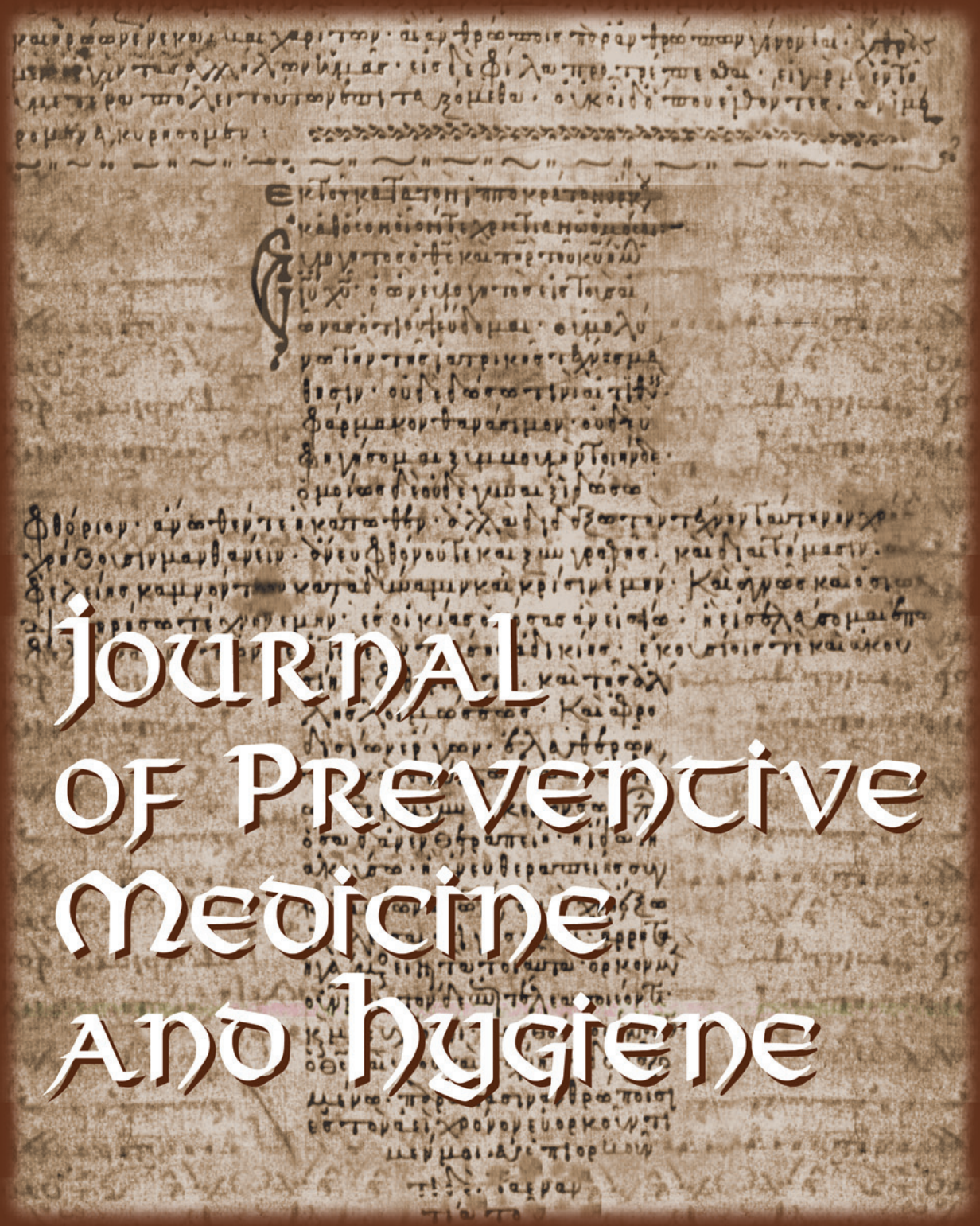
ISSN 1121-2233

vol. 55 n. 2

June 2014

Cited in Index Medicus / Medline
NLM ID 921440 (Pub-Med)



Journal of Preventive Medicine and Hygiene

The original document of Hippocrates' oath

Periodico trimestrale. - Aut. Trib. di Genova n. 507 del 6/10/60

The Journal has been accreditated, on occasion of the 17th December 2004 Meeting of the Executive and Scientific SItI Councils, by the Italian Society of Hygiene, Preventive Medicine and Public Health

PACINI EDITORE MEDICINA

JOURNAL OF PREVENTIVE MEDICINE AND HYGIENE

http://www.jpmh.org

Volume 55 - Issue 2 - June 2014

CONTENTS

Editorial note

L'autorizzazione in Europa e in Italia di un vaccino contro il meningococco di tipo B (Bexsero) allestito con metodica biotecnologica ha innescato un vivace dibattito riguardo alle modalità di impiego di suddetto vaccino. Il dibattito si è inizialmente sviluppato fra gli addetti alla sanità pubblica e si è successivamente esteso a tutta la classe medica e alla popolazione in generale. È sembrato pertanto utile al comitato scientifico del *Journal of Preventive Medicine and Hygiene* di chiedere al professor G. Gabutti una review sull'argomento e di pubblicarla in lingua italiana in modo da coinvolgere anche le fasce professionali culturalmente più distanti da questo tipo di problema.

Review

Meningococco B: controllo di due focolai epidemici mediante vaccinazione

G. GABUTTI
Dipartimento di Scienze Mediche, Università degli Studi di Ferrara

Parole chiave

Meningococco B • Vaccino • Focolaio epidemico

Riassunto

La problematica di un efficace approccio vaccinale nei confronti del Meningococco B (MenB) è stata superata identificando con la metodica della "reverse vaccinology" alcuni antigeni capaci di indurre una risposta verso la maggior parte dei ceppi di MenB circolanti nel mondo. Il nuovo vaccino MenB a 4 componenti (4CMenB) è stato autorizzato in Europa, Australia e Canada, ed è entrato nei calendari di immunizzazione pediatrica internazionali: Australia, Canada, UK. In Italia, le prime regioni che hanno raccomandato la vaccinazione contro il MenB sono state Basilicata e Puglia. La gestione di epidemie/focolai epidemici richiede la messa in atto di una risposta rapida da parte delle autorità sanitarie nei confronti di una emergenza sanitaria ad elevato impatto, anche emotivo, sulla popolazione, come recentemente dimostrato in due università americane. Alla dichiarazione di focolaio epidemico in atto, in entrambi i contesti si è attivata una procedura per l'uso del vaccino 4CMenB non ancora autorizzato negli USA. È stato così possibile organizzare gli interventi di profilassi attiva nei due campus universitari, adottando il primo impiego su larga scala del nuovo vaccino 4CMenB e conseguendo, in tempi relativamente brevi, elevati tassi di copertura vaccinale. A fronte di circa 14000 studenti immunizzati con almeno una dose, non è stata segnalata alcuna problematica di eventi avversi conseguenti all'immunizzazione; ad oggi non si sono verificati casi nei soggetti che hanno ricevuto il vaccino. Come conseguenza dei due focolai descritti, è oggi in corso la valutazione da parte dell'FDA per l'estensione dell'uso del vaccino 4CMenB negli Stati Uniti negli adolescenti e giovani adulti.

Meningococcus B: control of two outbreaks by vaccination

Summary

The issue of an effective vaccine against Meningococcus B (MenB) has been overcome by identifying, with the "reverse vaccinology" methodology, some antigens able of inducing a response to the majority of MenB strains circulating in the world. The new 4-components MenB vaccine (4CMenB) has been approved in Europe, Australia and Canada, and included in international pediatric immunization schedules: Australia, Canada, UK. In Italy, the first regions that have recommended vaccination against MenB were Basilicata and Puglia. The management of epidemics/ outbreaks requires the implementation of a rapid response by health authorities in respect of a medical emergency with a high impact, even emotional, on the population, as recently demonstrated in two American universities. The declaration of outbreak in place has been followed in both contexts by the adoption of a procedure for the use of the 4CMenB vaccine not yet licensed in the USA. It was thus possible to organize interventions of active prophylaxis in the two campuses, establishing the first large-scale use of the new 4CMenB vaccine and achieving, in a relatively short time, high rates of vaccination coverage. With around 14,000 students immunized with at least one dose, no safety issues have been reported following immunization. Besides, to date there have been no cases in subjects who have received the vaccine. As a result of the two outbreaks described, FDA is now evaluating for the extension of the use of the 4CMenB vaccine in adolescents and young adults in USA.

Background

La *Neisseria meningitidis* (meningococco) rappresenta un importante problema di Sanità Pubblica ed è la principale causa di meningite e setticemia (compresa la polmonite batteriemica) in tutto il mondo. Sebbene i sistemi di sorveglianza adottati a livello internazionale non permettano una stima precisa del *burden* dell'infezione, vi è un consenso unanime sul rilevante ruolo epidemiologico svolto da questo agente eziologico [1].
La *N. meningitidis* è classificata in 12 sierogruppi sulla base dei polisaccaridi capsulari; è possibile un'ulteriore classificazione in sierotipi e sotto-sierotipi sulla base delle proteine della membrana esterna di classe 1 (PorA) e di classe 2 o 3 (Por B) ed in immunotipi sulla base della struttura dei lipo-oligosaccaridi. È anche possibile una classificazione dei ceppi in base alla sequenza (tipi ST) utilizzando la metodica *MLST* (*multi-locus sequence typing*) [2]. I ceppi di meningococco risiedono abitualmente a livello nasofaringeo senza causare alcun quadro patologico; la transizione dallo stato di portatore asintomatico alla forma clinica invasiva è un fenomeno poco conosciuto, probabilmente correlato ad una serie di fattori quali la struttura genetica e capsulare dei ceppi patogeni [3].
Il meningococco, come tutti i componenti della specie *Neisseria*, alberga in modo asintomatico nel nasofaringe dell'uomo e viene trasmesso per via aerea, soprattutto attraverso *droplet* respiratori. Lo stato di portatore a livello nasofaringeo è stato riscontrato in un *range* pari a 4-35% dei soggetti adulti sani. Livelli anche più elevati dello stato di portatore sono stati documentati in comunità ristrette (ad es. *college*, caserme) [4, 5].
A livello internazionale esiste un consenso unanime sul ruolo epidemiologico sostenuto dai sierogruppi A, B, C, W135 ed Y. I meningococci dotati di questi polisaccaridi capsulari hanno il potenziale di sostenere sia lo stato endemico che i focolai epidemici; tuttavia, il ruolo svolto da ciascuno di essi varia notevolmente in relazione al periodo temporale ed all'area geografica presa in considerazione [6].
Frequentemente la patologia meningococcica assume un andamento sporadico o di focolai epidemici limitati; tuttavia in determinati contesti geografici (ad esempio Africa sub-sahariana) lo stato endemico può esitare in epidemie di notevole gravità sia in termini di morbosità che di mortalità [7].
Nei paesi industrializzati la patologia colpisce tipicamente i bambini nei primi anni di vita (ed in particolare in neonati di 3-12 mesi) e gli adolescenti; durante i focolai epidemici la morbosità può essere più elevata nei bambini più grandi e nei giovani adulti. I neonati ed in generale i bambini molto piccoli presentano un livello di immunità naturale nei confronti del meningococco basso e sono quindi più a rischio di contrarre l'infezione [8, 9]. Gli adolescenti sono a rischio in rapporto a comportamenti e stili di vita tipici dell'età che implicano stretti contatti personali; inoltre negli stessi vengono registrati i tassi più elevati sia dello stato di portatore che di letalità. Sono da considerare a rischio di malattia invasiva da meningococco i soggetti asplenici (asplenia anatomica o funzionale), i talassemici ed i soggetti affetti da anemia falciforme, soggetti con alterazione della cascata del complemento o con immunodepressione, pazienti affetti da malattie epatiche croniche, diabete mellito tipo I, insufficienza renale [10].
La patologia meningococcica invasiva è particolarmente temibile in quanto correla con elevati tassi di sequele permanenti e di letalità. Una caratteristica rilevante è costituita dal fatto che presenta una tipica evoluzione temporale del quadro clinico con rapidissima progressione [11, 12].
Dal punto di vista immunologico, i polisaccaridi capsulari, in quanto antigeni timo-indipendenti, non sono dei buoni immunogeni e presentano caratteristiche che hanno limitato l'uso dei vaccini di prima generazione che li contengono: assenza di induzione della memoria immunologica, produzione di anticorpi di classe IgM a bassa affinità, induzione di una buona risposta anticorpale solo dopo i 2 anni di età, nessun impatto sullo stato di portatore [13]. È stata inoltre documentata una iporesponsività indotta dai vaccini polisaccaridici correlata ad una deplezione delle cellule B della memoria che comporta una risposta immunitaria a dosi successive inferiore rispetto alla risposta primaria [13, 14].
La coniugazione degli antigeni polisaccaridici ad una proteina *carrier* ha permesso di ottenere una risposta T-dipendente con il vantaggio di generare anticorpi ad alta affinità, memoria immunologica, responsività a eventuali dosi di richiamo [15].
La disponibilità dei vaccini coniugati contro i meningococchi di sierogruppo A, C, W135 e Y ha permesso di implementare campagne di immunizzazione conseguendo importanti risultati in termini di controllo e riduzione della patologia meningoccica invasiva.
Questi successi sono stati conseguiti in molti Paesi a livello mondiale intervenendo con strategie di immunizzazione specificamente rivolte ai nuovi nati ed agli adolescenti/giovani adulti [16, 17].

Il ruolo epidemiologico del Meningococco B

Secondo i dati raccolti dal SIMI-ISS, in Italia – come nel resto d'Europa - la gran parte delle meningiti e sepsi meningococciche è storicamente associata ai sierogruppi B e C. Valutandone l'andamento a partire dagli anni '90, emerge un'alternanza dei due sierogruppi: fino al 2001 ha prevalso il meningococco B, mentre nel 2003-2004 c'è stata un'inversione di tendenza e quindi prevalenza del meningococco C. Dal 2006, grazie all'uso estensivo e all'implementazione nei calendari regionali prima e nazionale poi del vaccino coniugato contro il MenC, il quadro epidemiologico è nuovamente cambiato lasciando come principale causa di meningite e setticemia il meningococco di tipo B, il cui andamento epidemiologico vede un impatto prevalente nei bambini nei primi anni di vita e negli adolescenti [6, 18]. In particolare, l'incidenza del MenB nei bambini sotto l'anno di vita è più di 3 volte superiore a quella riscontrata nei bambini di 1-4 anni di età, con un picco massimo registrato nei primi sei mesi di vita [19, 20].

Per molti anni si è tentato di sviluppare un vaccino anche contro questo sierogruppo, ma non sono stati ottenuti risultati soddisfacenti in relazione al fatto che la capsula del MenB è un altigene-self, cioè i polisaccaridi B contengono epitopi che possono cross-reagire con antigeni umani; tra l'altro gli stessi polisaccaridi B risultano poco immunogeni [21]. Per ovviare a queste criticità sono stati sviluppati ed utilizzati in specifici contesti epidemiologici alcuni vaccini contenenti vescicole della membrana esterna *(OMV)* del meningococco B. Le *OMV* pur essendo immunogene, sono clone-specifiche, e quindi hanno la caratteristica di essere efficaci solo nel contesto epidemiologico in cui circola uno specifico clone [20, 22]. Pur con queste limitazioni, il loro utilizzo ha fornito buoni risultati in termini di contenimento di focolai epidemici, anche rilevanti, a Cuba, Nuova Zelanda, Norvegia e Francia (Normandia) [23]. L'approccio *OMV*, proprio per le sue caratteristiche, impedisce di ipotizzarne un uso estensivo in tutto il mondo. Per questo sono state cercate soluzioni alternative utilizzando antigeni sottocapsulari multipli, previa identificazione di componenti batteriche capaci di indurre una risposta protettiva. L'applicazione della tecnologia innovativa, denominata *reverse vaccinology*, ha permesso di identificare nuovi antigeni del MenB e di poterne testare la capacità di indurre una risposta battericida. In questo modo sono stati identificati 3 antigeni proteici, denominati *fHbp*, *NadA* e *NHBA*, che sono stati inclusi insieme alle *OMV-NZ* (utilizzate nell'*outbreak* in Nuova Zelanda) nel nuovo vaccino prodotto da Novartis e recentemente licenziato in Europa, Canada e Australia [24-26].
L'antigene *fHbp* (*factor H binding protein*) lega il fattore H consentendo la sopravvivenza del batterio nel sangue, l'antigene *NadA* (*neisserial adhesin A*) promuove l'adesione e l'invasione delle cellule epiteliali, l'antigene *NHBA* (*neisserial heparin-binding antigen*) è in grado di legare l'eparina aumentando la resistenza del batterio nel siero; le **OMV-NZ** inducono una robusta risposta anticorpale. Nel loro complesso gli antigeni inclusi nel vaccino 4CMenB, commercializzato con il nome di Bexsero, sono importanti per la sopravvivenza, funzione e virulenza del MenB. Grazie all'inclusione di più componenti immunogeniche nella formulazione finale del vaccino, oltre a determinare ampia copertura, si minimizza la possibilità di insorgenza di *escape mutants* [27].

Gli *outbreak* di meningite di tipo B in due *college* americani

Recentemente negli USA si sono verificati due *outbreak* epidemici di meningite da MenB. Questi due eventi, differenti tra di loro per caratteristiche e tempistica di insorgenza, hanno fatto emergere con chiarezza le difficoltà operative che devono essere sostenute per gestire un'emergenza di questo tipo [26].
Il primo *outbreak* epidemico si è verificato presso l'Università di *Princeton* ed è stato definito come tale quando il 4° caso di meningite da MenB è stato identificato nel Maggio 2013. Il primo caso si è verificato in una ragazza che era stata lontana dal *campus* universitario per le vacanze primaverili ed aveva presentato i sintomi di malattia meningococcica il 22 marzo 2013 al rientro al *campus*. Il secondo caso ha coinvolto un soggetto che aveva visitato il *campus* dal 6 all'8 aprile 2013 a cui la meningite batterica era stata diagnosticata al rientro in un altro stato. Il terzo caso ha coinvolto uno studente con diagnosi di meningite batterica in data 7 maggio 2013 ed il 4° caso ha interessato uno studente residente al di fuori dello stato che aveva presentato i sintomi il 19 maggio 2013 durante il rientro a casa per le vacanze estive.
Il Dipartimento della Salute del New Jersey *(NJDOH)* alla dichiarazione di *cluster* epidemico, avvenuta all'insorgenza del terzo caso, ha attivato un'ampia campagna educativa per rendere edotti tutti gli studenti sulle possibili modalità di trasmissione dell'agente patogeno (poster, avvisi nelle mense, brochure, meeting, email e siti web dedicati) e di profilassi antibiotica ai contatti stretti dei casi. Con l'insorgenza del 4° caso e la dichiarazione dello stato di focolaio epidemico, conseguente alle analisi condotte presso il *Center for Disease Control and Prevention (CDC)* che indicavano che tutti i casi sopra riportati erano stati causati dallo stesso ceppo di meningococco B, le attività di educazione sanitaria e di profilassi sono continuate con crescente preoccupazione di un'ulteriore diffusione del batterio in relazione alle attività (consegne dei diplomi, ecc.) che avrebbero coinvolto circa 20000 persone nel *campus*. D'altra parte si confidava nell'imminente sospensione estiva delle attività per bloccare completamente la circolazione dell'agente patogeno.
Alla fine di giugno (29 giugno) è stato identificato il 5° caso in uno studente in viaggio all'estero (Grecia) insieme ad altri 15 studenti dell'Università di *Princeton*. Ai primi di luglio *CDC*, *NJDOH* e lo *staff* dell'Università di *Princeton* avevano iniziato a valutare l'opportunità di intrapendere una campagna vaccinale nella consapevolezza di dover comunque richiedere alla *Food and Drug Administration (FDA)* l'autorizzazione all'utilizzo di un vaccino contro il MenB non ancora licenziato negli USA. La pianificazione di un eventuale intervento vaccinale, iniziata nell'agosto 2013, è proseguita mantenendo in atto e rafforzando gli altri interventi sopra riportati alla ripresa dell'attività didattica. I giorni 1 ottobre, 8 novembre e 20 novembre sono stati identificati altri 3 casi (2 femmine e un maschio). Poco prima dell'identificazione dell'ottavo caso, è stata rilasciata l'autorizzazione all'uso del vaccino sperimentale e le prime vaccinazioni sono state pianificate ad inizio dicembre.
La procedura adottata per divulgare la notizia dell'inizio dell'attività vaccinale e per la somministrazione del vaccino è stata analoga a quella usualmente utilizzata per la campagne di immunizzazione contro l'influenza. La strategia di intervento prevedeva la somministrazione di due dosi; trattandosi di un vaccino ancora non autorizzato, la procedura richiedeva la firma del consenso informato da parte dei soggetti se maggiorenni o dei loro tutori legali, se minorenni. La prima fase della campagna vaccinale, svoltasi nel periodo 9-12 dicembre 2013, ha permesso di somministrare la prima dose a oltre 5000

studenti. La seconda fase, condotta nel febbraio 2014, ha permesso di somministrare la seconda dose a più di 4700 studenti. Pertanto, complessivamente si è raggiunta una copertura vaccinale con due dosi superiore al 90%. Si ritiene che questo risultato sia stato ottenuto in conseguenza dell'elevata percezione del rischio da parte degli studenti e dei loro genitori. Degno di nota il fatto che non è stato segnalato alcun problema di tollerabilità o sicurezza del vaccino. Gli aggiornamenti disponibili non segnalano alcuna incidenza particolare di eventi avversi correlati con il vaccino.

Al momento non vi è sicurezza che la problematica sia risolta; infatti nel marzo 2014 si è registrato il nono caso. Questo caso ha interessato una studentessa proveniente dalla *Drexel University*, non vaccinata quindi durante la campagna di *Princeton*, che aveva avuto contatti stretti con studenti dell'Università di *Princeton* circa una settimana prima di ammalarsi. All'inizio dei sintomi in data 9 marzo 2014 è seguito un rapido peggioramento del quadro clinico con decesso in data 10 marzo 2014. Le ricerche di biologia molecolare hanno confermato che anche questo caso è stato causato dallo stesso sierogruppo di MenB coinvolto in tutti gli altri casi all'Università di *Princeton*. Per questo motivo, è stato deciso di continuare ad offrire la somministrazione del vaccino per il MenB a tutti i nuovi studenti del primo anno nel mese di settembre 2014.

In conclusione, l'*outbreak* epidemico verificatosi all'Università di *Princeton* ha avuto un andamento particolare, coprendo un arco di circa un anno, coinvolgendo 9 soggetti, con 1 caso mortale ed 1 con sequele (amputazione dei piedi) [28-30].

Il secondo *outbreak* epidemico si è invece verificato all'Università di Santa Barbara in California ed ha avuto un andamento "più tipico" con 4 casi di meningite da meningococco in 2 settimane nel novembre 2013.

Le indagini epidemiologiche hanno permesso di collegare questi casi con un caso verificatosi nello stesso ambito circa 7 mesi prima. I cinque casi non sembravano avere particolari collegamenti tra di loro avendo coinvolto 2 studenti del primo anno, due del secondo ed un laureando. I casi risiedevano in ambiti completamente diversi rappresentati da un dormitorio per 1300 persone, un appartamento privato ed una comunità femminile. I 4 casi verificatisi a novembre hanno coinvolto soggetti con un fattore di rischio identificato per l'acquisizione della patologia meningococcica avendo partecipato tutti ad una festa per *Halloween* in una comunità densamente abitata vicino al *campus* universitario. Il quinto caso presentava solamente un contatto con un compagno di stanza che era componente di una squadra sportiva in cui si era verificato un altro caso.

La criticità della situazione era elevata sia per le dimensioni della popolazione afferente all'Università di Santa Barbara (circa 19000 studenti nei primi 4 anni dei corsi universitari) sia per il fatto che a pochi chilometri di distanza dal campus universitario esiste un *college* altrettanto grande. La popolazione universitaria e del *college* hanno elevate possibilità di socializzazione reciproca, anche con la comunità della città vicina. Analogamemte a quanto verificatosi nel focolaio epidemico di *Princeton*, il Dipartimento della Salute di Santa Barbara *(SBPHD)* ha coordinato l'attività di prevenzione organizzando la profilassi antibiotica per i contatti stretti con la complicazione derivante dal fatto che un caso aveva esordito come shock settico e quindi l'indagine epidemiologica era risultata complessa. Successivamente si è verificato che questo caso aveva avuto contatti stretti con la sua squadra di lacrosse ed anche con i componenti delle squadre di altri sport.

L'attività concertata tra *SBPHD*, lo *staff* dell'università ed il *CDC* ha portato alla somministrazione di circa 1200 dosi di ciprofloxacina in relazione alla preoccupazione presente nel campus ed alla difficoltà nel definire con sufficiente precisione i contatti stretti dei casi.

Nei dieci giorni seguenti al verificarsi del quarto caso, si è iniziato la procedura per poter utilizzare il vaccino per il MenB non ancora autorizzato negli USA. L'iter approvativo di questo uso del vaccino sperimentale e per l'organizzazione delle prime vaccinazioni è durato circa 3 mesi, anche perché l'attività di stoccaggio e somministrazione doveva essere totalmente a carico dell'Università, non avendo il Dipartimento risorse sufficienti per intervenire su una popolazione dimensionalmente così grande. Lo schema vaccinale a due dosi è stato analogo a quello adottato a *Princeton*.

L'avere impiegato circa tre mesi per ottenere l'autorizzazione, per l'organizzazione dell'intervento vaccinale, l'acquisto e l'installazione dei frigoriferi per lo stoccaggio del vaccino, l'installazione della rete internet e dei computer all'interno dello stadio per l'*hochey*, identificato come sede dell'ambulatorio vaccinale, la creazione di uno staff sanitario ed amministrativo ad hoc, hanno certamente impattato negativamente sulla *compliance* alla vaccinazione, facendo percepire meno l'urgenza e l'importanza dell'intervento vaccinale da parte degli studenti.

Nonostante queste problematiche è stato possibile immunizzare circa 9000 studenti con la prima dose conseguendo un tasso di copertura vaccinale pari a 51% (con tassi pari al 60% dei residenti nel grande dormitorio e dei membri della comunità greca). Il 37% degli studenti dei primi 4 anni di università, il 50% degli studenti del primo anno ed il 45% di quelli del secondo anno hanno ricevuto 2 dosi; complessivamente sono stati vaccinati con due dosi circa 7000 studenti.

Anche in questo caso va notato che non è stato segnalato alcun problema di tollerabilità o sicurezza del vaccino. Gli aggiornamenti disponibili non segnalano alcuna incidenza particolare di eventi avversi correlati con il vaccino [30, 31].

Conclusioni

La ideazione e successiva disponibilità dei vaccini coniugati contro quattro tipi di meningococco (A, C, W135, Y) e il loro inserimento nei calendari vaccinali di molti paesi ha permesso di impattare in modo significativo su una patologia il cui rischio è da sempre percepito, non solo dagli operatori sanitari, ma anche dalla popolazio-

ne, come molto elevato. Per molto tempo la problematica di un efficace approccio vaccinale nei confronti del MenB è rimasta insoluta. La particolare composizione antigenica dei polisaccaridi del MenB, identica a quella dell'acido sialico delle cellule umane, ha impedito lo sviluppo di un vaccino polisaccaridico coniugato verso questo patogeno per le potenziali problematiche di reazioni autoimmuni ad esso correlate.
Laddove si sono verificati epidemie o focolai epidemici sostenuti da MenB, l'approccio vaccinale utilizzato si è basato su vaccini contenenti vescicole della membrana esterna (*OMV*) contenenti porina A. I vaccini contenenti *OMV* sono "costruiti su misura" per una specifica situazione epidemiologica e non possono indurre alcuna reazione crociata. Questa criticità è stata recentemente superata identificando con la metodica della *"reverse vaccinology"* alcuni antigeni capaci di indurre una risposta verso la maggior parte dei ceppi di MenB circolanti nel mondo [20].
Il nuovo vaccino è stato autorizzato in Europa, Canada e Australia e permette di completare la possibilità di prevenzione vaccinale fornendo protezione verso l'attuale principale causa di malattia meningococcica nei paesi industrializzati: il meningococco B [32].
Generalmente l'inserimento di un nuovo vaccino nel calendario vaccinale richiede non solo la valutazione della sua efficacia, immunogenicità, tollerabilità e sicurezza ma anche una analisi farmacoeconomica [10]. Ad un anno dalla sua approvazione da parte di diversi enti regolatori, il vaccino contro il meningococco B è entrato nei calendari di immunizzazione pediatrica internazionali: Australia, Canada, UK.
In Italia, le prime regioni che hanno raccomandato la vaccinazione contro il meningococco B sono state Basilicata (Tab. I) e Puglia (Tab. II) con una schedula vaccinale intercalata 3+1, ovvero di tre dosi, più una dose booster.
Differente è il caso di epidemie o di focolai epidemici in cui l'importanza della valutazione farmacoeconomica passa in secondo piano e prevale la definizione del rischio elevato di malattia meningococcica, la percezione del rischio da parte della popolazione, la pressione esercitata dai *media* sulla spinta dell'insicurezza/paura della popolazione stessa in una situazione di emergenza.
Tutto questo è emerso chiaramente nel corso dei due focolai epidemici, tra loro distinti in quanto sostenuti da ceppi diversi di MenB (ST409 a *Princeton* e ST32 a Santa Barbara), verificatisi recentemente in due università americane [28, 31]. L'identificazione di primi casi ha fatto attivare immediatamente sia le autorità delle università sia il Dipartimento della Salute competente con l'implemetazione di attività informative per la popolazione e l'organizzazione degli interventi di profilassi antibiotica per i contatti stretti. È importante sottolineare come alla dichiarazione di focolaio epidemico in atto, in entrambi i contesti si sia attivata una procedura per poter utilizzare, in accordo con l'Istituto nazionale per la salute *(NIH)*, il *CDC* e la *FDA*, il vaccino MenB a 4 componenti (4CMenB) non ancora autorizzato per l'uso negli USA. Grazie a questa procedura è stato possibile organizzare gli interventi di profilassi attiva nei due *campus* universitari [30].
Di fatto si è trattato del primo impiego su larga scala del nuovo vaccino contro il MenB. L'esperienza ha permesso di acquisire alcune importanti informazioni. Prima di tutto è emerso chiaramente quale sforzo organizzativo richieda la messa in atto di una risposta rapida da parte delle autorità sanitarie nei confronti di una emergenza sanitaria ad elevato impatto, anche emotivo, sulla popolazione. L'organizzazione di una intervento vaccinale in risposta ad un focolaio epidemico di malattia menigococcica richiede un notevolissimo dispendio di risorse umane ed organizzative in tempi relativamente brevi. Come dimostrato dai fatti è però possibile conseguire in tempi rapidi elevati tassi di copertura vaccinale.

Tab. I. Calendario Vaccinale della vaccinazione antimeningococco B, Regione Basilicata [33]

3° mese (61° giorno di vita)	3° mese + 15/30 gg (75°/90° giorno di vita)	5° mese (121° giorno di vita)	5° mese + 15/30 gg (135°/150° giorno di vita)	7°+ 15/30 gg (181°/210° giorno di vita)	11° mese	Dopo il 13° mese
Esavalente		Esavalente			Esavalente	
Pneumococco 13-valente		Pneumococco 13-valente			Pneumococco 13-valente	Meningococco C
	Meningococco B		**Meningococco B**	**Meningococco B**		**Meningococco B**

Tab. II. Calendario Vaccinale della vaccinazione antimeningococco B, Regione Puglia [34].

3° mese (61° giorno di vita)	3° mese + 15 gg (76° giorno di vita)	4° mese (106° giorno di vita)	5° mese (121° giorno di vita)	6° mese (151° giorno di vita)	12° mese	15° mese
Esavalente			Esavalente		Esavalente	
Pneumococco 13-valente			Pneumococco 13-valente		Pneumococco 13-valente	Meningococco C
	Meningococco B	**Meningococco B**		**Meningococco B**		**Meningococco B***

* in co-somministrazione con il vaccino antimeningococco C

Gli interventi messi in atto nei due *campus* non erano strutturati in modo da poter valutare l'efficacia vaccinale. Considerata la composizione del vaccino utilizzato, la probabilità di ottenere un controllo delle epidemie è molto elevata, malgrado l'elevata variabilità dei ceppi B del meningococco. Al momento tuttavia non si è in grado di verificare se l'utilizzo del vaccino abbia permesso di contenere i due focolai epidemici, anche se ad oggi non si sono verificati casi nei soggetti che hanno ricevuto il vaccino contro il Men B.

È invece dimostrato che è stato possibile conseguire elevati tassi di copertura vaccinale e che, a fronte di circa 14000 studenti immunizzati con almeno una dose, non è stata segnalata alcuna problematica di insorgenza di eventi avversi conseguenti all'immunizzazione.
Come conseguenza dei due focolai descritti, è ad oggi in corso la valutazione da parte dell'*FDA* per l'estensione dell'uso del vaccino contro il MenB negli Stati Uniti negli adolescenti e giovani adulti. [35].

Bibliografia

[1] WHO. *Meningococcal vaccines: WHO poistion paper. November 2011.* Wkly Epidemiol Rec 2011;86:521-40.

[2] Hill DJ, Griffiths NJ, Borodina E, et al. *Cellular and molecular biology of Neisseria meningitidis colonization and invasive disease.* Clinical Sciences 2010;118:547-64.

[3] Stephens DS. *Biology and pathogenesis of the evolutionarily successful, obligate human bacterium Neisseria meningitidis.* Vaccine 2009;27S:B71-B77.

[4] Durey A, Bae SM, Lee HJ, et al. *Carriage rates and serogroups of Neisseria meingitidis among freshmen in a university dormitory in Korea.* Yonsei Med J 2012;53:742-7.

[5] Gasparini R, Comanducci M, Amicizia D, et al. *Molecular and serological diversity of Neisseria meningitidis carrier strains isolated from Italian students aged 14 to 22 years.* J Clin Microbiol. 2014;52:1901-10.

[6] Halperin SA, Bettinger JA, Greenwood BG, et al. *The changing and dynamic epidemiology of meningococcal disease.* Vaccine 2012;30S:B26-B36.

[7] Jafri RZ, Ali A, Messonnier NE, et al. *Global epidemiology of invasive meningococcal disease*. Population Haelth Metrics 2013;11:17.

[8] Gasparini R, Rizzetto R, Sasso T, et al. *Seroprevalence of bactericidal antibody against Neisseria meningitidis serogroup C in pre-vaccinal era: the Italian epidemiological scenario.* Vaccine 2009;27:3435-8.

[9] Guzzetta G, Manfredi P, Gasparini R, et al. *On the relationship between meningococcal transmission dynamics and disease: remarks on humoral immunity.* Vaccine 2009;27: 3429-34.

[10] Presidenza del Consiglio dei Ministri. Intesa, ai sensi dell'articolo 8, comma 6, della legge 5 giugno 2003, n.131, tra il Governo, le Regioni e le Province autonome di Trento e Bolzano sul documento recante "Piano Nazionale Prevenzione Vaccinale 2012-2104", febbraio 2012.

[11] Pace D, Pollard AJ. *Meningococcal disease: clinical presentation and sequelae.* Vaccine 2012;30S:B3-B9.

[12] Strelow VL, Vidal JE. *Invasive meningococcal disease.* Arq Neuropsiquiatr 2013;71:653-8.

[13] Pollard AJ, Perrett KP, Beverley PC. *Maintaining protection against invasive bacteria with protein-polysaccharide conjugate vaccines.* Nature Reviews 2009;9:213-20.

[14] Zahlanie YC, Hammadi MM, Ghanem ST. et al. *Review of meningococcal vaccines with updates on immunization in adults.* Hum Vaccin Immunother 2014;10:995-1007.

[15] Hedari CP, Khinkarly RW, Dbaibo GS. *Meningococcal serogroups A, C, W-135, and Y tetanus toxoid conjugate vaccine: a new conjugate vaccine against invasive meningococcal disease.* Infect Drug Resist 2014;7:85-99.

[16] Snape MD, Kelly DF, Salt P, et al. *Serogroup C meningococcal glycoconjugate vaccine in adolescents: persistence of bactericidal antibodies and kinetics of the immun eresponse to a booster vaccine more than 3 years after immunization.* Clin Infect Dis 2006;43:1387-94.

[17] de Whalley PC, Snape MD, Kelly DF, et al. *Persistence of serum bactericidal antibody one year after a booster dose of either a glycoconjugate or a plain polysaccharide vaccine against serogroup C Neisseria meningitidis given to adolescents previously immunized with a glycoconjugate vaccine.* Pediatr Infect Dis J 2011;30:e203-8.

[18] Chang Q, Tzeng YL, Stephens DS. *Meningococcal disease; changes in epidemiology and prevention.* Clin Epidemiol 2012;4:237-45.

[19] Bettinger JA, Scheifele DW, Le Saux N, et al. *The disease burden of invasive meningococcal serogroup B disease in Canada*. Pediatr Infect Dis J 2013;32:e20-5.

[20] Panatto D, Amicizia D, Lai PL, et al. *New versus old meningococcal group B vaccines: how the new ones may benefit infants & toddlers.* Indian J Med Res 2013;138:835-46.

[21] Snape MD, Pollard AJ. *The beginning of the end for serogroup B meningococcus?* Lancet 2013;381:785-7.

[22] Holst J, Martin D, Arnold R, et al. *Properties and clinical performance of vaccines containing outer membrane vesicles from Neisseria meningitidis.* Vaccine 2009;27(S2):B3-B12.

[23] Holst J, Oster P, Ranold R, et al. *Vaccines against meningococcal serogroup B disease containing outer membrane vesicles (OMV). Lessons from the past programs and implications for the future.* Hum Vaccin Immunother 2013;9:1241-53.

[24] O'Ryan M, Stoddard J, Toneatto D, et al. *A multi-component meningococcal serogroup B vaccine (4CMenB): the clinical development program.* Drugs 2014;74:15-30.

[25] Andrews SM, Pollard AJ. *A vaccine against serogroup B Neisseria meningitidis: dealing with uncertainty.* Lancet Infect Dis 2014;14:426-34.

[26] CDC. *Meningococcal disease, Serogroup B meningococcal vaccine and outbreaks.* su: www.cdc.gov/meningococcal/outbreaks/vaccine-serogroupB.html (accesso Giugno 2014).

[27] Major M, Moss S, Gold R. *From genes to vaccine: a breakthrough in the prevention of meningococcal group B disease.* Paediatr Child Haelth 2011;16:e61-e64.

[28] CDC. *Princeton University meningococcal disease outbreak.* su: www.cdc.gov/meningococcal/outbreaks/princeton.html (accesso Giugno 2014).

[29] National Foundation for Infectious Diseases. *Addressing the challenges of serogroup B meningococcal disease outbreaks on campuses: a report by the National Foundation fro Infectiuos Diseases.* su: www.nfid.org/meningococcal-b (accesso Giugno 2014).

[30] Novartis meningitis B vaccine Bexsero® receives FDA Breakthrough Therapy designation in the US [news release]. Basel, Switzerland: Novartis, April 7, 2014. su: www.novartis.com/newsroom/media-releases/en/2014/1774805.shtml. (accesso Giugno 2014).

[31] CDC. *University of California, Santa Barbara meningococcal disease outbreak.* su: www.cdc.gov/meningococcal/outbreaks/ucsb.html (accesso Giugno 2014).

[32] Kaaijk P, van der Ende A, Luytjes W. *Routine vaccination against MenB. Considerations for implementation.* Hum Vaccin Immunother 2014;10:310-6.

[33] Regione Basilicata. Dipartimento Politiche per la Persona. Deliberazione n. 167 dell'11 febbraio 2014. Approvazione del documento tecnico.-scientifico dal titolo "Programma di campagna vaccinale per la prevenzione primaria della malattia invasiva da Meningococco di gruppo B". su: http://opendata.regione.basilicata.it/opendata/home.jsp?tile=DELIBERE.delibere.jsp&numAtto=167&oggetto=&year=2014 (accesso Giugno 2014).

[34] Regione Puglia. deliberazione della Giunta regionale n. 958 del 20 Maggio 2014. Commissione Regionale Vaccini. Modifica Calendario per la Vita 2012- DGR 241/2013. Approvazione nuovo Calendario Vaccinale per la vita 2014. su: http://www.regione.puglia.it/index.php?page=delibere&opz=view&id=12256) (accesso Giugno 2014).

[35] Midthun K. *FDA is working closely with manufacturers of meningitis B vaccines.* su: http://blogs.fda.gov/fdavoice/?s=bexsero (accesso Giugno 2014).

■ Ricevuto il 7/6/2014. Accettato il 30/6/2014.

■ Corrispondenza: Giovanni Gabutti, Dipartimento di Scienze Mediche, via Fossato di Mortara 64b, 44121 Ferrara - Tel. +39 0532 455568 - Fax +39 0532 205066 - E-mail: giovanni.gabutti@unife.it

ORIGINAL ARTICLE

Screening for diabetes mellitus and human immunodefiency virus infection in persons with tuberculosis

A.O. OGBERA[1], A. KAPUR[2], K. ODEYEMI[3], K. LONGE-PETERS[3], O.O. ADEYEYE[1], I. ODENIYI[4], B.E. OGUNNOWO[3]
[1] Department of Medicine, Lagos State University Teaching Hospital Ikeja, Lagos Nigeria; [2] World Diabetes Foundation Gentofte Denmark; [3] Department of Community Medicine, Lagos State University Teaching Hospital Idi-araba, Lagos Nigeria; [4] Department of Medicine, Lagos University Teaching Hospital, Idi-araba Lagos Nigeria

Key words

Diabetes mellitus • Tuberculosis • Screening • HIV

Summary

Introduction. *Nigeria is a country saddled with a high tuberculosis (TB) and human immunodeficiency virus (HIV) burden but the possible combination of these communicable diseases with diabetes mellitus (DM) has been overlooked. We undertook to determine the burden of HIV and DM in persons with TB by documenting the prevalence rates of these disorders.*
Methods. *This is a cross-sectional Study that was conducted within 54TB/DOT centers in Lagos State. A total of 3,376 persons with TB who were on antiTB drugs were screened for HIV and DM using standardized tests. Statistical analysis was performed using Students t test and chi square.*
Results. *The frequency of occurrence of DM in TB and that of HIV in TB were comparable (4.8% Vs 3.5%). The Study subjects with DM were older, had higher waist circumference measurements and had higher proportions of hypertension compared to the subjects without DM. The combination of HIV and DM in TB was found in (0.3%). We also noted that DM in TB and HIV in TB occurred more frequently in the third and fourth decades of life.*
Conclusion. *This study demonstrated the potential co existence of HIV, DM and Tuberculosis. It is therefore important that these two diseases are sought for in patients with TB considering the changing epidemiology of these diseases particularly in developing countries like Nigeria.*

Introduction

Diabetes mellitus (DM) is one of the four priority Non communicable diseases identified by the World Health Organisation (WHO) and is assuming epidemic proportions with devastating human, social and economic consequences [1]. DM affects 5-6% of the global population and in Nigeria, the estimated prevalence of DM was 2.2% as at 1997 with present estimates ranging from 6-8% [2]. Human immunodeficiency virus is a blood borne retrovirus typically transmitted via sexual intercourse, shared intravenous drug paraphernalia and via mother to child transmission. In Nigeria, the human immunodeficiency virus (HIV) prevalence among the general population is 3.6% with an estimated 3.1 million people affected [3]. Tuberculosis is a communicable disease caused by any of the several species of Mycobacteria usually Mycobacteria tuberculosis or tubercle bacillus. Nigeria is ranked fourth among the 22 worst affected countries with tuberculosis (TB) in the world and the first in Africa. In Nigeria, Lagos State carries 8.4% of the nation's TB burden and this consistently has been responsible for about 11% of the cases of TB registered in Nigeria [4].
In sub-Saharan Africa, the HIV epidemic is accelerating the already massive TB epidemic with a documented increase in the incidence of TB from 146 per 100,000 to 345 per 100,000 in 2003 [5]. The prevalence of HIV among TB patients increased from 2.2% in 1991 to 19.1% in 2001 and to 25% in 2010 thus underscoring the fact that the TB scenario in the country is HIV driven [6]. The multiple effects of HIV infection on the natural history of TB include an increase in the risk for reactivation of latent infection and for exogenous infection [7]. The existing link between DM and TB is well recognised with evidence that DM is not only an important risk factor for the acquisition of TB but also that TB might induce glucose intolerance and worsen glycaemic control in people with diabetes [8]. There is a global growing awareness and concern on the possible relationship between TB and DM thus informing the recent collaboration between WHO and the International Union Against Tuberculosis and Lung Disease (Union) to the effect of putting together a document for the care and control of both diseases [9]. In countries with high TB burden like China and India the reported prevalence rates of DM and TB are 16% [10] and 25-44% [11, 12] respectively. In SubSaharan Africa, specifically from Tanzania, the documented prevalence of DM in TB is 16.7% [13].
There is a growing body of evidence to substantiate the claim that some infectious diseases may be potential risk factors for some non communicable diseases[14]. TB and HIV are communicable diseases of public health significance that may be closely associated with the development of DM [14].

The objective of this Report is the determination of the burden of DM and HIV in persons with established diagnoses of pulmonary TB.

Methods

This was a cross sectional Study carried out in Lagos, Nigeria. Lagos State is a cosmopolitan state, located in the south Western region of Nigeria and has a population of about 20 million people.

Participant Recruitment and Data Collection: Patients with confirmed diagnoses of TB and on treatment for TB were recruited consecutively from 54 DOT centres during the Study period, which was from September 2010 to March 2012. Consenting patients with pulmonary TB of age ≥ 12 years and who had commenced anti-TB drugs were enrolled into the study. Patients who were pregnant, those with features of extrapulmonary TB and those who did not give Consent were excluded from the recruitment exercise. A diagnosis of TB was made if the patient presented with clinical symptoms suggestive of TB and either a positive sputum smear on Ziehl Nielsen or radiological indices of TB. Diagnosed patients were registered and treated with antiTB drugs in accordance with the WHO Guidelines [15]. The anti-TB drugs used in the intensive phase being Rifampicin, Isoniazid, Ethambutol and Pyrazinamide and in the maintenance phase being Rifampicin and Isoniazid.

Measurement of fasting plasma glucose concentration was performed in participants using capillary blood with glucose meters that provide plasma equivalent readings. (The Finetest Auto-coding™, Infopia Co., Ltd. Korea). The diagnosis of DM was made based on the 1999 World Health Organization (WHO) guidelines which state that a fasting plasma glucose of ≥ 7 mmol/ is in the diabetic range [16].

In Lagos State all TB patients receiving care at the DOT facilities are routinely screened for HIV. Blood samples were tested for the presence of HIV using Elisa kit (Genescreen HIV -1/2) and all reactive samples were confirmed with a repeat test (Gene 11 Sanofi, Pasteur, Paris).

Ethical approval for this study was obtained from the Lagos State Ministry of Health which directly oversees the DOT centers within the State and informed consent was obtained from all study participants.

Results

A total of 3,376 persons with tuberculosis were screened for HIV and DM. The males were 1932 and females 1444 thus making up 57% and 43% respectively of the Study population.

The Mean age of the Study population was 34.9 (12.97) years. Males were older than females and this difference was statistically significant (35.7(12.7) Vs 33.8(13.1), p = 0.001). The majority of the Study subjects had some form of education as only 120 (3.5%) were non-literates. Of the Study subjects who were literate, the proportion of persons with primary, secondary and tertiary education were 586(18%), 2101(65%) and 571(17%) respectively. Sputum smear positivity was noted in 2809 (83%) of the subjects. HIV infection was found to be present in 118(3.5%) of the Study subjects.

Diabetes mellitus in tuberculosis

Of the 3,376 TB patients screened, 162 (4.8%) were found to have DM. Of these 77 (47.5%) already had a previous diagnosis of DM thus about half of the patients with DM were newly diagnosed cases. A family history of DM was documented in 290 (8.5%) of the Study populace and hypertension in 59 persons making up 1.7% of the Study populace. Well over half-82%- of persons with TB and DM had sputum smear positivity for TB. The median age of persons with DM in TB was 40 years. A comparison between persons with TB and DM and those without DM showed that persons with DM were older and tended to have hypertension as a co-morbidity. These results are shown in Table I.

HIV/DM/TB

The number of TB patients who had HIV coinfection was 118 thus making up 3.5% of the Study group. Patients with TB and HIV were older than those without HIV and this difference was statistically significant. 38.2(10.1) Vs 34.8(13), p = 0.004. Well over half (70%) of persons with TB and DM were sputum smear positive for TB.

DM and HIV were present more commonly in patients between 40-60 years of age. The distribution of HIV and DM according to age decades is shown in Figure 1.

The HIV/DM/TB prevalence was 11-(0.3%). Patients with DM and HIV were older than those with HIV and no DM. A comparison of the clinical parameters of persons with TB/HIV/DM versus those with TB/HIV and no DM are shown in Table II.

Discussion

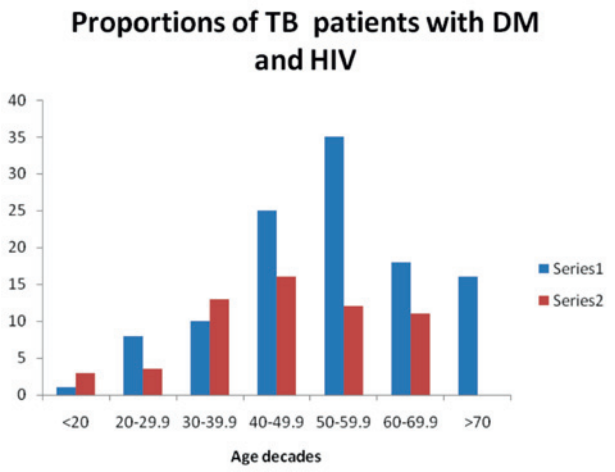
Nigeria like most developing countries is experiencing an epidemiological transition with the burden of

Tab. I. Comparison of Sociodemographic parameters between TB patients with DM and TB patients without DM.

Variable	DM	Non DM	p
Age (years)	40.7 (12.8)	34.6 (12.9)	0.0001
Waist circumference	74.5 (9.8)	72.6 (9.1)	0.017
Gender (M:F) %	57:43	59:41	0.7
Hypertension	12 (7.4%)	47 (1.5%)	0.001

Fig. 1. Distribution of the proportions of TB patients with DM and HIV.

Series 1: TB patients with DM, Series 2: TB patients with HIV infection.



Non communicable diseases (NCD) like DM poised to overwhelm the healthcare system that is already overburdened by HIV/AIDS, TB and other communicable diseases such as malaria.

The relation between HIV and TB has been long established and in Africa, the percentage of persons with HIV co-infection has steadily risen in geometric proportions from 4% in 2004 to 68% in 2011 [17]. Sub-Saharan Africa has borne the brunt of the HIV and TB co-epidemic and accounts for 79% of the global burden of HIV infection-associated TB cases in 2007 [18]. We report the frequency of occurrence of HIV in persons with TB to be 3.5% and notably occurring in the fourth and fifth decades of life. This is against a background median prevalence of HIV of 17% which had earlier been documented in Nigerians with TB [19]. The implications of this entwined infections are oft times grave as they may result in multidrug and extreme drug resistance TB, development of other opportunistic infections and ultimately a reduction in both quality and quantity of life.

Diabetes mellitus is a chronic metabolic disease that is of public health significance in developing and developed countries. In sub- Saharan Africa objective documentation of the predisposition for persons with TB to develop DM was done by Mugusi et al [20] who noted that DM tended to occur four times more in persons with TB in comparison to persons without TB. The projected global geometric increase in DM is unfortunately bound to occur more in developing countries of which an estimated 70% are TB endemic countries [21]. In this Report we determined from our screening that 50% of the persons with DM were newly diagnosed. Our data is comparable to that from Indonesia where 61% of persons with DM with TB were noted to be newly diagnosed [22]. The clinical correlates of DM in TB included older age and higher mean waist circumference and this pattern was also documented in persons with DM and HIV compared to those with HIV but no DM. We did not classify our DM patients but given the median age of 40 years of persons with TB who had DM and the clinical correlates we are of the opinion that a large majority of these persons, have type 2DM. Reports from Africa and elsewhere indicate that patients with DM and TB are usually older and have higher body mass indices than TB patients without DM [21]. From the foregoing it is pertinent to note that the documented clinical correlates in our Report are essentially known risk factors for the development of type 2 DM.It is instructive to note that DM was also documented in persons who were less than 20 years of age. Sputum smear positivity in patients with TB and DM was high and same scenario obtained for persons with TB and HIV. The association of DM with particularly for smear positive cases of TB has been reported in Indian populations with TB [11]. The importance of this finding though unclear may be related to delayed sputum conversion which has been reported [22] as a feature of persons with TB and concomitant DM.

Screening for hypertension though was not a stated objective of this Report, we undertook to screen for hypertension. The reason for this is because hypertension is a co-morbidity that is oft reported in Nigerians with DM – a recent Nigerian Report found that as many as half of persons with DM also have hypertension [23]. The prevalence of hypertension in our Study populace is 1.7% but that in the subjects with DM was 7.4%.

Although the combination of DM and HIV in persons with TB was less than one percent, the implication of these comorbidities may translate into increased morbidity and mortality. In resource poor settings like Nigeria with high burden of TB, opportunistic screening for DM should be a priority. We have shown that the combination of DM and TB is comparable to that of HIV and DM but unfortunately DM often goes undiagnosed in persons with TB.

The impact of DM in TB has been found to portend poor treatment outcome which could be in the form of drug resistance, relapse of TB and death. The presence of TB

Tab. II. Comparison of the clinical parameters of persons with TB/HIV/DM versus those with TB/HIV and no DM.

Variable	DM/HIV	Non DM/HIV	p
Age (years)	42.6 (13.2)	34.9 (12.9)	0.04
Waist circumference	80 (12.8)	72.7 (9.1)	0.009
Gender (M:F) %	6:5	59:41	0.7
Hypertension	2 (18.2%)	3 (2.5%)	0.001
Smear positivity	9 (72.7%)	(82.6%)	0.3

in persons with DM may lead to poor glycaemic control. Nigeria is facing a dual burden of communicable and NCDs. The magnitude of DM in TB and HIV and DM in TB is not yet known.
Conclusion: The dual burden of DM and HIV in persons with TB is of moderate magnitude. Given that the DM/TB combination is comparable to that of HIV/TB, we recommend that opportunistic screening for DM be offered to persons with TB.

Acknowledgement

I wish to acknowledge the Staff of Structured Healthcare Initiatives and Staff of the DOT centres in which these TB patients were recruited for the Study. I also acknowledge the World Diabetes Foundation who sponsored this work.

References

[1] Zimmet P, Shaw J, Alberti KG. *Preventing Type 2 Diabetes and the Dysmorphic syndrome in the real world: a realistic view*. Diabet Med 2003;20:693-702.

[2] Akinkugbe OO, Akinyanju OO. *Final Report-national Survey on non-communicable diseases in Nigeria.* Lagos: Federal Ministry of Health 1997, pp. 65-8.

[3] www.unaids.org. HIV and AIDs Estimates, Nigeria

[4] *Global Tuberculosis Report*, 2012. Geneva: World Health Organization 2012.

[5] WHO. *Global tuberculosis control: surveillance, planning, financing. WHO report 2005.* Geneva: World Health Organisation 2005.

[6] Nigeria Tuberculosis Fact Sheet. Photos.state.gov.libraries/Nigeria/487468/pdfs/January Tuberculosis Fact Sheets.pdf.

[7] DeReimer K. *Quantitative impact of human immunodeficiency virus infection on tuberculosis dynamics*. Am J Respir Crit Care Med 2007;176:936.

[8] Dooley KE, Chaisson RE. *Tuberculosis and diabetes mellitus: convergence of two epidemics.* Lancet Infect Dis 2009;9:737-46.

[9] Stop TB Initiative (World Health Organization); World Health Organization, Department of Chronic Diseases and Health Promotion; International Union against Tuberculosis and Lung Disease. *Collaborative framework for care and control of tuberculosis and diabetes.* Geneva: World Health Organization 2011.

[10] Li L, Lin Y, Mi F, et al. *Screening of patients with tuberculosis for diabetes mellitus in China*. Trop Med Int Health 2012;17:1294-301.

[11] Viswanathan V, Kumpatla S, Aravindalochanan V, et al. *Prevalence of diabetes and pre-diabetes and associated risk factors among tuberculosis patients in India*. PLoS One 2012;7:e41367.

[12] Balakrishnan S, Vijayan S, Nair S, et al. *High diabetes prevalence among tuberculosis cases in Kerala, India*. PLoS One 2012;7:e46502.

[13] *Global Tuberculosis Report, 2012.* Geneva: World Health Organization 2012.

[14] Young F, Critchley JA, Johnstone LK, et al. *A review of co-morbidity between infectious and chronic disease in Sub Saharan Africa: TB and diabetes mellitus, HIV and metabolic syndrome, and the impact of globalization.* Global Health 2009;5:9.

[15] World Health Organization. *Treatment of tuberculosis: guidelines for national program.* Geneva: World Health Organization 2006.

[16] The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus.* Diabetes Care 1997;20:1183-97.

[17] World Health Organization. *Tuberculosis and HIV data and statistics*. www.who.int/hiv/topics/tb/data/en/index1.html

[18] Bekkee L-G, Wood R. *The changing natural history of Tuberculosis and HIV coinfection in an urban area of Hyperendemicity.* Clin Infect Dis 2010;50:S208-S214.

[19] Odaibo GN, Gboun MF, Ekanem EE, et al. *HIV infection among patients with pulmonary tuberculosis in Nigeria*. Afr Med J Sci 2006;35:S93-8.

[20] Mugusi F, Swai AB, Aberti KG, et al. *Increased prevalence of diabetes mellitus in patients with pulmonary tuberculosis in Tanzania.* Tubercle 1990;71:271-6.

[21] Ruslami R, Aarnoutse RE, Alisjahbana B, et al. *Implications of the global increase of diabetes for tuberculosis control and patient care.* Trop Med Int Health 2010;15:1289-99.

[22] Jimenez-Corona ME, Cruz-Hervert LP, Garcia-Garcia L, et al. *Association of diabetes and tuberculosis; impact on treatment and post-treatment outcomes.* Thorax doi:10.1136/thoraxjnl-2012-201756.

[23] Alisjahbana B, van Crevel R, Sahiratmadja E. *Diabetes mellitus is strongly associated with tuberculosis in Indonesia.* Int J Tuberc Lung Dis 2006;10:696-700.

[23] Unadike BC, Eregie A, Ohwovoriole AE. *Prevalence of hypertension amongst patients with diabetes mellitus in Benin City, Nigeria.* Niger J Clin Pract 2011;14:300-2.

■ Received on April 6, 2014. Accepted on April 12, 2014.

■ Correspondence: Anthonia Ogbera, Department of Medicine, Lagos State University Teaching Hospital Ikeja, Lagos Nigeria - E-mail: oogbera@yahoo.co.uk.

ORIGINAL ARTICLE

Breast self examination and mammography in cancer screening: women health protective behavior

Z. GHODSI[1], S. HOJJATOLESLAMI[2]
[1] Department of Midwifery, Toyserkan Branch, Islamic Azad University, Toyserkan, Iran;
[2] Department of Nursing, Hamedan Branch, Islamic Azad University, Hamedan, Iran

Key words

BSE • Breast cancer • Preventive behaviors

Summary

Background. *Breast cancer (BC) is one of the leading causes of death among women. Secondary prevention may enable early detection, but this is suboptimal among all Iranian women.*
Methods. *This was a descriptive, analytic cross sectional study on 385 women 35 years old or more with no history of BC. Participants were selected by simple randomized method and were assessed through a two-part self-administered questionnaire and a self-examination checklist with content validity and test-re-test reliability.*
Results. *14.8% of women carried out breast self examination (BSE). Among them 5.7% was done in adequate timing and 9.4% performed it on a regular basis. The average age of BSE onset was 20.1 ± 7.6 and mean of Score was 6.25 ± 2.26 (2-11). 2.3% of participants performed BSE poorly, 7.5% fairly and 1.6% performed it well. 25.84% of samples had a history of mammography that 13% of whom received it as a result of prescription. The average age for mammography was 36 ± 7.2 (20-50) years and the frequency of mammography was 1.8 ± 1.4 (1-8) of times. Due to the low percentage of breast cancer preventive behaviors, in this study knowledge towards breast cancer was also measured because they are factors that are crucial in performance.*
Conclusion. *The results highlight the need to educate Iranian women to recognize the risk factors to promote early detection of breast cancer. Creation of health behavioral by focused educational programs might cause decrease of breast cancer prevalence.*

Introduction and background

Breast cancer is the second common cancer in women [1]. The incidence of breast cancer in women is about 15% in the UK [2]. In the United States about 230,480 new cases of invasive breast cancer was diagnosed in women [3]. Based on the latest statistics, the statistics of cancer incidence is increasing in Iran [4]. A variety of screening tests are used to detect breast cancer e.g., mammography, ultrasound, MRI, clinical breast examination, and BSE [5].There are only three methods for early detection of breast cancer including mammography, clinical examination, and breast self-examination (BSE) [6].
Early detection of breast cancer is crucial not only to the survivorship of a patient, but to her quality of life while treating the cancer, and thereafter [7]. Breast self-exam or a clinical exam allowed many breast cancers to be diagnosed and successfully treated. They with an annual mammogram starting at age 40 can help to early detection of breast cancer, when it's most treatable [8]. A study in China on 267,040 women, who did BSE, revealed that after about 10 years of follow-up, BSE enabled women to find their cancers earlier [9]. Another study in Canada on 290,000 women showed that screening included both a clinical exam and a mammogram was 95% effective at detecting breast cancer [8]. Despite efficacy of these methods, only 49.5% of women act in accordance with these guidelines [10] and women do not perform them on a regular basis [11,12]. One of the factors influencing the success of these programmes is women's acceptance, their motivations and attitudes [13].
There are many risk factors that affect the chance of developing cancer. Some of them like a person's age or race have a great impact and can't be changed. Others are related personal behaviors and may be controllable [14]. Understanding probably affecting factors can help develop a breast health plan [1, 15]. Considering the high prevalence of breast cancer in Iran, and considering that improving women's knowledge can be a base for improving their motivation and performance, present study aimed to assess women's knowledge, and their health behaviors about breast cancer.

Methods

Study design and Subjects

Present study was a descriptive, analytic cross sectional. By using simple randomized method and after approving of the Research Ethic Committee of Islamic Azad University of Hamedan, 358 women were selected from Gynecology clinics in Hamedan city from April 10 to July 10, 2012. The including criteria were 35 years of

age or more who were eligible to take part in the study, and without any history for breast cancer. The excluding criteria were unwillingness to attend the research during the study, and having a series of diseases.

Procedure and data analysis

Data collection tools included a two-part self-administered questionnaire and a self examination checklist. The first part of the questionnaire included some socio-demographic data (age, marital status, parity, etc); and the second section included questions relating to practices of Mammography, knowledge of breast cancer signs and relative risk factors, and the attitude of the participant toward BSE.

The knowledge questions were asked in three parts. Part 1) general question about knowledge of breast cancer in women and preventive behaviors were included: the prevalence of breast cancer in Iran, the kind of disease, easiest and cheapest way to detect, the onset of self-examination, breast self-examination in breast cancer prevention, breast self-examination time, the frequency of breast self-examination, mammography onset age, annual mammography information, the role of mammography in preventing breast cancer, the most common site of breast cancer, the most common age of breast cancer, symptoms (10 cases) and risk factors for breast cancer (9 cases). Part 2) knowledge about the symptoms of breast cancer were measured with ten questions including sinking part of the breast, orange peel form part of the breast, touching the wall and movable painless lump found in the breast, breast deformity, breast pain, bloody or watery discharge from the nipple, nipple of a breast sinking, sinking and breast skin lesions, breast enlargement, an enlarged auxiliary lymph nodes. Part 3) Knowledge about risk factors for breast cancer in women was measured by 9 items including a history of cancer in one breast, low fat diet, onset of menstruation at the age of 12 years, women who have not had children, women with first pregnancy before age 30, obesity, a history of cancer in a mother or sister or a close relative, history of benign breast tumors, menopause before 50 years of age. The questionnaire was set based on questions mean and sample score on three levels: poor, moderate and good. Participants received self-administered questionnaire. Answers were "correct" or "incorrect". If participant ticked "correct" she got 1 score (suitable) and if she ticked "incorrect", 0 score was considered (unsuitable). Total number of knowledge questions was 30. Mean of score was 12 ± 4.7. Total number of questions about cancer signs and risk factors were following 30 and 9. Mean of score for breast cancer symptoms was 2.39 ± 4.76 and for risk factors was 1.39 ± 4.2.

Check list also included how to do BSE. Initially, all subjects were taught breast self-examination technique by a physician. Then to each person was given a self examination check list. Checklists were controlled by trained researchers. If the person did the examination correctly, she got "1" score, and otherwise she got "0" score.

Content validity and test re-test reliability was done for the questionnaire and check list. To determine the validity of research tool, they were reviewed by 10 experts in this field. The reliability was determined by the measurement carried out by two researchers. Finally, the results were analyzed using the SPSS version 17.0. The level of $p \leq 0.05$ was considered for all statistical analyses. Chi-square test, Fischer's exact & V Cramer test were used to determine Knowledge.

Results

83.8% of participants were married; 56.8% were housewife. 64.3% had a diploma degree or more. 51% of participants belonged to middle-class and had average family income. Popularity of prior information about breast cancer was 72.4%. 13% of all samples reported a family history of breast tumors.

14.8% of participants stated that they carry out BSE. Among them, 5.7% started in adequate time and only 9.4% had done it monthly. 25.84% of samples had a history of mammography that in 13% was done as a result of prescription. The average age of BSE onset was 20.17 ± 7.6. Mean of Score was 6.25 ± 2.26 with a ranged of 2-11. Among them, 1.6% carried out BSE correctly, 2.3% poor and 7.5% quite correctly. 25.84% of samples had a history of mammography that was done as prescribed in 13%. The mean age onset of mammography was 36 ± 2.7 (20-50) years and the frequency of mammography was 8.1 ± 4.1 (1-8) of times. Tab. I shows some demographic characteristics.

64.9% of participants did not have previous information about BSE and 62.2% had information about mammography.

Fig. 1 shows knowledge level of participants about breast cancer in women and preventive behaviors, breast cancer signs and risk factors. There was specific statistical relationship between: knowledge and participant's age ($PV < 0.002$), family history ($PV < 0.009$), prior information about mammography, BSE or breast cancer ($PV < 0.005$), Knowledge and mammogram doing ($PV < 0.001$). However there was not specific statistical relationship between knowledge and BSE practice.

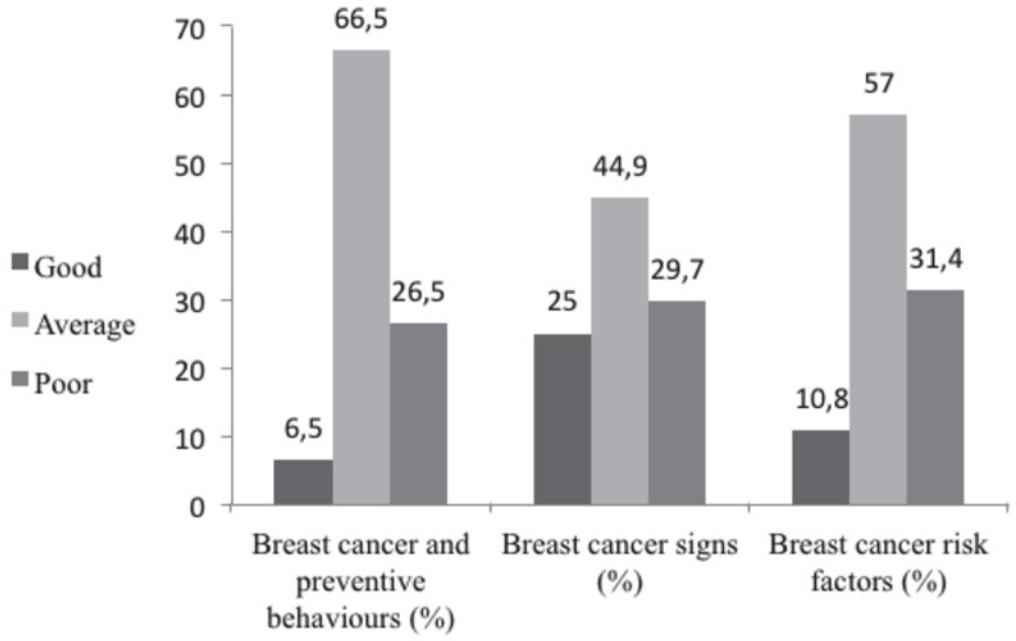
Conclusion and discussion

In this study few women had undergone breast screening, so that most participants did not perform BSE and mammography. Moreover, most participants did not per-

Tab. I. Some demographic characteristics of participants.

Variable	Ranged	Mean ± SD
Age pregnancy	35-80	45.63 ± 9.04
First age pregnancy	14-29	22 ± 3.36
Number living child	1-13	3.5 ± 2.1
Number abortion	1-9	3 ± 1.7
Number night work	1-4	1.5 ± 0.8
History	1-14	6.33 ± 3.7

Fig. 1. Knowledge of a: breast cancer and preventive behaviors, b: signs and c: risk factors.
a: Poor = 10 or less, Average= 10-20, Good=20 or more.
b: Poor = 3 or less, Average= 3-6, Good=6 or more.
c: Poor = 3 or less, Average= 3-6, Good=6 or more.



form BSE and mammography on the timing and regular basis. It can was affected by the low prior information about BSE and breast cancer. These results are in line with Radi study that found only 20.5% of participants had undergone breast screening, and 47.5% knew how to perform BSE [16]. In Yurdakos et al. study on 500 health personnel from 7 public hospitals with a mean age of 32 years old, only 22.2% of the health personnel had undergone mammographic evaluation [17].

At present study most participants did not have any prior information about breast self examinations. However, prior information about mammography was widespread. In Radi study 79% of participants heard about BSE [16]. Mean of awareness for breast cancer recognition, breast cancer signs and its risk factors among participants were low. A study in Egypt showed that total breast cancer knowledge scores had an average of 13.318 [18]. Moreover, only 44% of participants recognized the concept of breast self examination and 60% of them did not recognize mammography as an early detection method [18]. Another study on 45 breast cancer patients in Saudi indicated that Saudi women level of knowledge about breast cancer is very inadequate [16]. In contrast, in a survey, breast cancer and BSE awareness among nurses was relatively high [19]. This may be because of the key role they played in cancer information. It seems essential to increase women's awareness about benefits of breast cancer early detection.

Findings of present study highlight high educational need about breast cancer in Iran. Public awareness interventions are needed in order to promote early detection of breast cancer and overcome an ever-increasing burden breast cancer with emphasis on prevention and screening. In a program known as "Circle of Sisters", a breast cancer education initiative of free mammography was performed on 37 American Indian women. As a result, percentage of those expressing an intention to get a mammogram annually grew from 81.1% to 94.6% [20]. An educational program should be considered for health care providers in order to train BSE effectively to women and mammograms guidelines. Physicians, health providers, health pamphlets, and other information sources should assist in clarifying the benefits [21]. Such training can lead to an increased awareness so that in a survey in Iran significant increases were observed after the educational program [22].

Implications for practice

Government should design women education programs by health care professionals in order to recognize the role of them in cancer preventing. El-Shinawi et al. in their study found that 97% of breast cancer patients were willing to participate in spreading awareness among their community and their own families [18]. Programs should be augmented in daily living pattern of women, as Sadler et al. found that program initiation time is the most important factor in participation in cancer education programs among Korean people [23]. It seems to be effective if the cultural factors are considered

Because of low prevalence of self-care interventions for early detection of breast cancer, and high prevalence of breast cancer in Iranian society, increasing the awareness

about it is a necessity and using this type of interventions based on the costs versus benefits may be of special interest in health care system. The data were collected from one geographic region. It is recommended other studies in other regions, countries and with larger samples.

Acknowledgments

We would like to thank president of research of Islamic Azad University, Toyserkan and Hamedan branches for their cooperation in this study. Also we thank the staff in Gynecology clinics in Hamedan city, Dr. Parisa Jalaly, Mr. Salman Khazaei, and Mr. Abbas Mehrpoya for their contributions to the research.

References

[1] Chisti MA, Alfadley AA, Banka N, et al. *Coetaneous Metastasis from Breast Carcinoma: a brief report of a rare variant and proposed morphological classification*. Gulf J Oncolog 2013;1:90-4.

[2] *Cancer incidence statistics, Incidence cases and rates for males, females and persons in the UK 2010, England, Wales, Scotland and Northern Ireland*. Online July 26 2010, Available from: http: www.cancerresearchuk.org

[3] Wender R. *Ways to Increase Cancer Screening Rates of Documentation*. Department of Family & Community Medicine, Thomas Jefferson University, Philadelphia, PA. Online2011. Available from: www. The guidelines Advantages.org

[4] Olfatbakhsh A. *New statistic of breast cancer in Iran*. Online 2011 [Cited 2011 July17]. Iranian Center of Breast Cancer. Available from: www.icbc.ir /index.aspx?ID_ News=82

[5] Allen TL, Van Groningen BJ, Barksdale DJ, et al. *The breast self-examination controversy: what providers and patients should know?* J Nurse Pract 2010;6:444-51.

[6] Maurer F. *A peer education model for teaching breast self-examination to undergraduate college women.* Cancer Nurse 1997;20:49-61.

[7] Weiss C. *New Guidelines against Breast Self-Examination Could Seriously Endanger Women's Health*. Online 2012 [Cited 2008 July15], Available From: www.Breast Cancer.org / About Us/ The Press Room /Press Releases/ Press Releases 2008.

[8] *Breast Cancer, Mammography Plus Exam Better at Finding Cancer, But Produce More False Positives.* Online 2013 [Cited 2009 Aug 31]. Available From: www.Breast Cancer.org.

[9] Thomas DB, Gao DL, Ray RM, et al. *Randomized Trial of Breast Self-Examination in Shanghai: Final Results*. Journal of the National Cancer Institute 2002;94:1445-57.

[10] Park K, Hong WH, Kye SY, et al. *Community-based intervention to promote breast cancer awareness and screening: The Korean experience*. BMC Public Health 2011;11:468.

[11] Friedman LC, Nelson DV, Webb JA, et al. *Dispositional optimism, self-efficacy, and health beliefs as predictors of breast self-examination.* Am J Prev Med 1994;10:130-5.

[12] Murray M, McMillan C. *Health beliefs, locus of control, emotional control and women's cancer screening behaviour*. Br J Clin Psychol 1993;32:87-100.

[13] Bowling A. *Implications of preventive health behavior for cervical and breast cancer screening programmes: a review*. Family Practice Journal 1989;6:224-31.

[14] American Cancer Society. *Breast cancer: early detection*. Online2011. Available From: http://www.cancer.org /Cancer/ Breast Cancer /More Information /Breast Cancer Early Detection.

[15] Rosen L, Rosen G. *Breast cancer: early detection. The importance of finding breast cancer early.* Online 2011. Available From: American Cancer Society/ Learn about cancer/Breast Cancer/Early Detection.

[16] Radi SM. *Breast Cancer Awareness among Saudi females in Jeddah*. Asian Pac J Cancer Prev 2013;14:4307-12.

[17] Yurdakos K, Gulhan YB, Unalan D, et al. *Knowledge, attitudes and behaviour of women working in government hospitals regarding breast self examination 2013*. Asian Pac J Cancer Prev 2013;14:4829-34.

[18] El-Shinawi M, Youssef A, Alsara M, et al. *Assessing the level of breast cancer awareness among recently diagnosed patients in Ain Shams University Hospital*. Breast 2013;22:1210-4.

[19] Chong PN, Krishnan M, Hong CY, et al. *Knowledge and Practice of Breast Cancer Screening Amongst Public Health Nurses in Singapore*. Singapore Med J 2002;43:509-16.

[20] Chilton JA, Downing C, Lofton M, et al. *Circle of sisters: raising awareness of Native American women to breast cancer*. J Health Care Poor Underserved 2013;24:1167-79.

[21] Gigerenzer G, Mata J, Frank R. *Public knowledge of benefits of breast and prostate cancer screening in Europe*. J Natl Cancer Inst 2009;101:1216-20.

[22] Moodi M, Baladimood M, Sharifirad GR, et al. *Evaluation of breast self-examination program using Health Belief Model in female students.* J Res Med Sci 2011;16:316-22.

[23] Sadler GR, Ryujin LT, Ko CM, et al. *Korean women: breast cancer knowledge, attitudes and behaviors.* BMC Public Health 2001;1:7-18.

■ Received on October 6, 2013. Accepted on May 26, 2014.

■ Correspondence: Simin Hojjatoleslami, Department of Nursing, Hamedan Branch, Islamic Azad University, Hamedan, Iran - Tel. +98 0811 449 4001 - E-mail: Simineslami@yahoo.com

ORIGINAL ARTICLE

Molecular identification of *Pseudomonas aeruginosa* recovered from cystic fibrosis patients

M. DOURAGHI[1,2], F. GHASEMI[1], M.M. SOLTAN DALLAL[1,2], M RAHBAR[3,4], A. RAHIMIFOROUSHANI[5]
[1] Division of Bacteriology, Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran; [2] Food Microbiology Research Center, Tehran University of Medical Sciences, Tehran, Iran; [3] Department of Microbiology, Iranian Reference Health Laboratory Research Center Ministry of Health and Medical Education, Tehran, Iran; [4] Antimicrobial Resistance Research Center, Iran University of Medical sciences, Tehran, Iran; [5] Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

Key words

Pseudomonas aeruginosa • cystic fibrosis • *oprI* • *oprL*

Summary

Objective. *Precise identification of various morphotypes of Pseduomonas aeruginosa which developed during cystic fibrosis (CF) is of prime importance. We aimed to identify the isolates of P. aeruginosa recovered from CF patients at the genus and species level through primers targeting oprI and oprL genes via PCR.*
Methods. *Sputum samples or throat swabs were taken from 100 CF patients and plated on cetrimide agar. All suspected colonies were primarily screened for P. aeruginosa by a combination of phenotypic tests. Molecular identification of colonies was performed using specific primers for oprI and oprL genes.*
Results. *Based on phenotypic tests, P. aeruginosa isolates were recovered from 40% of CF patients. Forty isolates yielded amplicon of oprI gene using genus-specific primers confirming the identity of fluorescent pseudomonads. However, 37 of 40 isolates yielded amplicon of oprL gene using species-specific primers, verifying the identity of P. aeruginosa.*
Conclusion. *This study showed that the species-specific PCR targeting oprL gene can be used as accurate test for identification of highly adaptable P. aeruginosa in CF patients. This procedure may provide a simple and reliable method for identification of various morphotypes.*

Introduction

Pseduomonas aeruginosa is considered as the most common recovered bacterium from respiratory infections in cystic fibrosis (CF) patients [1]. Cystic fibrosis is a genetic disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) encoding gene. CFTR primarily acts as a chloride ion channel and mutation in this gene affects the chloride transport and sodium absorption, leading to thickness of mucus in lungs [2]. CFTR is also recognized as a cellular receptor for binding and clearance of *P. aeruginosa* from the lungs [3], therefore malfunction of CFTR may lead to persistence of this species and ultimately severe pulmonary disease. CF patients are more prone to colonization by a variety of bacteria during their life [4] but the majority of CF patients endure *P. aeruginosa* chronic lung infections [5]. The early colonization of patients may occur by susceptible and non-mucoid strains of *P. aeruginosa*, but as early as the colonized bacteria are covered by thickened and dehydrated mucosa in lung, the bacterial microenvironment will be established. This microenvironment creates a way for evasion of bacteria and a barrier to antibiotics and consequently selection of variants of *P. aeruginosa* [6]. Despite the prolonged antibiotic therapy, *P. aeruginosa* is not eradicated in such patients and the formation of various morphotypes of bacteria during chronic and sustained infection is reported worldwide. The major morphologic alterations of *P. aeruginosa* in CF patients are conversion to mucoid variants, loss of pigments, overproduction of alginate, formation of small-colony variants, and evolution of mutator strains [7-10].
The emergence of mucoid variants *P. aeruginosa* is usually considered as a poor prognostic indicator in CF patients [11]. One of the critical control measures in CF patients is accurate species identification. The broad phenotypic variation of *P. aeruginosa* may lead to misindentification of these strains in CF patients. Therefore, precise identification of various morphotypes which developed during chronic infections and differentiation of *P. areugionsa* from other non-fermentative strains involving in the pathogensis of CF is of prime importance. Due to drawbacks of phenotypic methods for identification of *P. areuginosa* morphotypes, we aimed to identify *P. aeruginosa* at the genus and species level through primers targeting *oprI* and *oprL* genes via PCR.

Materials and methods

In this cross-sectional study, the cases that had a sweat chloride equal to or greater than 60 mmol/L and were diagnosed as CF patients were included. The patients who

treated with antibiotics within the previous two weeks were excluded. One hundred patients suffering from CF, aged 1 to 23 years were studied from three health centers in Tehran during 2011 to 2012. Demographic characteristics and medical histories were collected from medical records. The study was approved by the local ethical committees.

Sputum samples or throat swabs were taken and cultured on cetrimide agar, blood agar and MacConkey agar and the plates were incubated for 72 h at 37 °C. Regardless of morphology of colonies, all suspected colonies primarily were screened for *P. aeruginosa* by a combination of tests including growth on cetrimide agar, growth at 42 °C, and biochemical tests such as oxidase, citrate, OF glucose, and arginine dihydrolase.

Genomic DNA extraction was performed using the standard phenol-chloroform extraction method [12]. Specific primers targeting the genes *oprI* and *oprL* were used to amplify 249 base pair (bp) and 504 bp products [13], respectively (Tab. I). Amplification of *oprI* and *oprL* was carried out in a total reaction volume of 20 µl containing 2 µl 10x PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM deoxynucleotide, 0.25 mM each primer (Bioneer, Seoul, South Korea), 0.5 U *Taq* polymerase, and 10 ng DNA. The Thermocycler (Peqlab, Germany) was set with the following conditions: Initial denaturation for 5 min at 95 °C and 30 cycles consisted of denaturation for 30s at 95°C, annealing for 30s at 57 °C, extension for 1 min at 72 °C, and final extension for 10 min at 72 °C. Electrophoresis was performed in a 1.5% agarose gel along with GeneRuler 100 bp DNA Ladder (Fermentas, Lithuania) and was stained with 0.5 µg/ml ethidium bromide. *Pseudomonas aeruginosa* ATCC 27853 was used as a positive control in all experiments.

Statistical analysis

The descriptive analysis was performed to calculate the frequency and percentage of variable using SPSS version 11.5.

Results

Among 100 patients with CF, 54 were females and 46 were males. Forty two patients were younger than 7 years old, 38 aged 7 to 14 years, and 20 aged more than 14 years. Demographic characteristics of CF patients including age and gender is shown in Fig. 1.

Based on phenotypic tests, *P. aeruginosa* isolates were recovered from 40% of CF patients. The distribution of CF patients colonized with *P. aeruginosa* in relation to demographic characteristics is shown in Fig. 2. *P. aeruginosa* were recovered from females (n = 19, 47.5%) and males (n = 21, 52.5%) in approximately similar frequency and no statistically significant difference was found. The colonization rate by *P. aeruginosa* among various age groups was different. An increasing trend of *P. aeruginosa* colonization was seen ranging from 23.8% in the 1-7 years age group to 36.8% in the 7-14 years age group, and 80% in cases older than 14 years.

For each isolate, the distinct morphotypes either mucoid or non-mucoied colonies grown on cetrimide agar were primarily identified as *P. aeruginosa* which oxidase- and citrate-positive but were OF glucose nonfermenter. Subsequently, all morphotypes were distinguished from oth-

Fig. 1. Distribution of patients with cystic fibrosis in relation of age and gender.

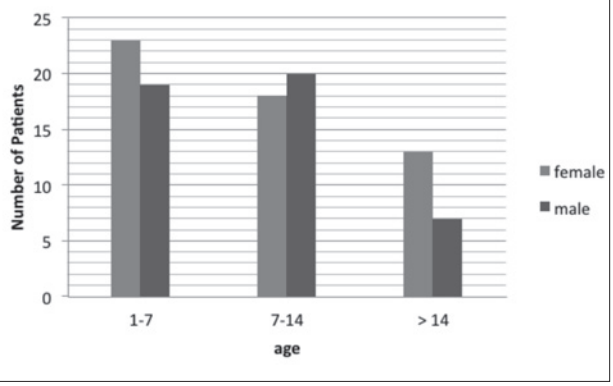
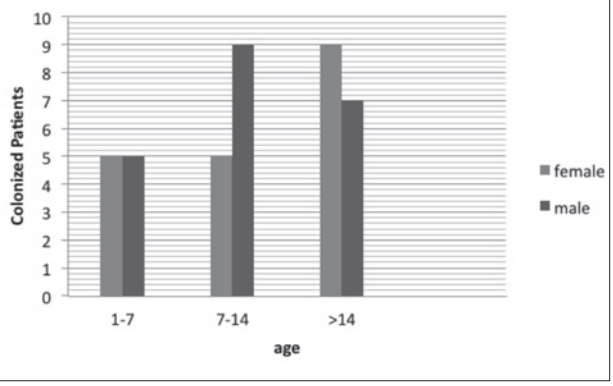


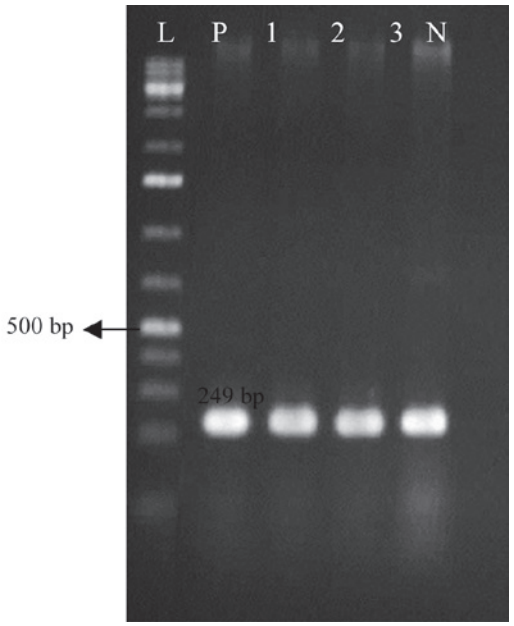
Fig. 2. Distribution of *P. aeruginosa* colonized patients suffering from cystic fibrosis in relation of age and gender.



Tab. I. Specific primers sequences targeting *oprI* and *oprL*.

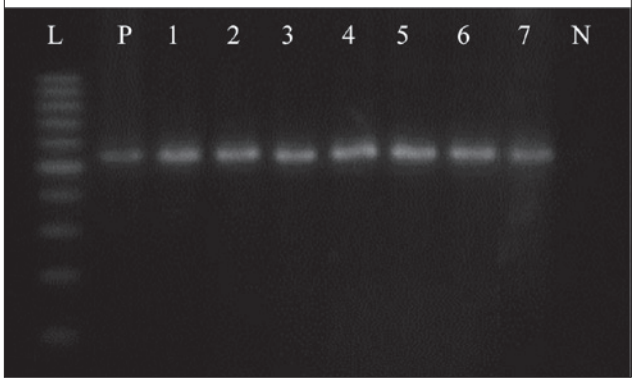
Amplicon size (bp)	Primer sequence (5′-3′)	Target region
249	5′- ATGAACAACGTTCTGAAATTCTCT -3′ 5′- CTTGCGGCTGGCTTTTTCCAG -3′	oprI
504	5′- ATGGAAATGCTGAAATTCGGC -3′ 5′- CTTCTTCAGCTCGACGCGACG -3′	oprL

Fig. 3. The *oprI* gene amplification using specific primers yielded a product of 249 bp typical to Speudomonas genus. L: 100 bp ladder (Fermentas, Lithuania); P: Positive control; 1-3: Pseudomonas isolates of CF patients; N: Negative control (distilled water).



er members of fluorescent pseudomonads by growth at 42 °C. Among 40 isolates of *P. aeruginosa*, 19 (47.5%) and 17 (42.5%) were mucoid and non-mucoid, respectively. In remaining isolates (n = 4, 10%) both mucoid and non-mucoid colonies were observed. Four (10%) and 17 (42.5%) isolates were arginine dihydrolase negative and non-pigmented, respectively. According to the combination of phenotypic and biochemical tests, 40 CF isolates were identified as *P. aeruginosa* and were considered for further identification via PCR-based assays. Forty isolates yielded 249 bp (Fig. 3) amplicon through amplification of *oprI* gene using genus specific primers confirming the identity of fluorescent pseudomonads. However, 37 of 40 isolates yielded 573 bp (Fig. 4) amplicon through amplification of *oprL* gene using species specific primers, verifying the identity of *P. aeruginosa*. Three isolates were identified as *P. aeruginosa* by phenotypic tests but did not confirmed via species specific PCR.

Fig. 4. The *oprI* gene amplification using specific primers yielded a product of 504bp typical to *P. aeruginosa*. L: 100 bp ladder (Fermentas, Lithuania); P: Positive control; 1-7: *P. aeruginosa* isolates of CF patients; N: Negative control (distilled water).



Disscusion

P. aeruginosa is not a fastidious organism and the identification of clinical isolates of *P. aeruginosa* is usually based on phenotypic methods. The phenotypic tests including macroscopic characteristics and biochemical tests are the most reliable tests for identification of typical isolates of *P. aeruginosa* [14]. However, extensive alterations in phenotype of *P. aeruginosa* may occur during chronic infection. For instance, the microenvironment of the CF lung may provide suitable conditions for mutation and selection of unique population of colonized bacteria [15]. Conversion to mucoid and non-pigmented colonies is common in *P. aeruginosa* recovered from CF patients. On the other hand, CF lungs may be colonized with other non-fermentative Gram-negative bacilli which are not easily differentiated from *P. aeruginosa*. These limitations may result in misidentification of *P. aeruginosa* as the most frequent pathogen in CF respiratory samples [16]. Inaccurate identification may affect the antibiotic susceptibility testing, administration of effective antipseudomonal antibiotics, and patient care. In the current study, we identified some of isolates with atypical phenotype; the isolates which non-pigmented and some isolates display a negative reaction for arginine dihydrolase. A possible explanation for these findings might be the plasticity and adaptation of *P. aeruginosa* in response to the unusual environment in CF lungs during chronic infection. As the isolates of *P. aeruginosa* with atypical phenotype were observed, we considered both mucoid and non-mucoid as well as pigmented and non-pigmented colonies for molecular identification. In this study, we determined the identity of multiple morphotypes of *P. aeruginosa* recovered from Iranian patients suffering from CF based on PCR assasys. To minimize the potential error of single-target assays, we used two targets for molecular identification of *P. aeruginosa.* We used PCR targeting two genes; *oprI* and *oprL* which are peptidoglycan associated outer membrane lipoproteins. *oprI* gene was previously identified as a conserved gene in members of fluorescent pseudomonads and in *Pseudomonadaceae* family. As reported previously, *oprI* gene sequence is highly conserved in *P. aeruginosa* isolates [18]. We found that amplicon of *oprI* gene were detected in all the phenotypically identified isolates including mucoid and non-pigmented Pseudomonas. This finding indicates that all isolates are more likely a member of fluorescent pseudomonads or Pseudomonas genus. These results are consistent with previous studies that applied *oprI* gene for identification of Pseudomonas genus [13, 19]. Moreover, we identified the *P. aeruginosa* at the species level using *oprL* gene specific primers. Our findings indicate

that the majority of isolates were *P. aeruginosa* and three isolates belonged to Pseudomonas genus as amplified only through genus-specific primers and these isolates were misidentified via phenotypic methods. This study also demonstrated that non-aeruginosa species may isolated from CF patients. The discrepancy between the results of phenotypic assays and molecular tests was not statistically significant, but species-specific PCR is more reliable and sensitive method for identification of morphotypes of *P. aeruginosa*.

This study showed that the species specific PCR targeting *oprL* gene can be used as accurate test for identification of highly adaptable *P. aeruginosa* in CF patients. This procedure may provide a simple and reliable method for identification of various morphotypes which misidentified via phenotypic methods.

References

[1] Rowe SM, Miller S, Sorscher EJ. *Cystic fibrosis*. N Engl J Med 2005;352:1992-2001.

[2] Ratjen F, McColley SA. *Update in cystic fibrosis 2011*. Am J Respir Crit Care Med 2012;185:933-6.

[3] Pier GB, Grout M, Zaidi TS. *Cystic fibrosis transmembrane conductance regulator is an epithelial cell receptor for clearance of Pseudomonas aeruginosa from the lung*. PNAS 1997;94:12088-93.

[4] Hart CA, Winstanley C. *Persistent and aggressive bacteria in the lungs of cystic fibrosis children*. Br Med Bull 2002;61:81-96.

[5] Starner TD, McCray PB. *Pathogenesis of early lung disease in cystic fibrosis: a window of opportunity to eradicate bacteria.* Ann Intern Med 2005;143:816-22.

[6] Hassett DJ, Sutton MD, Schurr MJ, Herr AB, et al. *Pseudomonas aeruginosa hypoxic or anaerobic biofilm infections within cystic fibrosis airways.* Trends Microbiol 2009;17:130-8.

[7] Mathee K, Ciofu O, Sternberg C, et al. *Mucoid conversion of Pseudomonas aeruginos by hydrogen peroxide: a mechanism for virulence activation in the cystic fibrosis lung*. Microbiology 1999;145:1349-57.

[8] Worlitzsch D, Tarran R, Ulrich M, et al. *Effects of reduced mucus oxygen concentration in airway Pseudomonas infections of cystic fibrosis patients*. J Clin Invest 2002;109:317-25.

[9] Oliver A, Cantón R, Campo P, et al. *High frequency of hypermutable Pseudomonas aeruginosa in cystic fibrosis lung infection*. Science 2000;288:1251-53.

[10] Ciofu O, Mandsberg LF, Bjarnsholt T, et al. *Genetic adaptation of Pseudomonas aeruginosa during chronic lung infection of patients with cystic fibrosis: strong and weak mutators with heterogeneous genetic backgrounds emerge in mucA and/or lasR mutants.* Microbiology 2010;156:1108-119.

[11] Emerson J, Rosenfeld M, McNamara S, et al. *Pseudomonas aeruginosa and other predictors of mortality and morbidity in young children with cystic fibrosis*. Pediatr Pulmonol 2002;34:91-100.

[12] Sambrook J, Fritsch EF, Maniatis T. *Molecular cloning*, 2nd ed. New York: Cold Spring Harbor Laboratory Press 1989.

[13] De Vos D, Lim A, Pirnay JP, et al. *Direct detection and identification of Pseudomonas aeruginosa in clinical samples such as skin biopsy specimens and expectorations by multiplex PCR based on two outer membrane lipoprotein genes, oprI and oprL.* J Clin Microbiol 1997;35:1295-9.

[14] Miller MB, Gilligan PH. *Laboratory aspects of management of chronic pulmonary infections in patients with cystic fibrosis*. J Clin Microbiol 2003;41:4009-15.

[15] Burns JL, Emerson J, Stapp JR, et al. *Microbiology of sputum from patients at cystic fibrosis centers in the United States*. Clin Infect Dis 1998;27:158-63.

[16] Kidd TJ, Ramsay KA, Hu H, et al. *Low rates of Pseudomonas aeruginosa misidentification in isolates from cystic fibrosis patients*. J Clin Microbiol 2009;47:1503-9.

[17] Wessel AK, Liew J, Kwon T, et al. *Role of Pseudomonas aeruginosa peptidoglycan-associated outer membrane proteins in vesicle formation.* J Bacteriol 2013;195:213-9.

[18] De Vos D, Bouton C, Sarniguet A, et al. *Sequence diversity of the oprI gene, coding for major outer membrane lipoprotein I, among rRNA group I pseudomonads*. J Bacteriol 1998;180:6551-6.

[19] De Vos D, Lim Jr.A, De Vos P, et al. *Detection of the outer membrane lipoprotein I and its gene in fluorescent and non fluorescent pseudomonads: implications for taxonomy and diagnosis.* J Gen Microbiol 1992;139:2215-23.

■ Received on January 19, 2014. Accepted on May 26, 2014.

■ Correspondence: Mohammad Mehdi Soltan Dallal, Food Microbiology Research Center and School of Public Health, Tehran University of Medical Sciences, Tehran, Iran - Tel. +98 21 8899297 - Fax +98 21 88954913 - E-mail: msoltandallal@gmail.com

ORIGINAL ARTICLE

Candiduria in children: a first report from an Iranian referral pediatric hospital

P. GHOLAMIPOUR[1], S. MAHMOUDI[2], B. POURAKBARI[2], M. TAGHI HAGHI ASHTIANI[3], F. SABOUNI[1], M. TEYMURI[2], S. MAMISHI[1,2]

[1]Department of Infectious Diseases, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran; [2] Pediatrics Infectious Diseases Research Center, Tehran University of Medical Sciences, Tehran, Iran; [3] Department of Pathology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Key words

Candiduria • Children • Iran

Summary

Candida spp. especially Candida albicans is considered as one of the most common cause of fungal infections. The aim of our study was to determine epidemiology of candiduria in children who were referred to an Iranian referral hospital. During May 2011 to February 2013, among 4813 urine culture positive, 209 candida spp. isolates (4.3%) was found. Forty-one percent of cadiduria infection was seen in patients between 1 month and 1 year, 24% in neonatant and 24% in patients 1 to 5 years. Cadiduria was mainly found in patients who had received more than 2 or 3 antibiotic during their hospitalization (37% and 24%, respectively). In our study, the highest frequency of cadiduria was seen in patients who had received more than 2 antibiotics and more than 3 antibiotics during their hospitalization; therefore, the strategic goals to optimize antimicrobial use including optimizing choice and duration of empiric therapy as well as monitoring and providing feedback regarding antibiotic resistance are recommended.

Introduction

Candida spp. especially *Candida albicans* consider as one of the most common cause of fungal infections leading to a range of life-threatening invasive to non-life-threatening mucocutaneous diseases [1].

According to nosocomial Infection Surveillance systems of the United State, *candida* spp. are the 7th most common nosocomial pathogens [2]. Several reports over the last 30 years has been reported not only in dramatic increase in the prevalence of candiduria but also in the incidence of candida urinary tract infections (UTI) [3].

The recognition of differences in incidence, populations at greater risk, species distribution is important in order to establish appropriate measures of infection control and the management of this disease [4]. The aim of our study was to determine epidemiology of candiduria in children in an Iranian referral hospital.

Method

The diagnosis of a UTI due to Candida species is much more difficult and it has not been established the importance of quantitative urine cultures for UTI due to Candida [5]. In our study, urine samples were collected from patients who had symptoms suggesting a UTI.

However, there is no consensus cut-off limit to define candiduria and investigators use different definitions, quantitation of the number of organisms in the urine to define infection was considered $\geq 10^4$ yeast cfu/ml.

Each urine sample was cultured on CHROM agar candida plates and incubated at 37°C for 24-48h aerobically. The number of colonies on each plate were counted and recorded based on colony colors. Furthermore, a direct smear was prepared from each colony and confirmed as yeasts. Candida isolates were identified based on colony morphology on CHROM agar candida and germ tube production. The following data were collected from the medical records of patients: gender, age, hospital unit, duration of hospitalization, use of central venous and urinary catheters during the hospitalization, the use of antibiotics at time of diagnosis of candiduria, previous usage of antifungal drugs and clinical finding.

Statistical analyses

The Statistical Package for the Social Sciences (Windows version 16.0; SPSS Inc, Chicago, US) was used for all analyses. Descriptive statistics were used to summarize patient variables.

Results

From May 2011 to February 2013, among 4813 urine culture positive, 209 *candida* spp. isolates (4.3%) was found (150 *Candida albicans* (72%) and 57 *Candida*

spp. (28%)). The demographic data of patients with candiduria was shown in Tab. I. Candiduria was found in 66 girls (32%) and 143 boys (68%). The majority of cadiduria infection was seen in patients less than 5 years. Forty-one percent of reported infection was in patients between 1 month and 1 year, 24% in neonatant and 24% in patients 1 to 5 years (Tab. I).

The highest frequency of cadiduria was seen in patients who had been hospitalized over a month (73 cases, 35%), between 2 weeks to 1 month (57 cases, 27%), between 5 day to 2 week (52 case, 25%) followed by 27 cases (13%) that were hospitalized less than 5 day.

The highest frequency of cadiduria was seen in patients who received more than 2 antibiotics (37%) or more than 3 antibiotics (24%) during their hospitalization (Tab. I).

Tab. I. Demographic data of patients with candiduria.

		N	%
Sex	Male	143	68
	Female	66	32
Age	Neonate	49	24
	1 month to 1 year	86	41
	1 year to5year	50	24
	6year to10year	13	6
	11year to15year	11	5
Ward	PICU	51	24.5
	Urology	30	14
	NICU	26	12
	Surgery	21	10
	CICU	20	10
	Gastroenterology	14	7
	Rheumatology	10	5
	Nephrology	10	5
	Infectious	9	4
	Neonatal	6	3
	Cardiology	5	2
	Emergency	4	2
	Oncology	2	1
	Neurology	1	0.5
Duration of hospitalization	<5 days	27	13
	5-10 days	27	13
	10-15 days	25	12
	15-30 days	57	27
	>30 days	73	35
Antibiotic usage	No	14	7
	2 or more antibiotic	78	37
	3 or more antibiotic	51	24
	Cephalosporins	37	18
	Meropenem	8	4
	Penicilin	7	3
	Piperacillin/tazobactam	4	2
	Vancomycin	1	0.5
	Aminoglycoside	1	0.5
	Others	8	4

PICU: Pediatric intensive care unit, NICU: Neonatal intensive care unit, CICU: Cardiovascular Intensive Care Unit.

Tab. II. Underlying diseases of patients with candiduria.

Diagnosis	N	%
Cardiovascular disease	37	18
Respiratory disease	22	10
Anomaly of urinary tract	21	10
UTI or nephrogenic disease	21	10
Gastrointestinal and liver diseases	19	9
Infectious disease	18	8.5
Nervous system	18	8.5
Neurosurgery	15	7
Metabolic diseases	8	4
Rheumatologic disease	7	4
Endocrine diseases	7	4
Immunodeficiency	4	2
Leukemia or Lymphoma	3	1
Prematurity and RDS	3	1
Other blood diseases	2	1
Surgical-site infections	2	1
Others	2	1
Total	209	100

UTI: Urinary Tract Infections, RDS: Respiratory Distress Syndrome.

The majority of children with candiduria were hospitalized in ICUs (pediatric intensive care unit (24.5%), neonatal intensive care unit (12%), and coronary care unit (10%) (Tab. I). Among underlying diseases, the highest frequency belonged to cardiovascular disorder (18%), respiratory diseases (10%), anomaly of urinary tract (10%), UTI or nephrogenic disease (10%), Gastrointestinal and liver diseases (9%), infectious diseases (8.5%) and neurologic disorders (8.5%) (Tab. II).

Among all patients, 38 (18%) and 25 (12%) had central catheter and urine catheter, respectively.

Diaper rash and oral thrush was reported in 70 (34%) and 12 (6%) of patients, respectively. In addition, 25 cases (12%) had genital anomalies and candidemia was present in 5 cases (2%). Thirty- four patients (16%) were treated with systemic antifungal drugs. Fluconazole was prescribed for 23 cases (11%), clotrimazol for 16 patients, nistatin for 12 cases and amphotericin B for 7 cases. Four cases were treated with combination of fluconazole and amphotericin B and the others did not receive any treatment for their infection.

Discussion

In the present study, candiduria was diagnosed in 4.3% of the patients with UTI. It has been reported that 11 to 52% of nosocomial urinary tract infections (UTIs) are caused by *Candida* spp. [6-11]. Increased age, female sex, antibiotic use, urinary drainage devices and prior surgical procedures are considered as risk factors for candiduria [12, 13]. Although females have higher risk for developing candiduria, In our study, similar to Jain et al. report, candiduria was more common in

males (68%) than females (32%) [14]. Candiduria has been dramatically increased among hospitalized patients especially among those patients with indwelling drainage devices [15, 16]. In our study, long-term indwelling urethral catheters or other urinary drainage devices were present in 40% of patients and all of the patients with candiduria had a known underlying illness. A study performed by Platt et al. showed that 26.5% of all urinary infections related to indwelling catheters were caused by fungi [15]. Philips et al. reported that *Candida* spp. were responsible for 25 of 60 (42%) UTI in infants admitted to a neonatal intensive care unit [17]. The frequency of candidemia in our study was 2% that was less than Philips et al. study that reported 52% [17]. Our result, confirm the Binelli et al. report which was reported that in the majority of patients the urinary tract was not the source of candidemia [16].

It has been reported that the prevalence of candiduria in the ICU population is increasing ranges from 19 to 44% of urine specimens depending upon different population and definition of candiduria [18-20]. Candiduria and candidemia occur commonly in neonatal and pediatric ICUs and particularly in premature infants [12, 17, 21-25]. *Candida* spp. were the pathogens identified in 42% of hospital-acquired urinary tract infections in a neonatal intensive care unit [17]. In this study, the majority of children with candiduria were hospitalized in ICUs (PICU (24.5%), NICU (12%), CICU (10%). The higher number of candiduria cases in patients of ICUs might be due to concurrent factors that contribute to the selection of these pathogens such as underlying diseases, immunodeficiency, and multiple manipulations by health care personnel and altered bacterial flora as well as use of antibiotics and long hospital stay [18, 19].

Use of antibiotics consider as a chief risk factor to develop *Candida* urinary tract infection [26]. In our study, the highest frequency of cadiduria was seen in patients who had received more than 2 antibiotics and more than 3 antibiotics during their hospitalization;

Although *C. albicans* is frequently reported as the most prevalent species infecting the urinary tract [16], non-albicans *Candida* spp. which better adapted to the urinary tract environment accounted for more than 50% of urinary *Candida* isolates [13, 27]; consequently, identification of *Candida* spp. isolates as well as monitoring and providing feedback regarding antifungal resistance is suggested. In addition, the strategic goals to optimize antimicrobial use including optimizing choice and duration of empiric therapy as well as monitoring and providing feedback regarding antibiotic resistance are recommended.

References

[1] Achkar JM, Fries BC. *Candida infections of the genitourinary tract*. Clin Microbiol Rev 2010;23:253-73.

[2] Saha R, Das SD, Kumar A, et al. *Pattern of Candida isolates in hospitalized children*. Indian J Pediatr 2008;75:858-60.

[3] Sobel JD, Fisher JF, Kauffman CA, et al. *Candida urinary tract infections-epidemiology*. Clin Infect Dis 2011;52(suppl. 6):S433-S6.

[4] Nucci M, Queiroz-Telles F, Alvarado-Matute T, et al. *Epidemiology of Candidemia in Latin America: a laboratory-based survey*. PloS one 2013;8:e59373.

[5] Kauffman CA. *Candiduria*. Clin Infect Dis 2005;41(Suppl. 6):S371-6.

[6] Febre N, Silva V, Medeiros E, et al. *Microbiological characteristics of yeasts isolated from urinary tracts of intensive care unit patients undergoing urinary catheterization*. J Clin Microbiol 1999;37:1584-6.

[7] Weinstein RA, Lundstrom T, Sobel J. *Nosocomial candiduria: a review*. Clin Infect Dis 2001;32:1602-7.

[8] Richards MJ, Edwards JR, Culver DH, et al. *Nosocomial infections in combined medical-surgical intensive care units in the United States.* Infect Control Hosp Epidemiol 2000;21:510-5.

[9] Brindha S, Jayashree M, Singhi S, et al. *Study of nosocomial urinary tract infections in a pediatric intensive care unit.* J Trop Pediatr 2011;57:357-62.

[10] Pourakbari B, Rezaizadeh G, Mahmoudi S, et al. *Epidemiology of nosocomial infections in pediatric patients in an Iranian referral hospital*. J Prev Med Hyg 2012;53:204-6.

[11] Bouza E, San Juan R, Munoz P, et al. *A European perspective on nosocomial urinary tract infections II. Report on incidence, clinical characteristics and outcome (ESGNI− 004 study).* Clin Microbiol Infect 2001;7:532-42.

[12] Rivett A, Perry J, Cohen J. *Urinary candidiasis: a prospective study in hospital patients.* Urol Res 1986;14:183-6.

[13] Kauffman CA. *Candiduria.* Clin Infect Dis 2005;41(Suppl. 6):S371-S6.

[14] Jain M, Dogra V, Mishra B, et al. *Candiduria in catheterized intensive care unit patients: Emerging microbiological trends.* Indian J Pathol Microbiol 2011;54:552.

[15] Platt R, Polk BF, Murdock B, et al. *Risk factors for nosocomial urinary tract infection*. Am J Epidemiol 1986;124:977-85.

[16] Binelli C, Moretti M, Assis R, et al. *Investigation of the possible association between nosocomial candiduria and candidaemia*. Clin Microbiol Infect 2006;12:538-43.

[17] Phillips JR, Karlowicz MG. *Prevalence of Candida species in hospital-acquired urinary tract infections in a neonatal intensive care unit.* Pediatr Infect Dis J 1997;16:190-4.

[18] Richards MJ, Edwards JR, Culver DH, et al. *Nosocomial infections in pediatric intensive care units in the United States.* Pediatrics 1999;103:e39-e.

[19] Alvarez-Lerma F, Nolla-Salas J, Leon C, et al. *Candiduria in critically ill patients admitted to intensive care medical units.* Intensive Care Med 2003;29:1069-76.

[20] Passos XS, Sales WS, Maciel PJ, et al. *Candida colonization in intensive care unit patients' urine*. Mem Inst Oswaldo Cruz 2005;100:925-8.

[21] Shay AC, Miller LG. *An Estimate of the Incidence of Candiduria Among Hospitalized Patients in the United States*. Infection control and hospital epidemiology 2004;25:894-5.

[22] Hamory B, Wenzel R. *Hospital-associated candiduria: predisposing factors and review of the literature.* J Urol 1978;120:444.

[23] Triolo V, Gari-Toussaint M, Casagrande F, et al. *Fluconazole therapy for Candida albicans urinary tract infections in infants.* Pediatr Nephrol 2002;17:550-3.

[24] Hitchcock R, Pallett A, Hall M, et al. *Urinary tract candidiasis in neonates and infants*. Br J Urol 1995;76:252-6.

[25] Pappu LD, Purohit DM, Bradford BF, et al. *Primary renal candidiasis in two preterm neonates: report of cases and review of literature on renal candidiasis in infancy*. Archives of Pediatrics & Adolescent Medicine 1984;138:923.

[26] Bukhary Z. *Candiduria: a review of clinical significance and management.* Saudi J Kidney Dis Transpl 2008;19:350.

[27] Bochicchio GV, Joshi M, Shih D, et al. *Reclassification of urinary tract infections in critically ill trauma patients: a time-dependent analysis.* Surg Infect 2003;4:379-85.

■ Received on March 9, 2014. Accepted on April 13, 2014.

■ Correspondence: Setareh Mamishi, Department of Pediatric Infectious Disease, Children Medical Center Hospital, School of Medicine, Tehran University of Medical Sciences No.62, Gharib St., Keshavarz Blvd., Tehran, Iran - Tel: +98 021 6642 8996 - Fax: +98 021 6642 8996 - E-mail: smamishi@sina.tums.ac.ir

ORIGINAL ARTICLE

Ownership and utilisation of insecticide-treated mosquito nets among caregivers of under-five children and pregnant women in a rural community in Southwest Nigeria

A.M. ADEBAYO[1], O.O. AKINYEMI[2], E.O. CADMUS[1]
[1] Department of Preventive Medicine and Primary Care, College of Medicine, University of Ibadan, Ibadan, Nigeria; [2] Department of Health Policy and Management, College of Medicine, University of Ibadan, Ibadan, Nigeria

Key words

Ownership • Utilisation • Insecticide Treated Nets • Nigeria

Summary

Introduction. *Malaria still constitutes a serious public health problem in Nigeria despite control efforts. The use of Insecticide Treated Net (ITN) has been proven to be an effective preventive modality in the control of malaria but its utilisation has been shown to be low. This study assessed the ownership and utilisation of ITN in Igbo-Ora, a rural community in Ibarapa Central Local Government Area (LGA) of Oyo State, Southwest Nigeria.*
Methods. *A descriptive cross-sectional survey among female caregivers of under-five children and pregnant women was conducted using semi-structured interviewer-administered questionnaire. Data were analyzed using SPSS version 16.*
Results. *Among 631 respondents that participated, 84.9% were caregivers of under-five children. Mean age was 27.7 ± 6.3 years with 53.4% between 20-29 age group. Majority, 91.1% had at least primary education, 60.2% were traders and 69.7% were married. Most respondents, 71.8% had at least one type of mosquito nets. Among those that had, 85.4% had window/door net, 25.2% untreated mosquito net while only 15.5% had ITN. Overall, 11.1% of the respondents had ITN among which 78.6% had ever slept under an ITN. Among those that had ever slept under an ITN, slightly less than half, 49.1% slept under an ITN the previous night. Less educated respondents were five times more likely to use ITN (95% CI = 1.24-21.28).*
Conclusions. *This study revealed very low ownership and utilisation of ITNs. There is need to improve on the knowledge of community members of the relevance of ownership and utilisation of ITN in malaria prevention.*

Introduction

Malaria still constitutes a serious public health problem in Nigeria. Malaria is endemic in the poorest countries in the world, causing 400 to 900 million clinical cases and up to 2.7 million deaths each year [1]. More than 90% of malaria deaths occur in Sub-Saharan Africa, resulting in an estimated 3,000 deaths each day. Almost all the deaths are among children younger than five years of age. Other high-risk groups include women during pregnancy, non-immune travelers, refugees and other displaced persons, and people of all ages living in areas of unstable malaria transmission [2]. In highly endemic countries, malaria poses a serious danger to pregnant women and their unborn children. Malaria in pregnancy causes maternal anaemia, miscarriage, and low birth weight. In endemic countries, it is the leading cause of maternal mortality and one of the primary causes of neonatal deaths [1, 2].
In Nigeria, malaria is the leading cause of under-five mortality contributing 33% of childhood deaths and 25% infant mortality. As a child will typically be sick of malaria between 3-4 times in one year, the disease is a major cause of absenteeism in school aged children, thus impeding their educational and social development [3].
Several global and regional attempts have been made at controlling the disease in the past with little success as a result of ineffective strategies used and insufficient resources. However, the most recent launching of Roll Back Malaria initiative has generated a lot of resources for the control of the disease with simple and cost effective interventions, with special focus on the most at risk. At the malaria summit hosted by Nigeria in 2000, African Heads of States made a declaration to halve the burden of malaria by the year 2010. One of the targets set for the first five years was to ensure that 60% of the vulnerable groups, children under five years of age and pregnant women, have access to and sleep under insecticide treated nets (ITNs) [3, 4] and to have 80% of this group covered by ITNs by 2010 [5].
Insecticide treated nets reduce human contact with mosquitoes and are effective malaria prevention intervention. ITNs have been shown to reduce severe disease due to malaria in endemic regions and reduce all-cause mortality by approximately 20%. Studies of ITN effectiveness suggest a reduction in malaria episodes by 45 to 50% [6-10].
Despite the knowledge that ITNs are effective in the prevention of malaria, ITN coverage and utilisation still

remain low in many African countries [11]. Fewer than 10% of children and pregnant women regularly sleep under ITNs in most malaria endemic regions [12, 13]. In Uganda Demographic and Health Survey (UDHS), it was estimated that only 13% of households in Uganda owned a mosquito net and 8% of under-fives usually used them [14].

The 2003 National Demographic Health Survey in Nigeria reported a 12% household ownership of any net and 2% of ITN; under-five children's utilisation of ITN was 1.2% while 5.9% of them used any net. Netmark [15], a United States Agency for International Development (USAID) funded project, reported overall household ownership of any net of 12% in 2000 and 27% in 2004 while ITN ownership increased from 0 to 9%. Similar finding was also reported by Oresanya et al [3] with overall ownership of any net of 23.9% and 10.1% ownership of ITN.

Few studies have documented household net coverage and utilisation in Nigeria. Most of the published studies available were conducted in other malaria endemic countries in Sub-Saharan Africa and the few published studies in Nigeria were from the urban centres of other states. This study therefore aimed at assessing ownership and utilisation of ITN among under-5 caregivers in a rural community in Southwest Nigeria.

Methods

The study was conducted at Igbo-Ora, a rural community and headquarters of Ibarapa Central Local Government Area of Oyo State, Southwest Nigeria. It has a population of about 60,000 and it is located about 80 Km west of Ibadan, approximately 20km east of Eruwa, 32 Km from Abeokuta and 128km from Lagos. Igbo-Ora is divided into six census areas with each census area subdivided into enumeration areas (a total of 62), each with an average population of 600 people. Individual enumeration area is further divided into compounds; each compound has about 100 women in the reproductive age group.

The study population comprised of female caregivers of under-five children and pregnant women in their reproductive age (15-49 years), who have been resident in the community for a minimum of one year.

A descriptive community based cross-sectional survey was conducted using multistage cluster sampling technique was used to select participants. A minimum sample size of 126 was estimated using Leslie and Kish formula for estimating sample size for cross sectional study at 10.1% prevalence for ITN ownership [3], 95% confidence interval, 90% power, 5% precision and 10% non-response rate; this was adjusted by a factor of two for clustering effect. Three enumeration areas were selected by balloting from each of the six census areas. Two compounds were then selected from each of these enumeration areas by balloting. All the eligible and consenting caregivers of under-five children and pregnant women in the households within the selected compounds were subsequently interviewed.

A semi-structured interviewer-administered questionnaire consisting of five sections was used to obtain information on socio-demographic characteristics and ownership and utilisation of ITN. The questionnaire was translated to Yoruba, the predominant local language in the community, in order to enable proper understanding by respondents and back-translated to English to ensure that the original meaning was retained. This questionnaire was pre-tested among caregivers of under-five children and pregnant women in Idere, the second town in Ibarapa Central LGA. The questionnaires were administered by four trained research assistants.

Data obtained was entered, cleaned and analyzed using the Statistical Package for Social Sciences (SPSS version 16). Frequency tables were generated. Mean and standard deviation were computed. Univariate analysis between dependent variables and independent variables was also explored. Associations between variables were tested with Chi-square and Fischer's Exact test for qualitative variables. Level of statistical significance of 5% was used. Multivariate logistic regression analysis was done to identify factors predicting adequate use of ITN in malaria prevention in the study population.

Ethical clearance and approval was obtained from the Oyo State Ethical Review Committee. Informed verbal consent was also obtained from individual research participant during data collection. The respondents were given the right to refuse to take part in the study as well as to withdraw any time during the interview. Privacy and confidentiality were maintained throughout the study. Participants were made to understand that their participation in the study will contribute towards future policy making and assist in the design of programmes that will help to improve utilisation of ITN in Nigeria.

Results

A total of 631 respondents were interviewed. Among these, majority, 536 (84.9%) were caregivers of under-five children. The mean age of respondents was 27.7 ± 6.3 years with a little above half, 53.4%, between 20-29 years age. Majority (96.8%) was of Yoruba ethnic group and more than half, 57.6%, were Muslims. Most respondents, 69.7%, were married and living with their spouses. A larger proportion, 71.9%, of those that were married was in a monogamous relationship. Overwhelming majority, 91.1%, have at least primary education while trading 60.2% formed the largest occupation group. The median average income was ₦5,000. Most respondents, 64.7%, earned less than ₦10,000 monthly. Only 8.4% earned ₦30,000 and above (Tab. I).

Tab. II shows respondent ownership of mosquito nets and by type. Most respondents, 71.8%, had mosquito nets and most of them, 75.1%, had only one. Among those that had, 85.4% had window/door net, 25.2% ordinary mosquito net while only 15.5% had ITN.

Overall, 70 (11.1%) of the respondents had ITN among which 55 (78.6%) had ever slept under an ITN. Among those that had ever slept under an ITN, slightly less than

Tab. I. Socio-demographic characteristics of the respondents (N = 631).

Socio-demographic Characteristics	Number (n)	Percentage (%)
Respondent		
Pregnant	65	10.3
Under-five caregiver	536	84.9
Both	30	4.8
Age group		
< 20	36	5.7
20-29	337	53.4
30-39	230	36.5
≥ 40	28	4.4
Religion		
Christianity	268	42.5
Islam	363	57.6
Ethnic group		
Yoruba	611	96.8
Igbo	7	1.1
Hausa	5	0.8
Others	8	1.3
Marital status		
Single	30	4.8
Co-habiting	85	13.5
Married, living together	440	69.7
Married, living alone	67	10.6
Separated	8	1.3
Divorced	1	0.2
Family type		
Monogamous	454	71.9
Polygamous	177	28.1
Level of education		
No formal education	56	8.9
Primary	206	32.6
Secondary	275	43.6
Tertiary	94	14.9
Occupation		
Trading	380	60.2
Civil servant	78	12.4
Farming	15	2.4
Monthly income (₦)*		
< 10000	408	64.7
10000-19999	107	17.0
20000-29999	63	10.0
≥ 30000	53	8.4

*As at the time of data collection, $1= ₦150.

half, 49.1% slept under an ITN the previous night. Also, among the respondents that owned ITN, 92.9% reported that their under-five children had ever slept under an ITN while 43.1% of them stated that their under-five children slept under it the previous night. See Tabs. II and III.

Tab. IV shows respondents' socio-demographic characteristics and utilisation of ITN at least once. Significantly higher proportion, 75%, of respondents with low level of education utilized ITN compared with 35.9% with high level ($p < 0.05$). There was no significant association between utilisation of ITN and age group, marital status, family type, occupation, average income, total number of children as well as under-five children. The knowledge of cause of malaria was statistically significant with utilisation of ITN. Higher proportion, 63% of respondents with good knowledge of cause of malaria used ITN last night compared with 32.1% with poor knowledge ($p < 0.05$). Higher proportion of those with good knowledge of ITN in malaria prevention also used ITN compared to respondents with poor knowledge but was not statistically significant ($p > 0.05$).

Tab. V shows predictors of utilisation of ITN. The model included age, educational level and knowledge of cause of malaria. Only the level of education was found to be a significant predictor of ITN utilisation. Respondents with low educational status were significantly more likely (OR = 5.00; 95% CI = 1.24-21.28; P = 0.02) to use ITN. Respondents < 30 years of age were more likely (OR = 3.13; 95% CI = 0.92-10.87; P = 0.07) to use ITN compared to those of ≥ 30 years, but the association was not statistically significant.

Tab. II. Respondents' ownership of mosquito nets by type.

Variable	Number (n)	Percentage (%)
Owned any mosquito net (N = 631)		
Yes	453	71.8
No	178	28.2
Number of mosquito nets owned (N = 453)		
1	340	75.1
2	105	23.2
3	8	1.8
Types owned* (N = 453)		
Window/door net(s)	387	85.4
Ordinary mosquito net(s)	114	25.2
(Untreated)	70	15.5
ITN(s)		

*Multiple response

Tab. III. Ownership, source and utilisation of ITN.

Variable	Number (n)	Percentage (%)
Owned ITN (N = 631)		
Yes	70	11.1
No	561	88.9
Number of ITN owned (N = 70)		
1	63	90.0
2	6	8.6
3	1	1.4
Source of ITN (N = 70)		
Bought	22	31.4
Given free	47	67.2
Both	1	1.4
Ever slept under ITN (N = 70)		
Yes	55	78.6
No	15	21.4
Slept under ITN last night (N = 55)		
Yes	26	49.1
No	29	50.9
Reasons for non-use last night (N = 29)		
Discomfort	5	17.2
Difficult to hang	7	24.2
Waiting for delivery	5	17.2
No reason	12	41.4
If no, last time slept under ITN (N = 29)		
≤ 7 days	5	17.2
8 – 29 days	4	13.8
≥ 30 days	20	69.0
Under-five children ever slept under ITN (N = 70)		
Yes	65	92.9
No	5	7.1
Under-five children slept under ITN last night (N = 65)		
Yes	28	43.1
No	37	56.9

Discussion

This study evaluated the ownership and utilisation of ITN among caregivers of under-five children and pregnant women in Ibarapa Central Local Government Area (LGA) of Oyo State.

Over three quarters of the respondents in the study were of the opinion that ITN was useful in malaria prevention and majority thought ITN was useful in preventing mosquito bites and killing mosquitoes. The observed ITN awareness level in the study is similar to that found in other parts of the continent [16, 17]. However, a Ugandan knowledge, attitude and practice survey reported a very low level of awareness [18]. The sources of ITN

Tab. IV. Association between socio-demographic characteristics and utilisation of ITN.

Respondents' socio-demographic characteristics and other factors	Utilisation of ITN Yes, n (%)	No, n (%)	X^2	p-value
Age group				
< 30 years	15 (60.0)	10 (40.0)	2.98	0.08
≥ 30 years	11 (36.7)	19 (63.3)		
Marital Status				
Never married	2 (50.0)	2 (50.0)	Fischer's Exact test = 1.00	
Ever married	24 (47.1)	27 (52.9)		
Family type				
Monogamous	18 (45.0)	22 (55.0)	0.30	0.58
Polygamous	8 (53.3)	7 (46.7)		
Level of education				
Below secondary (Low)	12 (75.0)	4 (25.0)	6.96	0.01
Secondary (high)	14 (35.9)	25 (64.1)		
Occupation				
Employed	21 (42.9)	28 (57.1)	Fischer's Exact test = 0.09	
Unemployed	5 (83.3)	1 (16.7)		
Average income				
< 10000	16 (59.3)	11 (40.7)	3.11	0.21
10000-19999	4 (33.3)	8 (66.7)		
≥ 20000	6 (37.5)	10 (62.5)		
No. of children				
< 3	11 (42.3)	15 (57.7)	4.49	0.49
≥ 3	15 (51.7)	14 (48.3)		
No. of under-fives				
< 2	14 (42.4)	19 (57.6)	0.78	0.38
≥ 2	12 (54.5)	10 (45.5)		
Knowledge of cause of malaria				
Poor	9 (32.1)	19 (67.9)	5.24	0.02
Good	17 (63.0)	10 (37.0)		
Knowledge of prevention of malaria				
Poor	15 (50.0)	15 (50.0)	0.20	0.66
Good	11 (44.0)	14 (56.0)		
Knowledge of ITN in malaria prevention				
Poor	2 (25.0)	6 (75.0)		
Good	24 (51.1)	23 (48.9)	Fischer's Exact test = 0.26	

information were health education sessions from health workers and radio programs similar results were obtained in Ethiopia [19]. Another study carried out in the central part of Nigeria by Blackburn et al. [20] identified traditional birth attendants as the major source of information about ITN. This may be because level of education in this part of the country is lower than that in the southern part of the country where our study was carried out.

Despite the high level of awareness documented in this study, ownership of ITN was extremely low. Among the few that possess ITNs, less than half of the mothers and children slept under the ITN the previous night. Reasons given for non-use include discomfort due to heat, difficulty in hanging up the nets and among the pregnant respondents some said they were waiting to deliver before they started using the nets as they felt it will be more beneficial to the newborn. Similar results were documented by Baume et al. [21] for Nigeria in his study of the use of bed nets in various parts of Africa and Oresanya et al. [3] in his study on utilisation of ITNs by under-five children in Nigeria.

This study reported that those with low education are significantly more likely to use ITN, suggesting that education does not necessarily translate to utilisation. This may be because of the perceived vulnerability which is higher among the poor or those with little or no education. Such may be compelled to use ITN because of the limited options left to them when the child gets sick. So it is likely that community with low income and education as documented in this study will sleep under an ITN once it is available to them. However, this finding did not support an Ethiopian study [22] where higher education was reported to be significantly associated with use of ITN. This may be due to the fact that the Ethiopian study was conducted in an urban area.

Reasons given for the use of ITN in the study include keeping mosquitoes and flies away, malaria prevention, to keep rodents away and for fishing. Other advantages mentioned include protection against other bugs and avoiding roof debris falling on the bed and provision of warmth during the cold season. Similar findings were documented in a study carried out in Ghana [17]. In the study, majority of the respondents said they would like to sleep under the ITN and that it was comfortable and there were no cultural limitations to its use. Respondents' perceived disadvantages of bed nets include dis-

Tab. V. Adjusted Odds Ratio of predictors of ITN utilization.			
Variables	Odds	95% CI	p-value
Age < 30 ≥ 30	3.13	0.92-10.87	0.07
Level of education Below secondary Secondary and above	5.00	1.24-21.28	0.02
Knowledge of cause of malaria Poor Good	0.35	0.10-1.17	0.09

comfort due to heat, chemical smell, and the need to mount the nets daily. Similar results were obtained by Blackburn [20] in a study carried out in the central part of Nigeria.

Less than a third of the respondents felt that ITNs were available. This may grossly affects utilisation of ITN. In a study conducted in selected malaria prone area in Ethiopia, utilisation of ITN was high because of free distribution of ITNs by the Ministry of Health to community members. The resultant mean utilisation rate of ITNs based on the history of sleeping under nets in the previous night was 81.6% [23].

Among respondents who had ITN, more than half were given free of charge at ANC and infant welfare clinics. This is higher than what was reported by Yared et al [22] who stated that only 3.8% of the respondents obtained their ITN free of charge in a study done in Western Ethiopia. Several other studies focusing on who uses the household net were intervention studies where nets were given free and it was documented that adults were slightly more likely than young children to use an ITN. Similarly, Baume et al, in a multi-country study, identified the health facility as the major source from where ITNs were obtained [21].

Conclusions

This study has generated information on ownership and utilisation of ITN in a rural community in Southwest Nigeria where ownership and utilisation were found to be very low among the respondents. There is need to improve on the knowledge of community members with regards to the importance of ITN in malaria prevention and enhance ownership and utilisation. This could be done through free ITN distribution to community members in order to achieve the MDG goals relating to malaria control especially among the vulnerable groups.

Acknowledgements

This study was funded by the National Primary Health Care Development Agency, Abuja, Nigeria. The authors extend their appreciation to community members in Igboora, Ibarapa Central Local Government Area of Oyo state, Nigeria who participated in this study. The supports of Drs. Pelumi Adebiyi and Eme Owoaje of the Department of Preventive Medicine and Primary Care, as well as Professor O. Omotade of Institute of Child Health, University of Ibadan, Nigeria are deeply appreciated.

References

[1] Breman JG, Egan A, GT K. *The intolerable burden of malaria: a new look at the numbers*. Am J Trop Med Hyg 2001;64(Suppl. iv-vii);1-2.

[2] Prothero RM. *Migration and malaria risk*. Health Risk Soc 2001;3:19-38.

[3] Oresanya BO, Hoshen M, Sofola TO. *Utilisation of insecticide-treated nets by under-five in Nigeria: Assessing progress towards the Abuja targets*. Malar J 2008;7:145.

[4] The Global Malaria Action Plan [Accessed March 2, 2014]; The Roll Back Malaria Partnership. 2008 Available at: http://www.rbm.who.int/gmap/gmap.pdf.

[5] World Health Organization. *Roll Back Malaria: Global Strategic Plan, 2005-2015.* Geneva: Roll Back Malaria Partnership, 2005.

[6] Pettifor A, Tailor E, Nku D, et al. *Bed net ownership, use and perceptions among women seeking antenatal care in Kinshasa, Democratic Republic of the Congo (DRC): Opportunities for improved maternal and child health*. BMC Public Health 2008;8:331.

[7] Schellenberg JR, Abdulla S, Nathan R, et al. *Effect of large-scale social marketing of insecticide-treated nets on child survival in rural Tanzania.* Lancet 2001;357:1241-7.

[8] Binka FN, Indome F, Smith T. *Impact of spatial distribution of permethrin-impregnated bed nets on child mortality in rural northern Ghana*. Am J Trop Med Hyg 1998;59:80-5.

[9] Alonso PL, Lindsay SW, Armstrong JRM, et al. *The effect of insecticide-treated bed nets on mortality of Gambian children*. Lancet 1991;337:1499-502.

[10] Abdulla S, Schellenberg JA, Nathan R, et al. *Impact on malaria morbidity of a programme supplying insecticides nets in children aged under 2 years in Tanzania: community cross-sectional study.* BMJ 2001;322:270-3.

[11] Minja H, Schellenberg JA, Mukasa O, et al. *Introducing insecticide-treated nets in the Kilombero Valley, Tanzania: the relevance of local knowledge and practice for an Information, Education and Communication (IEC) campaign.* Trop Med Int Health 2008;6:614-23.

[12] Wiseman V, Scott A, McElroy B, et al. *Determinants of bed net use in the Gambia: Implication for malaria control.* Am J Trop Med Hyg 2007;76:830-6.

[13] UNICEF, WHO. *African Malaria Report 2003*.

[14] Mugisha F, Arinaitwe J. *Sleeping arrangements and mosquito net use among under-fives: results from Uganda Demographic and Health Survey.* Malar J 2003;2:40.

[15] NetMark Survey on Insecticide-Treated Nets (ITNs) 2000, Nigeria. 2004.

[16] Tyagi P, Roy A, Malhotra MS. *Knowledge awareness and practices towards malaria in communities of Rural, semi-rural and bordering areas of east Delhi, India*. J Vector Borne Dis 2005;42:30-5.

[17] Adongo PB, Kirkwood B, Kendall C. *How local community knowledge about malaria affects insecticide treated net use in northern Ghana*. Trop Med Int Health 2005;10:366-78.

[18] Adera TD. *Beliefs and traditional treatment of malaria in Kishe settlement area, South West Ethiopia.* Ethiopian Medical Journal 2003;41:25-34.

[19] Legesse Y, Tegegn A, Belachew T, et al. *Knowledge, Attitude and Practice about Malaria Transmission and Its Preventive Measures among Households in Urban Areas of Assosa Zone, Western Ethiopia.* Ethiop J Health Dev 2007;21:157-65.

[20] Blackburn BG, Eigege A, Gotau H, et al. *Successful integration of insecticide-treated bed net distribution with mass drug administration in central Nigeria*. Am J Trop Med Hyg 2006;75:650-5.

[21] Baume CA, Marin MC. *Intra-household mosquito net use in Ethiopia, Ghana, Mali, Nigeria, Senegal, and Zambia: are nets being used? who in the household uses them?* Am J Trop Med Hyg 2007;77:963-71.

[22] Yared L, Ayalew T, Tefera BTK. *Knowledge, attitude and practice about malaria transmission and its preventive measures among households in urban areas of Assosa Zone, Western Ethiopia.* Ethiop J Health Dev 2007; 21(2).

[23] Animut A, Gebre-Michael T, Medhin G, et al. *Assessment of distribution, knowledge and utilisation of insecticide treated nets in selected malaria prone areas of Ethiopia*. Ethiop J Health Dev 2008;22(3).

■ Received on March 15, 2013. Accepted on May 6, 2014.

■ Correspondence: O.O. Akinyemi, Department of Health Policy and Management, Faculty of Public Health, College of Medicine, University of Ibadan, Ibadan, Nigeria - Tel. +234 803 502 0136 - E-mail: seunakinyemi@hotmail.com

ORIGINAL ARTICLE

Hand hygiene behavior among urban slum children and their care takers in Odisha, India

S. PATI, S.S. KADAM, A.S. CHAUHAN
Indian Institute of Public Health, Bhubaneswar, Public Health Foundation of India

Key words

Hygiene • Hand washing • Slum • Knowledge • Attitude

Summary

Objective. *To study the knowledge and practice of hand washing among mothers and children of shikharchandi slum of Bhubaneswar, Odisha and to recommend possible measures to improve the current practices.*
Methodology. *Present cross-sectional study was carried out in the Shikharchandi slum located in the Bhubaneswar city of Orissa state in India. 150 women and 80 children were interviewed. Children questionnaire were prepared to suit to their age and according to local context. Components of sanitation like food handling and hand washing were covered in this questionnaire.*
Results. *Hand washing before preparing food is being practiced by 85% of women. Of all women interviewed, 77% wash hands before serving food. Only 15% children said soap was available in their school to wash hands. Out of total children interviewed, 76% told that their teachers tell about sanitation and hand washing in the class. Only 5% children told they were consulted by doctor/health worker during last 3 months. As many as 81% children told that they wash their hands before taking food and 19% children said they take their food without washing hands. Though most of the children told that they wash hands before taking food, but only 17.5% told that they use soap for hand washing. Only 29% children told that their teachers check hand washing in school. When asked about critical timing of hand washing, 44% children told about at least two critical timings and 56% were unaware about the critical timings of hand washing.*
Conclusion. *Inadequate knowledge on this among our study participant is a point of concern. Systematic integration of health and hygiene education in schools through curricular modifications could be an appropriate strategy.*

Introduction

Communicable diseases continue to be the major contributor to global morbidity and mortality [1]. Sixty two percent and 31 % of all deaths in Africa and south-Asia, respectively are due to infectious diseases [2]. According to WHO estimates, 3.8 million children aged less than five die each year from diarrhea and acute respiratory tract infections [3]. An estimated 88 percent of diarrheal deaths worldwide are attributable to unsafe water, inadequate sanitation and poor hygiene [4]. Clean water and hand-washing are viewed as the most cost effective intervention for preventing diarrheal diseases [5]. Various studies have highlighted that simple act of hand-washing and basic hygiene behavior could prevent diarrhea, acute respiratory infection and skin infections [6,7]. Despite much evidence supporting the effectiveness of personal hygiene behavior, they are yet to be practiced widely. It is observed that young children and their mothers in developing countries fail to wash their hand adequately after fecal contact [8]. Magnitude of the problem is more in urban slums with reduced access to safe water and sanitation.
Children from poorest urban are three times more likely to die before the age of five than children from wealthiest urban and rural areas [9]. A study conducted in Mumbai slum shows that 30% of all morbidity can be accounted for by water related infection [10]. Understanding usual hand-washing is an important baseline assessment for any programme intended to improve sanitation, hand hygiene and water quality. However, there are limited data that have assessed the hand hygiene behavior of children and their mothers particularly in slums.
Keeping this in view the present study was taken up to understand the knowledge, attitude and practices relating to hand-washing of urban slum children and their mothers. The objective of the study was to access hand-washing behavior among the participants so as to identify and overcome barriers to proper hand hygiene practices.

Methodology

The present cross-sectional study was carried out in the Shikharchandi slum located in the Bhubaneswar city of Orissa state in India. Sikharchandi slum is authorized and the largest slum of the city with 1,500 hundred household and total population of around 6,000. It was decided to take 10% of all households for the study purpose. Thus a total of 150 households were selected. This slum is divided into three clusters. There are total 600 households each in cluster one and cluster three and 300 households in cluster two. Stratified random sampling was carried out to select the households

Tab. I. Demographic Characteristics of participant (Mother).

S. No.	Characteristics	Response	Number	Percentage (%)
11	BPL Cards	Yes	54	36
		No	96	64
22	Education	Literate	80	53
		Illiterate	70	47
43	House	Rent	53	35
		own	97	65
54	Household condition	Kutcha	83	55
		Pakka	67	45
65	Rooms in house	Less than 2	45	30
		Two	74	49
		More than two	31	21
76	Caste	SC	36	24
		ST	10	7
		OBC	72	48
		Other	32	21
87	Language	Oriya	76	51
		Hindi	19	13
		Telugu	52	34
		Bengali	3	2
98	Native state	Orissa	94	63
		Andhra Pradesh	41	27
		West Bengal	13	9
		Other states	2	1
19	Size of family	Less than 5	91	61
		6 to 9	48	32
		10 and above	11	7
110	Income(in rupees/month)	Less than 5000	33	22
		5000 to 10000	86	57
		Above 10000	31	21
111	Occupation	Housewife	57	38
		Skilled*	14	9
		Unskilled**	79	53

from each of these three clusters proportionately. For the study 10% of households are selected from each cluster. Total selected houses were 60 households each from cluster one and three and 30 households from cluster two. 150 women and 80 children were interviewed. Children between age group of 6 to 12 were separately interviewed. Questionnaire was prepared by adopting the theme of core questionnaire on sanitation by WHO and EHP. Semi structured questionnaire was developed which was suitable to local context. Children questionnaire were prepared to suit to their age and according to local context. Components of sanitation like food handling and hand washing were covered in this questionnaire. The questionnaire was pretested in non study area and necessary changes were made accordingly. Data was entered in MS Excel and analyzed using statistical software SPSS Version 17.0. Verbal consent was taken before interview of mothers and they were well informed about purpose of the study and confidentiality. Verbal consent of parents was taken prior to interview of children.

Results

A total of 150 participants were selected for study, out of which 36% were having the BPL card. Mean age of women participant was 31 years and the range was from 17 to 55 years. Participants were comprised of all castes, 24% of them belong to SC, 7% were from ST community, 48% belongs to OBC and 21% belongs to other caste. Main languages spoken in the community were Oriya, Telugu, Hindi and Bengali. Out of all households interviewed, 55% live in kaccha house and 45% live in pakka house; 30% families lived in single room, 49% living in two room house and 21% lived in more than two room house; 63% were from Orissa and 37% were migrated from neighboring states like 27% were migrated from Andhra Pradesh, 9% were migrated from West Bengal and 2% are migrated from Bihar. Participants are grouped as housewives and working women. Working women were either skilled or unskilled profession. Pre-primary teacher, ASHA, tailor were labeled as skilled workers. Those who are working as daily laborer, maid servant, sari seller, vegetable seller and rag pickers were grouped as unskilled workers. Among participants

Tab. II. Hand-washing Practices among women.

S. No	Characteristics	Options	Numbers (%)
11	Hand washing before preparing food	Yes No	128 (85) 22 (15)
22	Hand washing before serving food	Yes No	116(17) 34(23)
33	Hand wash with soap after toilet	Yes No	108(72) 42(28)
44	Use of slipper	Yes No	93(62) 57(38)
55	Hand washing with only water is as good as hand washing with water and soap	Yes No	7(4) 143(96)

38% are housewives, 9% were skilled workers and 53% were unskilled workers. Twenty two percent households have income less than 5,000 rupees per month, 57% households earn 5,000 to 10,000 rupees per month and remaining 21% households have monthly income more than 10,000 rupees (Tab. I).

Hand washing before preparing food is being practiced by 85% of women. Still 15% reports that they were not practicing hand washing. Of all women interviewed, 77% wash hands before serving food. When asked about who serves, 43% reported that mother serves the food while in 42% families children take food themselves and remaining 15% told other members like grandmother, sister or aunt serves the food (Tab. II). When asked about availability of soap in school, 15% children said soap was available in their school to wash hands but for 85% students soap was not available in school to wash hands. Out of total children interviewed, 76% told that their teachers tell about sanitation and hand washing in the class while 24% told that their teacher doesn't tell about sanitation and hand washing. Only 5% children told they were consulted by doctor/health worker during last 3 months. As many as 81% children told that they wash their hands before taking snacks in school and 19% children said they take their snacks without washing hands. Though most of the children told that they wash hands before taking snacks in school, but only 17.5% told that they use soap for hand washing. Only 29% children told that their teachers check hand washing in school. When asked about critical timing of hand washing, 44% children told about at least two critical timings and 56% were unaware about the critical timings of hand washing.

Discussion

In this study of urban slum mothers we assessed the knowledge attitude and practices of hand hygiene. Of the mother surveyed, seventy two percent were found to practice hand washing by soap after defecation. This is lower than the WHO study where they found this was practiced by 84% women. The lower level could be due to non availability of soap and decreased perceived susceptibility to diarrhea. Although, 96% of the women were of the opinion that hand

Tab. III. Hand-washing Practices among children.

S. No.	Characteristics	Options	Number (%)
1.1	Soap is available in school to wash hands.	Yes No	12 (15) 68 (85)
2.2	Teacher tells about sanitation and hand washing in class.	Yes No	61 (76) 19 (24)
3.3	Visited by health worker/Doctor in school during last 3 months.	Yes No	4 (5) 76 (95)
64	Hand washing before taking food.	Yes No	65 (81) 15 (19)
75	Use soap for hand washing before taking food.	Yes No	14 (17.5) 66 (82.5)
86	Teacher checks hand washing in school.	Yes No	23 (29) 57 (71)
97	Who enforce to wash hands.	Mother Sister Other family members teacher	47(59) 20 (25) 18 (22) 23 (29)
18	Tells at least 2 critical time of hand washing.	Yes No	35 (44) 45 (56)
19	Hand washing with soap after toilet.	Yes No	49 (61) 31 (39)

washing with water and soap is better compared to simple hand washing, it is not reflected in their practice. This could be explained by the fact that women are not able to link infections like diarrhea directly with their own hand washing behavior. Limited knowledge appears not to be constraint in this case. However, the translation of knowledge into sustainable behavior needs to be reinforced. Behavioral Intervention aimed to improve hand hygiene practices should focus on this important issue should be taken up in order to improve the hand hygiene practices of the respondents. Earlier studies by Ray et al. have also highlighted similar findings [11]. In our study area 85% of the mother use hand washing before preparation of food, which encouraging. This differs from the study by Ray *SK* in two communities of eastern India where hand washing was not practiced before "preparing food" and after handling "raw vegetables" [12]. Another encouraging finding of the present study was 77 % women practicing hand washing before serving food. These behaviors need to be reinforced for preventing Fecal-Oral transmission of infectious agent. Use of slippers was found to be practiced by 62% of the mothers, which could be taken as satisfactory, considering their socio-economic condition.

Our study additionally explored the knowledge, attitude and practices of hygiene among school children (6-12 years) from the same households. We attempted to find the contextual factors contributing to hand washing practices of the children. It included both school and family level influencers. As many as 81% children practiced hand washing before taking food out of which only 17.5% use soap for hand washing. Sixty one percent children used soap for hand washing after toilet. This could be due to Non or limited availability of soap in both school and household. In a similar study on hand washing among school children in Colombia, it was observed that only 33.6% of children were washing hands with soap before eating and after defecation [13]. Our participants have better hygiene practice which could be attributed to increased awareness. There appears to be low supervision by the teachers when compared to mothers for enforcing hand washing. This might lead to decreased motivation among the students for regular hand washing. Educating teachers to inculcate hygiene behavior among the students is of prime concern. Future school based hand hygiene interventions should take this into account. Health educators (physician, nurse, health worker) play an important role in this regard. Bearing in mind that, the school has been recognized as a vital setting for health promotion, our findings display a strong deficit of such initiatives. When asked about at least two critical times of hand washing only 44% of surveyed students could answer correctly. In a KAP study of hygiene in Ethiopia found that 52% of the students have adequate knowledge of proper hygiene, which is higher than the present study [14]. Critical times of hand washing are crucial in breaking the chain of fecal oral contamination, a major cause of diarrheal diseases. Inadequate knowledge on this among our study participant is a point of concern. Systematic integration of health and hygiene education in schools through curricular modifications could be an appropriate strategy.

Due to the restricted time period and resource constraint, the study was conducted only in one slum and it cannot represent the entire situation of the other slums of the Bhubaneswar and that of entire state. So the results of this study cannot be generalized to the other slums. So it is suggested that more similar studies should taken up to assess sanitation status of slum areas in future.

References

[1] World Health Organization. *Better Health for Poor Children*. [Accessed August 4, 2009]. Available at: http://www.who.int/child_adolescent_health/documents/a91061/en/index.html

[2] Curtis VA, Danquah LO, Aunger RV. *Planned, motivated and habitual hygiene behavior: an eleven country review*. Health Educ Res 2009;4:655-73.

[3] World Health Organization. *World Health Statistics. 2009*.

[4] Murray CJ, Lopez AD. *Global mortality, disability, and the contribution of risk factors: Global Burden of Disease Study*. Lancet 1997;349:1436-42.

[5] Jamison DT, Breman JG, Measham AR, et al. (eds). *Disease Control Priorities in Developing Countries,* 2nd ed. Oxford: Oxford University Press, 2006.

[6] Luby SP, Agboatwalla M, Feikin DR, et al. *Effect of handwashing on child health: A randomized controlled trial*. Lancet 2005;366:225-33

[7] Shahid NS, Greenough WB, Samadi AR, et al. *Handwashing with soap reduces Diarrhoea and spread of bacterial pathogen in a Bangladesh village*. J Diarrhoeal Dis Res 1996;14:85-9.

[8] LiKosek M, Bern C, Guerrant RL. *The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000.* Bulletin of the World Health Organization 2003;81:197-204.

[9] World health organization/UNICEF (2009), *Diarrhea why children are still dying and what can be done.*

[10] Kumar S, Harada H. *Field survey on water supply, sanitation and associated health impacts in urban poor communities, a case from Mumbai city, India*. Water Sci Technol 2002;46:269-75.

[11] Ray SK, Dobe M, Maji S, et al. *A pilot survey on hand washing among some communities of West Bengal*. Indian J Pub Health 2006;50:227-30.

[12] Ray SK, Zaman FA, Laskar BN. *Hand washing practices in two communities of two states of eastern India: An Interventional Study*. Indian J Pub Health 2010;54:126-30.

[13] Lopez-Quintero, Freeman P, Neumark Y. *Hand washing among school children in Bogotá, Colombia*. Am J Public Health 2009;99:94-101.

[14] Vivas AP, Gelaye B, Aboset N, et al. *Knowledge, Attitudes and Practices (KAP) of hygiene among school children in Angolela, Ethiopia*. J Prev Med Hyg 2010;51:73-9.

■ Received on August 24, 2013. Accepted on May 13, 2014.

■ Correspondence: Sanghamitra Pati, Indian Institute of Public Health, Bhubaneswar, Public Health Foundation of India - Tel. +91-8010 335 915 - E-mail: sanghamitra.pati@iiphb.org

INSTRUCTIONS TO AUTHORS

Authors submitting papers for publication should adhere to the format described below. Failure to do so may result in the return of the paper for revision before assessment, with inevitable delay in publication.

General
The *Journal of Preventive Medicine and Hygiene* is published on a three-monthly basis and covers the field of epidemiology and community health. The Journal publishes original papers and proceedings of Symposia and/or Conferences which should be submitted in English with the exception of other languages. Papers are accepted on their originality and general interest. Ethical considerations will be taken into account.

Submission of manuscripts
Papers submitted for publication should be sent by E-mail as attachment to: jpmh@jpmh.org

A letter signed by every Author must accompany the manuscript which should include a statement that the submitted material has not been previously published, and is not under consideration (as a whole or partly) elsewhere, that its content corresponds to the regulations currently in force regarding ethics research and that copyright is transferred to the Publisher in case of publication. If an experiment on humans is described, a statement must be included that the work (also to be included in the methods section of the paper) was performed in accordance to the principles of the Declaration of Helsinki (1975, rev. 2000), and Authors must state that they have obtained the informed consent of patients for their participation in the experiments and for the reproduction of photographs. As regards the studies performed on laboratory animals, Authors must state that the relevant national laws or institutional guidelines have been observed. The Authors are solely responsible for the statements made in their article.
Authors may suggest up to two potential referees, (including complete addresses, fax number and e-mail addresses) expert in the specific fields.

Conflict of Interests
In the letter accompanying the article, Authors must declare if they got funds, or other forms of personal or institutional financing – or even if they are under contract – from Companies whose products are mentioned in the article. This declaration will be treated by the Editor as confidential, and will not be sent to the referees. Accepted works will be published accompanied by a suitable declaration, stating the source and nature of the financing.

Format for original articles

The article should be saved in .RTF format.
Do not use, under any circumstances, graphical layout programmes such as Publisher™, Pagemaker™, Quark X-press™, Adobe Indesign™. Do not format the text in any way (avoid styles, borders, shading …); use only character styles such as italics, bold, underlined.
Do not send the text in PDF.
The text should not exceed 4,000 words including the abstract (maximum 250 words), references, figures and tables. Papers will not be returned, so a copy must be retained by the author.

Title page
The *first page* should contain the title of the paper; a short running head; a set of key words (maximum three); the names of all the authors; the authors' institutions (only one affiliation per author); the category under which the authors intend the work to be published; the name and mailing address of the author to which correspondence should be sent, and a telephone and fax number for the corresponding author.

Abstract and the remainder of the paper
The *second page* should contain the abstract which must be structured as follows: Introduction, Methods, Results and Discussion.
The rest of the paper should be laid out along standard lines:
Introduction, Methods, Results, Discussion, Conclusions, References, Figures and Tables.

Tables must be limited in number (the same data should not be presented twice, in both text and tables), typewritten one to a page, and numbered consecutively with Roman numbers.

Illustrations
Send pictures in separate files from text and tables.
Software and format: preferably send images in .TIFF or .EPS format, resolution at least 300 dpi (100 x 150 mm). Other possible formats: .JPEG, .PDF. If possibile avoid .PPT (Powerpoint files) and .DOC (images included in .DOC files). Insert an extension that identifies the file format (example: .Tif; .Eps).

References
References must be limited to the most essential and relevant, identified in the text by Arabic numbers in square brackets and listed at the end of the manuscript in the order in which they are cited. The format of the references in the bibliography section should conform with the examples provided in N Engl J Med 1997;336:309-15. The first three authors must be indicated, followed by et al. Journals should be cited according to the abbreviations reported on Pubmed. Examples of the correct format for bibliographic citations:

The following styles are used:

JOURNAL ARTICLES:
Cellesi C, Sansoni A, Casini S, et al. *Chlamydia pneumoniae antibodies and angiographically demonstrated coronary artery disease in a sample population from Italy.* Atherosclerosis 1999;145:81-5.
Gasparini R, Pozzi T, Fragapane E. *Immunity to diphtheria in Siena.* Epidemiol Infect 1997;119:203-8.

BOOKS:
Smith DW. *Recognizable patterns of human malformation.* Third Edition. Philadelphia: WB Saunders Co. 1982.

CHAPTERS FROM BOOKS OR MATERIAL FROM CONFERENCE PROCEEDINGS:
Krmpotic-Nemanic J, Kostovis I, et al. *Aging changes of the form and infrastructure of the external nose and its importance in rhinoplasty.* In: Conly J, Dickinson JT, eds. *Plastic and Reconstructive Surgery of the face and Neck.* New York, NY: Grune and Stratton 1972, pp. 84-95.

Do not use "*et al.*" unless there are more than three authors, in which case list the first three.

Drugs
Drugs should be referred to by their chemical name; the commercial name should be used only when absolutely unavoidable (capitalizing the first letter of the product name and giving the name of the pharmaceutical firm manufacturing the drug, town and country).

Units
All units must be in International System of Units (SI) except blood pressure values, which continue to be reported as mmHg.

Statistics
Standard deviations and standard errors are given in parentheses after the values they qualify; & is not used. In statistical evaluation, it is desirable to quote 95% confidence intervals. A full description of the statistical methods used must be provided in the methods section.

Abbreviations
On the whole the Journal does not use abbreviations. There are very few exceptions so if in doubt always give the names or terms in full.

Spelling
English spelling is used.

Specific instructions for the various categories of papers
- **Editorials** (written on the invitation of The Editor or a member of the Editorial Board) should be written in English; no abstract is required.
- **Reviews** may be submitted for consideration by authors, or may be written on the invitation of The Editor. The text should not exceed 4,000 words including the references, tables and figures. No abstract is necessary.
- **Original articles** represent reports of new and original work, or descriptions of a consolidated body of experience (even if not entirely original) in a given field.

Proofs
The Authors are required to correct and return (within 3 days of their being sent) the first set of galley proofs of their paper. If proofs are not returned within a reasonable period it will be assumed that there are no corrections and the paper will be considered approved.

Reprints
Request of reprints and all other correspondence should be sent to: *Journal of Preventive Medicine and Hygiene*, c/o Pacini Editore S.p.A., Via Gherardesca 1, 56121 Pisa, Italy. Tel: +39 050 3130285; Fax +39 050 3130300.

Published online October 2014.

Journal registered at "Registro pubblico degli Operatori della Comunicazione" (Pacini Editore SpA registration n. 6269 - August 29, 2001).

CONTENTS