compared in the different seasons of the year, it is noticed that number of the detected organisms in the summer is higher than the winter season (Tab. V). The reason could be that in the summer the ambient temperature is high and lacking of refrigeration in the situation of long distance milk transportation helps the situation. It agrees with the report documenting difficultly in obtaining high quality milk during summer season [23].

Considering the results of this study and the similar investigations, it could be concluded that the presence of *coliforms* bacteria may not necessarity indicate a direct fecal contamination of milk, but it is a precise indicator of poor sanitary practices during milking and further handling process. In this condition, awareness about the source of contamination is very important. Considering the rate of raw milk contamination to *E. coli* and *coliforms*, in the Sari Township, practice of hygienic condition and also supervision to the milk processing, transportation and preservation, particularly during the summer season is recommended.

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Paediatric nurses' perception of the child-family dyad's autonomy in managing a chronic disease situation: the experience of an Italian Paediatric Department

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Key words

Paediatric chronic disease • Adherence • Autonomy

Summary

Introduction. *Chronically ill patients have to take several medications and non-adherence to treatment can lead to severe and negative outcomes. Therefore, several interventions are suggested in literature to improve adherence rates in clinical practice. Adherence to treatment can be particularly troublesome in adolescents, who strive for autonomy and self-care independence. Literature suggests that improving adherence is useful to guarantee positive outcomes and reduce costs.*
Aim. *To explore how nurses perceived autonomy in parents, adolescents, and children related to the management of chronic disease.*
Materials and methods. *A qualitative study including 1 focus group and 7 semi-structured interviews conducted between September 2011 and October 2011. The qualitative date were analysed with the thematic analysis method. The sample included 12 paediatric nurses working in a Children's Cystic Fibrosis Unit and Neuromuscular Disease Unit.*
Results. *The 5 main categories that emerged from this qualitative study after he process of categorization were: 'Changes in daily lifestyle', 'Nurses' attitude towards educating the dyad', 'Adolescence and transition', 'Parents' attitudes towards chronic disease', and 'Availability of information'.*
Discussion. *Correct information and education is crucial for families who have a chronically ill child. Internet can be a misleading source of information and provide wrong information also in relation to prevention.*

Introduction

Chronically ill patients are obliged to take several medications and the success of the therapy depends on patient's ability and willingness to engage in and maintain beneficial health behaviours such as taking medications correctly. However, it is also strongly influenced by the supportiveness of the patient's and family's environment, healthcare providers' practices, and by the quality of the healthcare system [1].
Literature suggests that improving adherence can be useful to ensure positive outcomes and reduce costs. On the contrary, the consequences of non-adherence to treatment can be disastrous for patients [2] and depend on forgiveness of the drug regimen (i.e. how much deviation from the dosing schedule is tolerated before an adverse event occurs).
K.K. Marciel [3] suggests various interventions to improve adherence in clinical practice in chronically ill children, such as the use of technological devices including automated reminders (e.g. text messages, alarms) and use of videos, which were well accepted by especially by adolescents. However, adherence can be particularly difficult during adolescence. In two large, multicentre studies, adolescents scored 55-60% on a knowledge measure focusing on Cystic Fibrosis (CF) care management, showing a significant knowledge gap. However, Marciel did not evaluate the relationship between knowledge [4, 5] of disease management and adherence to treatment, the positive association between knowledge of prescribed treatments and treatment-related behaviours was described in other populations [6].
Despite the effectiveness of psychological interventions in improving adherence in chronically ill children [7], few behavioural interventions to improve adherence in CF patients have actually been developed and studied.
Recently, an educational intervention utilizing a CD-ROM for children with CF was planned to fill gaps in knowledge and improve coping skills, but this study unfortunately did not evaluate changes in adherence behaviours [8].
Different previous research has consistently shown that education alone is not sufficient to change behaviour [9]. Behavioural interventions conducted in a group format, including the dyad, successfully increased adherence to prescribed caloric intake in young children with CF [10]. Some of the success of this program may be due in part to the much-needed peer support between parents, which has been related to improved adherence [11]. However, programs including interactive group sessions are no longer considered to be safe because of the potential for patient-to-patient transmission of respiratory tract pathogens [12, 13]. Thus, elimination of group-based activities and minimizing contact between patients has led to a

loss of peer support. Although studies have not evaluated the impact of the current infection control guidelines on patients' social and emotional functioning [2, 13, 14], social support has been shown to play a major role in facilitating adaptation to chronic diseases, including CF, and adherence to daily medical regimens [14, 15].
Although adherence to treatment and autonomy in self-care can prevent possible complications [16], to date, there is a paucity of studies in the Italian context regarding the correlation between adherence to treatment and self-care autonomy in the field of paediatric chronic diseases. To improve future quality improvement interventions it is crucial to have a better understanding of the context [17] and determine what is the nurses' perception regarding the autonomy of the dyads (parents, children and adolescents) in the management of chronic disease in an Italian Paediatric Department.
Autonomy refers to 'the parent's, child's or adolescent's freedom of choice, self-sufficiency and independence, the extent to which the child/adolescent feels able to shape his/her own life, as well as being able to make decisions about day-to-day activities' [18].

Materials and methods

Design

This is a descriptive study with a qualitative approach including one focus group and semi-structured interviews [19]. Since the aim of the study was to investigate the healthcare professionals' perceptions of the autonomy of the dyad (parents and sons) in the management of a chronic disease, the qualitative approach was the most appropriate choice, so that participants could describe their personal experiences, feelings and thoughts.
In addition, literature suggests that qualitative data can contribute to provide answers to specific research questions also in epidemiological studies, by focusing on the understanding of meanings, beliefs, and attitudes from the point of view of the participants [20-23], allowing the analysis of human behaviour.

Setting

The present study was conducted at the 'Giannina Gaslini' Children's Hospital in Genoa (Italy) and it involved paediatric nurses working in the two units of Cystic Fibrosis and Neuromuscular Diseases. The study was carried out from September 2011 to October 2011 and was approved by the Hospital Direction.

Participants

A total of 12 paediatric nurses agreed to participate in this study: 5 paediatric nurses took part in a focus group and 7 answered to a semi-structured interview.
The focus group participants were: 5 paediatric nurses; 1 experienced moderator, and 1 person taking notes and the participants were all full-time staff nurses working in the Neuromuscular Disease Unit.
Seven nurses working in the Cystic Fibrosis Unit were individually interviewed. We personally contacted the participants to explain the objective of our study and if they agreed to take part in our study we fixed an appointment with our interviewers.
Since the semi-structured interviews would have been audiotaped, a priori written consent was obtained from the participants.
The participants were all females aged between 25-37 years and their experience in the Unit ranged between 2-15 years. All participants were enrolled on a voluntary basis.

Data collection

To define the questions, the research team met several times and additional demographic data were included (age, gender, years of experience in the Unit, education).
The semi-structured in-depth interviews were administered to 3 nurses working in the Diabetes Outpatients Clinic to test acceptability and comprehensibility.
All the interviews were conducted in rooms inside the Units, in a relaxed atmosphere and no interruptions. Notes were taken at the end of each interview.
The first question was of a general nature to investigate how nurses perceived the autonomy of the dyad facing the chronic disease. The other questions aimed at identifying the major factors influencing the field accordingly to the evidence cited in the introduction.

Data analysis

The second, third, and fourth authors listened to and verbatim transcribed the recordings, analysed and coded the transcriptions 'line by line' according to the 'thematic analysis' [22]. The three researchers analysed them independently, and then compared the codes they had identified to reach an agreement on the emerging categories.

Validity and reliability

To ensure the trustworthiness of the present study we adopted Lincoln and Guba's" Four criteria" [24]: credibility, confirmability, transferability and dependability.
Credibility was demonstrated by the length of the interviews, data saturation, and independent analysis of the three researchers [23]. At the end of the interviews and after the data analysis, the researchers gave nurses the possibility to review the emerging themes.
Variation in sampling ensured the confirmability of the conclusions.
Reliability was confirmed by the negotiation of the codes done by the three researchers. Finally, the results were discussed with another 2 colleagues to confirm the transferability of the results who were not on duty during the interviews

Results

The results include the following 5 main categories, considered to be important factors influencing the nurse's

perception of the dyad's autonomy in managing a chronic disease in Paeditric Cystic Fibrosis Unit and Neuromuscular Disease Unit:

- Changes in everyday life.
- Attitude of nurses in educating the dyad.
- Adolescence and transition.
- Attitudes of parents in facing the chronic disease.
- Availability of information.

Changes in everyday life

The majority of the nurses said that the chronic disease totally changed the family's everyday life. This change is triggered by the diagnosis that generates a strong sense of guilt, stress and anxiety in parents. Parents felt overwhelmed by a strong sense of disorganization, anger, fear, and often struggled with depression: they felt guilty for their child's condition. Initially, *"there are difficulties related to the lack of knowledge about the disease and its management practices, especially at home."*
However, nurses reported that it was a *"fleeting moment",* because in a short time they became very skilled and autonomous in managing their child's disease, and sometimes were even *"better than the health workers themselves"*.
In addition, *"The parents recognize all types of symptoms* and *they understand if their child needs the application of non invasive ventilation, during the night"*.
The required changes regard not only the parents' and their ill child's personal behaviours, but also the house they live in has to be adapted to facilitate the autonomous management of the disease.

Attitude of nurses towards educating the dyad

All nurses stated that they play a crucial role in helping parents and their children to increase the level autonomy and safety. *"Our job is to educate parents to help them increase both their self-esteem and their confidence in our competences and in nursing techniques*".
The nurses pointed out that another key element is their ability to communicate. Nurses declared that it is possible to learn "communication skills" by attending special courses and with their own professional experience.
In addition, all nurses stated that it is instrumental to assess the different types of parenthood. In fact, some parents are totally alienated by their child's disease, whereas others "serenely accept their child's disease". The nurses' ability to communicate with the parents and the children can improve the parents' and children's participation in the care plan to achieve their autonomy with the chronic disease.

Adolescence and transition

All nurses are aware that adolescence is considered the most critical period of the individual's life, because it entails a series of changes that mark the transition to adulthood. Chronic diseases are challenging at any age, but even more in childhood and adolescence.
"In our practice, we meet adolescents who underestimate the disease, and refuse therapies".
Despite their condition, however, nurses reported that the social life of these patients continues, thanks to the steady progress of technology that provides them with innovative devices:
"They go out, have a pizza with friends, go to the cinema...".
It should be emphasized that their parents, who can be more or less permissive according to their health conditions, always influence their social life. It seems that the parents' age influences the adolescent's life:
"The degree of anxiety and fear makes parents more open-minded and permissive or more prohibitive"
*"Attitude also changes depending on the age of the par*ents"
All nurses reported that, at least in the hospital, they tend to give greater independence to boys, and reduce the role of the parents.
Admission is the first step to win their trust:
"You have to communicate with him/her as if he/she wasn't ill, for example, you have to ask simple questions related to his/her hobbies, favourite movies / books. This relationship based on mutual trust helps us to make fun of the disease""
The interviewed nurses unanimously reported that in adolescents another critical moment concerning the chronic disease is the transition to the *"adult hospital"*.
"The "adult hospital" is another world."
Indeed, the first element of destabilization in this "*Transition"* is the "*change"* of the nurses, when the adolescents lose their relationship made of friendship with their former caregivers.
The fact that they have to take on all the responsibilities that used to be shared with the family and nurses, is the second element of destabilization. "*They want to grow up but they refuse responsibilities"*

Parents' attitude in facing the chronic disease

All nurses reported that children are less rebellious than adolescents. Their life is influenced by their parent's perspective and knowledge
"The child's knowledge about the disease comes from his parents"
"The acceptance or denial of the disease of the child are related to the parents' perspective and ideas"
"If the parent does not accept the disease, it will be more difficult to educate the child."
In the long term, chronic illness can interfere with the child's habits, conditions, and it can affect them.
The interviews show that, in very young children, the burden of the disease is mitigated by the reassuring presence of an adult.
"The children face the disease as their parents do."
Subsequently, the patient acquires a sharper awareness of his status.
The patient gets to understand the explanations about the treatments, received from the nurses.
The ability to explain the disease to the child, and to make him/her more aware using simple words or play is very important. Not being honest could lead to confusion and mistrust.

All nurses pointed out the importance of helping them lead a life as normal as possible, and to encourage their participation in various activities.

Availability of information

Nurses pointed out that Internet is accessible to everyone, including ill children, adolescents, and parents.
Nurses stated that *"Patients and parents already know everything about the disease when they meet us for the first time"*.
Nurses had the perception that information provided by the hospital is not considered as important as the one taken from the Internet. Certainly Internet can be dangerous and misleading, "*A guy asked me when he would have been transplanted, but he did not need it. He read it on the Internet"*
"The Internet is the most consulted tool for the resolution of their cares, although in some cases, it is a source of misunderstanding". Nurses believed that any material used to educate patients must be selected by *"experts"*, such us physicians and senior nurses. One senior nurse suggested *"Educational material should be evidence-based"*
All nurses agreed on the definition of the parent *as "one of the figures for the patient's development of autonomy."*

Discussion

The following 5 categories emerged from our qualitative study after categorization of the verbatim transcriptions:

- Changes in every day life.
- Nurses' attitude towards educating the dyad.
- Adolescents and transition.
- Parents' attitudes.
- Availability of information.

This study confirmed and extended previous findings regarding the factors influencing the autonomy of the dyad.
Living with a chronic disease can profoundly affect the life of all the family members in terms of the everyday biological and psychosocial functions. Literature shows that comparing the Quality of Life (QoL) of a chronically ill child with that of a healthy child can be useful to assess the impact of a chronic disease. Health-Related Quality of Life (HRQoL) [25] can be conceptualized as a multidimensional construct which describes physical, psychological and social functioning. Impaired HRQoL was found in children and adolescents with several chronic conditions, e.g. diabetes, gastrointestinal diseases, cardiac conditions, asthma, obesity, end-stage renal disease, psychiatric disorders, cancer, rheumatologic conditions, and cerebral palsy [26].
Nurses perceived that parents played an important role in the life of the children and adolescents included in our study. Literature points out that parents play a key role in ensuring that their child's treatment plan properly implemented and in preventing complications.
Overanxious parents may attempt to further restrict their children's autonomy. These children could be inadvertently isolated from their peers and siblings due to concerns related to their illness, the fear of pain and the need for constant monitoring [27].
Nurses agreed that parents sometimes override their child's decisions. This overstepping attitude probably derives from a situation of stress, and generally dictated by fear of the disease and the consequences it may entail.
Nurses underlined the impossibility, especially for the mothers, to lead a normal life, due to their child's disease. For this reason, it is very important to support parents and give them the help they need. For instance, it is instrumental to inform parents that the hospital offers them the possibility to speak to a psychologist.
The state of anxiety can *'be transmitted to their children"*. Nurses try to facilitate the adherence of the child and the family to treatment to both improve their health status and gain their confidence through high quality personalized care and constant evaluation. Constant evaluation prevents misunderstandings related to treatment, can improve adherence and facilitates the achievement of a more autonomous lifestyle.
In relation to this, nurses' communication skills play a key role in the way they handle their relationship with these families.
Literature identifies two different types of adolescents with chronic diseases: a) those who are autonomous in spite of the disease; and b) those who are totally dependent on their caregivers [28].
These 2 types of adolescents require different approaches to build a strong sense trust with health professionals [28]. Nurses should firstly focus on the acceptability of the disease, then move on to encourage autonomy and adherence to treatment so that complications are prevented and a good quality of life is ensured.
Some adolescents instead have an excessive feeling of autonomy and tend to be indifferent towards the disease, because their unconscious desire to be autonomous prevails. On the contrary, adolescents forced to sit in a wheelchair are fully aware of the disease and their autonomy is limited by the constant presence of their parent. Obviously, it is necessary to negotiate the objectives of the care plan to be sure that personal preferences are respected. From this perspective, the education provided to parents and their chronically ill children also has a preventive purpose.
Transition to the adult hospital is another critical point, because they are never keen to leave the paediatric hospital even if they have grown up to be young adults. This could be an interesting point that would deserve to be investigated to prevent conflict between adult and paediatric unit/staff and rethink paediatric nurses' education so that they may also deal with adolescent patients [29-33].
Our study showed that patients often use Internet to search for medical information but they do not share this information with physicians and nurses [34]. Adolescents in particular seem to be receptive to online health information and often use this information for their deci-

sion-making processes. Yet, online health information is often incomplete, inaccurate, or unreliable [35].
As reported in literature, the role of the patient has shifted from being a passive recipient of care to that of and active consumer of health information [36].
Health professionals are reacting to more 'Internet-informed' patients in the following ways: a) health professional feel threatened by the information patients have and respond defensively by asserting their 'expert opinion' (health professional-centred relationship); b) Health professionals and patients collect and analyse the information together (patient-centred relationship); c) Health professionals guide patients so that they may retrieve health information from reliable websites (Internet prescription). In our study, nurses feared the Internet because they found that patients often did not share the information they had found with them. Our findings highlighted the need to establish quality standards for health website content, adequately train health care providers to help their patients retrieve reliable health information from the Internet, and maybe strengthen e-health literacy skills among online-information seekers, including health professionals themselves.

Study limitations

The major limitation of this study is that is was conducted only in one centre. In addition, only nurses were included in the survey, excluding the different perspectives of physicians and other healthcare workers, which could have provided further interesting data.

Conclusions

This is a first study to explore the factors that could affect autonomy management practice patterns in paediatric chronic patients of Cystic Fibrosis and Neuromuscular Diseases units. According to our findings, we would suggest to plan educational interventions so that nurses gain a better understanding on how to facilitate the integration of more effective strategies into daily practice (e.g. multi-level behavioural and psychological/affective interventions).
A Family Centred Care approach [37] could enhance the involvement of the child-family dyad in the care plan. Although nurses are often aware of his concept they sometimes have great difficulty in putting it into practice.
Our study confirmed that nurses play an important role in terms of providing education/information, because they stay with the patient on a 24/7 basis.
This study would benefit from further research to explore the views of the child-family dyad concerning the disease.

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