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CONTENTS OF THE VOLUME

- i. Copyright Notice
 - ii. Editorial Board Members
 - iii. Chief Author and Dean
 - iv. Table of Contents
 - v. From the Chief Editor's Desk
 - vi. Research and Review Papers
-
1. A Novel Elicitor (PiPE) from *Phytophthora Infestans* Induces Active Oxygen Species and the Hypersensitive Response in Potato. **1-14**
 2. Amphibia, Anura, Microhylidae, Gastrophryninae, *Elachistocleis Bicolor* Guérin Méneville, 1838: Distribution Extension and Geographic Distribution Map. **15-18**
 3. Tongue-Tie in Children; Mothers Perceptions, Attitudes and Practices in Port Harcourt, Nigeria. **19-23**
 4. Romania and the Crisis in the Health System. Migration of Doctors. **25-32**
 5. Objectivity, Reliability, and Validity of the 90° Push-Ups Test Protocol Among Male and Female Students. **33-37**
 6. Posterior Urethral Valve Presentation, Management and Outcome. **39-40**
 7. Cross Section Study of Malnutrition in Children of 1-10 Years Age Group in Urban Slums of Aligarh. **41-44**
 8. Managements of Exposure to Blood and Body Fluids Among Healthcare Workers and Medical Students in University of Gondar Hospital, Northwest of Ethiopia. **45-52**
-
- vii. Auxiliary Memberships
 - viii. Process of Submission of Research Paper
 - ix. Preferred Author Guidelines
 - x. Index



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A Novel Elicitor (PiPE) from Phytophthora Infestans Induces Active Oxygen Species and the Hypersensitive Response in Potato

By Naotaka Furuichi, Kazutoshi Yokokawa, Hisakazu Okamura
& Masahiro Ohta

Niigata University,

Abstract - A novel elicitor (PiPE) from the oomycete *Phytophthora infestans* (Pi) stimulates the hypersensitive response (HR) in potato. The PiPE, purified by anion-exchange chromatography from a water-soluble extract of Pi caused cell death, characteristic of HR, and enhanced active oxygen species (AOS) generation in tuber tissues. The partial amino acid sequence, and the sequence of the PiPE cDNA derived by PCR had homologous domain to fructose 1,6 biphosphate aldolase (FBA) genes. To demonstrate that the PiPE cDNA encodes an active elicitor, we expressed PiPE in *Echerichia coli*, high five insect cells and purified the recombinant protein.

His-PiPE induced HR, browning and generation of AOS in potato tissues. The PiPE was produced in the germination fluid from Pi and was existing in the cell wall of Pi. The role of PiPE peptides in the induction of HR in an incompatible interaction between Pi and potato cells is a prerequisite for the AOS and HR induction.

Keywords : *hypersensitive cell death, PiPE elicitor, potato, Phytophthora infestans, signal transduction of HR, fructose 1, 6-bisphosphate aldolase.*

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A NOVEL ELICITOR PIPE FROM PHYTOPHTHORA INFESTANS INDUCES ACTIVE OXYGEN SPECIES AND THE HYPERSENSITIVE RESPONSE IN POTATO

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A Novel Elicitor (PiPE) from *Phytophthora infestans* Induces Active Oxygen Species and the Hypersensitive Response in Potato

Naotaka Furuichi^α, Kazutoshi Yokokawa^σ, Hisakazu Okamura^σ & Masahiro Ohta^σ

Abstract - A novel elicitor (PiPE) from the oomycete *Phytophthora infestans* (*Pi*) stimulates the hypersensitive response (HR) in potato. The PiPE, purified by anion-exchange chromatography from a water-soluble extract of *Pi* caused cell death, characteristic of HR, and enhanced active oxygen species (AOS) generation in tuber tissues. The partial amino acid sequence, and the sequence of the PiPE cDNA derived by PCR had homologous domain to fructose 1,6 biphosphate aldolase (FBA) genes. To demonstrate that the PiPE cDNA encodes an active elicitor, we expressed PiPE in *Escherichia coli*, high five insect cells and purified the recombinant protein.

His-PiPE induced HR, browning and generation of AOS in potato tissues. The PiPE was produced in the germination fluid from *Pi* and was existing in the cell wall of *Pi*. The role of PiPE peptides in the induction of HR in an incompatible interaction between *Pi* and potato cells is a prerequisite for the AOS and HR induction.

Keywords : hypersensitive cell death, PiPE elicitor, potato, *Phytophthora infestans*, signal transduction of HR, fructose 1, 6-bisphosphate aldolase.

1. INTRODUCTION

An incompatible interaction between plants and pathogens often leads to rapid and localized plant cell death, termed the hypersensitive response (HR), at the infection site. Induction of biochemical defense responses in the host cells (Ebel and Scheel, 1992, Furuichi, 1993, Xu and Heath, 1998) likely involves recognition events for both elicitor and suppressor molecules from the pathogen at the host plasma membrane (Ebel and Scheel, 1992, Furuichi and Tomiyama, 1980, Furuichi et al., 2008). Subsequently Ca^{2+} influx increases, pH decreases in the cytoplasm, and the cytosolic kinases are activated in the plant cells (Ebel and Scheel, 1992) (Furuichi et al., 2008) Furuichi et al., 1997) (Hamel et al., 2011). Electrolyte leakage contributing to host cell death (Goodman, 1968, Pavlovkin and Novacky, 1986, Tomiyama et al., 1983), resulting from the activation of a K^{+} efflux across the plasma membrane (Tomiyama et al., 1968, Tomiyama et al., 1983, Atkinson et al., 1985, Baker et al., 1987, Tomiyama and Okamoto, 1989). Active oxygen species (AOS) also are generated rapidly at the plasma membrane of host cells during the incompatible interaction (Doke, 1983, Doke and Miura, 1995). Many

of these responses associated with HR are duplicated by treatment of tissues with factors derived from pathogenic pathogens termed elicitors (Keen, 1975, Scheel et al., 1999). Elicitors from the oomycete pathogens, *Phytophthora* include 1,3- and 1,6-β-D-glucans (Ayers et al., 1976, Sharp et al., 1984), glycoproteins (Keenan et al., 1985, Parker et al., 1988), and arachidonic acid (Bostock et al., 1981). A family of extracellular proteins produced by *Phytophthora* species, termed elicitors, also induces defense responses in plant cells (Ricci et al., 1989). Elicitors are highly conserved, 10-kDa proteins that are produced by several *Phytophthora* and *Pythium* spp. (Kamoun et al., 1994, Pernollet et al., 1993, Ricci et al., 1989). However, a 13-mer oligopeptide within a 42 kDa glycoprotein secreted by *Phytophthora megasperma*, also caused ion fluxes across the plasma membrane, the oxidative burst and phytoalexin biosynthesis in parsley cells (Nurnberger et al., 1994, Sacks et al., 1995). From the findings, it was suggested in the present report that we isolated PiPE elicitor from the fungal cell wall fractions in the *Phytophthora*. We described previously the elicitor activity of a hyphal cell wall preparation (HW), from *Pi* (Furuichi and Suzuki, 1989). This HW has a protein content of about 22% (Ikeda and Furuichi, 1993). Treatment of the HW with pronase or trichloroacetic acid prior to its addition to potato tuber tissues reduced elicitor activity in a dose-dependent manner (Ikeda and Furuichi, 1993). Purification of the activity that induced HR and phytoalexin accumulation in the potato cells, correlated with proteinaceous materials. Response to a monoclonal Abs (Abs), selected from mice hybridoma immunized with the HW of *Pi*, was retained by the purified protein (Ikeda and Furuichi, 1993).

In this study, we have isolated the hyphal cell wall proteins and found that active elicitor activity correlated with a PiPE that also reacted with the anti-PiPE Abs. After determining a partial amino acid sequence of the purified protein(s); we used RT-PCR to generate a sequence corresponding to a gene encoding the putative elicitor peptide. We used a His-tagged expression system with the PiPE gene to generate a fusion protein that demonstrated elicitor activity. Using the cDNA sequence for the elicitor clone, we examined for the presence of homologue sequences in other *Phytophthora* spp.

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The reported nucleotide sequence appears in the DDBJ/EMBL/GenBank databases under the accession number AB051573.

II. RESULTS

a) Purification of antigens that recognize the-PiPE monoclonal Abs

Elicitor activity and the level of extractable proteins from mycelia (race 0 of *Pi*) grown in liquid culture increased for three weeks. We used homogenates of 15-days cultures as the initial PiPE source. Fractionation of elicitor activity by anion exchange chromatography at pH 8.1 (Fig. 1A) with elution of a linear gradient of NaCl (0-0.5 M) resulted in seven protein peaks. The results of assaying each fraction for Abs-binding activity are illustrated in Figure 1B. The maximum Abs-binding activity was detected in fractions F17 to F20, eluted at 0.35 M NaCl.

b) Elicitor activity of the fractions recognized by anti-PiPE Abs

Treatment of tubers from the resistant cultivar Eniwa (*R₁*) with the elicitor preparation (containing proteins and carbohydrates) showed that it was more active than on tubers from the susceptible cv. Irish Cobbler (*r*-gene) (Fig. 3B). Thus the initial preparation possesses the same specificity as the *Pi* isolate from which the cell wall elicitor was derived. The elicitor activity in the concentrated samples of fractions F15 to F21 from anion exchange chromatography showed similar response being more active on cv. Eniwa than on cv. Irish Cobbler. The intensity of the symptoms of browning and cell death characteristic of HR was much higher in fractions F19 and F20 than in the other fractions. Fractions F17-F20 were pooled and the proteins separated by SDS-PAGE and silver-stained (Fig. 2B). Several peptide bands were detected in each fraction. The anti-PiPE Abs recognized one clear band of protein (47 kDa) in fractions F17 and F18, and three protein bands (47, 38, and 34 kDa) in fractions F19 and F20 (Fig. 2C). Because no elicitor activity was detected in F17 and F18, containing 47 kDa peptides, we concluded that elicitor activity resided with either or both of the 38 kDa and 34 kDa protein bands.

c) Elicitor activity of affinity-purified PiPE

Affinity-purified PiPE peptides by using the anti-PiPE-Abs from fractions F19 and F20 had higher elicitor activity on tuber tissue from the resistant potato cv. Eniwa than on the susceptible cv. Irish Cobbler. The affinity-purified proteins transiently enhanced the production of AOS in suspension cultures of both potato cultivars after 30 min returning to the control treatment level after 150 min. AOS generation was higher in cv. Rishiri than in cv. Mayqueen. The cv. Eniwa showed similar enhanced production of AOS in potato tuber disks, whilst cv. Irish Cobbler showed lesser enhancement of the AOS production.

d) The effect of His-Strboh1 for the activation of AOS in potato plasma membrane

To examine the effect of His-Strboh1 to AOS generation in potato cells, entire sequence of Strboh1 was expressed with the *E. coli* (BL21pLysS) containing a C-terminal 6His-tag. Affinity purification yielded approximately 110 kDa translation product and His-Strboh1 was confirmed by SDS-PAGE and immunoblot analysis by using His-Abs. The effect of His-Strboh1 on the activity of AOS generation was measured by using luciferase substrate (CLA) in potato microsomal fraction (cv Eniwa). Because it was reported that activated NADPH oxidase was localized in plasma membrane in mammalian cells, AOS measurements were performed with microsomal fraction prepared from potato tuber tissue. *E. coli* expressed His-Strboh1 (1.2μM) was treated to potato microsomal fraction and measured its chemiluminescence. In potato microsomal fraction treated His-Strboh1, AOS generation was transiently increased 5 to 10 min after the treatment, whereas treatment of Tiron (1, 2-dihydroxy-3, 5-benzenedisulfonic acid disodium salt) which was a scavenger of AOS inhibited the generation. Ten min after AOS generation was declined to the basal level in the microsomal fraction. These results suggested that His-Strboh1 expressed in *E.coli* was activated after the treatment to microsomal fraction.

Expression vector containing a N-terminal 6His-tag construct was employed for the production of His-Strboh1. Affinity purification yielded approximately 120 kDa product and the production of His-Strboh1 was confirmed by immunoblot analysis by using His-Abs. The effect of insect cells expressed His-Strboh1 on the generation of AOS was performed. The AOS generation was transiently increased up to 10 min after treatment. However, AOS generation was increased until 40 min, and the peak of AOS generation was 7 times higher than with the fusion from *E. coli*. These results suggested that glycosylation of His-Strboh1 had effect on the generation of AOS in membrane fraction of potato.

e) Immunochemical analysis of germination fluid using anti-PiPE Abs

Immunoblotting of the germination fluid from zoospores of *Pi* with anti-PiPE Abs, detected proteins of 47 and 38 kDa (Fig. 3D, E). We have reported that the germination fluid from race 0 caused a typical HR response and browning in the potato tuber disks of cv. Rishiri (Furuichi et al. 1979). Thus one protein of a size associated with elicitor activity from mycelial extracts, the 38 kDa, was detected in the germination fluids. We have tested the localization of PiPE antigens by using PiPE Abs and by using electron microscope of germinating zoospores (Fig. 3D). Figure 3D shows the germinative zoospores at five hour after the germination(x 14800). The results show PiPE existed on the surfes of the cell wall of *Pi* by using the rhodamine anti-PiPE Abs. (Fig.3Eb).

f) Peptide mapping of the PiPE isolated from HW and cloning of a partial cDNA fragment encoding PiPE

We had examined if the PiPE protein is a glycoprotein or not, by using SDS-PAGE and the stain by periodic acid-Schiff's reagent. Digestion of the purified PiPE with *Staphylococcus aureus* V8 protease, to generate peptides for amino acid sequencing, generated two major bands. A sequence of 20 amino acids determined from the N-terminus of the native peptide was identical to the sequence of one of the V8 protease-digested fragments (Table 1).

We designed oligonucleotides based on the N-terminus amino acid sequence (Table1) as PCR primers. We derived cDNA from RNA extracted from a freshly harvested mycelium (for 10 days) of *Pi* race 0 and used this material as template in PCR. A 674-bp PCR product was obtained, cloned and sequenced (Fig. 4A).

Searches using the Blast program (Stephen et al. 1997) revealed significant similarity to fructose 1, 6 bisphosphate aldolase (FBA) genes, with the highest similarity to yeast FBA (Fig. 4B). A database survey of Blast searches against *Pi* ESTs in DDBJ revealed a full length EST of 674 bp to correspond to the cDNA sequence. Our finding that a protein with homology to a FBA produced by *Pi* has elicitor activity is novel because the PiPE is secreted by the germinating spores also and is detected in extracts from the mycelia.

g) Southern blot analysis of the PiPE

Southern blot hybridization was used to determine the number of copies of the cloned PiPE gene sequences in the genomic DNA of *Pi*, races 0 and 1. Using a probe containing a 674-bp fragment of the PiPE open reading frame from *Pi*, four *Pst*I fragments and five *Hind*III fragments from the *Pi* race and two *Pst*I fragments and three *Hind*III fragments, four *Pst*I fragments and five *Hind*III fragments were detected in the race of *Pi*, and two *Pst*I fragments and three *Hind*III fragments were detected in *Pi*831 (Fig. 5) were detected from *Pi*831 (Fig. 5). Because there is one *Pst*I site and no *Hind*III site in the cloned partial cDNA (674 bp) coding the PiPE (Fig. 4B), at least two copies of the PiPE gene occur in the *Pi* genome.

h) Elicitor activity and generation of AOS in functional analysis of the tagged PiPE

We generated a 6His-PiPE to demonstrate that the protein produced from the gene had elicitor activity. A His-PiPE band (27.5 kDa) was observed after SDS-PAGE and CBB-staining (Fig. 6A). A protein band of this size, was not obtained when the His- control from the plasmid vector without insert cDNA was analyzed (Fig. 6A, lane 1). The purified His-PiPE was recognized by both the anti-His Abs and anti-PiPE-monoclonal Abs (Fig. 6B).

The His-PiPE had stronger elicitor activity on Eniwa (*R₁*-gene) tuber cells than on Irish Cobbler

(*r*-gene) suspension cultured cells responded with a stronger oxidative burst than those of cv. Irish Cobbler after exposure to the His-PiPE.

i) Presence of PiPE genes in different Phytophthora species

To investigate whether sequences encoding PiPE are present in other *Phytophthora* isolates, RT-PCR was performed. The primers indicated in Table 1 was used with cDNA derived from total RNA from freshly harvested mycelium of *Pi*, DN101 (race 0) and E003 (race 0), *Pi*831 and St401 (race 1); *P. megasperma*; *P. nicotianae*; *P. cryptogea*; and *P. capsici* as templates. PCR products of 674-bp were generated from all tested samples (Fig. 8A). These RT-PCR products all hybridized with the probe from race 0 (Fig. 7B).

III. DISCUSSION

a) Immunochemical analysis of the localization of PiPE Abs in potato cells

Based on this similarity, we assume that fructose 1,6-bisphosphate aldolase peptides (FBA) may exist in the cell wall, and may be an elicitor of *Pi* against potato cells. Though FBA may exist in the cytosol of *Pi*, it could also be expressed and translocated into the cell wall of the germinating tube during the infection process. Secretion peptide from FBA was produced after germination of *Pi* spores. Aldolases are known to be glycoproteins.

We propose a model for the elicitation of HR mediated by recognition of the PiPE by the StCDPK (a Ca^{2+} dependent protein kinase) in the potato cell plasma membrane (Furuichi et al., 1997). It was suggested that the PiPE binds to a 6H-StCDPK2 from a resistant cv. Rishiri (*R₁*), based on an ELISA assay using a monoclonal Abs of PiPE. It remains to be clarified what domain of the PiPE interacts with the CDPK of the host cells.

The isolated PiPE is showing the activity for AOS generation and tissue browning; it induced HR and AOS generation in cv. Rishiri, a resistant potato (*R₁*-gene), but induced only AOS generation in cv. Irish Cobbler, a susceptible potato (*r*-gene).

During the infection process, the PiPE might be produced by the oomycete directly from the germ tubes and infection hyphae. It may be recognized specifically by CDPK of potato cells, causing some conformational change in the structure of the kinase domain, which would result in induction of CDPK activation within several minutes. It is possible that as a consequence of the PiPE binding, the catalytic domain of CDPK is now accessible to the substrates for the kinase in potato cells. However, the activation pattern of the kinase is different from the suppressor treatment of *Pi* which results in activation of the CDPK within 5-10 minutes.

The glycoprotein has 65% homology to FBA from yeast, which has a elicitor activity like as fungal cell

wall elicitor of *Pi*. As shown by Figure 3D, 3E PiPE was secreted from fungal cell wall surfs and that PiPE was detectable in the germination fluids by using immunochemical methods.

It was reported that ectopic expression of a heterologous CDPK (AK1-6H, an Arabidopsis calcium dependent protein kinase) in tomato protoplasts enhanced plasma membrane-associated NADPH oxidase activity (Tena et al., 2011). We examined the effect of CDPK Abs (Furuichi and Yokokawa, 2008), recognizing kinase domain-III, to AOS generation in potato microsomal fraction. It was observed that treatment of CDPK Abs to the microsomal fraction of potato, which was added with expressed His-Strboh1 protein from insect cells, inhibited approximately 50 % of AOS generation. It was suggested that CDPK kinase play an important role in the NADPH oxydase activation in potato microsome.

In the Cf9-Avr9 gene-for-gene interaction, a 68 to 70 kDa CDPK is activated in the plasma membrane fraction of tobacco cell cultures. The reported inhibitor studies were consistent with the evidence that CDPK was located upstream in the signal pathway that leads to the induction of AOS generation (Romeis et al., 2000), and it is in accordance with the results of quantitative RT-PCR in this study. Furthermore, arachidonic acid, an elicitor of *Pi*, induces activation of 78 kDa protein kinase C-like enzyme in potato tubers in calcium-dependent manner (Tena et al., 2011). These lines of evidence suggest that the NADPH oxidase is activated by the regulation of CDPK. Despite the importance of *Phytophthora* species as devastating plant pathogens, the basis of its specificity on potato cultivars is not resolved although elicitor active fractions have been isolated (Furuichi and Yokokawa, 2008, Furuichi and Suzuki, 1990, Kamoun et al., 1998, Joosten et al., 1999). The studies in the present results suggest that the PiPE with a sequence resembling that of fructose- 1, 6-bisphosphate aldolase could be functioning as an avirulence factor. This protein incited browning and AOS production to a greater extent on the resistant cultivars than the susceptible cultivars.

However all four (two race 0, avirulent and two race 1, virulent) of the *Phytophthora* species tested, possessed sequences that hybridized to the PiPE gene. Since the PiPE was recognized by anti-HW Abs, this PiPE was considered to exist in the cell wall of *Pi* cells. We observed that FITC-labeled Abs bound to the surfs of germinated spores of *Pi* by using fluorescent microscopy ($\times 800$) (unpublished data).

It was reported that ectopic expression of a heterologous CDPK in tomato protoplasts enhanced plasma membrane-associated NADPH oxidase activity (Tena et al., 2011, Furuichi and Yokokawa, 2010). We examined the effect of CDPK Abs, recognizing kinase domain-III, to AOS generation in potato microsomal

fraction (Furuichi and Yokokawa, 2008). It was observed that treatment of CDPK Abs to the microsomal fraction of potato, which was added with expressed His-Enrbh1 protein from insect cells, inhibited approximately 50 % of AOS generation. It was suggested that CDPK kinase play an important role in the NADPH oxidase activation in potato plasma membrane. In the Cf9-Avr9 gene-for-gene interaction, a 68 to 70 kDa CDPK is activated in the plasma membrane fraction of tobacco cell cultures. The reported inhibitor studies were consistent with the evidence that CDPK was located upstream in the signal pathway that leads to the induction of AOS generation (Tena et al., 2011), and it is in accordance with the results of quantitative RT-PCR in this study. Furthermore, arachidonic acid, an elicitor of *Pi*, induces activation of 78 kDa protein kinase C-like enzyme in potato tubers in calcium-dependent manner (Subramaniam et al. 1997). These lines of evidence suggest that the NADPH oxidase is activated by the regulation of CDPK (Furuichi et al., 2012).

IV. MATERIALS AND METHODS

a) *Phytophthora* strain and culture conditions

Phytophthora infestans (Mont.) de Bary isolates DN101 (presented by R. Bostock) and E003 (race 0), and St 401 (race 0) and P831 (race 1.2.3.4.5, presented by Tooley, Cornell University) were maintained on rye agar medium supplemented with 2% sucrose and 0.2% bacto yeast extract at 18°C in the dark. Other *Phytophthora* species were maintained in the dark. For liquid culture of the oomycete. The mycelia were grown in the dark at 18°C for 2-3 weeks on the synthetic medium as described by Furuichi and Suzuki (1990). The mycelial mat was harvested by gentle filtration, washed, and frozen at -20°C. Zoospores from the mycelial mat of *Pi* and the germination fluid were prepared using the methods reported previously (Doke and Tomiyama, 1977).

b) *Extraction and purification of HW*

Extractions of the HW were performed at 0 to 4°C either in an ice bath or cold room. The frozen mycelial mats of *Pi* (race 0), DN101 were ground in liquid nitrogen to a powder that was homogenized in five volumes of 0.2 M phosphate buffer (pH 7.2) containing 1 M NaCl and phenylmethylsulfonyl fluoride (PMSF) in a final concentration of 25 mM. The homogenate was sonicated for 3 min and centrifuged at 20,000 $\times g$ for 20 min at 4°C. The supernatant was dialyzed overnight against cold 25 mM Tris-HCl, pH 8.1 (Spectra/Por molecular porous membrane, Lincoln, molecular weight cut-off 1,000). The proteins in the crude dialyzed extract were purified by FPLC-anion exchange chromatography on a column (4.6 mm—100 mm, 1.7 ml, PoRos QE/M; PerSeptive Biosystems, Tokyo) equilibrated in the same buffer. The flow rate was 5 ml/min. Proteins were eluted

with a linear gradient of 0 to 0.5 M NaCl in 25 mM Tris-HCl, pH 8.1. The column eluate was monitored at 280 nm and recovered in fractions of 1 ml.

HW were isolated and purified after the homogenization and FPLC-anion exchange chromatography.

c) Protein Measurement

The protein contents of samples were measured according to Lowry et al. (1951) using bovine serum albumin (BSA) as the standard protein. Absorbance was measured at 595 nm.

d) Monoclonal Abs of the PiPE

The Abs used were monoclonal anti-PiPE Abs generated by mouse hybridomas as described in Ikeda and Furuichi (1993). PiPE was isolated from the *Pi* homogenate as reported previously (Furuichi and Suzuki, 1990).

Enzyme-linked immunosorbent assay (ELISA) was performed following the procedure described by McLaughlin et al. (1989). The wells of microtiter plates (Dynatech, Tokyo) were coated with the Abs diluted at 1/1,000 with 1% bovine serum albumin in phosphate-buffered saline (PBS). Immunoglobulin-alkaline phosphatase conjugates from rabbit were used as secondary Abs at 1/2,000 dilution. Absorbance due to alkaline phosphatase was measured with a Microplate Reader (Bio-Rad, Tokyo) at 595 nm.

e) Biological Assay

Induction of HR by the elicitor was assayed by using microscopic observation. 1) The cessation of cytoplasmic streaming, 2) Loss of stain ability by neutral red, and 3) Loss of ability for plasmolysis, of potato tuber tissue at 12 h after treatment.

The parenchymatous tissues of potato tuber from cvs. Eniwa (R₁) and Irish Cobbler (r) were aged for 16 h at 18°C prior to being treated with 30 µl of the PiPE (1 mg ml⁻¹ distilled water), which were isolated from fungal mat, and fractionated by FPLC then various FPLC fractions were used. The materials in the FLPC fractions were concentrated with a Centricon-30 micro concentrator (Amicon, Tokyo) (600 µg ml⁻¹). State replicates of studies here.

The cystospores were germinated by shaking in a flask with CaCl₂ (10⁻⁴ M).

f) Measurement of AOS generation from suspension of potato cells

A luciferase substrate, (CLA) was used to measure the concentration of AOS produced by suspension cultured potato cells. Suspension-cultured cells (5 ml of 3- to 4-day-old cells of potato cv. Eniwa (R₁-gene) and cv. Irish Cobbler (r-gene) were treated with His-tagged PiPE (500 µl of 600 µg ml⁻¹). At each time point, 500 µl of the treated suspension cells were centrifuged for 15 sec at room temperature to collect the supernatant. The supernatant (25 µl) was added to a 15

ml sample tube containing 426 µl of 39 mM HEPES (pH 7.0), 5 µl of 10 mM MgCl₂ or of 10 mM CaCl₂, 5 µl of 10 mM EGTA, 1.5 µl of 10 mM guanosine 5' triphosphate (GTP)-λ-S and 15 µl of 500 µl CLA(Cypridina Luciferin Analog; 2 Methyl-6-phenyl-3,7-dihydroimidazo[1,2-a]pyrazin-3-one). CLA was added to the tube last. The sample was then incubated at 37°C for 3 min. Radiated light was measured for 15 sec just after adding 23 µl of 3.3 mM NADPH (Luminescence Reader, Atto, Tokyo).

g) SDS-PAGE and immuno blotting

Proteins were separated by SDS-polyacrylamide gel electrophoresis as described previously (Laemmli, 1970) with a 12.5% acrylamide separation gel and 4.5% acrylamide stacking gel. Following electrophoresis, gels were silver-stained following the previously reported method or the proteins were transferred as described by Towbin et al. (1979) onto a polyvinylidene difluoride (PVDF) membrane (Immobilon-P, pore size 0.45 µm, Millipore, Tokyo) using a MilliBlot™-SDS System (Millipore) (Stephen et al.) at 2 mA per cm² for 30 min. The PVDF membrane was incubated for 1 h at 25°C in the blocking buffer (10 mM Tris-HCl, 150 mM NaCl (pH 7.5), and 5% skimmed milk). Membranes were washed twice in TBS-Tween 20 for 5 min, incubated for 1 h at room temperature in the primary HW-Abs diluted at 1/1,000 with TBS. After washing a third time in TBS-Tween 20 for 10 min, membranes were incubated for 1 h at room temperature in rabbit anti-mouse IgG conjugates with alkaline phosphatase (Bio-Rad, Tokyo, Japan) diluted at 1/2,000 with TBS. After three washes in TBS-Tween-20, each for 10 min detection of the antigen-Abs complexes was carried out with alkaline phosphatase color reagent (Bio-Rad, Tokyo, Japan). The reactive membranes were majored by using typhoon (GE-Science, Tokyo, Japan). Controls were recorded by using without anti-PiPE-Abs and with control antisera. The experiments were determined by using 3 times measurements. The membrane with antigen-Abs complexes were recording by using the typhoon gel scanner.

h) Peptide mapping by protease

PiPE protein was digested by *Staphylococcus aureus* V8 protease as described by Cleveland et al. (1977). Briefly, protein bands from an SDS gel, stained after SDS-PAGE with Coomassie brilliant blue, were digested by the V8 protease without prior elution, by placing gel slices containing these bands in the sample wells of a second SDS gel, then overlaying each slice with the V8 protease. Digestion proceeded directly in the stacking gel during the subsequent electrophoresis.

i) N-terminal amino acid sequencing

For N-terminal amino acid sequence determination, PiPE peptides were concentrated with a Centricon-30 micro concentrator in a final concentration

of 100 pmol and transferred to a PVDF membrane as described previously (Southerton et al., 1993). Automated Edman degradation of the PiPE peptides was performed with a Shimadzu PPSQ-21 sequencer (Shimadzu, Kyoto) using the reagents and method of the manufacturer.

j) Fungal RNA preparation and RT-PCR

Total RNA from freshly harvested *Pi* mycelia was isolated using the guanidine hydrochloride extraction method reported by (Logemann et al., 1987). Amplification of cDNA with the degenerate primers shown in Table 1 was carried out using Ready-To-Go™ RT-PCR beads (Amersham-Pharmacia, Tokyo) according to the methods of the manufacturer. Reverse transcription was carried out by adding 20 ng to 2 µg of total RNA and a final concentration of 1 pM oligo d (T)18 primer to dissolved beads in DEPC-treated water. The incubation conditions were as follows: 30 min, 72°C; 5 min, 95°C. After that, the degenerate primer indicated in Table 1 was added for PCR. The incubation conditions were as follows: 4 min, 94°C; 35 cycles (40 min, 94°C; 1 min, 50°C; 1.5 min, 72°C); 7 min, 72°C.

k) Genomic DNA preparation and Southern blot hybridization

Genomic DNA from freshly harvested *Pi* mycelia was isolated using reported methods (Ausubel et al., 1987; Sambrook et al., 1989). DNA was treated with RNase and digested with *Hind*III or *Pst*I. Approximately 3 µg of digested DNA was electrophoresed on a 1% agarose gel. Alkaline DNA was transferred to a nylon membrane (Hybond N⁺, Amersham-Pharmacia, Tokyo), and Southern blot hybridizations were performed at 55°C as reported (Ausubel et al., 1987). Probes for hybridization were synthesized by PCR using the primers described in Table 1 and comprised the nucleotide sequences (674-bp fragment — (Fig. 9). Probes were labeled using a AlkPhos Direct labeling and detection system (Amersham, Tokyo) according to the supplier's instructions. Membranes were washed twice at 65°C for 10 min in the primary wash buffer, then washed in secondary wash buffer (50 mM Tris base, 100 mM NaCl and 2 mM MgCl₂). Positive cDNA clones were detected using the CDP-*Star* chemiluminescent detection reagent according to the manufacturer's instructions.

l) Expression of recombinant PiPE

The *E. coli*, BL21 pLysS cells, harboring apCR T7/CT TOPO plasmid vector (Invitrogen) containing the insert cDNA the 674 base pairs. His tag added to C terminus. They were added to 10 ml LB medium. The 10 ml of LB medium was inoculated with 2 ml of the *E. coli* cultured, and then cultured for 4 h at 37°C with shaking. Isopropyl-λ-D-thiogalactopyranoside was added to a final concentration of 0.6 mM and cultured

for 24 h. The protein was harvested. Cells were harvested by centrifugation at 4000 rpm for 10 min at 4°C, and the pellet was resuspended in guanidinium lysis buffer (pH 7.8) and slowly shaken for 7 min before sonicated at ice-water temperature. The insoluble debris was removed by centrifugation at 6500 rpm for 15 min at 4°C. The supernatant was collected and stored at 4°C for subsequent His-tag purification by following the Xpress™ System protocol as described by Invitrogen. The polyhistidine-tagged fusion protein was loaded onto a ProBond™ histidine-bind resin column equilibrated with lysate buffer. The column was washed with denaturing binding buffer (8 M urea, 20 mM sodium phosphate, 500 mM sodium chloride, pH 7.8). Then, the column was washed with denaturing wash buffer, ranging at pH 6.0 and pH 5.3. Finally, the protein was eluted with denaturing elution buffer (8 M urea, 20 mM sodium phosphate, 500 mM sodium chloride, pH 4.0). The elute was dialyzed against 10 mM Tris-HCl, pH 8.0, and 0.1 % Triton X-100 overnight at 4°C to remove urea. Each samples were stocked at -30°C. What about the germination fluid also contains those PiPE. Affinity purified PiPE fraction were contained in a germination fluid.

m) Purification of recombinant protein

For purification of His-fusion protein, ProBond™ Protein Purification kit (Invitrogen) was used. The polyhistidine-tagged fusion protein was loaded onto a ProBond™ histidine-bind resin column equilibrated with lysate buffer. The column was washed with 8 ml of denaturing binding buffer (8 M urea, 20 mM sodium phosphate, 500 mM sodium chloride, pH 7.8). Then, the column was washed with 8 ml of denaturing buffer (8 M urea, 20 mM sodium phosphate, 500 mM sodium chloride) pH 6.0 and pH 5.3 successively. Finally, the protein was eluted with 5 ml of denaturing elution buffer (8 M urea, 20 mM sodium phosphate, 500 mM sodium chloride, pH 4.0). the elute was dialyzed against 10 mM Tris-HCl, pH 8.0, 0.1 % Triton X-100 overnight at 4 °C to remove urea. During this time, the dialysis buffer was replaced 4 times. The purified protein concentration was determined using the BCA protein assay kit (Pierce) with bovine serum albumin (BSA) as standard.

V. ACKNOWLEDGEMENTS

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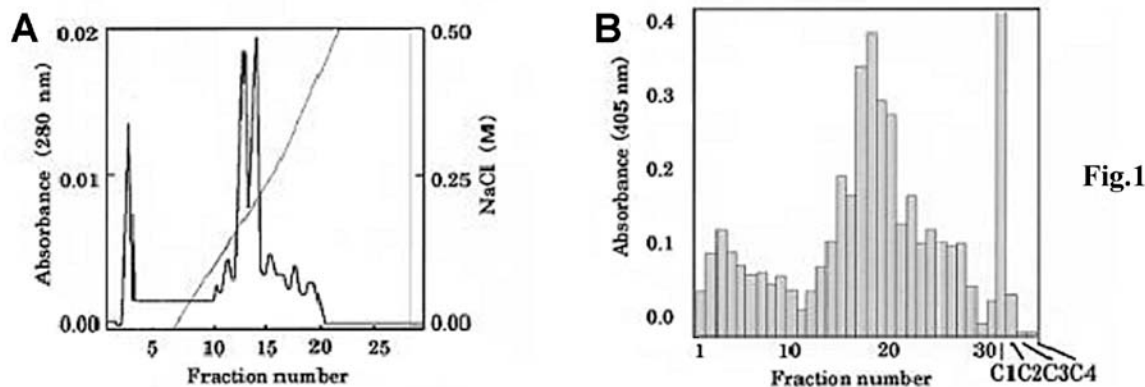


Figure 1 : Chromatogram of the crude extract prepared from *Phytophthora infestans* (Pi) (race 0) by FPLC-anion exchange chromatography (POROS QE/M column) at pH 8.1

A, Absorbance was monitored at 280 nm. The flow rate was 5 ml/min, and 1-ml fractions were collected after elution with a NaCl gradient. **B**, ELISA reactions using Abs against FPLC fractions of crude extract prepared from *Pi* (race 0). C1:Crude extract

prepared from race 0. C2: Elution buffer used for FPLC, C3: C1-treated with the first Abs (Abs only), C4: C1-treated with second Abs (anti-mouse IgG) only. Data are the average of four replications. A 405 nm on the axis was the value of ELISA.

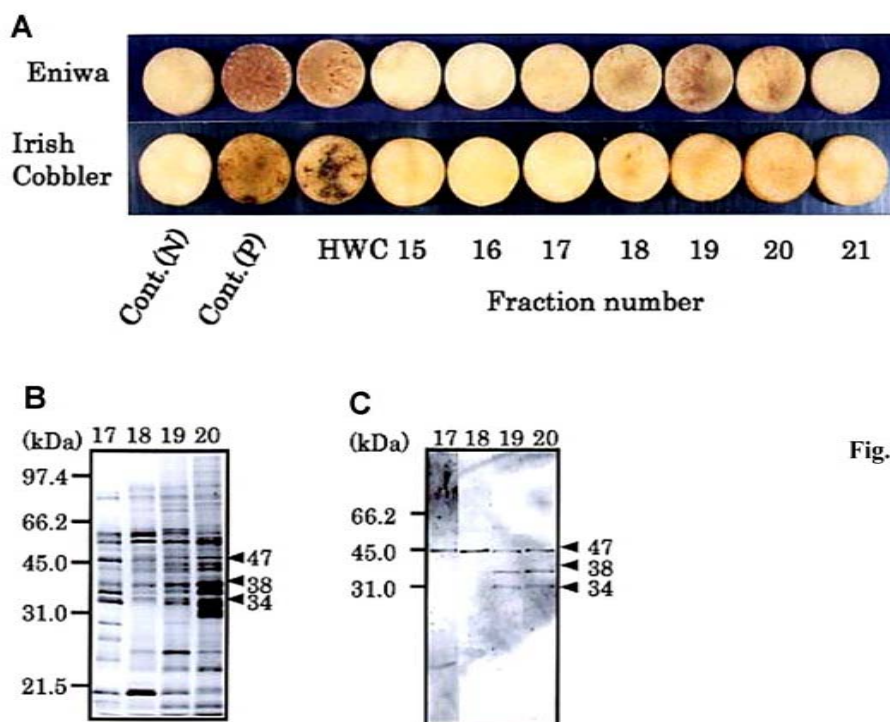


Figure 2 : Elicitor activity and peptide components of FPLC fractions

A, Elicitor activity as detected by browning after 30 min of treatment with preparations from *Pi* race 0. Each fraction (600 μ g/ml -1protein) was applied to the cut surface. Cont.(N): water treatment, Cont.(P): the extract prepared, HW: wall elicitor (1mg/ml -1distilled water) prepared from the treatment. **B**, Silver staining of peptides obtained by SDS-PAGE of FPLC fractions extracted from mycelium of *Pi* (race 0). Fraction numbers are given above lanes. Molecular mass

standards in kDa are designated. **C**, Western blot analysis of FPLC fractions with monoclonal Abs derived against HW. Samples and order of loading are identical to those in B.

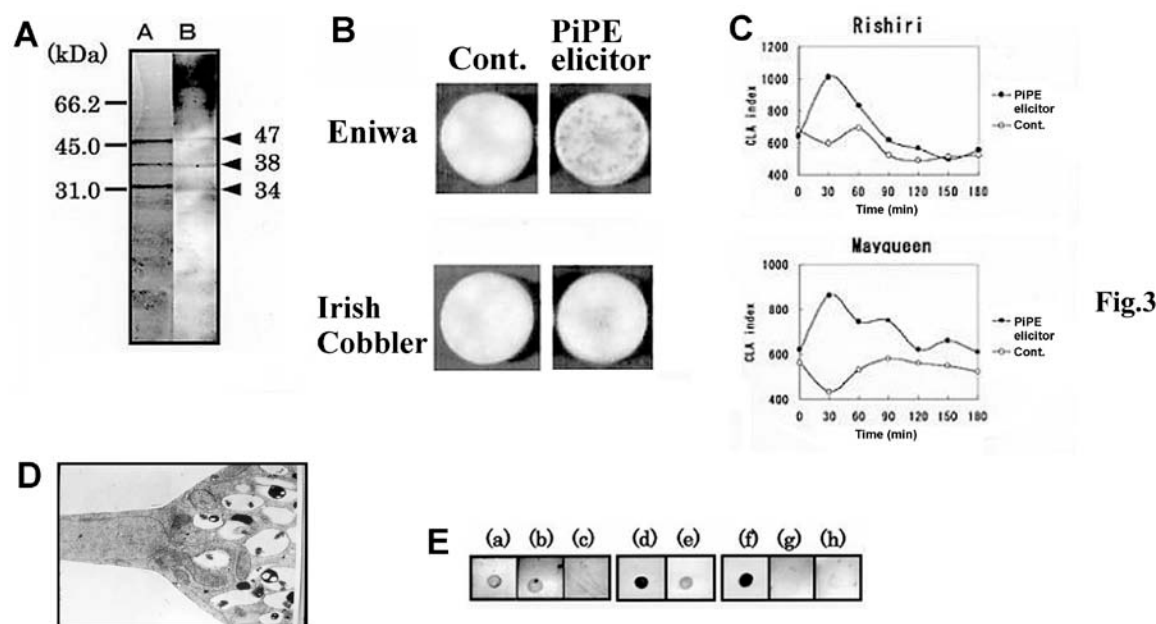


Fig.3

Figure 3 : Affinity purification of the Abs-binding protein

A, SDS-PAGE of affinity-purified Abs binding peptides. Gel was silver-stained. Molecular mass standards in kDa are given on the left. Western blot analysis of affinity-purified Abs-binding peptides. Molecular mass standards in kDa are given on the left. Germination fluid of *Pi* race 0, at 5h after shaking in flask. B, Elicitor activity of Abs binding protein on tuber tissues of potato and generation of active oxygen species in suspension culture cells of potato. Cvs. Eniwa (R_1) and Irish Cobbler (r) at 98 h after treatment. C, CLA index in culture cells of the resistance (R_1) and susceptible (r) potato cultivars was measured with a luminescence reader. The cultivar rishiri was used.

D, Electron microscopic observation of *Pi* ($\times 14,800$). Immunochemical assay of germination fluid of *Pi*. E, Immunochemical assay of the germination and culture-fluid of *Pi* (race 0). (a) Germination fluid. (b) The zoospore suspensions. (c) CaCl_2 as a control. (d) The culture fluid. (e) Rye medium as a control. (f) Homogenated soluble sample. Prepared from *Pi*. (g) The extract from *Pi* treated with the Abs 2A11. (h) The treatment with second Abs (anti-mouse immunoglobulin G).

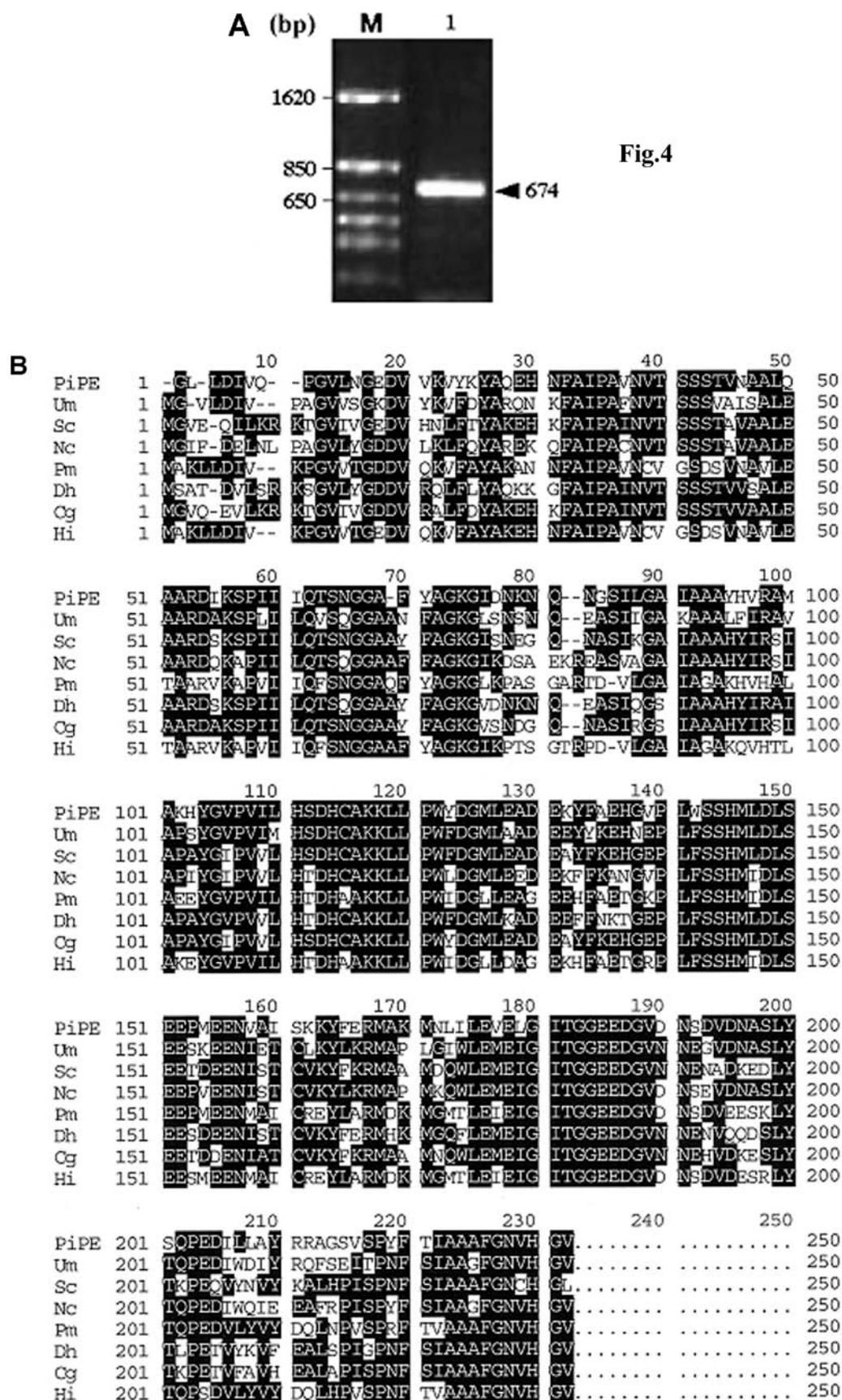


Figure 4 : Partial sequence alignment of the PiPE from Pi

A, Electrophoresis showed PiPE-gene (674 bp). **B**, Alignment analysis of PiPE-gene from *Pi*. It was shown that seven other FAB-genes were reported from fungus. Partial nucleotide sequence of the mRNA encoding the PiPE protein from *Pi* and deduced amino acid sequence. The arrowhead indicates predicted glycosylation sites. The alignments were made with the program DNAsys. Residue letters are indicated under the sequences. (PiPE: Peptide elicitor of *Pi*, Um: *Ustilago maydis*, Sc: *Saccharomyces cerevisiae*, Nc:

Neurospora crassa, Pm: *Pasteurella multocida*, Dh: *Debaryomyces hansenii*, Cg: *Candida glabrata*, Hi: *Haemophilus influenzae*).

Genomic DNA (3 μ g) of the *Pi* isolates Pi831 (race 1) (lanes 1 and 3) and DN101 (race 0) (lanes 2 and 4) and were digested with *Pst*I (lanes 1 and 2) or *Hind*III (lanes 3 and 4) and hybridized with a probe containing a 674-bp fragment internal to the PiPE open reading frame from *Pi* isolate DN101. Numbers on the left are DNA size markers in bp.

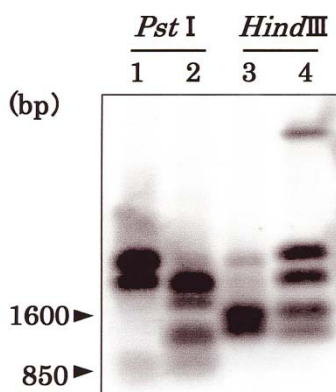


Fig.5

Figure 5 : Southern blot analysis of the PiPE gene from *Pi*

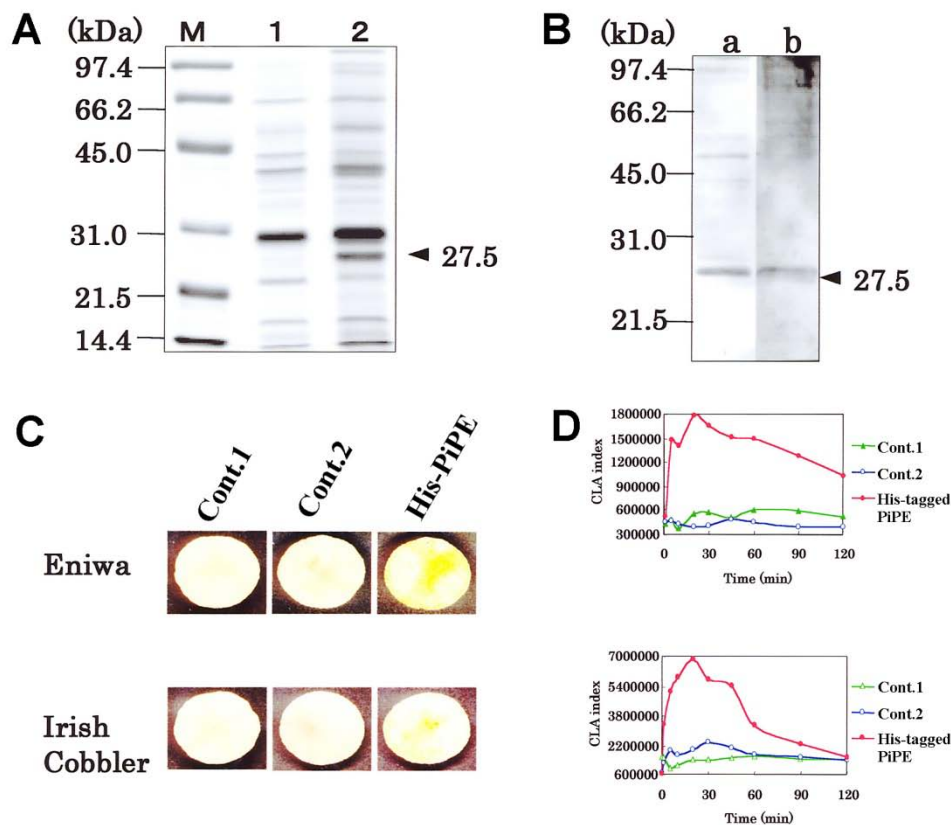


Fig.6

Figure 6 : Purification and immunodetection of the His-tagged PiPE protein

A, SDS-PAGE of the His-PiPE protein expressed in *E. coli*. Lane 1: control vector without PiPE gene sequence. Lane 2 : expressed proteins with the PiPE

gene. **B**, Western immunoblot analysis of fusion proteins with anti-His Abs (lane a) and anti-HW-Abs (lane b). **C**, Elicitor assay of the affinity purified protein

expressed from *E. coli* on tuber tissue of potato cultivars. Control 1: water treatment. Control 2: Expressed fusion protein and purified from the plasmid vector alone, then applied onto the tuber disks. Fusion protein: His-PiPE. The photograph was taken 96 h after

the treatment. **D**, Effect of the His-PiPE on the generation of active oxygen species in suspension cultured cells of potato cv. Rishiri (R₁-gene) and Mayqueen (r-gene). The CLA index was measured by a luminometer.

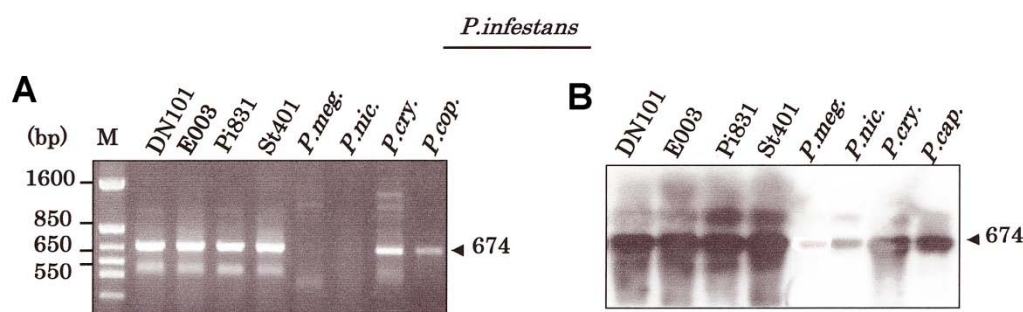


Fig.7

Figure 7 : Distribution of the PiPE genes in other *Phytophthora* strains

A, Agarose gel electrophoresis of the RT-PCR product. The templates were total RNA extracted from freshly harvested mycelial culture of (race 0) isolates, DN101 and E003, race 1 isolates Pi831 and St401; *P. meg.*, *P. megasperma*; *P. nic.*, *P. nicotianae*; *P. cry.*, *P. cryptogea*; and *P. cap.*, *P. capsici*. DNA size markers in bp are in the lane M. DNA size markers (in bp) are given in lane M. The experiments were repeated twice. **B**, RT-PCR clones were hybridized with a probe containing a 974-bp fragment internal to the PiPE open reading frame from

DN101. The samples and order of loading are identical to those in (a). Agarose gel electrophoresis of cDNA encoding the protein moiety of the PiPE from Pi using RT-PCR, lane 1. Total RNA extracted from freshly harvested mycelial culture of *Pi* was used as the template with the primers indicated in Table 1. DNA size (bp) markers are in lane M. RT-PCR products were hybridized with a probe containing a 674-bp fragment internal to the PiPE open reading frame from isolate DN101. The samples and order of loading are identical to those in Figure 7A.

Table 1 : Amino acid sequences of peptides derived from the PiPE elicitor and deduced degenerate oligonucleotides used for PCR

Antigenic peptide	Sequence
PiPE N-terminus	
	1 ^a M-G-L-L-D-I-V-Q-P-G-V-L-X ^b G-E-D-V-V-X-V-Y 21
	5' ATGGGNYTNYTNGAYATAGTG 3'
	5' ATHGTICARCCIGGIGT 3'
PiPE digest	
	207 L-G-S-V-S-P-Y-F-T-I-A-A-A-F-G-N-V-H-G-V 226
	3' GGNATRAARTGSTARCGNCG 5' 3' CGNAARCCNTTRCANGTRCC 5'

^a Numbers above ends of peptides refer to the coding sequence predicted in Fig. 5(b).

^b X stands for unidentified amino acid.

^c Arrows indicate sequences and orientation of the primer used for PCR.

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Amphibia, Anura, Microhylidae, Gastrophryninae, Elachistocleis Bicolor Guérin Méneville, 1838: Distribution Extension and Geographic Distribution Map

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Abstract - The geographic distribution of *Elachistocleis bicolor* (Anura: Microhylidae) extends over several biomes in Argentina, Bolivia, Paraguay, Peru, Uruguay and Brazil. In Brazil, this species was reported to occur in the States of Amazonas, Distrito Federal, Goiás, Mato Grosso do Sul, Paraná, Rio Grande do Sul, Santa Catarina and São Paulo. The current study reports the first record of *E. bicolor* for the state of Rondônia, Brazil, extending its distribution in 350 km eastwards from closest records in Beni, Bolivia and 715 km southwestwards from closest records in Amazonas.

GJMR-K Classification : NLMC Code: QY 60.A6



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Amphibia, Anura, Microhylidae, Gastrophryninae, *Elachistocleis Bicolor* Guérin Méneville, 1838: Distribution Extension and Geographic Distribution Map

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Abstract - The geographic distribution of *Elachistocleis bicolor* (Anura: Microhylidae) extends over several biomes in Argentina, Bolivia, Paraguay, Peru, Uruguay and Brazil. In Brazil, this species was reported to occur in the States of Amazonas, Distrito Federal, Goiás, Mato Grosso do Sul, Paraná, Rio Grande do Sul, Santa Catarina and São Paulo. The current study reports the first record of *E. bicolor* for the state of Rondônia, Brazil, extending its distribution in 350 km eastwards from closest records in Beni, Bolivia and 715 km southwestwards from closest records in Amazonas.

1. INTRODUCTION

The frog *Elachistocleis bicolor* Guérin Méneville, 1838 (Figure 1) belongs to the Microhylidae family (Lavilla *et al.* 2003; Rodrigues *et al.* 2003). The taxonomy of the species from the genus *Elachistocleis* are controversial and have undergone several changes (Lavilla *et al.* 2003; Thomé and Brasileiro 2007). Lavilla *et al.* (2003) remove *E. bicolor* from synonymy with *E. ovalis* Schneider 1799, which is widely distributed in South America (Frost 2009). The occurrence of *E. ovalis* in syntopy with other congeneric species of the genus and also taxonomically problematic can lead to unreliable identifications (Kwet and Di Bernardo 1998; Lavilla *et al.* 2003). According to De La Riva *et al.* 2000, considerable confusion took place in the past regarding the identity of *E. bicolor* vs. *E. ovalis*, whose main external difference is the ventral coloration (see Frost 1985). Today most authors apply the name *E. bicolor* to the frogs with an immaculate venter (De La Riva *et al.* 2000).

The geographic distribution of this species extends over several biomes, including Amazonia, Chaco, *Cerrado*, Pampa and Pantanal in Argentina (Céspedes *et al.* 2001; Lavilla *et al.* 2003; Aceñolaza *et al.* 2004; Kacoliris *et al.* 2006; Echeverría *et al.* 2007; Baldo *et al.* 2008; Duré *et al.* 2008; GBIF 2008), Bolivia (De la Riva *et al.* 1996, 2000; Reichle 1997; Reichle and Kohler 1998; GBIF 2008), Paraguay (Brusquetti and Lavilla 2006; GBIF 2008), Peru (MVZ 1999), Uruguay (Lavilla *et al.* 2004; Maneyro and Beheregaray 2007; Canavero *et al.* 2008) and Brazil. In Brazil, this species was reported to occur in the States of Amazonas (Gordo 2003; Lima *et al.* 2006; GBIF 2008), Distrito Federal (Lima and Costa 2006; Camargo and Aguiar 2007), Goiás (Bastos *et al.* 2003), Mato Grosso do Sul (Strüßmann *et al.* 2000; Gordo and Campos 2003; Rodrigues *et al.* 2003; Ávila *et al.* 2004; GBIF 2008), Paraná (Conte and Rossa-Feres 2006, 2007; Shibatta *et al.* 2009), Rio Grande do Sul (Braun and Braun 1980; Kwet and Di-Bernardo 1998; Colombo *et al.* 2008; Moreira *et al.* 2008; Santos *et al.* 2008; Quintela *et al.* 2009), Santa Catarina (GBIF 2008; Lucas and Fortes 2008) and São Paulo (Bernarde and Kokubum 1999; Vasconcelos and Rossa-Feres 2005; Silva *et al.* 2008) (Figure 2).

In this work we report the first record of *E. bicolor* for the state of Rondônia, in southwestern Brazil, in a transitional region between the *Cerrado* and the Amazonian forest biomes. One male and three female of *E. bicolor* were collected at the municipality of Porto Velho, state of Rondônia, in an area of forested land on a private farm (08°43'09" S, 63°51'64" W), on 13 February 2009 by DBH. Specimens were identified according to De La Riva *et al.* (1996, 2000), Frost (2009), Lima *et al.* (2006) and Rodrigues *et al.* (2003).

These new records extend the geographic distribution of *E. bicolor* about 350 km eastwards from closest records in Beni, Bolivia (10°58'59.98" S, 66°06'00" W) and 715 km southwestwards from closest records in Amazonas, Brazil (03°18'15" S, 60°37'03" W) (Figure 2). Voucher specimens were deposited in the anuran

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collection of the *Centro de Estudos de Biomoléculas Aplicadas a Medicina, Universidade Federal de Rondônia*, Porto Velho, Rondônia, Brazil (CEBio 090212-010527, CEBio 090212-010531, CEBio 090212-010532, CEBio 091016-0105134).

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Figure 1 : *Elachistocleis bicolor* (female) showing the ovoid body, small and triangular head, dark brown to yellowish brown back with a white to light yellow stripe that extends from the nose to the urostylus region, immaculate yellow belly (the gular region is black in males), and a thin reddish-brown line in the back of the thighs

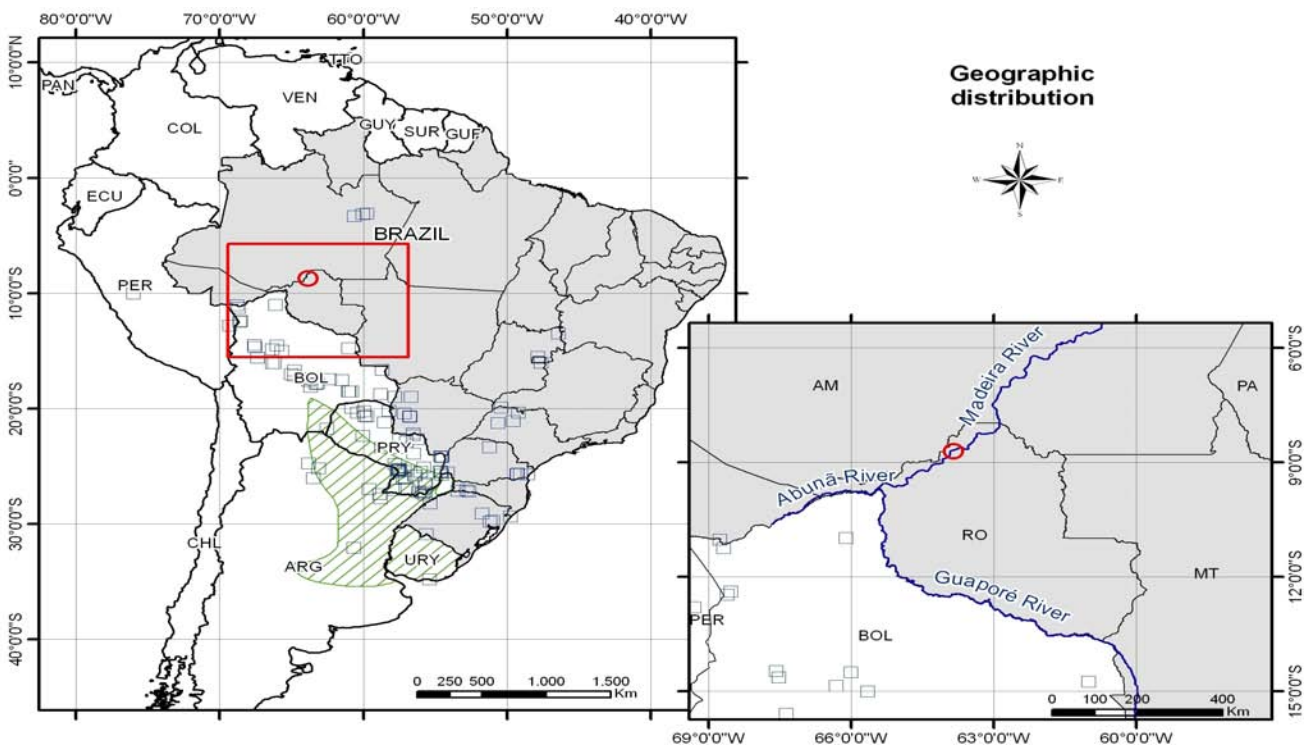


Figure 2 : Distribution map for *Elachistocleis bicolor* with the new record for the municipality of Porto Velho, state of Rondônia, Brazil (red circle). The blue squares correspond to records areas for *E. bicolor* obtained from the literature cited. The green area represents the distribution area for *E. bicolor* in the IUCN Red List of Threatened Species site (Lavilla *et al.* 2004)



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Tongue-Tie in Children; Mothers Perceptions, Attitudes and Practices in Port Harcourt, Nigeria

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Keywords : tongue tie, ankyloglossia, children, mothers, perceptions, practices, treatment, complications of treatment.

GJMR-K Classification : NLMC Code: WS 200, WS 205



TONGUE-TIE IN CHILDREN MOTHERS PERCEPTIONS, ATTITUDES AND PRACTICES IN PORT HARCOURT, NIGERIA

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RESEARCH | DIVERSITY | ETHICS

Tongue-Tie in Children; Mothers Perceptions, Attitudes and Practices in Port Harcourt, Nigeria

Opapa P.I. ^α & Eke G.K ^σ

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I. INTRODUCTION

Tongue tie, the common name for ankyloglossia results from the frenulum, a membrane under the tongue extending further than usual towards the tip of the tongue.^{1, 2} This limits upwards and forward movement of the tongue. It is a congenital condition which is often hereditary with a wide variation in incidence ranging from 1.7% to 10.9% in different localities.³⁻⁶

Restriction of tongue movement in an infant may prevent the infant from taking enough breast tissue into the mouth and hence result in breast feeding problems such as poor attachment with resulting bleeding painful nipples and poor milk supply for the mother, leading to frequent feeding, and poor weight gain for the baby despite frequent feeding.⁴ Breast feeding difficulties have been reported as the earliest complications associated with tongue tie.^{1, 3}

Other problems associated with tongue tie include difficulties with articulation of sounds, dental problems and inability to lick an ice cream or play wind instruments later in life. However, in many children it is asymptomatic and persistence beyond the first 2 to 3 years of life is uncommon compared with the higher incidence present in neonates. This suggests a

lessening of the degree of severity of the anatomical abnormality with growth and development.^{4, 7}

For many years, the subject of ankyloglossia has been controversial with practitioners of many specialties having widely different views regarding its significance.² This has led to unwillingness of many clinicians to intervene surgically even where indicated.^{1, 2} Some children do benefit from frenotomy which is the surgical treatment of tongue tie.

Although tongue tie is usually asymptomatic, it is surrounded by different myths and beliefs and may constitute a source of worry for parents who may seek help from professional and untrained personnel.⁸ Release by non-medical or inadequately trained medical personnel, may result in life threatening complications.⁸ The aim of this paper was to identify knowledge, attitudes and practices of mothers towards tongue tie.

II. METHODOLOGY

This study was a descriptive cross sectional survey carried out over a period of six months (November 2011- April 2012), amongst mothers in Port Harcourt. Port Harcourt City is cosmopolitan and host to major indigenous and multinational companies in the oil and gas, manufacturing, banking, telecommunications, construction and health sectors, employing staff from diverse ethnic nationalities.

Mothers who presented with infants to the Paediatric Outpatients Clinics of the University of Port Harcourt Teaching Hospital (UPTH) and gave consent participated in the study. The UPTH is a federal tertiary health institution serving Rivers State and the neighbouring states of Bayelsa, Abia, Imo, and Akwa-Ibom in the southern and eastern parts of Nigeria. The hospital serves as a general/referral centre for neonates and children in Port Harcourt and its environs. It also provides primary health care services as patients can and do walk in for consultation, treatment and other services such as immunisation and growth monitoring.

The Paediatric Outpatient clinics are run on week days and cater for children 0 – 17 years who do not have emergency/life threatening problems. These children are seen in the clinics and sent home or admitted into the Paediatric wards if indicated.

A closed-ended, anonymous and self-administered questionnaire was used to obtain information from the mothers and retrieved by the investigators immediately after they were filled. Data

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collected included biodata, knowledge of tongue tie, cultural beliefs, symptoms, treatment, and complications. Respondents could tick more than one option per question, where applicable. Those who had difficulty reading and/or writing were assisted by one of the investigators.

Data were entered into a Microsoft Excel Spread Sheet and analyzed using SPSS version 17.0. Results are presented using tables and text.

III. RESULTS

Two hundred and fifty mothers participated in the study. Of these 238 (95.2%) had heard about tongue tie. Mothers described tongue tie in various ways. (Table 1). Common descriptions were fleshy growth or mass under the tongue (28%) and something that prevents children from lifting up/moving the tongue (19.2%).

Table II shows sources of information on tongue tie. Most of the information on tongue tie had been handed down to the mothers by their own mothers (90; 36%) and other relatives. In the medical community, nurses (70; 28%) were the greatest source of information while doctors were the least (10; 4%).

One hundred and eighteen (47.2%) mothers reported speech difficulties as an adverse effect of tongue tie. Breast feeding difficulties were reported by only 6 (2.4%) mothers. (Table III).

Eighty six (34.4%) mothers had had frenotomies done for them in childhood. 96 (38.4%) had not had and the rest of them did not know whether or not they had frenotomies in childhood.

There was a strong cultural belief among mothers that all babies have tongue ties and should have them cut (Table IV).

Table V shows mothers perceptions of age for treatment of tongue ties and who should treat it. One hundred and fifty four (61.6%) mothers believed that tongue tie should be treated within the first month of life. Only 16 (6.4%) reported that tongue tie should not be treated.

About half of the mothers 124 (49.6%) reported that tongue ties should be treated by doctors.

Ninety two (36.8%) mothers admitted to having babies with tongue tie all of whom had treatment. Table VI shows who made the diagnosis of tongue tie on the baby and what problems were associated. In 38 (41.3%) of the babies the diagnosis was made by nurses while doctors made diagnosis in 6 (6.5%) of cases. 60 (65.2%) mothers reported inability to cry as the problem associated with tongue tie in their babies. A small number reported no associated problems.

Majority (72; 78.2%) of the babies were treated by nurses, 8 (11.1%) were treated by doctors while the others were treated by TBAs 10 (13.9%) and grandmothers 2 (2.8%).

Fifty eight (63.1%) of the babies were treated in health facilities while 20 (21.7%) were treated at home.

In majority of cases 64 (69.6%), treatment was carried out using an instrument while in 26 (28.3%), tongue tie was slashed with the finger nail. (Table VII)

Instruments used in the treatment of tongue tie included scissors (40; 43.5%), razor blades (18; 19.6%) and knives (4; 4.3%). Twenty six (28.2%) had it slashed with finger nails. Eleven (11.9%) mothers knew that the instruments used were new, thirty four (37%) knew that the instruments were not new while 14 (15.2%) did not know the status of the instruments. 26 (28.3%) mothers admitted that the instruments were boiled or sterilized whilst 34 (37%) were not boiled or sterilized and the others didn't know.

Eighteen (19.6%) babies had problems after the procedure. These included bleeding (12; 66.7%), fever (4; 22.2%), re-occurrence which necessitated a repeat of the procedure (2; 11.1%) and soft tissue injury (2; 11.1%).

Positive effects reported after the procedure included improvement in crying (56; 60.9%), ability to raise the tongue (2; 2.2%), improved breast feeding (6; 6.5%). Six (6.5%) mothers did not see any change after release of the tongue tie.

IV. DISCUSSION

Majority of the mothers in this study were aware of the subject of tongue tie. This is not surprising as this subject has been in existence for centuries in many parts of the world.⁹ The highest source of information were grandmothers and nurses.

In Nigeria and many other African societies, grandmothers are seen as custodians of wisdom and are very often responsible for passing on information and tradition to their daughters. This also includes information on child care practices.^{10, 11} Nurses are also usually the first point of care for patients and in most health care facilities in our region, are responsible for giving health talks to mothers on child care practices. Doctors are often too busy or probably not interested in giving health information to their patients. This has been reported in other authors.¹² Sadly, they often have to deal with complications that arise in patients due to wrong or poorly passed information. Mothers described tongue tie fairly well buttressing the fact that they did have good knowledge of the subject. This also may be a pointer to tongue-tie being a fairly common occurrence in our environment. The incidence of tongue tie has not been reported in Nigeria but studies show varying incidences in different parts of the world.³⁻⁶

The major adverse effects of tongue tie as perceived by mothers included speech difficulties, and inability to cry well. Some of these complications have been reported.^{2, 13} Breast feeding difficulties which have been reported^{1, 3} as the earliest problems associated with tongue tie were noted by very few mothers. The reason for this could not be readily ascertained, it may be possible that even if breast feeding problems exist,

mothers do not link them with tongue ties or that since breast feeding is a key child care practice in our society, reporting difficulties may be a reflection of failure on the mothers' part. However it is a well known fact that most babies with ankyloglossia are asymptomatic.¹⁻³ Furthermore obvious disabilities like speech difficulties may be more readily identified by mothers. This may also be influenced by cultural beliefs as also noted in the study.

Almost half of the mothers reported that their cultures supported clipping of the frenulum in all newborns. Before the 19th century, midwives were reported to have kept sharp finger nails to slash the membrane under the tongue of all newborns.⁹ So many years down the line cultural beliefs in our environment still support this practice. Routine clipping of the frenulum in newborns by traditional birth attendants was also reported in a case series by one of the authors.⁸ This highlights the effects of culture on child health practices.

Majority of the mothers also believed that tongue tie should be treated in the neonatal period. Inasmuch as treatment is indicated in the newborn period when there are indications such as breast feeding problems, treatment by untrained or inadequately trained personnel would contribute to morbidity and mortality in the newborn.⁸

Amongst mothers who reported having a baby with tongue tie, majority had the diagnoses made by nurses or themselves. A previous Nigerian study,¹⁴ showed that diagnostic accuracy by traditional and orthodox healthcare providers was very low, whilst parental curiosity and myth about tongue-tie were high. The diagnosis of tongue tie though still controversial should be done by adequately trained personnel in order to limit unnecessary interventions.^{2,8}

Again, even among mothers whose babies had tongue ties, breast feeding difficulties were mentioned by very few as an associated problem. Possible reasons for this have been highlighted.

Even though mothers perceived that tongue tie should be treated by doctors, among those whose babies had it, treatment was mainly done by nurses. The clipping of the frenulum without reason, which was common practice in earlier years, resulted in the surgical treatment of tongue-tie falling into disrepute amongst many in the medical community.^{1,2,9} This unwillingness of many clinicians especially doctors to intervene surgically has led mothers in our environment to seek help from both medical and non-medical personnel who are not trained to treat it; thus contributing to the morbidity associated with treatment.

Most of the tongue ties were cut with instruments like razors, scissors and knives but the study shows that the art of using finger nails to clip the frenulum still exists in our society. Some mothers actually knew that the instruments used on their babies

were not new and a few knew that the instruments were not boiled or sterilized. These methods are fraught with risks like bleeding and infection as previously reported.^{8,9} Some of these complications were reported by some of the mothers in this study. This shows that a relatively benign condition like tongue tie if not properly handled can contribute to morbidity and mortality in children especially newborns.

Although there was no way to ascertain that these babies actually had tongue ties, some of the positive effects reported after treatment included improvement in crying, ability to raise the tongue and improved breast feeding. Some of these effects have been reported in other studies following treatment of tongue tie. The fact that some reported recurrence or lack of improvement after treatment raises the question of whether these babies actually had tongue ties or received proper treatment.

V. CONCLUSION

Mothers are aware of tongue tie but need enlightenment on its mostly benign nature, and the need to seek professional advice when concerned about it. This will prevent unnecessary morbidity associated with improper treatment. Doctors should play an active part in this enlightenment campaign.

VI. ACKNOWLEDGEMENT

The authors wish to acknowledge the assistance given by the nursing staff of the Paediatric Outpatient Clinics of University of Port Harcourt Teaching Hospital during the data collection process for this study.

VII. DISCLOSURES

The authors declare that there are no potential conflicts of interest

VIII. FUNDING ACKNOWLEDGEMENT

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Table 1 : Mothers description of tongue tie

Description	Number	Percent
Growth/fleshy mass under the tongue	70	28.0
Rope under the tongue tying it down	20	8.0
Something that prevents children from talking	42	16.8
Something that prevents children from lifting up/moving the tongue	48	19.2
Something that prevents children from crying well	28	11.2
Non response	42	16.8
Total	250	100.0

Table 2 : Sources of knowledge on tongue tie

Source	Number	Percent
Child's grand mother	90	36.0
Other relatives	44	17.6
Nurse	70	28.0
Doctor	10	4.0
Other health workers	17	6.8
Others e.g. other mothers, friends etc.	19	7.6
Total	250	100.0

Table 3 : Adverse effects of tongue tie on babies as perceived by mothers

Adverse effect	Number	Percent
Speech difficulties	118	47.2
Inability to cry well	68	27.2
No effects	24	9.6
Difficulty breast feeding	6	2.4
Poor hearing	24	1.6
Don't know	42	16.8

Table 4 : Tradocultural beliefs/perceptions about tongue ties

Beliefs	Number	Percent
All babies have tongue tie and should have it cut	122	48.8
Tongue tie prevents normal speech development	96	38.4
Don't know	22	8.8
Others- causes deafness, stammering, poor feeding	10	4.0
Total	250	100

Table 5 : Mothers perception of age for treatment and who should treat tongue tie

Age	Number	Percent
≤ 1 month	154	61.6
1-2 Months	16	6.4
>2 months - 1 year	20	8.0
> 1 year	10	4.0
Treatment not required	16	6.4
Don't know	34	13.6
Total	250	100
Who should treat tongue tie?		
Doctor	124	49.6
Nurse	80	32.0
Traditional birth attendant	16	6.4
Others e.g. grandmother, other health workers	10	4.0
Non response	20	8.0
Total	250	100

Table 6 : Personnel diagnosing tongue tie and associated problems

Diagnosis made by	Number	Percent
Nurse	38	41.3
Doctor	6	6.5
Other health workers	6	6.5
Grandmother	6	6.5
Other relatives	16	17.4
Self (mother)	20	21.7
Total	92	100
Associated problems		
Could not cry well	60	65.2
Difficulty sucking at breast	12	13.0
Inability to lift up the tongue	4	4.4
No problems	16	17.4
Total	92	100

Table 7 : Shows place and mode of treatment of tongue tie

Place of treatment	Number	Percent
Hospital	34	37.0
Health centre	24	26.1
Home	20	21.7
TBA	14	15.2
Total	92	100
Mode of treatment		
Cut with an instrument	64	69.6
Cut with fingernail	26	28.2
Cut by applying pressure using cotton wool	2	2.2
Total	92	100

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Romania and the Crisis in the Health System. Migration of Doctors

By Petronela Daniela Feraru

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Abstract - Migration of doctors in Romania still continues today producing a range of long-term social, economic and demographic effects. Current data on the migration of these specialists from our country shows a negative trend, therefore according to the figures offered by the College of Physicians in Romania, only since 2007 10,000 doctors have chosen to work in the West, and 400 submit applications monthly to the Ministry of Health.

This paper brings to the attention issues related to the scale of the Romanian medical migration. For this purpose, we used different statistical data provided by specialized institutions in the country and abroad, information provided by the media, the results of studies published in the field. The goal is a better understanding of the dimensions of the phenomenon, for which the analysis is completed by means of the interactionist and interpretative method according the constructivist perspective. The results obtained in this study consist of the disclosure of certain current issues followed by the presentation of social effects and social, economic and demographic dimensions of the Romanian medical system from the perspective of medical migration

Keywords : *medical migration, brain drain, sanitation system, human resources, performance.*

GJMR-K Classification : *NLMC Code: WX 140, WY 108*



ROMANIA AND THE CRISIS IN THE HEALTH SYSTEM. MIGRATION OF DOCTORS

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I. THE HEALTH SYSTEM IN ROMANIA IN TERMS OF HUMAN RESOURCES MANAGEMENT

The main problems identified by the Ministry of Health of Romania in the public health system are human resources, finance and organizational details. An effective health system reform involves changing some aspects related to employment, working conditions, degree of decentralization of management skills, salary system and staff motivation. The issue of budgetary constraints may not be solved immediately without finding potential solutions for reorganizing the system, without solving the shortage of health professionals and without efficiency in the procurement field. The role of human resources in the health system is crucial. The quality performance in the field depends on the human resources management. It must comprise aspects mainly related to the development of a coherent training, the development and human resource allocation policy, the increase in the number of medical personnel and career development in the medical field.

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Poor management of the health system, political duels and the unsuccessful health system reform increases the distrust of young doctors in their professional future in Romania (Manea, 2011). Thus, we can see a major shortage of health workers in many counties due to the migration of doctors and of specialized health professionals. External migration of Romanian doctors is likely to increase even more the overall shortage of doctors in the regions and in some specialties, which are very popular in the European Union (anesthesia, intensive care, surgery, family doctors). Brain drain from Romania, including of the medical staff, is a growing phenomenon, as nationally no decisions are taken to reduce the phenomenon, while developed countries currently use different economic, budgetary and tax strategies in order to attract specialists from all fields coming from economically less developed countries. Ethical procedures of recruitment of health professionals should work as a factor protecting medical resources in developing countries where human resource is deficient (Cehan and Manea 2012:100-109, Dornescu 2012).

According to the *Dictionary of Sociology* migration is a phenomenon that consists in the movement of crowds of people from one area to another, followed by the change of residence and / or by employment in a field of activity in the arrival area (Vlăsceanu and Zamfir, www.dictsociologie.netfirms.com). If we look at the phenomenon with reference to a given population – a perspective mainly adopted by demography – as compared to this population, we can speak of two forms of migration: immigration (all inputs) and emigration (all outputs). In fact, any act of migration is at the same time, migration and emigration.

The term "brain drain" is the loss of skilled labor force intellectually and technically qualified through the movement of the labor force to more favorable media from a geographic, economic, or professional perspective (<http://www.answers.com/>).

The performance concept involves getting a great result. This result can be seen particularly in terms of three dimensions (Verboncu and Zalman, 2005, p 6): a result superior to the previously obtained results, a superior result compared with the achievements of others, a result superior to the objectives set and assumed. We can find numerous writings on the importance of human resources in public health

organizations. Experts in the field believe that of all health resources, human resources are the most important player. In the health sector, the role that the personnel has is more important than in case of other sectors. The improvement of the performance of human resource in the health sector is a goal pursued by all developed or developing countries (Pupăză, <http://www.utgjiu.ro>).

a) Migration of Romanian doctors: a motivated act

Romanian doctors want social recognition, professional achievement and respect from society. It is surprising that even patients show their dissatisfaction with the level of wages of doctors, and also with the conditions in which they work. The rate of patients' confidence in doctors is high, and even higher in regard to family physicians.

Doctors are dissatisfied with their salary, number of employed staff, technical equipment and provision of medicines. In the EU, the average income of a doctor in 12 countries is EUR 12,000 per month and the minimum income of a resident in France is EUR 1200 and EUR 1800 in the UK (<http://laurbadea.wordpress.com>). For example, several communes in France are willing to spend up to EUR 40,000 for each Romanian doctor brought in the region, after experiencing a phenomenon called by the media "medical desertification": no French is willing any longer to be a doctor in the country. This is specific to the medical situation in Romania. Dornescu and Manea present in a study the high level of polarization of doctors in urban areas (Dornescu and Manea, 2013, p.122). Thus, statistics are presented (NIS 2012 <http://www.insse.ro>) showing that in 2011, for example, the number of doctors in urban areas was 46 949 (89%) and that of doctors in rural areas was 5592 physicians (11%), which highlights the serious regional imbalances. Thus, the number of inhabitants per one rural doctor is now more than 6 times higher than in urban areas, and 100 towns in the country have no doctor available. The explanation is that urban areas - particularly university centers - absorb doctors from surrounding or rural areas, making them even more disadvantaged. A growing number of medical specialists prefer to work as representatives of drug companies, go abroad or choose to work in other fields than choose rural areas. In other words, in terms of the degree of coverage with medical personnel, Romania is facing a striking situation due to existing regional inequalities in terms of coverage and provision with medical personnel. Following recent analysis it was found that there is a shortage of doctors in remote rural areas where living and working conditions are difficult.

The degree of professional satisfaction of physicians depends on the conditions of their work (facilities, adequate labor protection equipment). Many doctors in Romania work in harsh conditions,

sometimes they do not even have the medicines or the supplies needed to administer appropriate treatment to patients. To this we can add the poor financing of the public health system, the conditions of stress, the strain at work, the lack of recognition and respect for the importance of the work they perform and the corruption in the system, which motivate emigration. Based on the degree of dissatisfaction of the medical staff in Romania we are dealing today with a challenge, namely the migration of labor force from the health sector. And all this despite the fact that Romania has a third less health personnel per one thousand people compared to the EU average. According to the CPR President Vasile Astarastoae' estimates, today we have more than 20,000 Romanian doctors who save lives abroad, even if the Romanian state paid for their training almost 230 million euros, while those remaining in the country are poorly paid, the responsibility belonging to the Romanian state, which drives them away (<http://www.ziaruldeiasi.ro>). Astarastoae said that according to statistics, the Romanian state spends with a medical student about RON 5,000 per year, and during his/her residency it pays almost RON 21,000. It follows that for the training of a physician for a period of six years the Romanian state spends RON 30,000, and for specialization, RON 21,000, approximately EUR 11,300 overall. The crisis accentuated the emigration rate, so that in six years over 20,000 specialists have left. A simple calculation shows that this time the state lost over 226 million euros.

i. Motivational factors in the emigration of Romanian doctors

The reasons given by doctors who choose to emigrate from Romania are wages, working conditions, promotion, facilities offered by the organization that provides the necessary comfort, socialization opportunities, public recognition of the value of their work. Satisfaction is based on other factors as stated by Abrudan (2010, p.51): satisfaction is achieved when there is a balance between whatever the employee brings to the organization (diplomas, professional experience) and whatever the organization offers (salary, position, level of complexity). Economic motivation represents the decisive factor in the choice of the medical staff of leaving abroad. In Romania, the average salary of a doctor is EUR 500-600 per month and a resident doctor earns between EUR 170 and EUR 340 per month, while abroad we can talk about salaries starting from EUR 2000-3000 per month for resident physicians and wages over EUR 5000 for medical specialists.

Another important motivation is the one related to poor working conditions in hospitals that hinder the work of doctors to which we can add the lack of materials, the technological deficit, the old utensils and appliances. Romanian doctors in our country are

working on average 5-6 times more in terms of quantity and difficulty than the European average. Although Romania is facing a shortage of medical personnel, very few positions are declared vacant where staff is needed. Although we have a shortage of doctors, Romania specializes a small number of the total number of graduate students from medical faculties (48% of graduates).

Doctors who left Romania did not do it out of pleasure or because they have had enough of their country, but because they were "tired of how things worked." Another reason why doctors leave is the pressure and blaming of the medical staff in Romania. "In Romania there were during the communist period attacks on the medical personnel, but not in such a manner as it began to exist since January 1, 2013. This has to do with Romanian society's reaction and therefore Romania is ill in this regard as well" said the President of CPR (<http://www.ziare.com>).

Also, the level of resources allocated to health care in Romania is low and affects the quality of medical act as it is a decision factor of the doctors' migration. Unfortunately, the health system is chronically underfunded, there is an inconsistency, inequality and lack of fairness in healthcare, Romania still allocating the least amount of GDP on health, less than 3%, while the EU allocates between 5-6% of GDP.

ii. *The Romanian health system financing*

The resources allocated to health care in Romania is low and it requires finding solutions to increase the level of public health funding because the reduced level of public resources allocated to health directly affects the quality of the medical act and determines more and more Romanian doctors to emigrate. Dornescu and Manea pursue the funding level of the health system by means of the analysis of two important indicators: *total expenditure on health and public expenditure on health / capita* (Dornescu and Manea, 2013, pp.123-126).

Total expenditure on health, expressed as a percentage of GDP in 2000 and 2007 were in Romania at a level of 5.2 percent, the lowest in the European Union, while the average for the 27 countries of EU has experienced much higher values and increasing values, respectively 8.4 percent in 2000 and 8.8 percent in 2007. According to estimates made by the World Health Organization WHO (2010) the highest values of total expenditure on health share of GDP were recorded in this period in Germany (10.3 percent), France (10.1 percent), Austria (9.9 percent), Belgium (9.1 percent), Sweden (8.2 percent). (See Figure 1).

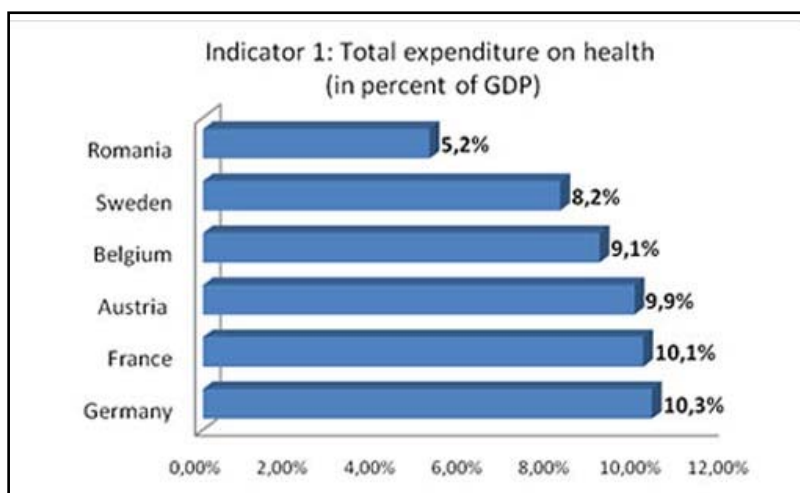


Figure 1 : Total expenditure on health (in percent of GDP)

Source : According to WHO, 2010, Statistiques Sanitaires Mondiales 2010, available online at http://www.who.int/whosis/whostat/FR_WHS10_Full.pdf.

Public expenditure on health / capita in the same period was also the lowest in comparison with EU countries (\$ 202 / capita, \$ 475 / capita respectively), while the average in the European region was \$ 901 /capita, \$ 1.401 / capita, respectively. Much higher levels and increasing levels were recorded in countries such as Luxembourg (\$ 2.800/ capita., \$ 5.212 / capita respectively), Austria (\$ 2.169 / capita, \$ 2.875 / capita respectively), Germany (\$ 2.128 / capita, \$ 2.758 / capita respectively), France (\$ 2.076 / capita, \$ 2.930 / capita

respectively), Malta (\$ 2.104 / capita, \$ 3.140 / capita respectively), Denmark (\$ 1.960 / capita, \$ 2.968 / capita respectively), Sweden (\$ 1.938 / capita, \$ 2.716 / capita respectively.) (WHO 2010). (See Figure 2).

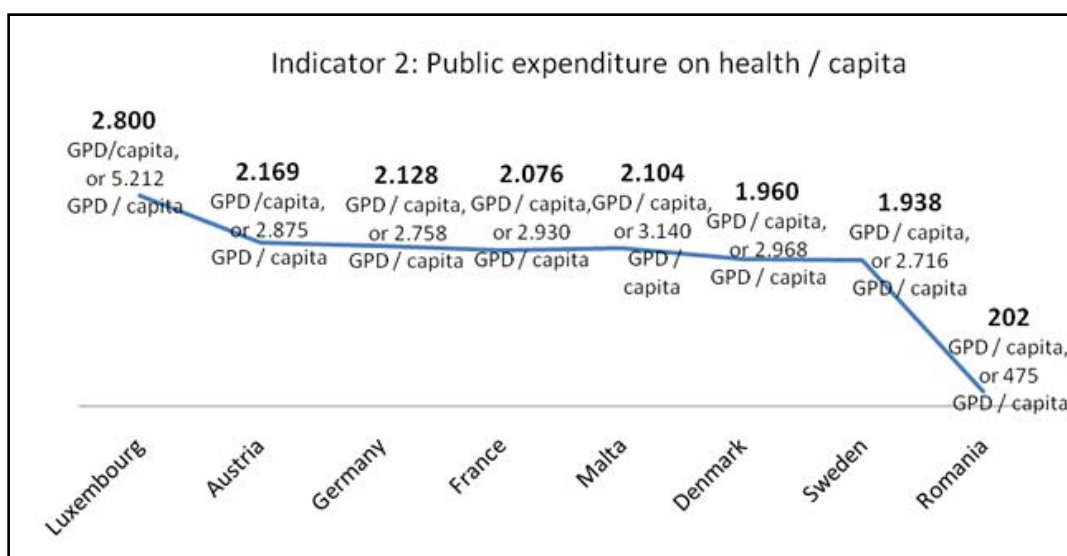


Figure 2 : Public expenditure on health / capita (in percent of GDP)

Source : According to WHO 2010, Statistiques Sanitaires Mondiales 2010, available online at http://www.who.int/whosis/whostat/FR_WHS10_Full.pdf.

Although there are economic differences between EU countries, the data presented as percentages of the gross domestic product shows the percentage of GDP allocated by each government to expenditure on health. These figures show how much the government cares to ensure the right to health of its citizens.

The financing degree of the health system directly affects the job satisfaction of physicians. The remuneration level of the medical personnel in Romania can be considered a "push" factor of migration, valid not only for physicians but also for the other categories of the medical staff. The degree of professional satisfaction of physicians in terms of income earned depends on the level of economic development of the country and can be determined by comparing the individual gross income of physicians to the average level of wages on overall economy.

II. MIGRATION OF ROMANIAN DOCTORS: SOLUTION OR PROBLEM FOR ROMANIA?

We included issues related to the migration of experts, including of physicians in the research conducted for the PhD thesis presented in 2011 which contains a qualitative case study and two integrated quantitative studies conducted in Italy in 2010. Thus, we have the research results with a sample of over 30 interviews and over 120 questionnaires conducted in groups of subjects according to the research objectives (Feraru, 2011). The case study is published, but without the two integrated studies in a volume that bears its name and contains aspects related to both approaches that refer to the stages of the migration process and to the levels of related analysis (Feraru, 2011b). Thus, the analysis of the research data showed that more than

50% of those surveyed were determined in their actual migration by objective economic factors out of the wish to earn more in Italy. Men were found to be more motivated than women professionally and educationally and aspired more towards the profession for which they were trained in Romania. Women can be regarded as more likely to accept a profession non-compliant with their training in Romania.

Social development can be enhanced by developing motivations for success and prospective motivations and by removing the dependency motivations. The development and evolution of social reality assumes first of all an intervention through programs of selection and guidance of the motivation of the individuals who make up that reality. The motivation for success and the prospective motivation have a positive role and the dependency motivations have a negative role. According to data obtained from the field, most interviewees said to be favored by motivations for success, being active people, passionate by their profession and excelling in the field, others are favored by prospective motivations being primarily concerned with the care for the common welfare of all, with the expansion of the self to the whole society or with altruism. Last were the interviewees underprivileged by dependency motivations, being people who feel the need for protection, help, support.

Professional dimension, as an important part of the integration of migrants at destination, is often considered in terms of satisfaction or dissatisfaction with life and work, as compared to the position held in Romania until departure from the country: "... I had worked as a nurse in Romania for three years of which the last year I was a chief nurse in an Emergency University Hospital from Cluj ... It depends on

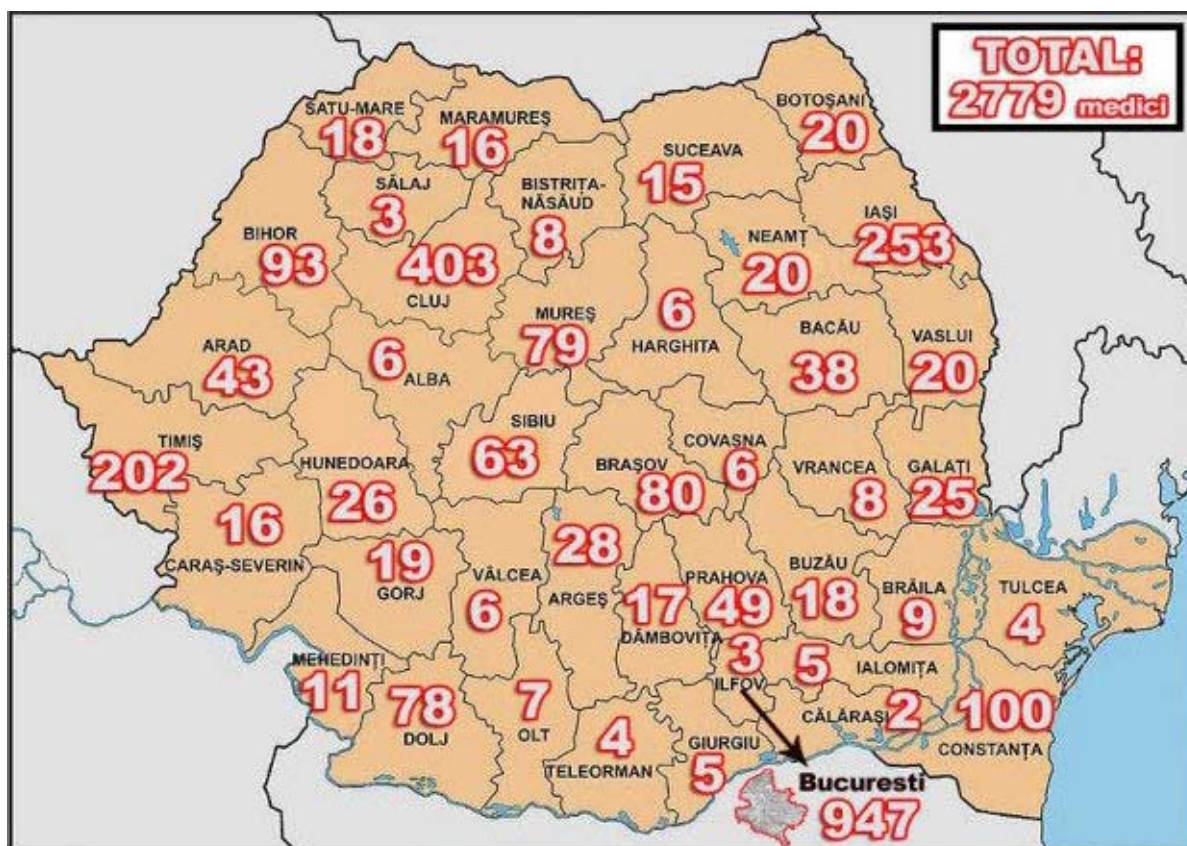
motivation, it depends on training. My chances as a nurse are higher than those of a young man of 20 who had graduated from high school and has other studies, that's the reality here and all over the world, my chances are higher ... Romanian nurses are trained, and anyway, the systems are very different, when you get to work here in a short time you are shocked by the differences, starting from patients and ending with the most complicated operations performed, operations that you cannot imagine in Romania, this is the turth, everything is different ... it is a very well developed medical system, here the patient is protected, here you must see the patient and give him everything ... If I were to return home I could not work in health care for the patient in Romania is not respected, I would probably do something else, but not in medicine, because the Romanian healthcare system doesn't work although the Romanian medical school is very good, the possibilities are great, doctors are very well-trained, but the system does not work effectively". (I.G., 33, a nurse at the Rivoli Hospital in Turin, for 5 years in Italy).

a) *Evolution of the migration of doctors since 1989 to the present*

According to data provided by the College of Physicians of Romania, in 1989 Romania had 56,000

physicians with the right to practice, in 2013 their number is 39,896. In the first three months of 2013, 580 doctors left and other 250 requested documents to go abroad in the first two weeks of April, claiming that this wave of departures recorded in 2013 can only be compared with that of 2007 after Romania joined the European Union.

According to data held by the College of Physicians, the large number of departures of Romanian doctors abroad is worrying, if we relate it to the number of the ones employed in the national health system. The number of employees in the health system was, on average, in recent years of approximately. 50,000 (52,541 doctors in 2011, of whom 41 171 doctors in the public sector (National Institute of Statistics 2011). Greates departures of doctors in Romania were from the academic centers of the country (from Bucharest 947, 403 Cluj, Iasi 253, Timis 202). (See Figure 1).



The most common destination countries were France (over 100 doctors left), United Kingdom (over 900) Germany (400), Italy and Norway (with 3 doctors

left), the Netherlands, Sweden and Finland (one doctor) Belgium (over 80) and Cyprus (2 doctors). (See Figure 2).



Figure 2 : The most common destination countries preferred by Romanian doctors

Source: <http://stirileprotv.ro/>

It is noted also that the specializations most required by foreign employers were that of general medicine, family medicine, general surgery, anesthesia and intensive care. According to statistics (College of Physicians in Romania 2012), the number of doctors who had left so far is over 20,000, of whom 10,000 doctors have left before Romania's accession to the European Union and over 10,000 doctors left in the period 2007-2011. (See Table 1).

Table 1 : The number of doctors who left Romania in 2007-2011

Year	No. of people
2007	2.200
2008	1.252
2009	1.900
2010	2.779
2011	2.841

Source: Medical College Romania, available at: <http://www.cmr.ro>

Some pessimistic scenarios lead to the idea that if the current migration balance is preserved, in 2021 there will be no doctors in Romania (News.ro 2011). To this we add the demographic factor, Romania's population fell dramatically after 1989, namely with about 3 million people, due to the influence of three important factors, namely: external migration, increase of mortality rates and birthrates rebound, which is below the mortality rate. We can also see a decrease in the number of young people, which further narrowed the age pyramid (National Institute of Statistics 2011).

Westward migration of doctors has become a phenomenon in Romania. Studies in the field and

predictions made by experts show that if Romania does nothing to keep doctors in the country, if migration and the number of pensioners are maintained at the pace of the last two years, over only 11 years, our country will be left without doctors. The trends of Romanian migration in recent years show a feminization and an aging of migrants which are highest in case of people aged between 26 and 40 years.

These developments are also reflected at the level of the medical staff which faces a phenomenon of aging.

III. CONCLUSIONS

Migration of doctors has been discussed ever since 2008 at the Assembly of professional medical organizations in Central and South-Eastern Europe (ZEVA) and raises very serious concerns. At present, all countries of the world face the migration of doctors, whether it is their departure from rural to urban areas, from their country to foreign lands, or migration to other professions.

Thus, the present paper attempts to attract attention on the extent and implications of the phenomenon of migration of doctors in Romania. The reasons for migration are always the same: conditions of work, better living and the financial aspect.

Along with the human dimension related to the human resources of the health system in Romania, an important role is played by the economic dimension. The migration of doctors has important economic connotations because it represents a loss to the country of origin, namely the cost of human capital training. This

loss consists of two components, namely "the cost of training" and "the cost of specialization."

In Romania, for example, annual expenditure on training a medical student is currently about RON 8,000 and for one year of residency the Romanian states spends about RON 21.000. It follows that the training and specialization of a physician for a period of 6-11 years (6 years of faculty and 3-5 years of residency) the Romanian state spends about RON 70,000 (about EUR 20,000). If over 20,000 physicians have left Romania so far, this means that the Romanian state lost more than EUR 400 million (Dornescu and Manea, 2013, p.132). The crisis accentuated the emigration rate, so that in six years more than 20,000 professionals have left and their number is growing.

The figures showing the performance of health systems in different European Union countries determined the Presidential Commission for the analysis and development of public health policies, established by decision of the President of Romania, to develop a public health policy proposal that can be used as a starting point in improving the functioning of the Romanian health system. However, the results fail to appear and the capacity of the Ministry of Public Health to engage in activities able to determine other sectors to comply with the health status of the population and with a healthy life environment is further reduced.

What kind of solutions can be applied to stop the phenomenon of migration of doctors? *Firstly*, regulations on working time and family situation of doctors can be developed. Here, the state can propose strategies for doctors who move from one area of the country to another in terms of education, mobility, spare time of the doctor and of his/her family. Not only money matters! *Secondly*, a strategy regarding praxis is required. In the past there was the doctor and his/her medical office and now we must consider the relationship doctor – praxis group, namely the association of several specialists in a medical office. This requires high costs, but the investment in health is the best investment for any country.

The entire documentation on the migration of doctors phenomenon, has revealed that there is no European country whose healthcare system can be considered an example for other countries. Advantages and disadvantages can be found everywhere although some countries have managed to make progress as compared to others. For example, there are problems in rural areas everywhere across Europe. If the rich countries in Europe do not to solve the problems so that they can keep their doctors, poor countries of the world will pay a price for this. Thus, both the patient and the doctor will do the same: will leave the system that does not meet their expectations.

A negative trend of the kind established in recent years becomes a factor that stimulates the

exodus of doctors and aggravates the already critical situation of the health system in Romania.

If we look at the figures we find that brain drain from Romania, including of the medical staff, is a phenomenon that cannot be stopped, which is why doctors say that migration is selective, controlled and used for a national interest.

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Objectivity, Reliability and Validity of the 90° Push-Ups Test Protocol Among Male and Female Students

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Abstract - This study was conducted to determine the objectivity, reliability and validity of the 90° push-ups test protocol among male and female students of Sports Science Program, Faculty of Sports Science and Coaching Sultan Idris Education University. Samples (n = 300), consisted of males (n = 168) and females (n = 132) students were randomly selected for this study. Researchers tested the 90° push-ups on the sample twice in a single trial, test and re-test protocol in the bench press test. Pearson-Product Moment Correlation method's was used to determine the value of objectivity, reliability and validity testing. The findings showed that the 900 push-ups test protocol showed high consistency between the two testers with a value of $r = .99$. Likewise, The reliability value between test and re-test for the 90° push-ups test for the male ($r = .93$) and female ($r = .93$) students was also high. The results showed a correlation between 90° push-ups test and bench press test for boys was $r = .64$ and girls was $r = .28$. This finding indicates that the use of the 90° push-ups to test muscular strength and endurance in the upper body of males has a higher validity values than female students.

Keywords : arm and shoulder-girdle strength and endurance, strength and endurance test, upper body strength and endurance.

GJMR-K Classification : NLMC Code: QT 260



Strictly as per the compliance and regulations of:



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1. INTRODUCTION

The 90° Push-ups test are used as a measure of arm and shoulder-girdle strength and endurance. Several different forms of 90° push-ups test s are used in physical fitness tests. In the past, 90° push-ups test s for males were executed with the toes and hands on the ground (full-body push-ups test), whereas females performed modified versions, including those with the knees and hands on the ground (bent-knee push-up). These modified versions were used because females tend to score lower than males on a full-body push-ups test. Also, the bent-knee push-up test was used with boys and girls who could not execute a full-body push-ups test. Presently, however, fitness tests like FITNESSGRAM® (Cooper Institute for Aerobics Research, 2007) have a 90° push-ups test for both boys

and girls in which the toes and hands are on the ground while the participant lowers the body to the ground until the arms are at a 90° angle. Although the FITNESSGRAM® are mainly designed for testing children and youth, they are used for testing young healthy adults such as college students in fitness programs.

The terms upper body strength and endurance and arm and shoulder-girdle strength and endurance (ASGSE) are used interchangeably. Because the former term could be incorrectly interpreted as from the waist up, the authors use the latter term. This is consistent with the terms used by Baumgartner and Jackson (1995) and Safrit and Wood (1995). A test of ASGSE has been a part of physical fitness test batteries for individuals. The push-up is the recommended test for ASGSE in nationally distributed physical fitness test batteries like FITNESSGRAM (Cooper Institute for Aerobics Research, 2007). Usually men execute push-ups on the hands and toes while women and very young individuals execute push-ups on the hands and knees. With FITNESSGRAM all individuals execute push-ups on the hands and toes.

Many fitness tests include versions of the push-ups test for females that appear to be easier and more discriminating among levels of arm and shoulder girdle strength and endurance for females than the male 90° push-ups test version. For example, in the past the Physical Fitness Index test required females to perform push-ups test s with the feet on the floor and the hands on a bench 13 in. high, touching the chest to the bench (Clarke, 1967; Mathews, 1978). The California Physical Performance test (Clarke, 1967), the Indiana Physical Fitness test (Clarke, 1967), and the Division for Girls' and Women's Sports test (Mathews, 1978) were several tests that included the bent-knee push-up for females. Safrit and Wood (1995) suggested using the bent-knee push-up for females examinees, stating that the full-body 90° push-ups test may not be a discriminating measure for some groups because some examinees cannot execute one execution. Tritschler (2000) suggested that girls and women perform a bent-knee push-up, whereas boys and men perform a full-body 90° push-ups test. The University of Massachusetts (2002) also includes a bent-knee push-up for women

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participants in the school's health and fitness assessments.

Recently, some programs like FITNESSGRAM® (Cooper Institute for Aerobics Research, 2007) required the 90° push-up for both males and females. Based on the FITNESSGRAM® standards, most females performing this 90° push-ups test could complete at least one. In the FITNESSGRAM®, intraclass reliability coefficients ranging from $r = .64$ to $.96$ were reported for 90° push-ups test scores of females (Cooper Institute for Aerobics Research, 2007); however, the reliability coefficient of $r = .96$ was obtained using bent-knee push-up scores. McManis, Baumgartner, and Wuest (2000) found low objectivity and reliability for both males and females in the ages from elementary school to college for 90° push-up scores. They also found that it was difficult to discern correct and incorrect forms with this 90° push-ups test, and low strength college-age women had difficulties performing the 90° push-up correctly. Based on these findings, they stated that a modified version of the 90° push-up might be needed for adequate measurement of low strength individuals. In light of these findings, Baumgartner, Oh, Chung, and Hales (2002) developed a 90° push-up test, defining the down position as the body from the chest to the knees contacts the floor. This 90° push-ups test protocol is a full-body push-ups test. Strong validity evidence was also found with correlations between revised 90° push-ups test and bench press scores of $.80$ for women and $.87$ for men. Despite this strong evidence, many women had scores of zero on this 90° push-ups test.

The developers of the FITNESSGRAM® (Cooper Institute for Aerobics Research, 2007) reported in their literature review that the push-ups test scores for college-aged women had good reliability but the push-ups test were done with the knees on the floor. The developers also found that, for youth taking the 90° push-up test in the FITNESSGRAM®, 5% of both boys and girls over 8 years of age and 10% of both boys and girls ages 6 to 8 years completed zero 90° push-ups. These zero scores on the 90° push-ups test may indicate that the 90° push-ups test on the hands and toes is too difficult for females to perform. Due to the difficulty of the 90° push-up and modified push-up tests for some participants, especially females, the tests may lack the discrimination needed in a fitness test. The bent-knee push-up may be easier than the full-body push-ups test for females to perform. McManis et al., (2000) found that low strength females had difficulties performing the 90° push-up correctly, but were more successful performing bent-knee push-ups as an exercise in their class.

Objectivity, reliability, and validity must be acceptable for a test to be considered a good test. Baumgartner, Jackson, Mahar, and Rowe (2003) stated that, to have validity, a test must have reliability; however, to have reliability a test must first have

objectivity. Validity exists if the interpretation of the test scores is correct (Baumgartner, Strong, Hensley, 2002). Validity evidence can be obtained by the criterion approach, determining the correlation between test scores and scores for a criterion or standard measure of the attribute being studied (Baumgartner et al., 2003). Baumgartner et al., (2003) defined reliability as the consistency of test scores; a test has objectivity if the scores are not dependent on who administered the test. At least two scores for each person being tested must be gathered to provide evidence of reliability or objectivity. These two scores can be collected from two different scorers, on two different trials in one day, or on two different days (Baumgartner et al., 2003).

For the measurement of arm and shoulder-girdle strength and endurance, a standard measure or criterion is the bench press. Baumgartner, Oh., et al., (2002) used the bench press as the criterion to estimate the validity for the 90° push-ups test. Jackson, Fromme, Plitt, and Mercer (1994) and Pate, Burgess, Woods, Ross, and Baumgartner (1993) also used the bench press to provide evidence of criterion validity for the 90° push-ups test. Because the 90° push-ups test requires moving a person's body weight up and down, the bench press should also require moving a percentage of a person's body weight. Baumgartner, Oh., et al., (2002) used 70% of body weight for men and 40% of body weight for women for the bench press test. Each person executed as many repetitions as possible for the bench press. The score of a participant was the number of bench presses continuously executed correctly before the participant stopped due to fatigue or before body position was changed.

Based on the findings of Baumgartner, Oh., et al., (2002) there is good objectivity, reliability, and validity for the 90° push-ups test; however, some women could not execute one 90° push-ups test. Possibly the bent-knee push-up test should be the alternative to the 90° push-ups test for college-age women. The purpose of this study was to determine the objectivity, reliability, and validity the 90° push-ups test protocol among male and female students of Sports Science Program and to determine the relationship between 90° push-ups test and bent press scores.

II. METHOD

Since objectivity is necessary for reliability, the objectivity study was conducted to determine if objectivity for the 90° push-up test protocol was high enough to warrant conducting a reliability study. Further, reliability is necessary for validity so a reliability study was conducted to determine if reliability for the revised push-up test protocol was high enough to justify conducting a validity study.

a) Participants

Data were collected from samples ($n = 300$), consisted of males ($n = 168$) and females ($n = 132$) students of Sports Science Program. Students were randomly selected for this study. Students volunteered to participate in the research study. They were accustomed to executing 90° push-ups and bench press. Most of the data were collected before classes began each day.

b) Procedures

During the initial meeting with the participants, all of the participants signed an informed consent form, were told the purpose of the study, and were familiarized with tests they would be performing. The participants were tested a total of 2 days. The first day, all participants were weighed and performed 90° push-ups. The 90° push-up required the person being tested to lie face down on the floor with the hands placed under the shoulders, fingers pointed forward, and elbows pointed backward along the sides of the body.

The person pushed up to full arm extension so the body weight was resting on the hands and toes; this was the up position. Then, keeping the body straight, the participant lowered herself until all of the body from the chest to the thighs touched the floor. The participant then pushed up to full arm extension, back to the up position. These down and up steps counted as one 90° push-ups test. The person being tested continued these steps at a comfortable rate with no rest until fatigued. One 90° push-ups test was counted when the participant started at the up position, went to the down position, and then returned to the up position. The score was the number of 90° push-ups test s executed correctly before stopping or before body position was changed (Clarke, 1967). Two raters, trained in the methods to be used, tested the participants for objectivity purposes. Both raters independently scored the number of 90° push-ups test s performed correctly. The two raters were used in only half of the classes. These classes were randomly selected.

On the 2nd day of testing, all the participants performed a bench press test. The bench press test protocol was based on lifting used 70% of body weight for men and 40% of body weight for women for the maximum number of repetitions possible (Baumgartner, Oh, et al., 2002). The participant's hands were placed on the bar approximately shoulder length apart. The participant pushed the bar up to full arm extension. This was the starting position. The bar was then lowered with a controlled motion to the chest. One press was counted once the bar was raised back up to the starting position. The score of a participant was the number of full presses continuously executed with correct form before the participant stopped due to fatigue or body position was changed. An attempt to make up for a missed test was made after each testing day.

c) Data Analysis

Objectivity for the 90°push-ups test scores was estimated for the score of one rater (in the future, one rater scored the test) using an intra class correlation coefficient (R) based on Pearson Product Moment model as presented by Baumgartner et al. (2003). Reliability for the 90° push-ups test scores was estimated for a score collected on one day by one rater (in the future, one rater scored the test on one day) using on intra class correlation coefficient (R) based on Pearson Product Moment model as presented by Baumgartner et al. (2003). For criterion validity, a Pearson correlation coefficient was calculated to determine the relationship between the 90° push-ups and bench press scores. Also, a Pearson correlation coefficient was calculated to determine the relationship between 90° push-up and bench press. The SPSS version 14.5 package of statistical computer programs was used to do all the calculations.

d) Results

A total of 300 participants were included in this study were tested with both the 90° push-ups test and bench press test. All participants were used in the objectivity of the 90° push-ups test scores, reliability of the 90° push-ups test scores, and criterion validity of the 90° push-ups test portions of the study. Descriptive information for the scores on the 90° push-ups test and bench press tests are presented in Table 1.

There were 132 women and 168 male who completed 90° push-ups tests on the 1st day when two raters were present. Presented in Table 1 is descriptive information concerning the scores for raters A and B. The score for a person varied only by one 90° push-ups test when variations occurred between the two raters. Participants (male = 84, female = 66) had 90° push-ups test s scores for both Day 1 and Day 2. The same rater tested the participants both days. One hundred and sixty eight male and one hundred and thirty two female performed the 90° push-ups test and bench press tests. For descriptive information on this group, see Table 1 and 2. The same rater administered both tests. The intra class correlation coefficient using the scores of the two raters and estimating the objectivity for the score of one rater (Baumgartner et al., 2003) was $r = .99$ (male) and $r = 0.98$ (female).



Table 1 : Descriptive Statistics for the 90° push-ups test and Bench Press Scores of the Participants in the Study (Male)

Variable	N	M	SD	Minimum	Maximum
90° push-ups test Rater A	84	36.42	4.05	26	45
90° push-ups test Rater B	84	36.50	3.96	28	44
Overall 90° push-ups test	168	36.93	3.469	26	45
Bench Press	168	7.75	1.87	1	11

Table 2 : Descriptive Statistics for the 90° push-ups test and Bench Press Scores of the Participants in the Study (Female)

Variable	N	M	SD	Minimum	Maximum
90° push-ups test Rater A	66	22.14	2.76	17	32
90° push-ups test Rater B	66	21.70	3.34	16	34
Overall 90° push-ups test	132	22.58	2.58	18	34
Bench Press	132	3.85	0.85	1	6

This correlation coefficient suggests high objectivity between raters. Ninety percent confidence limits for the correlation coefficient are presented in

Table 3. The confidence limits values are high and quite close together.

Table 3 : Ninety Percent Confidence Limits for the Intra class and Pearson Correlation Coefficients Calculated as Objectivity, Reliability, and Validity Evidence

Evidence Use	R (Male)	R (Female)	r (Male)	r (Female)	n (Male)	n (Female)
Objectivity	0.99	0.98	-	-	84	66
Reliability	0.93	0.93	-	-	84	66
Validity (90° push-ups test)	-	-	0.64	0.28	168	132

The intra class correlation coefficient using the scores from two days and estimating the stability reliability for 1 day was $r = .93$ (male) and $r = .93$ (female). This is an acceptable value for the stability reliability evidence (Baumgartner et al., 2003). The Pearson correlation coefficient between the 90° push-ups test and bench press scores was $r = .64$ (male) and $r = .28$ (female). This value is moderate criterion validity for the 90° push-ups test scores.

III. DISCUSSION

The objectivity inter scorer coefficients obtained (.98 - .99) for the 90° push-ups scores are considered very good. Baumgartner et al., (2003) stated that the inter scorer objectivity coefficient should be at least .80. The 90° push-ups test appears to be easily administered by different raters without the raters having different scores. The protocol for the 90° push-ups seems to be simple enough for the raters and the participants to follow easily. The stability reliability coefficient obtained (.93) was moderate for the 90° push-ups scores. Baumgartner et al. (2003) suggested that the stability reliability coefficient should be at least .80. Baumgartner, Oh., et al., (2002) found higher stability reliability (.90-.95) than was found in this study for the 90° push-ups test. The moderate stability reliability suggests that the scores may change slightly

between days for each participant even if the scorer remains the same. The confidence limits for a correlation coefficient are the degree of confidence the researcher has that the population value of the correlation coefficient is between the lower and upper values of the confidence limits.

The criterion validity evidence, a correlation coefficient between the 90° push-ups scores and the bench press scores, of $r = .64$ (male) was moderate and $r = .28$ (female) was below moderate. The correlation between the 90° push-ups test scores and bench press scores was .64. Baumgartner, Oh., et al., (2002) found a higher correlation ($r = .80$) than was found in this research study between the 90° push-up scores and the bench press scores for women. Differences in the correlation coefficients between their study and this study could be due to sample differences or size of the samples. Notice in Table 2 the upper limit value of the confidence limits for the validity of the 90° push-ups test is .80, the value found by Baumgartner, Oh., et al., (2002). Scores of zero were not found in this study on the 90° push-ups test. The 90° push-ups tests seem appropriate for use in fitness testing test protocol among male and female students of Sports Science Program, Faculty of Sports Science and Coaching.

In conclusion, the inter scorer objectivity for the 90° push-ups test scores in this study is very good for

male students. The stability reliability of these scores is acceptable. The validity obtained in this study for the 90° push-ups test is acceptable. Baumgartner, Oh., et al., (2002) developed the 90° push-ups test because he found good objectivity, reliability, and validity for the 90° push-ups test. In this study, the many zero scores of women do not seem to have been the cause of the high inter scorer objectivity and stability reliability values obtained because men had very few zero scores and objectivity and reliability values are high for men. The many zero scores for women may be an indication that the push-up test performed on the hands and toes is too difficult for women. Before the 90° push-up protocol was commonly used, women, children, and low strength people often performed push-ups on the hands and knees. This is easier than performing push-ups on the hands and toes.

Good inter scorer objectivity and stability reliability for Sport Science Programme students does not guarantee good inter scorer objectivity and stability reliability for other populations. Younger students may not follow test directions as well and/or make more push-up test form errors than Sport Science Programme students. This could result in many attempted 90° push-ups not being counted as correctly performed. The inter scorer objectivity and stability reliability of scores using the protocol in this study should be examined for students in Sport Science Programme. The authors recommend that this 90° push-up test protocol be utilized when evaluating ASGSE of Sport Science Programme and college-aged students. In fact, if participants follow this 90° push-up testing protocol when executing push-ups as an exercise to increase ASGSE, they will be developing the form required when taking the push-up test.

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Posterior Urethral Valve Presentation, Management and Outcome

By Mosaab Hussein Dahab & Abdalazeem Hussein Khalafallah

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Abstract - The most common cause of lower urinary tract obstruction in male infants is posterior urethral valves with an incidence about one patient in each 5000–8000 infants [1]. A better understanding of the exact cause of the congenital obstruction of the male posterior urethra, prevention of postnatal bladder and renal injury, and the development of safe methods to treat urethral obstruction prenatally (and thereby avoiding the bladder and renal damage due to obstructive uropathy) is the goals for the care of children with posterior urethral valves [2].

GJMR-K Classification : NLMC Code: QY 120



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Posterior Urethral Valve Presentation, Management and Outcome

Mosaab Hussein Dahab ^α & Abdalazeem Hussein Khalafallah ^ο

I. INTRODUCTION

The most common cause of lower urinary tract obstruction in male infants is posterior urethral valves with an incidence about one patient in each 5000–8000 infants [1]. A better understanding of the exact cause of the congenital obstruction of the male posterior urethra, prevention of postnatal bladder and renal injury, and the development of safe methods to treat urethral obstruction prenatally (and thereby avoiding the bladder and renal damage due to obstructive uropathy) is the goals for the care of children with posterior urethral valves [2].

II. OBJECTIVES

- ✓ To know the presenting symptoms and signs of the PUV.
- ✓ To know the trends of management of PUV in Alribat military hospital.
- ✓ To estimate the post operative complication.
- ✓ To evaluate the outcome of the management.

III. PATIENTS AND METHODS

This is a retrospective prospective study descriptive done in Alribat National hospital in the period from January 2010 to June 2013. The data collected by filling questionnaire from patients records, follow up charts and by direct interview. The data were analyzed using statistical package for social sciences (SPSS).

IV. RESULTS

This study included forty four patient diagnosed as having PUV by VUCG and the diagnosis confirmed by cystoscopy. Routine investigations done for all patients. Most patients presented during the first year of life (84.1%). There was wide range of presenting complains with painfull micturition was the most frequent one in 84.1%. VUR found in 31.1%, hydronephrosis in 29.5%, and abnormal RFT in 15.9%. Renal scan done for 2.3% and represented unilateral nonfunctioning kidney.

Most patients treated by primary valve ablation (93.2%) using 8 F sheath and the valve ablated using 11 F resectoscope with a hook of cold knife, and the valves incised in position 5 and 7 o'clock. Most of the patients

operated during their first year of life (68.2%). From those with abnormal RFT, 57.1% returned to normal RFT postoperatively. VUR disappear in 78.6%. Postoperative complications were septicemia, persistent ureamia, recurrent UTI, urethral stricture, and residual valve with frequency less than 7% for each. Mortality rate was 6.8%. Patients followed by VUCG, RFT, UG, and cystoscopy for few patients. Short term outcome was good in 84.8% in the sense of clinical, biochemical and radiological recovery.

V. DISCUSSION

In this study we tried to evaluate the common presentations, models of management, and the short term outcome of management of PUV. We found that most patients presented during the first year of life 29 neonates (65.9%), 8 infants (18.2%), and 7 old children (15.9%). Cass A *et al*, and Egami *et al* reported that most children with PUV present within the first year of life 50–70% of boys and 25–50% are initially seen in the neonatal period [3, 4].

There was wide range of clinical presentation of PUV as illustrated by our study with painfull micturition being the most common presenting complain in 84.1% of the patients. Lopez Pereira P (2004) and Agarwal S (1999) reported that patients of PUV may present with diurnal enuresis, infections, and severe voiding complaints, such as dribbling and retention, or hematuria [5, 6].

Lissauer D *et al* (2007) declared that VCUG is the gold standard for diagnosis of PUV [7]. In our study VUCG demonstrated a dilated posterior urethra due to obstructing membrane (PUV) and the presence of PUV is confirmed by cystoscopy, so VUCG showed 100% accuracy in diagnosing PUV. VUR internationally found in 19-72% of patients as reported by Kurth *et al* (1981)[8], while in this study VUR was found in 38.1%. Hydronephrosis found in 29.5% and this is quite different from that reported by Egami K *et al* in his series which is 90% [4]. Abnormal RFT was found in 15.9% of the patients, 57.1% of them returned to normal RFT postoperatively.

Smith GH *et al* (1996) concluded that primary valve ablation with surveillance was the preferred management for PUV. They proposed that by avoiding diversion in most cases, bladder function was preserved and the need for bladder augmentation decreased [9]. Most of our patients treated by primary valve ablation

(93.2%). Unfortunately there were no antenatal interventions because of the lack of these experiences in our country. Vesicostomy was the initial management in (6.2%) of patients with additional 4.5% treated by vesicostomy after the primary valve ablation because of persistent VUR. Most of the patients operated in their first year of life (68.2%), while the other operated in older age (31.8%).

Nijman RJM *et al* (1991) and Mayer DA *et al* reported that the percentage of complications post valve ablation is 5-25% for each [10,11]. We found our complication rate was less than 7% for each. Three patients died in the post operative period (6.8%) and this was slightly higher than the percentage reported by Connor JP (1990) [12] which is less than 5% and this difference because of lacking of full team work consisting of paediatric urologist, paediatric nephrologist, and neonatologist. VUR disappeared in 87.5%, Scott JRE reported that VUR resolves in more than 30% postoperatively [13].

The overall out come in this study was excellent. Most of the cases ended with good outcome (81.8%), reasonable outcome in (6.8%), and poor outcome in (11.4%). We found that the early the presentation of PUV, the worst the outcome. From those with poor outcome, 80% presented in the neonatal period, 20% infant. Hendren WH *et al*/reported that early presentation of PUV was viewed as a poor prognostic sign and suggestive of a severe degree of obstruction. Late presentation suggested a lesser degree of obstruction with little clinical significance [14].

We tried to evaluate the association between the presence of VUR and the outcome and we found that the Presence of VUR does not significantly affect the outcome of management. Parkhouse HF *et al* showed that presence of VUR is poor prognostic sign [15]. This difference because in our study we did not differentiate between the presence of unilateral or bilateral VUR.

VI. CONCLUSION

Most of the patients were treated by primary valve ablation and few by initial vesicostomy followed by valve ablation. Adjunctive therapy offered for those with renal insufficiency, septicemia and so. The outcome of management was good in most cases with few patients ended with poor outcome.

There is still much to be learned about PUV. There are many areas still deficient in our country. Long term follow up is not yet scheduled. This is important for long term assessment of outcome and complication of management. Also antenatal diagnosis and interventions are not well established.

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Cross Section Study of Malnutrition in Children of 1-10 Years Age Group in Urban Slums of Aligarh

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Abstract - Nutrition plays important role in development of growth and development of child. In many developing countries poor nutritional status is mainly due to illiteracy, poverty, least job opportunities etc. Poor hygiene, intestinal infection, worm infestation are another important groups leading malnutrition in India. Cross sectional study was conducted in Bhojpuri slum which is densely Muslim populated slum of Aligarh City, Uttar Pradesh on 300 children. Objective of the study was to assess the nutritional status of children below ten years of age. Measurement of height and weight was done by weighing machine and measuring scale removing shoes with minimal clothing. To measure the stunting of children enrolled in this study, height of the children measured during study was compared with expected height for age. The magnitude of stunting was decided on the basis of Water low classification and was statically analyzed using chi square test. Our study also revealed that incidence of grade III malnutrition in 7-10 year age group was highest (27.7%). Present study also concluded that prevalence of stunting among the children of Bhujpura Slum was more prevalent than wasting. It was found that out of 300 children 248 (83%) were stunted and male female proportion was 162 (65%) and 86 (34.7%) respectively. Malnutrition prevalent in these children can be attributed to their low socio economic status, poverty and early age of employment.

Keywords : malnutrition, stunning, children.

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Cross Section Study of Malnutrition in Children of 1-10 Years Age Group in Urban Slums of Aligarh

Shoaib Ahmad ^α & Paras Talat ^ο

Abstract - Nutrition plays important role in development of growth and development of child. In many developing countries poor nutritional status is mainly due to illiteracy, poverty, least job opportunities etc. Poor hygiene, intestinal infection, worm infestation are another important groups leading malnutrition in India. Cross sectional study was conducted in Bhojpuri slum which is densely Muslim populated slum of Aligarh City, Uttar Pradesh on 300 children. Objective of the study was to assess the nutritional status of children below ten years of age. Measurement of height and weight was done by weighing machine and measuring scale removing shoes with minimal clothing. To measure the stunting of children enrolled in this study, height of the children measured during study was compared with expected height for age. The magnitude of stunting was decided on the basis of Water low classification and was statically analyzed using chi square test. Our study also revealed that incidence of grade III malnutrition in 7-10 year age group was highest (27.7%). Present study also concluded that prevalence of stunting among the children of Bhojpura Slum was more prevalent than wasting. It was found that out of 300 children 248 (83%) were stunted and male female proportion was 162 (65%) and 86 (34.7%) respectively. Malnutrition prevalent in these children can be attributed to their low socio economic status, poverty and early age of employment.

Keywords : malnutrition, stunning, children.

I. INTRODUCTION

Nutrition is one of the most essential things of life and plays a crucial role in body growth, development and maintenance of health. Without adequate nutrition it is not possible to maintain health and protection of the body from ailments. Nutrition provides energy to the body which utilizes it to perform hundreds of biological and physical activities.

Though nutrition (food) is the basic need of life for human being, thousands of people are not able to get balanced or minimum food required for life across the world due to many reasons. Situation of food crisis is more serious in war torn countries like Sudan, Somalia, Rwanda, Uganda, Afghanistan, Iraq and Sri Lanka.

In many developing countries like India, Pakistan, Bangladesh, Nepal, poor nutritional status is mainly due to illiteracy, poverty, least job opportunities etc. Corruption at political and bureaucratic level is another leading cause of malnutrition in India and other Asian countries. Poor hygiene, intestinal infection, worm infestation are another important groups leading malnutrition in India.

In their several studies, National Nutritional Monitoring Bureau (India) found that prevalence of under nutrition is declining from 18-20.8% (1969-75) to <8.5% for the year 1976 onwards. This may partially be attributed to adaptation of lower standards of normal adopted after 1975¹.

Prevalence of under nutrition across the world varies from country to country. In developing countries, war torn countries and countries with political unrest and ethnic conflicts, its prevalence is high enough. In Sudan various studies have disclosed some alarming truth. Francesco Grandesso (2005) reported that in South Darfur (Sudan) 24% children younger than 5 years were acutely malnourished².

Michael J. (1988) reported that, in eastern Sudan (Tigrayan) prevalence of undernutrition among children <5 years of age was 14-50 %³. Not only in developing countries, under nutrition in various developed countries is also prevalent. In China and Malaysia 10% and 12% children less than 5 years of age were undernourished respectively. In Cambodia, Micronesia, Philippines, Vietnam and Papua New Guinea prevalence of under nutrition among children under 5 years of age were (45.3%), (48%), (30.6%), (30%) and (45%) respectively. In India several studies have been done in various states to assess the nutritional status of children aged 0-12 years. L. Jeyaseelan & M. Lakshman (1997) reported that 8.2% children aged 5 –7 years living in urban and rural areas of south India were severely undernourished⁴.

In 2005, F.A.O. reported 46.7 % wasting and 44.9% stunting in children of < 5 years age. [17]

Saiman Khalil and Zulfia Khan (2004) reported that the prevalence of wasting of boys and girls were 32.76% and 28.12% respectively and stunting was observed as 79.73% of boys and 81.8% of girls⁴. It was reported that out of 1,10,00 persons 38% male and 30% female showed vitamin-A deficiency in Bihar⁵. As

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malnutrition is still prevalent in developing countries with this previous present study was done.

II. MATERIAL AND METHODS

Cross sectional study was conducted in Bhojpura slum which is densely Muslim populated slum of Aligarh City, Uttar Pradesh. In this study 300 children (1-10 years) were selected randomly from the slum area. General information like name of the child, father's name, age, sex, religion and monthly income of their parents were recorded. Measurement of height and weight was done by weighing machine and measuring scale removing shoes with minimal clothing.. During measurement of weight and height norms of anthropometry were followed. The grading of malnutrition was done as per the recommendation of the nutrition subcommittee of pediatrics⁶.

To measure the stunting of children enrolled in this study, height of the children measured during study was compared with expected height for age. The magnitude of stunting was decided on the basis of **Water low classification**⁷. Children were also studied for different nutrition related symptoms, signs and ailments like Angular Stomatitis, Cheilitis, Diarrhea, Dermatitis, Night blindness, Pallor, edema and Respiratory tract infections. For statistical analysis, student t-test and Chi-square Test were applied.

III. RESULTS AND DISCUSSIONS

Present study was carried out in Bhojpura Slum of Aligarh City in 2006 by the department of Dietetics and Hospital Food Services, Food Craft Institute, University polytechnics, Aligarh Muslim University, Aligarh. Three hundred children of 1-10 year of age were enrolled in this study. Out of 300 children 195 (65%) were male and 105 (35%) were female and 285 (95%) were Muslim and 15 (5%) were Hindu .It revealed that Bhojpura Slum is a Muslim dominated Slum area of Aligarh. Children were divided in four age groups and it was found that maximum number of children was from 7-10 year age group while least number of children was from 3-5 year age group.

The overall occurrence of malnutrition in children of 1-10 years of age was found to be 68%. However it was found to be significantly higher (72.5 %) in the age group of 1-3 years and lower (62.1%) in the age group of 5-7 years. This age group (1-3 year) also exhibited significantly higher prevalence of grade I, and IV malnutrition .And prevalence of malnutrition was highest in age group of 7-10 in II and III grades only. Present data also revealed that prevalence of malnutrition in under five children was highest in 1-3 year age group (**table-1**). Chakraborty et al⁸ (2006) also reported higher prevalence of Protein Energy Malnutrition in the age group of 1-3 years while Saxena et al.¹⁴ (1997) reported a higher prevalence of

malnutrition in the age group of 0-1 year. It was found that males had an overall higher prevalence (69.7%) of malnutrition than females (64.8%) in our study. Saxena et al.(1976)⁹ and Srivastava (1985)¹⁰ also reported similar results as overall higher prevalence of malnutrition in males in comparison to females. While Chakraborty et al.(2006)⁸ reported contradictory results as overall higher percentage of malnutrition among females (70.6%) than in males (62.6%). However grade II and grade IV malnutrition was found to be higher in males (19.5% and 9.7 %) than in females (12.4% and 3.8% respectively) in our study. And prevalence of malnutrition was higher in females with grade I and grade III (28.5% and 20% respectively) than males (22.6% and 17.9 % respectively (see table-1).

The children were also analyzed for their height for expected age. It was found that 52 (17%) children [63.4% male and 36.5% female] were healthy while 248 (83%) children were stunted. Out of 248 stunted children, 162(65%) were male and 86 (34.7%) were female. Out of 195 male and 95 female children enrolled in this study 162 (83%) male and 86 (82%) female were stunted. It indicated that prevalence of stunting in male and female was almost equal. Age wise stunting of children was also observed and it was found that the onset of stunting was highest (87.5%) among the children of 3-5 year age group and lowest (79%) among the children of 1-3 year age group.

Magnitude of stunting was also studied and it was found that highest level of marginal stunting (19%) was prevalent in 7-10 year age group and lowest level of marginal stunting (6%) in 1-3 year age .Medium stunting was highly prevalent (33%) in children of 3-5 year age group and lowest (24%) among 7-10 year age group Similarly, severe stunting was highly (48%) prevalent in 3-5 year age group and lowest (38%) was observed in 7-10 year age group.It was also found that marginal (15%) and severe (46%) stunting were more prevalent in females in comparison to males (13% and 41.5% respectively), while medium stunting was more prevalent in males (29.7%) than females (25.7%). (**Table1**)

Prevalence of different malnutrition related diseases and symptoms & signs among the children enrolled in this study was also studied and it was found that Angular Stomatitis which is caused by vitamin B2 and/ or pyridoxine deficiency was present in 63 (21%) [35 male and 28 female] children. Cheilitis caused by vitamin B2 and/ or pyridoxine deficiency was present in 60 (20%) [34 male and 26 female] children. Diarrhea and Dermatitis caused by Nicotinic acid deficiency were present in 78 (26%) [47 male and 31 female] and 45 (15%) [29 male and 16 female] children respectively. Night blindness caused by vitamin A deficiency was present only in 25 (8.3%) [16 male and 9 female] children. Similarly Pallor mainly caused by Iron deficiency mainly was present in 95 (32%) [59 male and 36 female] children, while Edema caused by severe

protein deficiency (Kwashiorkor) was found in 26 (8.7%) [19 male and 7 female] children. And in 108 (36%) children (71 male and 37 female) respiratory tract infection was found. Respiratory tract infection is highly prevalent in overcrowded family, undernourished and immune compromised children. Saxena S.C. et al. (1997) found that 8.6% children aged 0 – 6 years in a slum area of Kanpur were undernourished⁹. Joseph B. et al. (2002) reported that wasting (31.2%) and stunting (9.4%) were more prominent among younger children of rural Karnataka¹¹. Mridula, D. et al (2004) reported that 65.5% and 48.5% children of > 5 years of Urban Slum of Varansi were wasted and stunted¹². Ehtisham et al, (2005) reported 56.39 % malnutrition among children aged 1-5 in Aligarh City¹³ which was comparable with our study.

IV. CONCLUSION

Our study concluded that prevalence of grades I, II and IV malnutrition (wasting) was highest in children of the age group 1-3 year. Male children had a higher incidence of grade II and grade IV, and Female children had a higher prevalence of grade I and grade III malnutrition (wasting). Our study also revealed that incidence of grade III malnutrition in 7-10 year age group was highest (27.7%). This might be due to work load and psychological stress because most of the children (male and female) of this age group were working in lock factories, on Dhaba and Restaurants. Our results were similar to other studies who stated that prevalence of malnutrition (wasting) was highest in the age group of 1-3 year⁸⁻¹¹.

Present study also concluded that prevalence of stunting among the children of Bhujpura Slum was more prevalent than wasting. It was found that out of 300 children 248 (83%) were stunted and male female proportion was 162 (65%) and 86 (34.7%) respectively. Prevalence of stunting in male (83%) and female (82%) was almost equal with a nominal superiority of 1% in male children. Stunting was highly prevalent (87.5%) in 3-5 year age group and it was lowest (79%) in 1-3 year age group. It was also found that highest incidence of marginal stunting (19%) was observed in 7-10 year age group while highest medium (33%) and severe (48%) stunting was observed in 3-5 year age group. Marginal stunting (15%) and severe stunting (46%) were highly prevalent in female children. And medium stunting (29.7%) was prominent in male children.

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Table 1 : Showing Prevalence of malnutrition (wasting) among the children of 1-10 years of age

Age group (Years)	Total Children	Normal	Grade-I	Grade-II	Grade-III	Grade-IV	Total
Age wise							
1-3	62	17(27.4%)	19(30.6%)	8(12.9%)	11(17.7%)	7(12.6%)	45(72.5%)
3-5	45	18(37.5%)	12(25%)	6 (12.5%)	8 (16.6%)	4 (4.8%)	30(62.5%)
5-7	82	31(37.8%)	25(30.4%)	10(12.2%)	7(8.5%)	9(10.9%)	51(62.5%)
7-10	108	30(27.8%)	18(16.6%)	27(25%)	30(27.7%)	3(2.8%)	78(72.2%)
Sexwise							
Male	195	59(32.3%)	44(22.6%)	38(19.5%)	35(17.9%)	19(9.7%)	136(69.7%)
Female	105	37(35.2%)	30(28.5%)	13(12.1%)	21(20%)	4(3.8%)	68(64.8%)
Total	300	96 (32%)	74(24.7%)	51(17%)	56(18.7%)	23(7.6%)	204(68%)

Table 2 : Showing Prevalence of malnutrition (stunting) among the children of 1-10 years of age

Age group (Years)	Total Children	Normal	Marginal	Medium	Severe	Total
Age wise						
1-3	62 (20.7%)	13 (21%)	04 (6%)	17 (27%)	28 (45%)	49 (79%)
3-5	48(16%)	06 (13%)	03 (6%)	16 (33%)	23 (48%)	42(88%)
5-7	82(27.3%)	13 (16%)	11 (13%)	26 (32%)	32 (39%)	69 (84%)
7-10	108 (36%)	20(18.5%)	21(19%)	26 (24%)	41(38%)	88 (82%)
Sexwise						
Male	195 (65%)	33 (17%)	23 (12%)	58(30%)	81 (41.5%)	162 (83%)
Female	105 (35%)	19 (18%)	16 (15%)	27 (25.7%)	43 (46%)	86 (82%)
Total	300	52 (17%)	39 (13%)	85 (28.3%)	124 (41%)	248 (83%)



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Abstract - Introduction: Occupational exposures to blood and body fluids in healthcare settings have the potential to transmit hepatitis B virus (HBV), hepatitis C virus (HCV), or human immunodeficiency Virus (HIV). Adherence to standard infection control practices is best way to prevent blood born infections in health care setting. This study tried to assess exposure management practices of HCWs and medical students to blood and body fluids (BBFs) in University of Gondar Hospital.

Methods: A cross sectional survey was conducted from September 6 to October 2, 2012, in University of Gondar hospital. Two hundred eighty five participants (including health professionals, janitors and medical students) were participated in the study. Stratified simple random sampling technique was used to select the participants. Data was collected through Self-administered questionnaire and interview using structured questionnaire.

Keywords : occupational exposure, health care workers, blood and body fluid, post exposure management.

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Managements of Exposure to Blood and Body Fluids Among Healthcare Workers and Medical Students in University of Gondar Hospital, Northwest of Ethiopia

Zelege Yimechew Nigussie ^α, Tadesse Ejigu Tafere ^σ & Gebeyaw Tiruneh Kassa ^ρ

Abstract - Introduction: Occupational exposures to blood and body fluids in healthcare settings have the potential to transmit hepatitis B virus (HBV), hepatitis C virus (HCV), or human immunodeficiency Virus (HIV). Adherence to standard infection control practices is best way to prevent blood born infections in health care setting. This study tried to assess exposure management practices of HCWs and medical students to blood and body fluids (BBFs) in University of Gondar Hospital.

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Result: The one-year prevalence of occupational exposure to BBFs at least once during the study period was 157 (62.3%). Majority, 109(71.7%) of source patient for occupational exposure were identified and 29(26.6%) of patients were positive at least one of the three viral diseases (HB, HC and HIV). In this study, only 38.2% of HCWs washed the exposed body part with soap and water after facing BBFs exposure. The 28-day mandatory post-exposure prophylaxis was used by only 12.1% of HCWs after facing BBFs exposure.

Conclusion: BBFs exposure among HCWs was a widespread occupational hazard. *Safety Precuations (SPs) were poorly applied for exposure prevention and post exposure managements to BBFs among participants of this study.* Altering the behavior of HCWs to apply Safety Precautions and an establishment of a surveillance system for registering, reporting and early management of occupational exposure are required.

Keywords : occupational exposure, health care workers, blood and body fluid, post exposure management.

1. INTRODUCTION

Occupational exposure to Blood and body fluid (BBF) to health care workers and the infectious complications associated with it is a global issue (1, 2, 3). Each day thousands of healthcare workers (HCWs), around the world, suffer accidental occupational exposures during the course of their role of caring for patients. These injuries can result in a variety of serious and distressing consequences ranging from extreme anxiety to chronic illness and premature death for the individual involved (4, 5).

HCWs in developing countries are at serious risk of infection from blood-borne pathogens particularly hepatitis B virus (HBV), hepatitis C virus (HCV) and Human Immunodeficiency Virus (HIV) because of the high prevalence of such pathogens in many poorer regions of the world, especially they are endemic in sub-Saharan Africa (3,6).

Adherence to standard infection control practices is best way to prevent blood born infections in health care setting. The exposed person shall inform an appropriate person as soon as possible after exposure so assessment and follow-up can be undertaken, an assessment of risk shall be undertaken as soon as possible after every incident of occupational exposure and Post-exposure prophylaxis, where indicated, should be prescribed as soon as possible after exposure (7).

Occupational hazards faced by healthcare personnel in University of Gondar hospital have received increasing attention but existing surveillance systems and HCWs responsiveness for Safety Precautions are inadequate to describe the scope and magnitude of occupational exposures to infectious agents that HCWs experience, the outcomes of these exposures and injuries, and the impact of preventive measures (8).

a) General Objective

- To assess exposure management practices of HCWs and medical students to blood and body fluids (BBFs) in University of Gondar Hospital.

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b) *Specific Objective*

- To assess precaution measures taken by HCWs and medical students to prevent exposure and
- To identify post exposure practices of HCWs and medical students to BBFs exposure.

II. METHODS

a) *Study Setting*

The study was conducted in University of Gondar Hospital; Gondar was one of the metropolitan cities of Ethiopia, founded in 1636 by Emperor Fasilades, 748 kms northwest from Addis Ababa.

b) *Participants*

Two hundred eighty five participants (including health professionals, janitors and medical students) of Gondar University Hospital were included in the study.

c) *Study Population*

Sampled health professionals, janitors and medical students

III. INCLUSION AND EXCLUSION CRITERIA

Health Care Workers that are assigned for clinical services, academic staffs that had regular program for patient care and graduate batch interns were included in the study.

Health Care Workers who were on leave (maternity, annual, sick, fieldwork) during the study period as well as HCWs who had not worked at least six months within the last one year, were excluded in this study.

a) *Sample Size and Sampling Technique*

Sample size was calculated using a single population proportion. Considering 20.2% of one-year prevalence of at least one BBFs exposure in previous study in Ethiopia (13), a sample size of 285 was required with sampling error of 5%, at a 5% confidence level.

A stratified sampling technique was used to select the participants using lists of monthly payroll as a sampling framework and the number of participants selected from each stratum was determined by proportional to size.

IV. STUDY VARIABLES

a) *Dependent*

Exposures of HCWs and medical students to blood and body fluids.

b) *Independent*

Age, gender, marital status, religion, occupation, work experience in the area, uses of Personal Protective equipments, Infection Prevention trainings, educational status and working shift.

V. DATA COLLECTION PROCEDURES AND QUALITY ASSURANCE

The data collection was done via self-administered questionnaire and interview using structured questionnaire. Self-administered questionnaire was used for Health professionals and interns, and data from janitors was collected by interview using the structured questionnaire.

Quality of data was assured through intensive training for data collectors and supervisors, pre-testing the questionnaire on similar setting that was not included in the study, close supervision and assistance of data collectors, checking filled questionnaires on daily basis for completeness, clarity and accuracy of data. In addition data cleaning was also made before commencement of the analysis.

VI. DATA PROCESSING AND ANALYSIS

Data was coded and entered into the SPSS version 20. Descriptive statistics was made using frequencies, tables, and figures and narrative explanations. Associations were examined using binary and multiple logistic regressions. P-value less than 0.05 was taken as a cut-off point to say significant at 95% confidence level.

a) *Ethical Considerations*

Ethical approval was obtained from the Research and Ethics Review Committee at Bahir Dar University. Written consent was also obtained from Amhara-regional health Bureau and University of Gondar hospital to conduct the study. All the study participants were informed about the purpose of the study and finally verbal consent was obtained before data collection. The respondents had the right to refuse participation or terminate their involvement at any point during the study. Information provided by each respondent was kept confidential. Furthermore, report writing did not refer a specific respondent with identifiers.

VII. RESULT

Out of the 285 selected HCWs and graduate batch interns, 252 responded giving response rate of 88.4%. Twelve incomplete questionnaires were discarded. Among the respondents, 127 (50.4%) were males. From the total participants 158 (62.7%) were health professionals, (24.6%) were housekeeping staffs, and 32 (12.7%) were graduated interns. Respondents age range from 19 to 54 years with a mean age of 29 years. Among participants in this study 113(44.5%) had less than two years of work experiences. HCWs who had experiences two to four years were 62(24.6%) and only 45(17.7%) were five years and more experienced in their current position. Other demographic information is presented in Table 1.

Among participants 137(54.4%) of HCWs reported that they were trained on infection prevention and 91(64.4%) of them whispered that the training was satisfactory for prevention of work related BBF exposure. A total of 202 (80.1%) reported that they used gloves and gown during patient care or related activities indiscriminately for all duty. Only 51(20.2%) of them said they used all necessary PPEs based on the characteristics of the procedure during work. Reason of HCWs for deficient usage of PPEs was presented in Figure 1.

The one-year prevalence of occupational exposure to BBFs at least once during the study period was 157 (62.3%). Majority, 109(71.7%) of source patient for occupational exposure were identified and 29(26.6%) of patients were positive at least one of the three viral diseases (HB, HC and HIV). Of those 24(82.8%) were HIV and 5(17.2%) were HB and HC. Only 7(6.4%) confirmed free from all the three viral diseases and 56(51.4%) were free from HIV but unknown status for HB and HC. Other, 17(15.6%) patients were refusal to be tested. There was no one who had been vaccinated or took prophylaxis against HBV among participants in this study. Post exposure practices of healthcare workers and medical students to BBFs are presented in figure 2.

Despite 157 NSI and BBF splash exposures within the last-one year, the 28-day mandatory post-exposure prophylaxis was used by no more than 19 (12.1%) HCWs and only 8 (42.1%) of them completed full course. The main reason given for non-compliance was intolerance to the side effects of the medication.

In multivariate logistic regression occupation, experience and satisfactory training on IP were also associated with BBF exposure (Table 2).

VIII. DISCUSSION

Healthcare workers have increased chance of acquiring blood borne pathogens through occupational exposure in developing countries due to a combination of increased risk and fewer safety precautions (3,9). Most exposures are caused by a departure from standard precautions (10). One of the factors for occurrences of BBFs exposure in this finding was lacks of use of PPEs (39.5%), non-consistent use of PPEs was found to be associated with chance of sustaining NSI previous studies in Ethiopia (11,12,13). Wearing gloves may reduce (>50%) the volume of blood introduced through an injury (7,10). A major reason in this finding for not using PPEs was inadequate supply (59.7%). Previous study of Ethiopia showed that Seventy-nine percent of HCWs reported that they did not wear any of the PPEs because of unavailability (14). Exacerbating the risk to health care workers in developing countries is a lack of gloves, gowns, masks, and goggles to protect them from contact with blood(3).

Study in India explored that exposure was inversely related to training, a sizeable number of those trained were subsequently exposed (15). In this study satisfactory training, but not training by itself, was highly associated with preventions of BBFs exposures. HCWs who was satisfied with trainings given was less likely exposed for BBFs than none trained and unsatisfied one (AOR=0.501, 95% CI=0.23-0.91).

In previous studies in Ethiopia and abroad, needle recapping was major causes of NSI (3,8,17,18), promisingly, it was of interest that needle recapping was not listed as a cause for accidental exposure in university of Gondar hospital, suggesting adherence to the hospital's protocol and guidelines(19).

Viral status of the source patient was unknown in 51.4% of exposures which was similar in study in Iran (50%) (18).

Of all exposure 27% involved source patient were testing positive for one blood borne viruses (HB, HC and HIV) but it was only 12% in NaSH and 15% in Iran (18,20). This difference might be due the high prevalence blood borne pathogens among patients. HBV and HCV are endemic in sub-Saharan Africa (3). In developing countries, patients are admitted with disease states that are associated with high risk, exposing workers to increased risks (21).

Among all known source patients, 7% were con-infected with HBV and HIV, 10% of HBV and HCV and 83% of HIV infected sources. There was no HCW who had been vaccinated for HBV and prophylaxis against HBV among participants in this study. In an unvaccinated person, the risk of HBV infection from single needle stick injury to HBV infected blood ranges from 6%-30 % (3, 22, 23). The estimated risk for Hepatitis C virus transmission (HCV) are up to 3% and for HIV is 0.03%. Indeed it is important to note that the lower risk of HCV and HIV transmission compared with HBV is offset by the greater risk of chronic infection. Eighty percent of all those infected will develop chronic HCV infection leading to further complications (3, 22).

Wounds and skin sites that have been in contact with blood or body fluids should be washed with soap and water; mucous membranes should be flushed with water and immediate evaluation must be performed by a qualified health care professional (7,22). In this study, only 38.2% of HCWs washed the exposed body part with soap and water. HCWs reported to the PEP focal persons to get service were 17.2%, which were 46% in study by NaSH (20). Accessibility of vaccination and PEP might encourage HCWs to report blood and body fluid exposures in developed countries. Hepatitis B vaccination coverage was 86% in Iran as free vaccination strategy program (18). The major reasons for under reporting and deficient use of VCT services were negligence by HCWs (83.1%). Study in India found that, less than a quarter of the exposed HCWs took a course of PEP against HIV. This low rate

of PEP was due to under-reporting to concerned hospital authorities (5). Unreported needle-stick and sharp injuries are a serious problem and prevent injured HCWs from receiving PEP against HIV, which is shown to be 80% effective against HIV infection (1,5).

Despite high prevalence of NSI and BBF splash exposure within the last one year in this study, the 28-day mandatory post-exposure prophylaxis was used by only 12.1% of HCWs, of those only eight workers (42.1%) completed full course. In developed country, NaSH reported that in 2007 the median duration of taking PEP by all HCWs was 28 days after exposure to HIV positive sources, consistent with U.S. public health service Guidelines (20). The main reason given in this study for non-compliance was intolerance to the side effects of the medication. Persons receiving PEP should complete a full regimen. However, as a result of toxicity and side effects among HCWs, a substantial proportion of HCWs have been unable to complete a full course of HIV PEP (1, 5, 10, 24).

a) *Strength and Limitations of The Study*

Including both health professionals and non health professionals like janitors/cleaners to assess occupational exposure to BBF could be taken as the strength of the study but not screening of viral infections like HIV and hepatitis for exposed HCWs and since the study was based on self report about previous one year and life time occupational exposure to BBF the result might be affected by recall bias.

IX. CONCLUSION

BBFs exposure among HCWs during patient care was a common occupational problem in the study area and *SPs were poorly applied for exposure prevention and post exposure managements to BBFs among participants of this study*. Only 38.2% of HCWs washed the exposed body part with soap and water and the 28-day mandatory post-exposure prophylaxis was taken by 12.1% of HCWs after facing BBFs exposure. In industrialized countries; the cost of protective devices and equipment that reduce blood exposure may be offset by lower expenditures associated with post exposure testing and prophylaxis, medical treatment of infected workers, institutional insurance premiums, and workers' compensation payments (3). In most developing countries, however, similar economic incentives do not exist; there are costs associated with failing to protect scarce number of health care workers in developing countries (2, 3).

X. RECOMMENDATIONS

1. It is supposed to be focused on altering the behavior of HCWs to follow standard operating procedures during care for patient and consistent availability of PPEs.

2. The institution is advised to ensure a mechanism is in place and made known to all healthcare workers and medical students about incidence reporting and recording to early manage infections, identify emerging problems, monitor trends, and evaluate preventive measures.
3. Healthcare workers are recommended to give attention for the standard precautions and other preventive measures and to develop habits of early reporting of blood and body fluids exposures and took full courses of PEP.

a) *Competing Interest*

The authors declare that they have no any financial or non-financial competing interests (political, personal, religious, ideological, academic, intellectual, commercial or any other).

b) *Authors' Contributions*

ZY was involved in the conception, design, data collection, analysis and interpretation, report and manuscript writing. TE and GT had been involved in the design, analysis and interpretation of the data, and report and manuscript writing.

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Table 1 : Socio-demographic variables of healthcare workers and medical students in University of Gondar Hospital, 2012

Demographic Variables	Frequency(N=252)	(%)
Age of respondent		
19-24 years	106	42.1
25-34 years	118	46.8
35 and above	28	11.1
Marital status		
Single	161	63.9
Married	83	32.9
Divorced & widowed	8	3.2
Religion		
Orthodox	215	85.3
Muslim	18	7.1
Other Christians	19	7.5
Educational status		
Intern	32	12.7
12/10 not completed	5	2.0
12/10 completed	41	16.3
Diploma	56	22.2
1 st degree and above	118	46.8

Table 2 : Multivariate logistic regression results of blood and body fluids exposure within the previous one year in University of Gondar hospital, Sep. 2012

Variable	Exposed (n=157)	Not Exposed (n=95)	AOR (95% CI)	P- Value
Occupation				
Intern	29(19)	3(3)	9.4(1.8-49.9)*	0.009
Health Prof.	100(64)	58(61)	1.2(0.37-3.69)	0.799
Housekeep.	28(18)	34(36)	1	
Experience in years				
< two years	86(55)	59(62)	0.94(0.5-2)	0.863
2-4years	47(30)	15(16)	3.2(1.4-7.5)*	0.008
Above four years	24(15)	21(22)	1	
IP training				
Satisfactory	49(31)	42(44)	0.5(0.3-0.9)*	0.023
Not satisfactory	108(69)	53(56)	1	

Figure 1 : Major reason for none using of personal protective equipments by healthcare workers and medical students in the last one year, University of Gondar Hospital, 2012

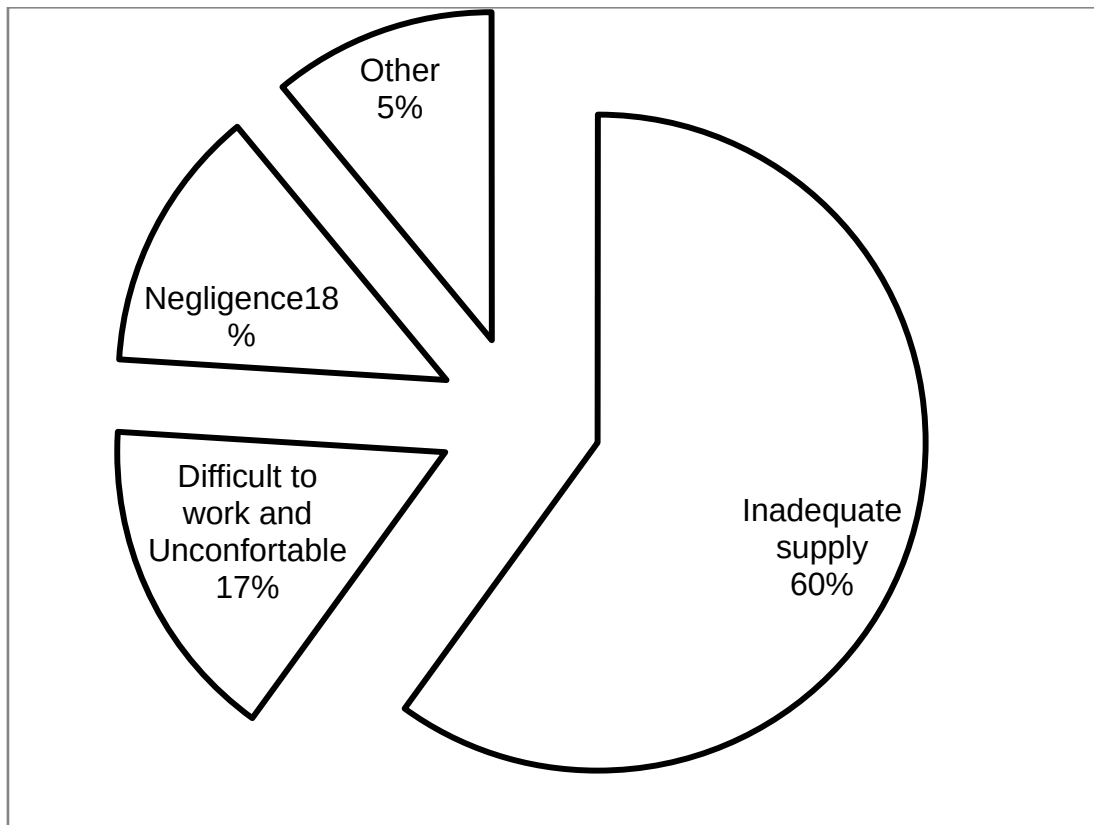
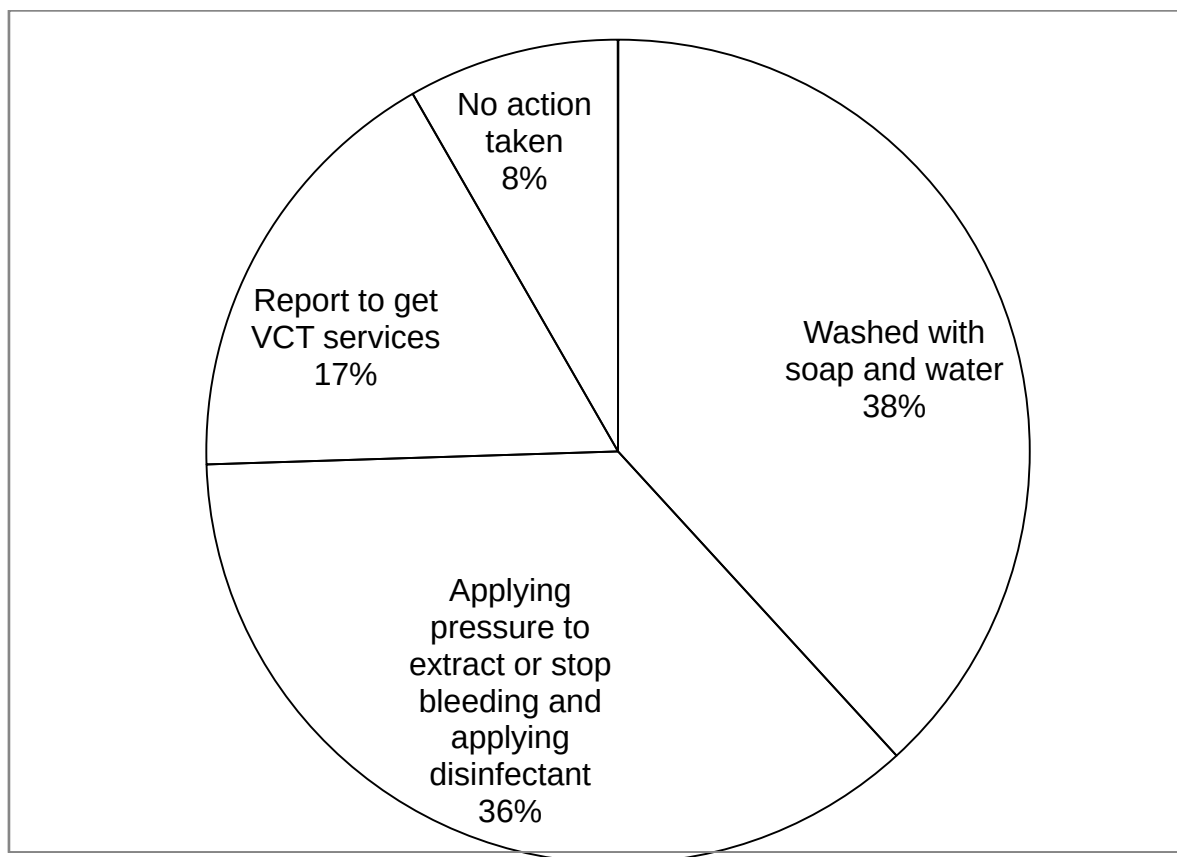


Figure 2 : Post exposure practices of healthcare workers and medical students to blood and body fluids in the last one year, University of Gondar hospital, Sep.2012



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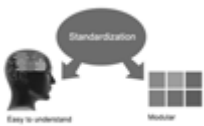
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21. Arrangement of information: Each section of the main body should start with an opening sentence and there should be a changeover at the end of the section. Give only valid and powerful arguments to your topic. You may also maintain your arguments with records.

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25. Take proper rest and food: No matter how many hours you spend for your research activity, if you are not taking care of your health then all your efforts will be in vain. For a quality research, study is must, and this can be done by taking proper rest and food.

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27. Refresh your mind after intervals: Try to give rest to your mind by listening to soft music or by sleeping in intervals. This will also improve your memory.

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29. Think technically: Always think technically. If anything happens, then search its reasons, its benefits, and demerits.

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Key points to remember:

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- Fundamental goal
- To the point depiction of the research
- Consequences, including definite statistics - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

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Approach:

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- If use of a definite type of tools.
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Approach:

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Approach

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Approach:

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References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring



INDEX

A

Ablation · 54, 55, 56, 57
Aldolase · 2, 6, 7, 8
Alkaline · 10, 11
Altruism · 44
Amylovora · 23
Anatomical · 34
Ankyloglossia · 34, 37, 40
Antenatal · 56
Anthropometry · 60
Anurofauna · 28
Arabidopsis · 8
Arachidonic · 2, 8, 9, 20
Argentina · 26, 28, 30
Astarastoare · 42
Avirulence · 8

C

Chemiluminescent · 12
Chromatography · 2, 4, 9, 10, 14
Cladosporium · 23
Cochraine · 40
Coomassie · 11
Cosmopolitan · 34
Curiosity · 37
Custodians · 36
Cystoscopy · 54, 55
Cytosolic · 2

D

Debaryomyces · 18
Denaturing · 13
Discolure · 41

E

Elachistocleis · 1, 26, 28, 30, 31, 32
Electrophoresed · 12
Elicitor · 2, 3, 4, 5, 6, 7, 8, 9, 10, 14, 18, 20, 22, 23, 24
Enuresis · 55
Ethiopia · 1, 64, 65, 67, 68, 69, 71, 72, 73, 74
Exacerbating · 67
Exodus · 47

F

Frenotomies · 36
Frenotomy · 34, 40
Frenulum · 34, 37

G

Gastrophryninae · 1, 26, 28, 30, 32
Girdle · 49, 50
Glycoprotein · 2, 6, 7, 24
Glycosylation · 5, 18
Guanosine · 11

H

Harboring · 12
Hepatitis · 64, 69
Herpetofauna · 28, 30
Hygiene · 58
Hyphal · 2, 3, 20, 22

I

Immaculate · 26, 32
Immunoglobulin · 10
Incubated · 11
Isopropyl · 13

L

Luciferase · 5, 10
Luminescence · 16

M

Maternity · 65
Megasperma · 2, 7, 20, 21, 23, 24
Microhylidae · 1, 26, 28, 30, 31, 32
Microsomal · 5, 8, 9
Micturition · 54, 55
Monoclonal · 3, 4, 6, 7, 10, 13, 14
Morbidity · 37, 38
Mycelia · 4, 6, 9, 12
Mycelial · 5, 9, 20, 24
Mycelium · 6, 7, 14

N

Neonates · 34, 35, 55, 57
Neonatologist · 56
Neotropica · 28, 30, 31
Nucleotide · 4, 12, 18, 24

O

Oligopeptide · 2
Oomycete · 2, 7, 9
Orthodox · 37

P

Paediatric · 56
Paediatric · 35, 38
Pantanal · 26, 28, 31
Paraguay · 26, 28
Parenchymatous · 10
Pasteurella · 18
Pavlovkin · 2
Pessimistic · 46
Phytoalexin · 2, 3, 23
Phytophthora · 1, 2, 3, 4, 6, 7, 8, 9, 10, 12, 14, 16, 17, 18, 20, 21, 22, 23, 24
Polyacrylamide · 11
Polyhistidine · 13
Polyvinylidene · 11
Prenatally · 54
Prevalence · 58, 60, 61, 62, 64, 65, 67, 68, 69
Prophylaxis · 64, 67, 68, 69
Protease · 6, 11
Proteinaceous · 3
Pyridoxine · 61

R

Reagent · 6, 11, 12, 23
Repetitions · 50, 51
Retrospective · 54, 71

S

Septicemia · 55, 56
Sonicated · 9, 13
Staphylococcus · 6, 11
Stratum · 65
Surgical · 34, 37
Susceptible · 4, 7, 8, 16
Syntopy · 26
Syringae · 20, 21

T

Tradocultural · 39
Trichloroacetic · 3

U

Urethra · 54, 55, 57
Urethral · 54, 55, 57
Urethral · 1, 54, 56, 57
Uropathy · 54
Urostylus · 32
Ustilago · 18

V

Vasconcelos · 27, 31
Vesicostomy · 56
Vesicostomy · 56

Z

Zoospores · 5, 20



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