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Nishimura (2000), Agindotan et al. (2003), (Kelebeni, 1983), (Usman and Smith, 2001), (Chege, 1998; Stein, 1987a,b; Tijani, 1993,1995), (Kumasi et al., 2001)
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Mussel RL, De Sa Silva E, Costa AM, Mandarim-De-Lacerda CA (2003). Mast cells in tissue response to dentistry materials: an adhesive resin, a calcium hydroxide and a glass ionomer cement. J. Cell. Mol. Med. 7:171-178.

Booth M, Bundy DA, Albonico P, Chwaya M, Alawi K (1998). Associations among multiple geohelminth infections in school children from Pemba Island. Parasitol. 116: 85-93.0.

Fransiscus RG, Long JC, (1991). Variation in human nasal height and breath, Am. J. Phys. Anthropol. 85(4):419-427.

Stanislawski L, Lefeuvre M, Bourd K, Soheili-Majd E, Goldberg M, Perianin A (2003). TEGDMA-induced toxicity in human fibroblasts is associated with early and drastic glutathione depletion with subsequent production of oxygen reactive species. J. Biomed. Res. 66:476-82.

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Full Length Research paper

Antimicrobial susceptibility of *Enterococcus faecalis* and a novel *Planomicrobium* isolate of bacterimia

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During nucleotide sequences of *16SrDNA* gene, *Enterococcus faecalis* was predominant in 322 samples; 11 isolates (100%) were from outpatients' vaginal swabs and 3 isolates (75%) from inpatients' blood samples. The three isolates from the blood showed two frame shift mutations "Deletion" at sequence positions 21 and 1076 bp to be 100% similar with type strain OG1RF. The isolates totally showed no resistance to gentamycin, streptomycin and erythromycin whereas, there was high resistance to amoxicillin/clavulanic acid (71.4%), methicillin and vancomycin (92.8%, for each). All isolates from the blood were completely resistant to penicillin and ampicillin. The study also detected *Planomicrobium mcmeekinii* from an inpatients' blood case (containing Gene or Point mutation type Transversion at position 358 bp resulting to 99% sequence identity to ATCC 700539) as first reporting and revealing it to be a new subspecies or species.

Key words: *Enterococcus faecalis, Planomicrobium mcmeekinii*, 16SrDNA and sequencing.

INTRODUCTION

In past years, Enterococci have rapidly emerged as important nosocomial and community acquired pathogens (Kapoor et al., 2005). These organisms can cause series of invasive infection including endocarditis, meningitis, urinary tract infection and bacterimia (Guardado et al., 2006; Nallapareddy et al., 2006; Peters et al., 2007; Singh et al., 2007). *Enterococcus faecalis* is responsible for 90% of enterococcal infection (Shepard and Gilmore, 2002). Furthermore, it is among the most common pathogens isolated from infected surgical sites, urinary tract infection, blood-stream and vagina (Dupre et al., 2003; Jahic et al., 2006). However, *E. faecalis* is associated with 6% mortality rate in early onset septicemia which rises to 15% in late-onset infection; in general, it is implicated in 7 to 50% of fatal cases (Jahic et al., 2005). Enterococci are considered as an important difficult-to-treat pathogens due to their intrinsic resistance to a wide range of antibiotics that most notably include beta-lactams and aminoglycoside frequently used to treat infections with Gram-positive cocci (Kapoor et al., 2005). In addition, enterococci have the ability to acquire resistance to antimicrobial agents through plasmid transferring, transposons and chromosomal exchange or mutations (Mundy et al., 2000; Tiwari and Sen, 2006). Moreover, management of vancomycin resis-tant enterococci infections poses a clinical challenge as these organisms may be resistant to several antimicrobials with unique mechanisms of action (Wong et al., 2000).

Detection and infection of microorganisms involved cultivation-based techniques, which has been a challenging task due to the mixed nature of the infection and the diverse physiological and nutritional requirements for culture. 16SrDNA-PCR-based diagnosis of *E. faecalis* has been reported by many workers (Simonsen et al., 2003; Carvalho et al., 2004; Sedgley et al., 2006).

Planomicrobium mcmeekinii a Gram-positive, rod-shaped bacterium (Reddy et al., 2002), is one of the five *Planococcus* species within the family planococcaceae (Tow and Cowan, 2003). *Planococcus* spp. have been isolated from a variety of marine environments, fermented seafood and some Antarctic lakes. In general many of these species have been reported to produce an orange or yellow pigment (Sheridan and Brenchley, 2000; Engelhardt et al., 2001). On the basis of the phenotypic and phylo-genetic data and the genomic distinctiveness, Yoon et al. (2001) proposed that *Planomicrobium*, a new

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genus belonging to the family planococcaceae, contains two previously assigned planococcal species, namely *P. mcmeekinii* and *Planomicrobium* okeanokoites.

The present study was undertaken to speciate and determine *E. faecalis* strains in blood and vagina with their antimicrobial susceptibility. Also to confirm the isolation of a new species or subspecies of *P. mcmeekinii* for the first time from blood samples.

MATERIALS AND METHODS

Source of isolation

Two hundred and thirty three samples were collected from an outpatients' vaginal swab (n = 101) and inpatients' blood sample (n = 132) from Al-Sadr Teaching Hospital, Al-Basrah General Hospital and Al-Basrah Hospital for birth and Child, from Basrah city, Iraq. These samples were enrolled from April 2006 to July 2006. Swabs of vagina and 2 ml of blood samples were added in sterile tubes of Brain Heart Infusion Broth (HIMEDIA) and streaked on Mannitol Salt Agar (ALPHA). Colonies grown after incubation were Gram stained and cultured into Nutrient Agar (ALPHA) for testing (Talan et al., 1989).

Genomic DNA extraction

Deoxyribonucleic acid (DNA) extraction was done according to Sambrook et al. (1989), Al-Badran (2003) and Japoni et al. (2004). 5 ml of Tryptic Soy Broth (ALPHA) was inoculated with tested bacteria and incubated at 37°C for 18 h. The grown bacteria were re-washed three times by Phosphate Buffer Saline (Oxoid). The washed bacteria was resuspended in 500 ml of Tris-EDTA buffer, 30 µl of 10% Sodium Dodecyle Sulphate and 30 µl of 25 mg/ml solution of Proteinase K (Promega) and then incubated for 1 to 3 h at 37°C. 100 µl of 5 M NaCl solution was added and incubated at 65°C for 10 min. DNA was purified by two extraction with phenol: chloroform: isoamyl alcohol (24:25:1) and precipitated with 70% chilled ethanol. The DNA was resuspended in 50 µl of Tris-EDTA buffer as stock. To check for DNA, the samples were loaded in 0.8% agarose gel 1 × TBE (54 g Tris-base, 0.5 M EDTA, 1- L distilled water, PH = 8, then diluted with 400 ml of distilled water) and electrophoresed at 60V for 30 min.

16SrDNA gene (PCR)

The 16SrDNA was detected by thermocycler apparatus (BioRAD Co.) according to the procedure and materials of MWG Biotech AG Co. Barker et al. (2005) used the primers: 27Forward 5'-AGAGTTTGA TCM TG GC TCAG-'3 and 1492Reverese 5'-TACGGYTACCTTGTTACG-'3 (Stackebrandt and Goodfellow, 1991). The polymerase chain reaction (PCR) is a mixture of the final volume of 50 µl containing 37.7 µl sterilized millipore water, 10 µl Taq-dNTPs buffer mix (5X), 2.5 µl of each primer (5 pmol), 2 µl $MgCl_2$ (50 mM), 1 µl DNA template and 0.3 µl of Taq DNA polymerase.

The PCR program involved initial denaturation at 95°C for 5 min, 30 cycle (denaturation at 95°C for 1 min, annealing at 56°C for 1 min and extension at 72°C for 2 min) and final extension at 72°C for 10 min, then soaking at 4°C to indefinite. The amplified PCR mixtures were resolved by electrophoresis through 1% agarose gel at 60V for 1.5 h prepared in 1 × TAE (242 g Tris-base, 57.1 ml Glacial acetic acid, 100 ml EDTA 5 M, 1-L distilled water, PH = 8, then 10 ml of this solution diluted with 490 ml of distilled water) containing 1 µl ethidium bromide in 100 ml agarose solution. Products were viewed under ultraviolet (UV) light system (UVi Co.). The band of 1000 to 1500 bp was indicative to 16SrDNA gene.

16SrDNA gene sequences

The 16SrDNA products sequencing and its preparation was according to MWG Biotech AG Co. with the procedure of Barker et al. (2005). For the purification DNA product, 60 µl of 20% polyethylene glycol applied Biosystem (ABI) was added to 30 µl of PCR product (*16SrDNA* gene) and mixed by vortex (Whirlimixer) and incubated at 4°C overnight after centrifugation (Scotlab) at 1200 rpm for 20 min (twice). The pelleted DNA was mixed with 0.5 ml of 70% chilled ethanol and the recentrifuged product was dried in a vacuum drier (Thermo) for 30 min. The DNA was resuspended in 15 µl of Millipore sterilized water and left overnight at 4°C and then send to the MWG Biotech AG Co. for sequencing.

Identification of bacteria

All bacterial species were identified (using the DNA sequencing products) in "BLAST" provided by the National Center for Biotechnology Information Service (NCBI)" http://www.ncbi.nlm.nih.gov" (Kerbauy et al., 2011).

Phylogenetic tree

The sequences data obtained from the present study (n = 15) and from type strains (n = 25) by GenBank (Sung et al., 2006) were aligned and concatenated at 1346 bp and compared to assign the differences using "CLUSTALW" http://www .ebi. ac.uk / clustalw/ (Kerbauy et al., 2011), then a phylogenetic tree by Neighbour Joining method was viewed by http://www.phylogeny. Fr/version2_ cgi/index.cgi/ (Dereeper et al., 2008).

Detection of *mecA* gene by PCR

The PCR mixture and procedure depend on Geha et al. (1994). The primers for *mecA* gene (Forward 5'-GTAGAAATGACTGAACGTCCGATGA-'3 and Reverse 5'-CCAATTCCACATTGTTTCGGTCTAA-'3) with the size of 300 to 310 bp and PCR mixture was 12.5 µl of Go Taq Green Master Mix.2x (Promega), 1 µl of each primer (100 pmol), 5 µl DNA template, 5.5 µl nuclease-free water and 25 µl mineral oil (FisherBiotech). A DNA thermocycler was programmed with the initial denaturation at 94°C for 4 min, 30 cycles (denaturation at 94°C for 45 s, annealing at 56°C for 45 s and extension at 72°C for 2 min. The PCR products electrophoresed by agarose (2%) was added to 1 × TBE buffer; the band of suitable size was indicative of *mecA* gene.

Detection of *vanA* and or *vanB* gene(s) by PCR

All PCR mixture and procedures have been discussed previously (above) in *mecA* gene with the exception of primers for *vanA* (Forward 5'-CATGAATAGAATAAAAGT TGCAATA-'3 and Reverse 5'- CCCCTTTAACGCTAATACGACGATCAA-'3) and *vanB* (Forward 5'-GTGACAAACCGGAGGCGAGGA-'3 and Reverse 5'-CCG CCA TCCTCCTGCAAAAAA-'3) genes with the size of 300 bp for each. A DNA thermocycler was programmed with initial denaturation at 94°C for 10 min, 30 cycles (denaturation at 94°C for 30 s, annealing at 50°C for 45 s and extension at 72°C for 30 s) and

extension at 72°C for 10 min. The PCR product was electrophoresed and visualized as previously.

Vancomycin resistance by agar screen method

From overnight culture, 2 pure colonies approximately 10^5 colony forming unit (CFU) were grown as straight lines in Muller Hinton (Bioanalyse) plates supplemented with 4 mcg/L, 8 to 16 mcg/L and 32 mcg/L vancomycin, then incubated at 37°C for 48 h. Any visible growth indicated vancomycin resistance (CLSI, 2007; Al-Hadithi and Abd Al-Abbas, 2003).

Disc diffusion method

Disc diffusion (Bioanalyse) of gentamycin (10 mcg), streptomycin (10 mcg), erythromycin (15 mcg), chloramphenicol (30 mcg), penicillin (10 mcg), ampicillin (25 mcg), amoxicillin/clavulanic acid (20/10 mcg), and 1 mcg of oxacillin (methicillin) was tested by spreading 0.1 of 1.5 ml BHIB cultured with bacteria (18 h) into mannitol salt agar. Each isolate was tested for growth with all antibiotic discs (NCCLS, 2000).

RESULTS

PCR amplification with 16SrDNA eubacterial primers (27 Forward and 1492 Reverse) showed the gene bands at 100 to 1500 bp for all bacterial isolates (n = 15). However, the sequencing of 16SrDNA genes (1346) with "BLAST" revealed that *E. faecalis* was a predominant (93.3%) enterococcal species (n=14) from infected specimens (n = 15) including 11(100%) from the vagina (n = 11) and 3 (75%) the blood (n = 4), whereas only one axenic isolate of *P. mcmeekinii* from the blood (Figure 1). The rooted neighbour joining phylogenetic tree showed the distribution and relationship between the tested bacteria (No.1 to 15) and the reference strains (n = 25) from the Gene Bank. All the *E. faecalis* isolates (No.1 to 11) from the vagina were 100% sequence identity with ATCC51299, while the other three identical *E. faecalis* isolates (No. 12, 13 and 14) from the blood were different from strain ATCC51299 in two positions of nucleotide sequences; the first one was a frame shift mutation (deletion of base A) from the sequence position 21 bp (Figure 2) and the second frame shift mutation (deletion of base A) was at 1076 bp (Figure 3). However, these three strains (No. 12, 13 and 14) were identical with the OG1RF strain (ATCC47077). Moreover, the single isolate of *P. mcmeekinii* was closely related with strain *P. mcmeekinii* ATCC700539 (99% sequences similarity) due to the single Gene or Point mutation (Transversion) of base C instead of A at position 358 bp (Figure 4) changing the amino acid Lys (AAA) to Gln (CAA), therefore, from our knowledge, the present report describes a first isolation of *P. mcmeekinii* from blood sample as an axenic culture, and according to the 16SrDNA sequences (1346bp), this isolate is revealed to be a new subspecies or species.

In general, all *E. faecalis* isolates showed no resistance to gentamycin, streptomycin and erythromycin (Table 1) and there were no differences in prevalence between vagina and blood *E. faecalis* isolates. In contrast, one isolate was resistant to chloramphenicol (7.1%) and 3, 4, 10 and 13 isolates were determine as resistant to penicillin (21.4%), ampicillin (28.5%), amoxicillin/clavulanic acid (71.4%) and oxacillin (92.8%), respectively with high prevalence in resistance to penicillin from blood isolates than vagina. According to agar screening method, vancomycin resistance depend on the concentration in the plate since all isolates were resistant in 4 and 16 µ/ml (100% for each), while one isolate was sensitive in 32 µ/ml (92.8%). The presence of the intrinsic *mecA* gene (92.8%), *vanA* and/or *vanB* genes (92.8%) were confirmed by PCR.

DISCUSSION

Conventional biochemical tests and commercial identi-fication system as well as phenotypic variants are not included in the level of subspecies and often miss identified (Seifert et al., 2003). In contrast, the high-quality of 16SrDNA sequence database provides excellent identifica-tion at the species and subspecies levels; furthermore, it can lead to the recognition of novel pathogens and non-cultured bacteria (Clarridge, 2004; Mellmann et al., 2006). In the present study, primers F27 and R1294 were used to amplify the 16SrRNA gene for all bacteria species to prevent losing of any species. *E. faecalis* is a predominant (93.3%) enterococcal species in the vagina (100%) and blood (75%) (Figure 1). *E. faecalis* is more frequent among the enterococcal species isolated from clinical sample especially vaginosis and bacteremia (Cetinkaya et al., 2000; Shepard and Gilmore, 2002; Karlowski et al., 2004; Jahic et al., 2005). Thus, any wound in the infected vagina might be the route to the bloodstream, since *E. faecalis* including proteases may help them to break down the normal barriers between the tissue and bloodstream (Huycke et al., 2002). Furthermore, enterococci have emerged as a major cause of nosocomial infection either locally or systematically including wounds, bacteremia and endocarditic infections (Nallaparedd et al., 2006; Peters et al., 2007) and it cause inflammation of the heart valves (Gilmore, 2002). Moreover, the three *E. faecalis* (No.12, 13 and 14) isolates from the blood were muted (Figures 2 and 3). This may be due to the foreignness of the abnormal environment "blood" (Satyanarayana et al., 2005); at the same time, they were identities with the *E. faecalis* strain OG1RF containing 3 Ebp (encoding for the endocarditis biofilm-associated pili) operons for producing surface pili which is used for attachment to the host surface and are antigenic in human during endocarditis (Nallapareddy et al., 2006). But, from a medical standpoint of pathogenicity, this species has the capacity to acquire a wide variety of antimicrobial resistance

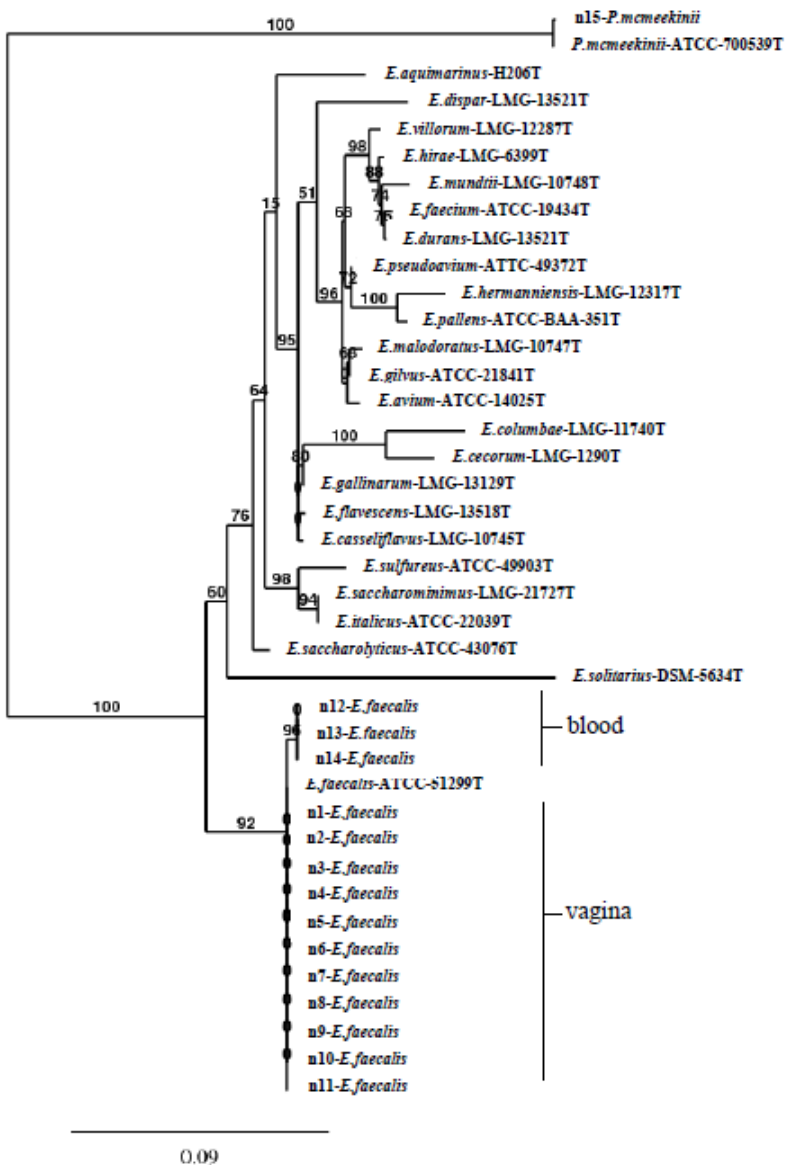

Figure 1. Rooted Neighbour-Joining tree based on 16SrDNA gene sequences (concaten- ated sequences at 1346bp.) showing the phylogenetic relationships of *E.faecalis* between the isolates (No. 1 to 14) and the reference strains (T) from GeneBank isolates. The tree has been rooted with *P.mcmeekinii*.
T: type strain, **ATCC** (American Type Culture Collection), **LMG** (Gent-Laboratorium voor Mirobiologie) and **DSM** (Deutsche Sammlung von Mikroorganismen and Zellkulturen) German Collection of Microorganisms and Cell Culture.

factors (Aakra et al., 2010), which present serious problems in the management of patients (Gilmore, 2002).

Table 1 shows a high resistance of *E. faecalis* to vancomycin (92.8%), oxacillin (92.8%) and amoxicillin/ clavulanic acid (71.4%) without different prevalence between in-(blood) and out-(vagina) patients. However, treatment should comprise a bacterial synergic combination of an aminoglycoside and a cell-wall active agent, such as vancomycin (Pupin et al., 2007). In general, enterococcal isolates with lowered susceptibility to vancomycin can be categorized as *vanA*, *vanB* and *vanC* genes (Chi et al., 2007). These genes lead to the production of an alternative structure D-alanine-D-lactate, instead of D-alanine-D-alanine found in the cell wall of susceptible bacteria (Courvalin, 2006). Furthermore, *vanA* and *vanB* are the most carried on plasmid and are readily transferable, thus *E. faecalis* can transfer these plasmids by conjugation (Cook et al., 2011).

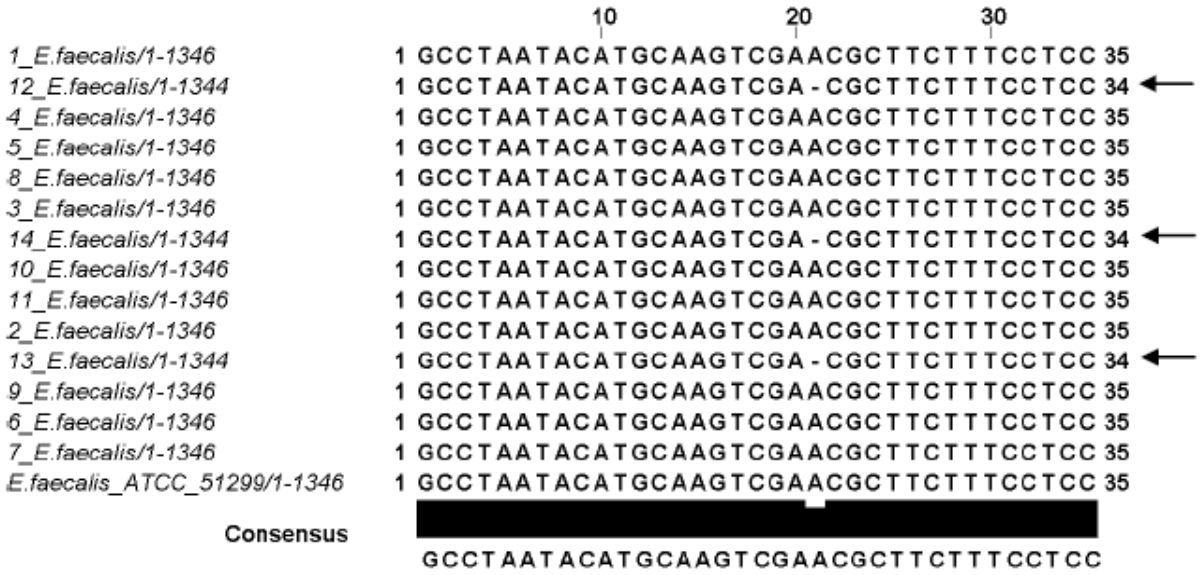

Figure 2. CLUSTALW for comparison of nucleotide sequences alignment (1346bp) for 16SrDNA gene of *E.faecalis* (No. 1 to 14) and ATCC51299. Isolates No. 12, 13 and 14 show Frame Shift Mutation (deletion nucleotide A) at the position 21bp.

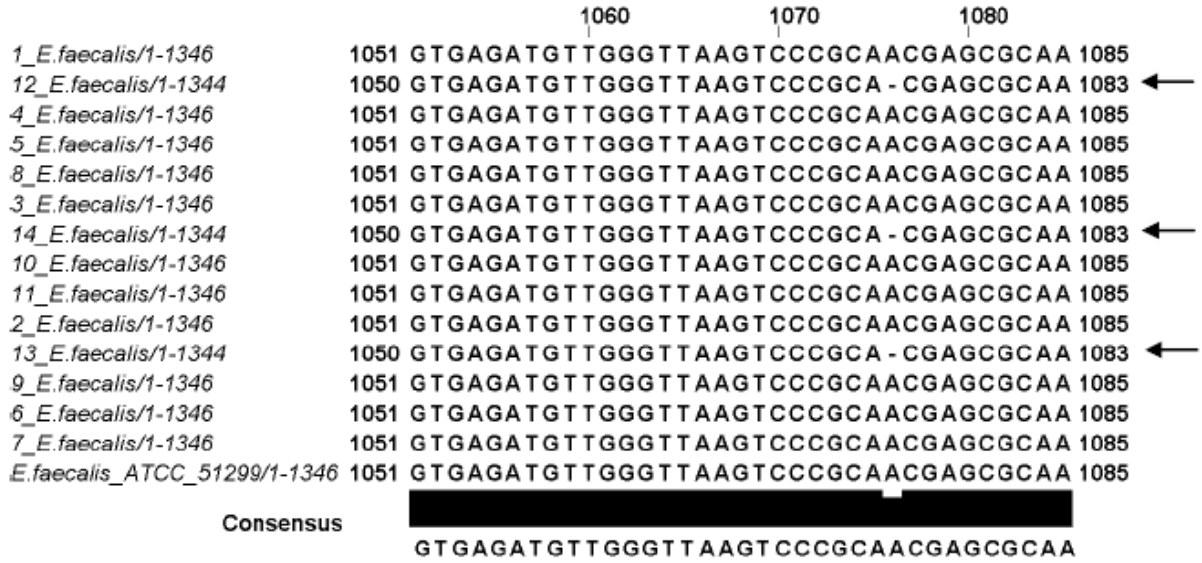

Figure 3. CLUSTALW for comparison of nucleotide sequences alignment (1346 bp.) for 16SrDNA gene of *E.faecalis* (No. 1 to 14) and ATCC51299. Isolates No. 12, 13 and 14 show Frame Shift Mutation (deletion nucleotide A) at the position 1076bp.

Although, the levels of resistance increase with the duration of exposure, but there is no methicillin or vancomycin using Basrah city. Therefore, the high resistance could be due to the plasmid transferring. Moreover, antimicrobial resistance is not a phenomenon restricted to a specific class of antimicrobials because of cross-resistance due to overlapping targets of different antimicrobials or co-selection related to genetic linkage between resistance genes (Simonsen et al., 2003).

Even the resistance to penicillin (21.4%) and ampicillin (28.5%) were low, but there was difference between the resistance of blood and vaginal isolates for both antimicrobial discs. However, the high susceptibility of gentamycin, streptomycin, erythromycin, chloramphenicol

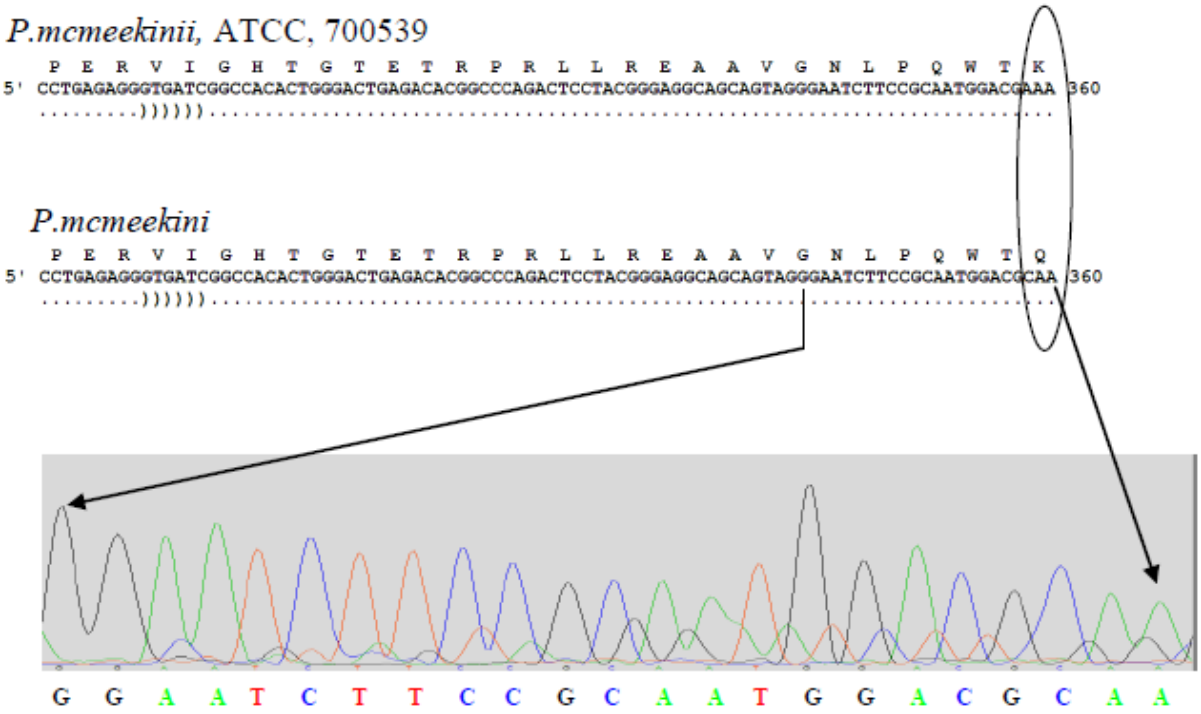

Figure 4. Comparison of 16SrDNA nucleotide sequences gene (1346bp.) for the isolate *planomicrobium mcmeekinii* (with peaks) and strain ATCC700539. A Gene or Point Mutation type Transvertion (C instead A) at the position 358bp. changing the amino acid Lys (AAA) to Gln (CAA).

col, penicillin and ampicillin (vaginal isolates) were in accor-dance with the results of Zhamel et al. (2003), Carvalho et al. (2004) and Aakra et al. (2010). The prevalence of resistance varied widely among laboratories (Simonsen et al., 2003); therefore, to detect a suitable antimicrobial drug, the susceptibility should be tested yearly. On the other hand, the present study appeared fortuitously a single isolate of *P. mcmeekinii* (1% difference in sequence of reference strain ATCC 700539) from blood patient which could be reported either a new separated species or subspecies. Since, according to some guidelines, a range of about a 0.5% to 1% difference (99.5 to 99% similarity) is often used for classification (Song et al., 2003). Bosshard et al. (2003) used ≥ 99% similarity to define species and ≥ 95 to < 99% to define a genus whereas, Hall et al. (2003) adopted a distance score of 0.00 to less than 1% as the criterion for species identity. While, Tang et al. (1998, 200), suggested a 0.5% difference as the limit for species designation. Furthermore, a strain with a small genotypic difference (less than 0.5%) has been considered as subspecies (Chen et al., 2002). When there is a clear phenotypic uniqueness, genogroups with less than 1% differences in sequence have in fact been named as a new species (Kattar et al., 2001; Roth et al., 2003; Tortoli, 2003). However, a comparison of sequences for several subspecies shows differences from 1 to 14 bp (Clarridge, 2004). Some of these variations among the researchers could be due to the fact, the percent difference can vary if it is calculated using only the first 500 bp or all 1500 bp of 16SrDNA gene sequences, and can also vary with the program used for calculations. Likely, the total sequence of *P. mcmeekinii* was 1346 bp after concate-nated. However, Clarridge (2004) appeared that isolates with a small genotypic difference (0.4 to 0.9%) but a definite phenotypic difference have been considered either separate species or subspecies. All the new species have been detected since 1990, most of which were grown from clinical samples and are potentially pathogenic, many of them differ from another by only a few base pairs, but seem to be correlated with unique phenotypic charac-teristics, clinical significance and niche, sometimes called sequevars (Tortoli, 2003; Takagi and Shin-ya, 2011). Because new sequences are found in almost all studies of clinical mycobacterial strains, the prospect is that many more sequevars will be detected, swelling the numbers of potential subspecies clades (Clarridge, 2004).

In conclusion, *E. faecalis* was observed as the predo-minant isolate from enterococcal bacteremia and vagina. The high resistance of *E. faecalis* to amoxicillin, methicillin and vancomycin revealed an alarming mark for uncon-trollable of these bacteria in future. Therefore, importance or rational use of antimicrobials in patient

Table 1. Antimicrobial susceptibility of *Enterococcus faecalis* isolated from vaginal outpatients' swab and blood inpatients' sample.

Source of sample	No.	Antimicrobial resistance / Intermediate										VN		
		CN	ST	E	C	P	AM	AMC	mecA	OX	vanA+B	ml/µ4	ml/µ16	ml/µ32
		10 mcg	10 mcg	15 mcg	30 mcg	10 mcg	25 mcg	20/10 mcg		1 mcg				
Vagina	1	S	S	S	S	S	S	R	+	R	+	R	R	R
	2	S	S	S	S	S	S	S	+	R	+	R	R	R
	3	S	S	S	S	S	S	R	+	R	+	R	R	R
	4	S	S	S	S	S	S	S	+	R	+	R	R	R
	5	S	S	S	S	S	R	R	+	R	+	R	R	R
	6	S	S	S	S	S	S	S	+	R	+	R	R	R
	7	S	S	S	S	S	S	R	+	R	+	R	R	R
	8	S	S	S	R	S	S	R	+	R	+	R	R	R
	9	S	S	S	S	S	S	R	+	R	+	R	R	R
	10	S	S	S	S	S	S	R	+	R	+	R	R	R
	11	S	S	S	S	S	S	S	+	R	-	R	R	S
Blood	12	S	S	S	S	R	R	R	+	R	+	R	R	R
	13	S	S	S	S	R	R	R	-	S	+	R	R	R
	14	S	S	S	S	R	R	R	+	R	+	R	R	R
	%	0	0	0	7.1	21.4	28.5	71.4	92.8	92.8	92.8	100	100	92.8

negative; **CN**, gentamycin; **ST**, streptomycin; **E**, erythromycin; **C**, chloramphenicol; **P**, penicillin**; AM**, ampicillin; **AMC**, amoxicillin/clavulanic acid; **,** resistant; **+,** positive; **- ,**Sensitive; **R ,S *mecA***, methicillin resistant gene; **OX,** oxacillin(methicillin); ***vanA+B***, vancomycin resistant *gene A* and *B*; **VN**, vancomycin.

management and infection control is needed. *P. mcmeekinii* was isolated from bacteremia case to appear in a new separate species or subspecies.

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Full Length Research paper

Frequency of rapid viral response (RVR) and influence of various factors on the response rates in chronic hepatitis C infected patients treated with interferon and ribavirin combination therapy

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The aim of this study was to determine the frequency of rapid viral response (RVR) and influence of various factors on the response rates in chronic hepatitis C infected patients treated with interferon and ribavirin combination therapy. This study was conducted in Isra University Hospital, Hyderabad-Pakistan and Liaquat University of Medical and Health Sciences, Jamshoro/ Hyderabad, Pakistan, from July 2007 to December 2008. All consecutive adult patients aged between 18 and 65 years who were naïve to interferon-based therapy and fulfilled the following criteria were eligible for this study: anti-HCV antibody, HCV RNA positive, genotype 3, and with elevated ALT (alanine aminotransferase) levels. Statistical analysis was performed using the statistical program for social sciences (SPSS 16.0 for window SPSS Inc: Chicago, IL).This descriptive case series study included 195 consecutive patients of which 113 (57.9%) were male and 82 (42.1%) female. The mean age of the patients was 37.3± 9.62 years. 150 (76.9%) patients were on conventional interferon. Rapid viral response was seen in 167 (85.6%) patients. In univariate analysis, only serum glutamic pyruvic transaminase (SGPT) quotient has shown a statistically significant difference as 96/107 (89.7%) patients with quotient < 2.3 went into RVR as compared to 71/88 (80.6%) patients with >2.3 quotient (p=0.03). In multivariate analysis, SGPT quotient has shown statistical significance with SGPT quotient < 2.3; this indicates that odds ratio of 0.40 (p=0.04) RVR is rapidly becoming a new tool for predicting treatment outcomes in patients with chronic hepatitis C and represents a key opportunity to individualize therapy according to treatment-related viral kinetics

Key words: Hepatitis C, serum glutamic pyruvic transaminase (SGPT), rapid viral response (RVR).

INTRODUCTION

Chronic infection with hepatitis C virus (HCV) is estimated to affect 170 million individuals worldwide (Alter, 1997). Hepatitis C virus infection is the major cause of chronic hepatitis and eventually liver cirrhosis and hepatocellular carcinoma in Pakistan (Farooqi and Farooqi, 2000; Durrani et al., 2001). The combination treatment with interferon and ribavirin for 6 to 12 months is the current treatment of choice for chronic hepatitis C infection (Poynared et al., 1998; Mc Hutchison et al., 1998).

In the treatment of chronic hepatitis C with interferon (IFN), there have been few reliable markers of viral infection that predict response to therapy. Genotype and baseline hepatitis C virus (HCV) RNA have been associated with the likelihood of a sustained response, while demographic information such as age, sex, liver

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enzymes, and histology have also been useful (Jenkins et al., 1996).

Although it is apparent that viral clearance (that is, undetectable HCV RNA) is the most reliable initial indicator of sustained biochemical and virological response, just how early an accurate prediction for virological response can be made is still not settled. The presence of early viral response (EVR) at week 12 was considered to be an important predictive factor of sustained viral response (SVR) by the National Institutes of Health (NIH) in 2002 and was a routine part of monitoring patients (National Institutes of Health, 2002). However, recent studies suggest that in patients with chronic hepatitis C treated with interferon and ribavirin, a rapid viral response was frequently an indication of early viral response at week 12, and can predict sustained viral response (Zeuzem et al., 1998). Civeira and Prieto (1999), while reviewing 18 studies involving 988 patients, concluded that undetectable levels of HCV RNA after 4 weeks of therapy correlate with a subsequent SVR in about 50% of patients (positive predictive value), whereas the presence of HCV RNA at 4 weeks correctly predicts failure to achieve SVR (negative predictive value) in over 97% of patients. This would be helpful in limiting unsuccessful treatment for patients with chronic hepatitis C, thus reducing side effects and cost. It would also allow other therapeutic options to be pursued sooner in the course of treatment.

The aim of this study was to determine the frequency of rapid viral response (RVR) and influence of various factors on the response rates in chronic hepatitis C infected patients treated with interferon and ribavirin combination therapy.

MATERIALS AND METHODS

Ethics

The study was conducted in accordance with the principles of the declaration of Helsinki and the International Conference on Harmonization for good clinical practice. All patients provided written informed consent before enrolment.

Patients

All consecutive adult patients aged between 18 and 65 years who were naïve to interferon-based therapy and fulfilled the following criteria were eligible for this study: presence of an anti-HCV antibody (Abbot HCV EIA 2.0 Abbot Diagnostic, Chicago, IL) and HCV RNA for than 6 months, genotype 3 and with elevated ALT (alanine aminotransferase) levels.

Patients were excluded from the study if they had non genotype 3 hepatitis C, neutropenia (neutrophil count < 1500/mm^3), thrombocytopenia (platelet count <90,000/ml^3), co-infection with hepatitis B virus (HBV), chronic alcohol abuse (daily alcohol consumption > 20 g/day), autoimmune liver diseases, decompensated cirrhosis (Child Pugh class B and C), neoplastic disease, organ transplantation or immuno- suppressive therapy, evidence of drug abuse, poorly controlled autoimmune diseases, cardio pulmonary diseases, neuropsychiatric disorders and were unwilling to take contraceptive during the study.

Study design

This descriptive case series study was started in two centers (Isra University Hospital and Liaquat University of Medical and Health Sciences) in Hyderabad-Pakistan from July 2007 and till December 2008. Eligible patients were assigned to receive either once weekly subcutaneous injection of 180 µg pegylated interferon α-2a (Pegasys, F. Hoffmann, La Roche, Basel, Switzerland) or thrice weekly subcutaneous injection of 3 MIU standard interferon α-2b (Bioferon, BIOSIDUS S.A. Constitucion, 4234 (C1254ABX) BuenousAires, Argentina) for 24 weeks. All patients received dosage of ribavirin mg/day according to body weight in two or three divisions. Participants received the study drugs on out patient basis. Furthermore, they received out patient visits to assess the efficacy and safety at monthly interval until the end of therapy. Laboratory tests including blood CBC and serum ALT levels were assessed at each out patient visit. Serum HCV RNA was evaluated qualitatively at base line, and at week 4 of the study (CobasAmplicor HCV monitor V2.0 Roche Molecular Systems Pleasanton CA; with detection cut off level of 50 IU/ml). HCV genotyping was performed at base line by a reverse hybridization technique (Inno-LIPA HCV II, Innogenetics, Ghent, Belgium). The RVR was defined as undetectable HCV RNA by a sensitive qualitative assay test at week 4 of the study.

Assessment of efficacy

The primary efficacy end point was RVR, defined as undetectable HCV RNA by a sensitive qualitative test at week 4 of the study by intention to treat (ITT) analysis. The secondary efficacy end point was to delineate the positive (favorable) predictors of RVR in this population of patients. The base line predictors were: age (<40 years), sex, body weight (<66 kg) at baseline, BMI (<25) at baseline, type of interferon (whether conventional or pegylated), ALT quotient (the average of the serum ALT values before treatment, divided by the upper limit of normal) (<2.3) AST (aspartate aminotransferase) quotient (the average of the serum AST values before treatment, divided by the upper limit of normal) (<2.3), and baseline platelets level (> 150,000 ml^3). The normal value of ALT and AST were taken 33 IU/L in males and 19 IU/L in females (R)

Statistical analysis

This was performed using the statistical program for social sciences (SPSS 16.0 for window SPSS Inc: Chicago, IL). The estimated sample size of 180 patients was based on type I error rate of $\alpha = 0.05$, with the assumption of 60 to 80% RVR in genotype 3 patients treated with conventional or pegylated interferon. The independent student t test was used to compare quantitative variables, and X^2 test or Fischer's exact test was used for qualitative variable. ITT analysis for efficacy was performed on the basis of patient who received at least one dose of the study medication. The secondary efficacy end point was to analyze only in those patients who had tested for pre treatment and week 4 HCV RNA. The relatedness of pre treatment variables to RVR was examined by univariate analysis, multivariate logistic regression analysis and X^2 test. A p-value of <0.05 was considered statistically significant. All the statistical tests were two-tailed.

Table 1. Baseline characteristics of patients.

Quantitative variable	Mean	±Std. deviation
Age of patients	37.3	9.62
SGPT IU/l	74.47	59.59
SGOT IU/l	50.11	34.92
Palelet count 10^9/L	236	62.17
Qualitative variable	**Frequency**	**Percentage (%)**
Male	113	57.9
Female	82	42.1
Diabetes mellitus	19	9.7
Conventional interferon	150	76.9
Rapid viral response	167	85.6

RESULTS

This descriptive case series study included 195 consecutive patients. There were 113 (57.9%) male and 82 (42.1%) female. The mean age of the patients was 37.3± 9.62 years. The mean serum glutamic pyruvic transaminase (SGPT) was 74.47 59.59 IU/l, serum-glutamate-oxaloacetate transaminase (SGOT) levels was 50.11± 34.92, and platelet count 236±62.17× 10^3. Diabetes mellitus was present in 19 (9.7%) patients. 150 (76.9%) patients were on conventional interferon. Rapid viral response was seen in 167 (85.6%) patients (p=0.0001). Table 1 shows the baseline characteristics of the patients. In univariate analysis, only SGPT quotient has shown a statistically significant difference as 96/107 (89.7%) patients with quotient < 2.3 went into RVR as compared to 71/88 (80.6%) patients with >2.3 quotient (p=0.07). Depending on age, type of interferon, sex, and SGOT quotient, diabetes mellitus has shown nonsignificant difference as 116/134 (86.5%) patients < 40 years of age showed RVR as compared to 51/61 (83.6%) patients > 40 years of age (0.66) and 126/150 (84%) patients on conventional interferon based therapy to 41/45 (88%) RVR on peg-interferon based therapy (p=0.33). Table 2 shows univariate analysis performed by Fisher's exact test of chi square. In multivariate analysis, SGPT quotient has shown statistical significance with SGPT quotient < 2.3 showed odds ratio of 0.40 (p=0.04). Table 3 shows multivariate analysis by logistic regression of all variables.

DISCUSSION

The primary endpoint in this study was a rapid virological response (SVR), that is, absence of serum HCV RNA after 4 weeks of the treatment.

In our study, the serum HCV RNA was cleared at 4 weeks in 167 (85.6%) patients. Our study confirms the results of Zeuzem et al. (2004) who saw RVR in 85% of cases. According to them, rapid viral response (RVR: undetectable HCV-RNA at 4 weeks of therapy) emerged as a strong predictor of SVR. In a randomized trial conducted by Von Wagner et al. (2005) involving six tertiary centers, and enrolling 153 patients, RVR was achieved in 92% of the patients with HCV 3; this again shows the importance of rapid viral response as a strong predictor of sustained viral response. According to ACCEL-ERATE study, RVR emerged as the strongest predictor for SVR as patients with RVR and low viral level (LVL) achieved SVR in 94% cases, while those with RVR and high viral levels (HVL) achieved SVR in 88% cases (Shiffman et al., 2007). Only 49% of non-RVR subjects achieved an SVR. Civeira and Prieto (1999), reviewing 18 studies involving 988 patients, concluded that undetectable levels of HCV RNA after 4 weeks of standard IFN and ribavirin therapy correlate with a subsequent SVR in about 50% of patients (positive predictive value), whereas the presence of HCV RNA at 4 weeks correctly predicts failure to achieve SVR (negative predictive value) in over 97% of the patients.

No data is available for prediction of RVR in patients undergoing treatment for HCV, although some data is available for SVR. One such study has been reported that at the age of 20 years, no cirrhosis/bridging fibrosis, ALT quotient ≤ 7,body mass index ≤20 kg/m, and viral load ≤40 × 1062 IU/L was associated with a 97% probability of SVR (Foster et al., 2007).

In our study, SGPT has been found as a statistically significant factor influencing the rapid viral response as SGPT < 2.3 has shown better RVR as compared to > 2.3 in accordance to Bader et al. (2008). One of the most important finding in our study was the nonsignificant difference between standard interferon and peg interferon as far as RVR is concerned in accordance to Mann et al. (2001) who observed 80% sustained viral response with both type of treatments in genotype 2 and 3 hepatitis C. More studies are needed for this important issue because peg interferon is very costly and if there is not much difference between peg interferon and standard interferon, then it would be cost effective in this economically poor country where genotype 3 is more preponderant.

In this study, no significant difference in response was found among the gender, age, weight and the presence of diabetes mellitus. Weaker associations have been reported for gender (women responding better than men) (Poynard et al., 2000; Hayashi et al., 1998) and body weight (Camps, 1993). According to Idress and Riazuddin (2009), age has been seen as a factor for predicting the sustained viral response in patients with hepatitis C.

In this study, the multivariate analysis of the variables associated with RVR found only one significant association with SGPT (odds ratio) and three significant associations with genotype non-1 (odds ratio, 3.25),

Table 2. Univariate analysis performed by Fisher's exact test of chi square.

Parameter	RVR (n = 195)		P value
	Positive n = 167 (%)	Negative n = 28 (%)	
Age in group			0.66
< 40	116 (86.5%)	18 (13.5%)	
> 40	51 (83.6%)	10 (16.4%)	
Type of INF			0.33
CONV	126 (84%)	24 (16%)	
PEG based	41 (88%)	4 (12%)	
Body mass index (kg/m^2)			0.92
< 25	70 (41.9%)	12 (42.9%)	
> 25	97 (58.1%)	16 (57.1%)	
Gender			0.58
Male	95 (84.1%)	18 (15.9%)	
Female	72 (87.8%)	10 (12.2%)	
Weight (in kg)			0.68
< 66	86 (51.5%)	13 (46.4%)	
> 66	81 (48.5%)	15 (53.6%)	
Diabetes mellitus			0.16
Present	14 (73.6%)	5 (27.3%)	
Absent	153 (86.9%)	23 (13.1%)	
SGPT			0.03
< 2.3	96 (89.7%)	11 (10.3%)	
> 2.3	71 (80.6%)	17 (19.4%)	
SGOT			0.75
< 2.3	133 (85.2%)	23 (14.8%)	
> 2.3	34 (87.1%)	5 (12.9%)	

Table 3. Multivariate analysis by logistic regression (n = 195).

Parameter	Adjusted odds ratio	95% Confidence interval	P value
Age > 40 years	0.79	0.34 – 1.83	0.58
Type of interferon(CONV/Peg)	1.90	0.58 - 6.18	0.28
BMI < 25 (kg/m^2)	1.14	0.44 – 2.96	0.78
Gender (male/female)	1.41	0.57 – 3.51	0.54
Weight < 66 (kg)	0.85	0.33 – 2.18	0.74
Presence of diabetes mellitus	2.15	0.66 – 6.98	0.20
SGPT < 2.3	0.40	0.16 – 0.98	0.04*
SGOT < 2.3	1.76	0.48 – 6.43	0.39

age<40 years (odds ratio, 2.60) and body weight < 75 kg(odds ratio, 1.91) (Fried et al., 2002).

Conclusion

RVR is rapidly becoming a new tool for predicting, which patients with hepatitis C have a high likelihood of attaining SVR. In addition, it may identify patients for whom a truncated course of therapy is appropriate. In patients infected with HCV G2 or G3, EVR has little usefulness. In contrast, RVR is an important point at which strategies for shortened treatment regimens can be

evaluated. Additional studies are necessary to improve understanding of the relationship between baseline viral load and RVR and how these factors can be used together to define optimal treatment duration. Shortened courses of treatment may be useful if adverse effects or costs are an issue and are particularly valuable in patients who experience substantial adverse effects that may pose a health risk if treatment is continued. Thus, RVR represents a key opportunity to individualize therapy according to treatment-related viral kinetics.

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Full Length Research Paper

Prevalence of dementia varied with age, education and social network in a Senegalese elderly population of patients utilizing the Medico-Social and University Center of IPRES, Dakar-Senegal

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With the ageing of the population, dementia is increasing. In Africa, studies on dementia of the elderly are seldom. However, dementia exists and is not well documented in Senegal especially among the elderly population. We conducted a study to estimate the prevalence of this disease among Senegalese elderly population utilizing the Medico Social and University Center of IPRES, Dakar-Senegal. The study was cross-sectional and intended, through a two-wave process of data collection, to collect data from March 2004 to December 2005 among Senegalese elderly population (aged 65 years) over utilizing the MedicoSocial and University Center of IPRES, Dakar-Senegal for health care. Sociodemographic, medical history, lifestyles and social network data were collected with a structured questionnaire completed with a clinical examination and neuropsychological testing. Diagnosis of dementia was based on DSM IV criteria. The population composed of 507 patients with a mean age of 72.4 years (±5.25), mostly male, married, and non-educated. Hypertension, arthritis, gastro-intestinal, respiratory and urinary diseases were the main health conditions reported. The elderly population had a high social network. 45 patients (8.87; 95% IC: 7.61 to 10.13) had dementia in which prevalence varied significantly with age, education and social network. The results confirm the variability of dementia with age, education and social network at the Medico Social and University Center of IPRES, Dakar-Senegal.

Keywords: Dementia, prevalence, education, elderly person, Senegal.

INTRODUCTION

With the ageing of the population worldwide, dementia is a real public health priority (Qui et al., 2007). In 2010, the estimated number of dementia cases was 35.5 millions people representing 0.4% of the worldwide population. This number will be 65.7 millions in 2030 and 115.4 millions in 2050. More or less, 2/3 of the cases lived in developing countries (Wimo and Prince, 2010). Dementia constitutes a real social, economic and medical burden. Its prevalence increases with age (Lobo et al., 2000). Dementia has been associated with institutionalization (Aguero-Torres et al., 2001), functional dependency (Aguero-Torres et al.,1998) and higher mortality (Aguero-Torres et al.,1999) among elderly. Anxiety and depression are of real concern within caregivers (Mahoney et al., 2005). Costs of care are too important

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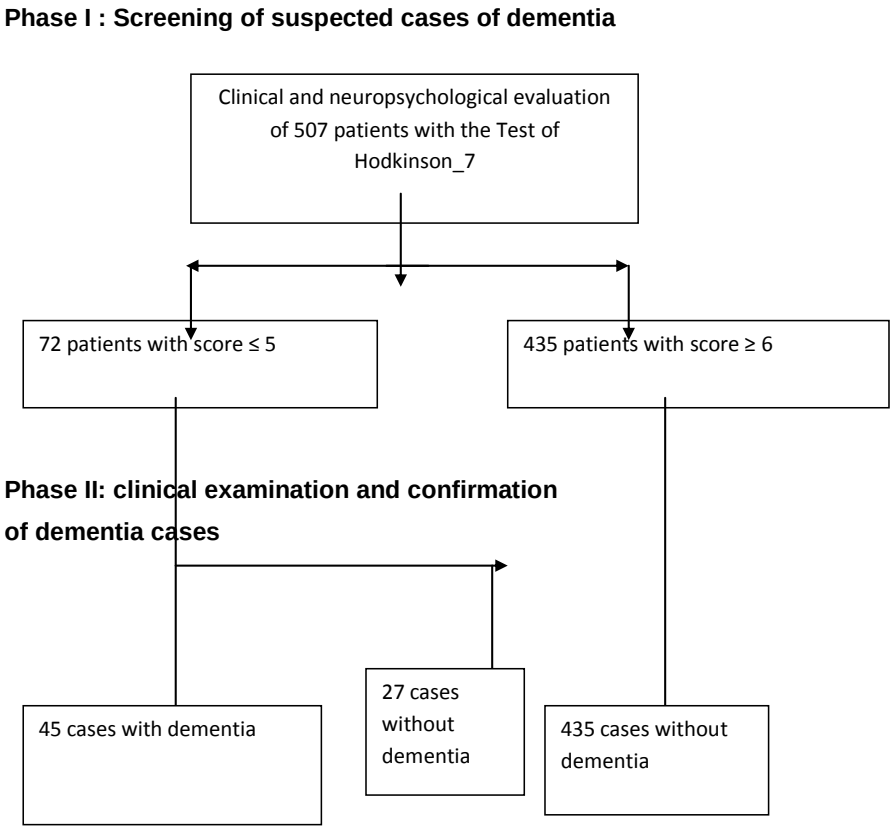

Figure 1. Description of the research process.

(Zhao et al., 2010). Several studies were conducted in developed countries to better understand the epidemiology of this new epidemic, few ones have been done in Africa (Toure et al., 2010). In Senegal, the estimated number of elderly 65 years and above was 421,305 in 2008 and 420,795 in 2009. It will be 426,443 in 2012 (ANSD, 2008). With the development of morbid conditions such as hypertension, diabetes and depression, dementia will become a real public health priority in the future (Toure et al., 2007). This means that the number of demented elderly will increase also. Considering the economic cost of dementia care, Senegal is not able to afford such cost. To plan for more accurate provision of social and medical services for the elderly population, it is important to have reliable information on the prevalence of dementia. Thus, a study was conducted to estimate the prevalence of dementia in an elderly population of dementia utilizing a primary health care service for retirees in Dakar, Senegal.

METHODS

The study population was composed of Senegalese elderly patients aged 65 years and over who came to the Medico Social and University Center of IPRES for health problems. This population is affiliated to IPRES. Those patients who were either less than 65 year old or were not able to fulfill interview are excluded (aphasia, delirium, coma, extreme visual and auditory impairment, cancer at terminal phase). The study was cross-sectional. From March 2004 to December 2005, 507 elderly patients aged 65 years and over or the relative who consulted a doctor for medical problem at the Medico Social and University Center of IPRES were first assessed with a screening interview questionnaire. Those who were considered as cognitively impaired underwent a clinical exam with neuropsychological testing. This study was the first step of a research on the validation of a screening tool to screen for dementia in a Senegalese elderly population called “The Test of Senegal” (Toure et al., 2008) (Figure 1). The screening interview questionnaire “Aging in Senegal” contained the following: socio-demographic variables (age, sex, marital status, education), medical history (vascular diseases [hypertension, heart diseases, vascular peripheral disease, stroke, diabetes], respiratory diseases, arthritis, cancer/benign tumour, Parkinson disease, epilepsy, genitor-urinary disease, cataract, glaucoma, hearing impairment, digestive disease (gastritis, constipation), anaemia, thyroid disease, head trauma, bone fracture), familial history of memory impairment, lifestyles (smoking, alcohol consumption, walking), social network (social ties with spouse, children, brothers/sisters, friends; frequency of weekly contacts with children, brothers/sisters, friends; members of community association, member of religious association), the patient’s functional autonomy (Fillenbaum et al., 1985) and the neuropsychological tests with the Abbreviated Mental Test (Jitapunkul et al., 1991) and the Test of Senegal (Toure et al., 2008). The Clinical assessment instrument had four components: 1) a historical review of the patient’s cognitive function, that is, the

onset and progression of any reported symptoms of cognitive impairment; 2) a review of the patient's medical, surgical and familial history, exposure to toxic products and medications; 3) a review of the patient's functional autonomy (Fillenbaum et al., 1985) 4) a review of the patient's clinical exam. Each patient underwent a screening interview with the questionnaire "Aging in Senegal" by four medical students at the MSUC who were trained for this issue. After the interview, each patient who had a score of 5 or less on the Abbreviated Mental Test (Jitapunkul et al., 1991) was referred for clinical assessment to the principal investigator. The clinical assessment consisted on a complete physical exam followed by a neuropsychological testing with the Mini Mental State Examination (Baiyewu et al., 1993). If a patient was suspected to have depression, the CES-D scale was administered to him/her to confirm the diagnosis (Radloff et al., 1997). All clinical assessments were made without knowledge of the screening status of the patient. At the end of the consultation, the team members met in a room to confirm the diagnosis of the patient. On the basis of the examination, patients with dementia were followed by the principal investigator. Appropriate laboratory exams and computerized tomography of the head were ordered and treatment of associated medical conditions proposed.

This study was approved by the ethical committee of the Senegalese Ministry of Health and university of Montreal, Quebec-Canada. Before the start of the study, informed consent was obtained from the patient and/or his/her relative.

Socio-demographic variables (age in 4 categories [65 to 69 years, 70 to 74 years, 75 to 79 years, 80 years and plus], sex, marital status, education were collected with the medical history and familial history of cognitive impairment. Lifestyles were divided into smoking habit (yes, no), alcohol consumption (yes, no) and walking (yes, no).

For the social network, we computed two indexes: diversities of social ties (score 0-4) and frequency of weekly contacts with relatives (score 0 to 6). Diversity of social ties were computed by summing "Having a spouse or husband, children, brothers/sisters and friends" and categorized into 3 levels: 0 to 2 ties, 3 ties and 4 ties. Frequency of weekly contacts with relatives were obtained by summing the frequency of weekly contacts with children, brothers/sisters and friends and categorized into 4 levels: 0 to 3 weekly contacts, 4 weekly contacts, 5 weekly contacts, 6 weekly contacts. The medical conditions related to medical variables were dichotomized into "yes or no". Dementia was defined according to the DSM-IV-R criteria (APA, 1994). All the data collected were analysed using the SPSS-13.0 version package for Windows. Univariate and bivariate analysis were performed and results expressed with a 95% confidence interval (CI).

RESULTS

The whole population (507 patients) with a mean age of 72.4 years (±5.2) were mostly male, married, and illiterates. Smoking was important (27.0%); alcohol consumption was rare (9.1%). But walking was the main physical activity (95.0%). The elderly population had a high diversity of ties and frequency of contacts with the relatives and friends (Table 1). Hypertension (58.6%), arthritis (49.5%), gastro-intestinal diseases (24.1%), respiratory diseases (14.7%) and cataract (14.4%) were the main health conditions reported in the past medical history (Table 2). 45 patients (8.87%; 95% CI: 7.61 to 10.13) had dementia. In the bi-variate analysis, age ($p<0.045$), education ($p<0.02$), diversity of ties with relatives ($p<0.001$), frequency of contact with relatives ($p<0.000$), stroke ($p<0.002$), epilepsy ($p<0.002$) and family history of dementia ($p<0.000$) were associated with dementia.

DISCUSSION

In our study, the prevalence of dementia was 8.87%. This prevalence is higher than expected in our population however the study was conducted in a geriatric service. But, frequency of dementia can be high especially in medical ward and institution. In Belgium, Kurz et al. (2001) found a prevalence of 11.3% in a population of 2234 elderly patients aged 65 years and over consulting in general practice. In Mexico, a prevalence of 16.1% was observed in a population of patients in a nursing home (Alvarado-Esquivel et al., 2004). In Denmark, it was 17.4% in a population of 793 patients followed by general practitioners (Waldorff et al., 2005). In USA, the prevalence was 48.2% in Maryland in a population of 2285 nursing home patient (Magaziner et al., 2000) and 7.3% among veterans followed through the Veterans Affairs Medical care System of Texas (Krishnan et al., 2005). However, the occurrence of dementia is considered rare in African population studies related to many factors (Toure et al., 2010). Lower prevalence was observed during populational studies conducted in Benin (2.6%) (Guerchet et al., 2009), Central Nigeria (6.4%) (Ochayi and Thacher, 2006) and Ibadan-Nigeria (2.2%) (Hendrie, 1995).

In our study, prevalence of dementia increases with age, literacy and social network. The role of age as a risk factor for dementia has been highlighted in several studies. In fact, the prevalence of dementia is increasing importantly with age as observed in clinical setting and during populational studies. So, the result we observed confirms the role of ageing (specially advanced age) in the occurrence of dementia as described worldwide and especially in Europe (Kokmen et al., 1998; Erkinjuntti et al., 1986), America (Breteler et al., 1998; Beard et al., 1995; Evans et al., 1989) and also Africa (Hendrie et al., 1995; Toure et al., 2008; Guerchet et al., 2010). Life expectancy is increasing in Africa and better ageing is possible through healthy lifestyle. Unfortunately, ageing is sometimes associated with the occurrence of cumulative risk factors for dementia as chronic diseases (hypertension, diabetes, hypercholesterolemia, and cancer), loneliness, isolation and poverty. Illiteracy which was frequent in our study population (57%) was associated with dementia. This result confirmed the role of illiteracy as a risk factor for dementia in elderly population as already seen in studies realized worldwide (Ravaglia et al., 2002; Katzman, 1993). So, it is important to set up and sustain an educational program dedicated to educate the youth at a higher level (till secondary school level at least) to prevent dementia in the ageing population.

African elderly occupied a central place in the society

Table 1. Sociodemographic characteristics, lifestyles and social network of the population of patients (N=507).

Variables	N	Percentage
Age : Mean (72,4 years ± 5,25: 65-90 years)		
65-69 years	160	31.6
70-74 years	178	35.1
75-79 years	118	23.3
80 years +	51	10.1
Sex		69.6
Male	353	
Marital Status		76.9
Married	390	
Education		47
Yes	240	
Alcohol		9.1
Yes	47	
Smoking		27
Yes	137	
Working		95.5
Yes	484	
Other activities		85
Absence	431	8.5
1 activity	43	6.5
2 activities +	33	
Diversity of social ties with relatives		6.1
0-2 liens	31	27.8
3 liens	141	66.1
4 liens	335	
Weekly contacts with relatives		11.8
0-3 contacts	60	17.4
4 contacts	88	14.2
5 contacts	72	56.6
6 contacts +	287	

where ageing was associated with respectfulness and dignity. He or she was living in a place where you could find 3 generations in the same place especially in the village where this family structure was the rule. His or her involvement in the societal development was well known and also securing the traditional values. But, with the modernization of the society and the economic crisis, the elderly seems to be alone and isolated. This situation could lead to depression and also dementing illness. In our study population, 6.1 and 11.8% of the elderly had respectively 0 to 2 ties and few weekly contacts with the relatives (at least 3 contacts). The prevalence of dementia varied significantly with the importance of social network. This result confirms what was described and observed in studies conducted worldwide showing that low social network is a great risk factor for dementia in elderly population (Fratiglioni et al., 2004; Bennett et al., 2006). In fact, low social network and its corollary loneliness is frequently associated with depression, cardiovascular diseases and vascular risk factors, which

Table 2. Prevalence of dementia and sociodemographic characteristics, lifestyles and social network of the population of patients (N=507).

Variables	Number of cases of dementia	Prevalence (%)	P-value
Age			0.045*
65-69	8	5.0	
70-74	16	9.0	
75-79	12	10.2	
80 et +	9	17.6	
Sex			
Male	31	8.7	0.515
Female	14	9.0	
Marital Status			
Married	31	7.9	0.125
No-married	14	12	
Education			0.02*
Yes	31	12.8	
No	14	53	
Smoking			0.732
Yes	12	8.8	
No	33	8.9	
Alcohol			0.556
Yes	3	6.5	
No	42	9.1	
Other Activities			0.398
Absence	31	8.8	
1 activity	3	6.7	
2 activities +	4	8.9	
Diversity of social ties			0.001*
0-2 liens	8	25.8	
3 liens	15	10.6	
4 liens	22	6.5	
Weekly Contacts with relatives			0.0001*
0-3 contacts	12	20	
4 contacts	14	16.9	
5 contacts	8	11.1	
6 contacts +	11	3.8	

are main risk factors for dementia (Stewart et al., 2003; Green et al., 2003).

Limits of the study

As a non-randomized cross-sectional study, under-estimation of dementia could be a limit because all the patients were not screened. First of all, the tool we used to screen for dementia was not fully sensitive, as such, demented patients could be missing thereby leading to an under-estimation of this disease among the sample. Secondly, some demented patients had been missing as they were not present during the consultation because of their disease. Thirdly, the study had a problem of external validity because the patients who were unrolled neither represent all the patients of the Medico-social and University Center of IPRES, nor the whole Senegalese

elderly population.

Conclusion

This study has shown a higher prevalence of dementia in this Senegalese elderly population of patients admitted at the Medico Social and University Center of IPRES, Dakar-Senegal. It confirmed the variability of the prevalence with age, education and social network. It is important to take into consideration these results for planning dementia prevention program in Senegal.

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Full Length Research Paper

Comparative study of pulmonary functions after administration of albuterol and levalbuterol in patients with moderate to severe bronchial asthma

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β_2 - adrenoceptor stimulants play a key role in the management of bronchial asthma. This study was carried out on 80 patients of moderate to severe bronchial asthma. Group I (n = 40) received Albuterol 2.5 mg/2.5 ml and Group II (n = 40) received Levalbuterol 0.63 mg/2.5 ml TDS for 4 weeks. Baseline and post-treatment evaluation of lung function, respiratory rate, Total leukocytes Count, Total eosinophil Count, Serum potassium and Heart rate were carried out. In group I, Forced Expiratory Volume in 1 s was increased from 1.565±0.53 to 1.74±0.64 L ($p>0.05$) and in group II it was increased from 1.48±0.91 to 2.10±0.70 L ($p<0.05$). Forced vital capacity and Peak Expiratory Forced Rate were also increased in both groups ($p<0.05$). Respiratory rate and Total eosinophil count were significantly decreased by both drugs. Total leukocyte count was decreased non-significantly by both drugs ($p>0.05$). Serum potassium was decreased in group I from 3.77±0.38 to 2.96±0.49 mEq/L ($p = 0.001$) and in group II from 3.79±0.57 to 3.51±0.56 mEq/L ($P = 0.017$). Heart rate was significantly increased by both drugs, but it was greater with Albuterol. Levalbuterol appears to be more effective with better tolerability in low dose as compare to Albuterol.

Keywords: Albuterol, Levalbuterol, bronchial asthma, bronchodilation, β_2-agonists.

INTRODUCTION

Asthma is a chronic inflammatory disease associated with airway hyper-responsiveness and episodic wheezing characterized by breathlessness, chest-tightness, and cough, particularly at night or in the early morning. Various cells like eosinophils, T-cells, mast cells, basophils and neutrophils play an important role in pathophysiology of asthma (Hamid et al., 2003). Asthma also involves contraction of airway smooth muscles, airway wall remodeling, edema and hyper secretion of mucus, contributing significantly to bronchial obstruction. As a result the use of bronchodilators remains at the fore front of modern approaches to asthma therapy (Fernandes et al., 2004). β_2- Agonists drugs are the most commonly used bronchodilators used in the treatment of asthma to relive bronchospasm (Dollery, 1999). The most commonly prescribed β_2- agonist is Albuterol (Salbutamol), was first described by Brittain et al. (1968). It is also known as racemic albuterol, 1:1 mixture of (R) - and (S) - albuterol, stereoisomers. R -and RS - albuterol have a 2:1 potency ratio for improvement in FEV_1 in asthmatic patients and shows that S - albuterol is clinically inactive. Because the RS - albuterol mixture

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contains only 50 % R - albuterol, it is clear that the clinical effect of albuterol resides with the R - enantiomer. Furthermore, the therapeutic ratios of R- and R,-S - albuterol are very similar, suggesting that the S - enantiomer of albuterol does not affect its therapeutic ratio (Lotvall et al., 2001). The new evidence suggests that (S) - albuterol is not inert, but rather may exaggerate airway reactivity and cause loss of asthma control. Specifically, (S) - albuterol increases intracellular calcium (Yamaguchi and McCullough, 1996; Mitra et al., 1998), enhances experimental airway hyper responsiveness to spasmogens (Morley, 1996; Johansson et al., 1996) and may have pro-inflammatory effects as gauged by eosinophil superoxide production in response to IL-5 (Volchek et al., 1998). (S) - albuterol is metabolized 10-fold more slowly than Levalbuterol (Walle et al., 1996; Boulton et al., 1996). With repeated frequent dosing, this slower metabolism increases the proportion of (S) - albuterol than Levalbuterol in vivo and exposes the patient to relatively more potential adverse effects of (S) - albuterol than the beneficial effects of Levalbuterol.

However, many studies shows the comparative effect of Albuterol and Levalbuterol on lung functions and checked the tolerability of these drugs (Khorfan et al., 2011; Maiti et al., 2011; Ali et al., 2010; Punj et al., 2009; Qureshi et al., 2005). But with the best of our knowledge there is no such study which shows the effect of both the drugs on lung functions (FEV1, FVC, PEFR) along with Respiratory rate, Total leukocyte count, Total eosinophil count, Serum potassium estimation and Heart rate evaluation in moderate to severe adult asthmatic patients in Indian settings, which was the aim of the study.

MATERIALS AND METHODS

Study population/subjects

This single blind prospective study was carried out on 80 patients of moderate to severe bronchial asthma in the Department of Respiratory Medicine, J.L.N. Medical College & associates group of hospitals, Ajmer, Rajasthan. Patients were selected according to the GINA guidelines, 2010 (Forced Expiratory Volume in 1 s between 40 to 60 % of the predicted value) with 6 months history of chronic stable asthma and who required pharmacotherapy at the time of the enrollment visit (V1). Patients of either sex (18 year or above ages) who were able to perform clinical assessment and previously not kept on regular inhaled corticosteroids or other bronchodilators like Methylxanthines or Anticholinergic group for last three weeks. Patients who required steroids for the treatment of asthma exacerbations were allowed to take low dose oral steroids therapy with prednisolone or its equivalent at 8 mg/day. If more than 8 mg/day was required, the patient was discontinued from the study. Patients must be nonsmoker and not suffering from any other chronic disease/condition, were included in this study. Patients of other acute or chronic pulmonary disease, cardiovascular disease, tremor, seizure or CNS disorder, history of carcinoma, drug abuse, hormonal or metabolite disorders, diabetes mellitus, sensitive to Albuterol or Levalbuterol, patients with unstable asthma and who have to change asthma therapy and unwilling patients were excluded from the study.

Study design

A total of 127 patients were enrolled in the study. Out of 127, forty seven patients withdrawn before randomization, 29 of whom did not meet enrolment criteria. 9 were withdrawn due to intolerable adverse events (including asthma exacerbations) and have to change asthma therapy. 6 patients were lost during follow up or withdrawn for other causes and 3 patients voluntary withdrawn from study and finally 80 patients were left who were randomized into two groups and successfully completed the study. Out of 80 patients, 40 patients (Group I) continued to use inhaled Albuterol (Salbiar) 2.5 mg/2.5 ml TDS for 4 weeks, remaining 40 patients (Group II) received inhaled Levalbuterol (Levolin) 0.63 mg/2.5 ml TDS for 4 weeks. During the enrollment, visit (V1); all patients underwent a complete clinical examination, respiratory function tests (PEFR, Peak expiratory flow rate; FEV_1, Forced Expiratory Volume in 1 second; FVC, Forced Vital Capacity), reversibility testing, TLC, TEC, respiratory rate, serum potassium and heart rate evaluation. After treatment, visit (V2); assessment included clinical examination of the respiratory system, spirometry, general objective examination, TLC, TEC, respiratory rate, serum potassium and heart rate evaluation. At least three spirometry maneuvers were performed and best reading were recorded and compared with pre-treatment values. Both group patients were asked about the need for rescue medication (low dose oral corticosteroids if necessary) during study period. Written informed consent was obtained from patients participating in this study. The study was approved by the institutional ethical review committee.

Statistical analyses

All results were expressed as mean ± SD. Differences between mean were calculated by sample Student's 't' test using SPSS version 17.0. Values of $p < 0.05$ were considered statistically significant. Results obtained were compared by paired't' test. Inter drug comparison was done by unpaired't' test.

RESULTS

Both the groups were identical, subjects in both groups comprise 80 cases out of which 44 patients (55 %) were male and 36 patients were female (45 %).

Table 1 shows effect of Albuterol and Levalbuterol on FEV_1. In group I FEV_1 was not significantly increased from 1.565±0.53 to 1.74± .64 L ($p > 0.05$) and in group II it was significantly increased from 1.48±0.91 to 2.10 ± 0.70L ($p < 0.05$). Inter drug comparison was also significant.

FVC was significantly increased in group I from 2.30±0.76 to 2.65±0.83 L and in group II from 2.22±0.90 to 3.10±0.98 L ($p < 0.05$; Table 2) respectively. Inter drug comparison shows effect was more significant with Levalbuterol as compare to Albuterol.

Before treatment, the mean value of PEFR was 3.32±1.03 and 3.40 ± 2.10 L/s in Group I and Group II respectively and after treatment it was 3.83±1.47 and 4.34 ± 0.93 L/s in Group I and Group II. The improvement in PEFR after therapy in both groups was statistically significant ($p < 0.05$) but there was greater improvement in PEFR in group II than group I (Table 3).

Table 4 shows the significant decrease ($p < 0.05$) in

Table 1. Effect of both drugs on FEV_1 (L).

Parameter	Albuterol	Levalbuterol	
Pre treatment	1.565 ± 0.53	1.48 ± 0.91	P**=0.612 (NS)
Post treatment	1.74 ± 0.64	2.10 ± 0.70	P**=0.021 (S)
	P*=0.145 (NS)	P*=0.001 (S)	

Table 2. Effect of both drugs on FVC (Litres).

Parameter	Albuterol	Levalbuterol	
Pre treatment	2.30 ± 0.76	2.22 ± 0.90	P**=0.619 (NS)
Post treatment	2.65 ± 0.83	3.10 ± 0.98	P**=0.03 (S)
	P*=0.03 (S)	P*=0.001 (S)	

Table 3. Effect of both drugs on PEFR (L/s).

Parameter	Albuterol	Levalbuterol	
Pre treatment	3.32 ± 10.3	3.40 ± 2.10	P**=0.830 (NS)
Post treatment	3.83 ± 1.47	4.34 ± 0.93	P**=0.007 (S)
	P*=0.05 (S)	P*=0.005 (S)	

Table 4. Effect of both the drugs on respiratory rate (per min).

Parameter	Albuterol	Levalbuterol	
Pre treatment	26.75 ± 4.63	25.78 ± 3.88	P**=0.309 (NS)
Post treatment	23.21 ± 3.26	22.77 ± 2.84	P**=0.51 (NS)
	P*=0.001 (S)	P*=0.001 (S)	

Table 5. Effect of both drugs on TLC (cells/mm^3).

Parameter	Albuterol	Levalbuterol	
Pre treatment	8511.10 ± 1179.09	8775 ± 1607.41	P**=0.407 (NS)
Post treatment	8381.87 ± 1683.89	8623 ± 1608.98	P**=0.516 (NS)
	P*=0.66 (NS)	P*=0.64 (NS)	

Table 6. Effect of both drugs on TEC (cells/mm^3).

Parameter	Albuterol	Levalbuterol	
Pre treatment	398.22 ± 125.90	404.72 ± 111.70	P**=0.808 (NS)
Post treatment	352.75 ± 124.71	356.25 ± 111.07	P**=0.895 (NS)
	P*=0.07 (NS)	P*=0.034 (S)	

respiratory rate by both the drugs which were increased during obstruction of airways. In group I it was from mean initial values 26.75±4.63 / minute to 23.27±3.26/minute ($p < 0.05$) and 25.78±3.88 to 22.77±2.84/ min ($p < 0.05$) in group II respectively.

As shown in Table 5 both the drugs did not significantly decrease the total leukocyte count. Total eosinophil count was not significantly decreased from 398.22±125.90 to 352.75 ± 124.71 cells/mm^3 in group I ($p > 0.05$, Table 6) and significantly decreased from 404.72±111.70 to 356.25±111.07 cells/mm^3 ($p < 0.05$, Table 6) in group II.

Before treatment, the mean value of serum potassium

Table 7. Effect of both drugs on serum potassium (m Eq/L).

Parameter	Albuterol	Levalbuterol	
Pre treatment	3.77 ± 0.38	3.79 ± 0.57	P**= 0.854 (NS)
Post treatment	2.96 ± 0.49	3.51 ± 0.56	P**=0.001(S)
	P*=0.001(S)	P*=0.017(S)	-

Table 8. Effect of both drugs on heart rate (beats/min).

Parameter	Albuterol	Levalbuterol	
Pre treatment	83.025 ± 9.09	83.875 ± 9.07	_
Post treatment	90.35 ± 5.85	89.30 ± 6.91	P**=0.5 (NS)
	P*=0.001 (S)	P*=0.003 (S)	-

p* for intra drug comparison and p** for inter drug comparison. (S) = Significant and (NS) = Non significant.

level was 3.77±0.38 and 3.79±0.59 mEq/L in Group I and Group II respectively and after treatment it was 2.96±0.49 and 3.51±0.56 mEq/L in Group I and Group II. The decrease in serum potassium level after therapy in both groups was statistically significant ($p < 0.05$). But decrease was greater in group I than group II (Table 7).

As shown in Table 8 both the drugs significantly increased the heart rate and caused tachycardia. It was increased from 83.025±9.09 to 90.35±5.85 beats/minute ($p < 0.05$) in group I and 83.875±9.07 to 89.30±6.91 beats/minute ($p < 0.05$) in group II.

DISCUSSION

In present study we have compared the effects and efficacy of Salbutamol/Albuterol and Levalbuterol/Levosalbutamol in patients of moderate to severe asthma. Levalbuterol causes more bronchodilation with less side effects as compare to racemic albuterol because Levalbuterol is free from deleterious effects of (S) - albuterol. S - Albuterol does not activate β_{2-} adrenoceptors and have no clinically meaningful ability to relax airway smooth muscle and also does not modify activation of β_{2-} adrenoceptors by Levalbuterol so that for many years it was thought to be biologically inert. It suggests that the S - albuterol contained within the racemic albuterol exerts deleterious effects on pulmonary function. Several researchers gave reasons for that it may be due to racemic albuterol increases basal levels of intracellular Ca^{++} and induces cell shortening and (S) - albuterol enhances the increase in intra cellular calcium induced by carbachol (Yamaguchi and McCullough, 1996). This is in direct contrast to the bronchodilator actions of Levalbuterol that has shown to decrease basal intracellular calcium (Baramki et al., 2002). The increase in intra cellular calcium caused by (S) - albuterol may hasten other adverse consequences. (S) - albuterol may cause an increase in Ca^{++} in the microvasculature (Chetham et al., 1997). Some studies have indicated that the airway hyperresponsiveness produced by racemic albuterol resides with (S) - albuterol and this induction is not a function of β_2- receptor down regulation (Perrin-Fayolle et al., 1996). Furthermore, exposure to racemic albuterol induces airway hyper-responsiveness to a variety of spasmogens or antigens in animals (Jafarian et al., 1996). This hyper-responsiveness persists longer than the bronchodilator effects of the compound. S - Albuterol was found to increase airway responsiveness to methacholine for three hour after administration (Kelly, 2007). S - Albuterol enhances and Levalbuterol inhibits the contractile response of histamine and leucotrine C_4 (Schmekel et al., 1999). S - Albuterol also causes facilitation of acetylcholine release from dysfunctional prejunctional muscarinic autorecptors (Zhang et al., 1998).

Thus, extensive evidence demonstrates that (S) - albuterol is not inert but might exacerbate airway reactivity and impairs the control of asthma. Because of the relatively slower metabolic sulfation of (S) - albuterol in comparison with (R) - albuterol, plasma concentrations of (S) - albuterol are several-fold greater and remain in circulation much longer after the administration of racemic albuterol (Gumbhir - shah et al., 1999). Moreover, (S) - albuterol appears to be preferentially retained in the lungs in comparison with (R) – albuterol (Dhand et al., 1999). Pharmacokinetically, it is well known that S - albuterol reaches higher circulating concentrations than R - albuterol after inhalation of the race mate. This is believed to be due to pre-systemic stereo selective metabolism of R - albuterol, which occurs in the gut and systemic circulation (Boulton et al., 1996) but not in the lungs (Ward et al., 2000).

In Our study, we observed that improvement in lung functions (FEV_1, FVC, and PEFR) is greater with Levalbuterol than Albuterol. Similar results were found by some other studies also (Jantikar et al., 2007; Nowak et al., 2006). We also observed a significant reduction in respiratory rate ($p < 0.05$) by both drugs. The decrease in rate of respiration was because of relief in bronchial obstruction as shown by improvement in pulmonary function tests after therapy. This improvement leads to better oxygenation of blood and reduced respiratory drive (Barnes, 2008). However, some studies showed that reduction is not significant (Punj et al, 2009)

In our study, both the Albuterol and Levalbuterol decreased the total eosinophil count, which was increased in bronchial asthma. The decrease with Albuterol was not significant ($p > 0.05$) but decrease with Levalbuterol was significant ($p < 0.05$). Ezeamuzie et al. (1998) also confirmed that human eosinophils could be directly modulated by β_2 - adrenoceptors agonists. Both the drugs decreases Total leukocyte count also but results were not significant. There was a lack of studies which shows the effect of both the drugs on total eosinophil count which is an important parameter of respiratory functions.

There was a significant ($p < 0.05$) reduction in serum potassium level observed after administration of both the drugs but hypokalemia was greater with albuterol ($p = 0.001$) as compared to levalbuterol ($p = 0.017$). Similar results were also observed by Punj et al. (2009), Nowalk et al. (2006) and Nelson et al. (1998). The possible mechanism behind hypokalemia is, intracellular uptake of potassium into skeletal muscle by stimulation of membrane bound Na/K ATP-ase pump by β_{2-} agonists (Lipworth et al., 1989).

In our study there was significant increase in heart rate observed after administration of both drugs ($p < 0.05$) but it was greater with albuterol. Our results were consistent with Nelson et al. (1998) and Milgrom et al. (2001). Lam et al. (2003) also observed an increase in heart rate but results were not significant. This increase in heart rate may aggravate tachyarrthmia because these agents tend to increase sympathetic activity and inhaled β_2 - agonists shows positive chronotropic effects which leads to increase in AV nodal conduction, decrease in AV nodal, atrial and ventricular refractoriness. These alterations can contribute to the generation of spontaneous arrhythmias (Kallergis et al., 2005).

Elevated heart rate and decreased serum potassium may lead to cardiomyopathy, coronary artery disease, sudden cardiac arrest so these parameters should considered seriously during administration of β_2 - agonist drugs.

Conclusions

This study concludes that Levalbuterol has better therapeutic index than Albuterol. The 0.63 mg/2.5 ml Levalbuterol (R - albuterol) dose provided better efficacy with reduced systemic β_{2-} agonist side effects as compared to 2.5 mg/2.5 ml of standard racemic albuterol or Albuterol. It also indicates that the bronchodilator effect of racemic albuterol (Albuterol) is due to R - albuterol and S - albuterol is considered as inactive but it is not yet clear and it needs further research.

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