ScienceMED

an International Journal of Medical Sciences

ISSN 2039-4632

MEDIMOND

INTERNATIONAL PROCEEDINGS vol. 5, n. 1.2014

ScienceMED
an International Journal of Medical Sciences
ISSN 2039-4632

Director and editor-in-chief
Musiani Irio
Medimond s.r.l. - Via G. Verdi 15/1, I-40065 Pianoro (Bologna)
e-mail: info@medimond.com - www.medimond.com

This journal was printed in July 2014 by EDITOGRAFICA s.r.l.,
www.editografica.com.

Contents

Otosclerosis: A Misnomer

Brookler K.

Aerospace Medicine Vestibular Research, Mayo Clinic, Phoenix, AZ, USA

The first description of ankylosis of the stapes is attributed to Valsalva in 1704 [1]. Toynbee in dissected the post mortem ears patients and connected the ante mortem findings with his dissections. In 1857 a catalogue description of the findings[2]. There were 1659 dissections, of which 135 revealed ankylosis of the stapes. He notes "the establishment of the existence as a disease of membranous and osseous ankylosis of the stapes to fenestra ovalis, one of the most common causes of deafness." Politzer had visited Toynbee in 1861 and published in 1861 "Ueber Ankylose des Steigbugels mit dem ovalen Fenster" (On ankylosis of the stapes in the oval window). The first published histopathology of "otosclerosis" was in 1890 by Katz that included the clinical picture as well.

Politzer attended the First PanAmerican Congress on Medicine in Washington, DC in 1893 and gave a paper entitled "Peculiar Affection of the Labyrinthine Capsule as a Frequent Cause of Deafness." He did not use the term otosclerosis and as far as can be seen never considered it. "The changes are due to a primary inflammatory process in the labyrinthine capsule." On the program was an American otologist Lawrence Turnbull practicing in London and presented cases some of which he termed "progressive or proliferous sclerosis" for which he performed surgery. This surgery he also gave the term "otosclerectomy."

While Politzer was proficient in the English language it appears that someone else wrote the paper in the proceedings of the 1893 meeting. In 1897, Politzer wrote regarding the term that he preferred the term "capsulitis labyrinthi", but understood that otosclerosis had become ingrained in the nomenclature and culture of otologists of the time. By the 1901 edition of his textbook there appeared a chapter for the first time entitled "Otosclerosis."[4] This topic was covered in 18 pages.

In 1903 Denker wrote a book Der Otosclerose (Otosclerosis). Another book was written by Gray in 1917, but published at the conclusion of the First World War in 1919. In the introduction he claims that the term "otosclerosis" is a misnomer. He was so convinced that otosclerosis was a misnomer he titled his book "Otosclerosis (Idiopathic Degenerative Deafness)![5]

In 1929, a two volume book was published on the topic of Otosclerosis entitled "Otosclerosis: A resume of the literature to July 1928."[6]

It organized and summarized all of the published papers on the topic of otosclerosis up to July1, 1928 in a complete resume. 1936, an additional volume was published updating the literature on the subject collected since 1928.

Guild in 1944 published the paper on the concept of "histologic otosclerosis."[7] This was otosclerosis identified in post mortem examinations at Johns Hopkins where the understood histopathology of otosclerosis was identified in a population of people some of whom had no hearing loss identified or present ante mortem. In this publication he does not indicate the histologic criteria. He may have been cognizant of a possible error in his conclusion and makes the following statement: "A clear distinction must be made, in all discussions of otosclerosis, between Clinical otosclerosis and Histologic otosclerosis. Failure to keep this distinction in mind can lead to confusion of ideas."

Forgotten were all of the clinical papers in the otosclerosis literature on the presentation of patients with other otologic disorders such as sensorineural hearing loss, tinnitus and vertigo as well the concomitant middle ear findings. [6]

A Guild authored publication in 1953 introduced the question whether otosclerosis can produce cochlear nerve degeneration? [7] He made the following statement: "The question is: Can a lesion other than "nerve degeneration" cause the clinical picture that has customarily been interpreted as "nerve degeneration secondary to otosclerosis"? To this question I believe the answer may prove to be yes." This question seemed to link the otosclerosis-like lesions he had described in the otic capsule

without a conductive hearing loss now with a sensorineural hearing loss. It became clear from the discussion that the 1944 report contained too few specimens to come to his conclusion compared to the experience of others. From this question grew the concept of "Cochlear otosclerosis" in 1965[8,9] and in 1969 also referred to as "Cochlear otospongiosis." [10]

"Radiologic otosclerosis" emerged first in 1970 [11] and was further characterized in 1985[12]. Computerized tomography was on the scene in 1985 but the slice thickness and resolution were insufficient to visualize the otic capsule and its density differences. As computerized tomography matured and the resolution of the images improved down to 0.3 mm and with a bone algorithm, the identification of otosclerosis improved. By 1993 into 2000 the computerized tomography imaging could almost overlay the equivalent slice histopathology.[13,14] The routine use of CT scan applied to many clinical otologic conditions revealed otic capsule disorders that were classified as otosclerosis because of demineralization but without oval window involvement. This resulted in a clinical quandary of clinical otosclerosis, histologic otosclerosis and radiologic otosclerosis. Other clinical situations that revealed radiologic otic capsule demineralization without footplate involvement were sensorineural hearing loss, tinnitus, vertigo, Meniere syndrome [15,16,17] and even semicircular canal dehiscences.[18,19,20] The presence of a disorder termed "cochlear otosclerosis" made possible by imaging was hotly debated from 1974 to 1992 with strong protagonists and even louder opponents.[21,22]

Now, about a century since the term otosclerosis was coined the evolution of the molecular biology of bone and the comprehension underlying mechanisms of bone physiology and pathology is evolving. These molecular mechanisms are emerging and explaining clinical otosclerosis and more importantly other clinical conditions related to otic capsule disorders. The first glimpse of molecular understanding of otic capsule bone disorders by Brookes revealed a role for vitamin D deficiency as a factor in otosclerosis.[23]In 2005 [24] osteoprotegerin (OPG) and TNF-alpha along with a lacunocanalicular network [25] were identified in the otic capsule. Simultaneously the effect of TNF-alpha on hair cells was described [26].

Fragments of stapes footplates were examined for measles virus mRNA, TNF alpha. (Karosi et al)[49-56]Not all of the stapes footplate fragments were positive for measles virus RNA, yet these were surgical specimens for the treatment of Clinical Otosclerosis.

Zehnder et al in 2006 reported active bone remodeling in the otic capsule of OPG knockout mice similar to the findings in human temporal bones. [31] The osteoclastogenesis terms of RANK (receptor activator of nuclear factor Kappa) and RANKL (RANK ligand) were introduced into the concept of the molecular basis of the remodeling. In 2012 Csomor reported the presence of Bone Morphogenetic Protein (BMP) in some footplates removed at stapedectomy with presumably the same surgical results.[32]

In 2014 from similar stapes fragments osteoblasts have been cultured and characterized for rate of proliferation, degree of mineralization and adhesiveness compared to osteoblasts cultured from normal bone specimens removed from patients without a history of hearing loss.[33]

The next frontier in the molecular basis for disorders of the oval window and otic capsule will revolve around many concepts in bone physiology and pathology.

In addition the genetics of these processes needs to be better understood. We also have the beginning of a genetic model of otic capsule disorders in the PHEX (phosphate regulating with homology to endopeptidases on the X chromosome) gene on the inner ear and the clinical entity of non-vitamin D deficient rickets as well endolymphatic hydrops in mice.

As McKenna suggested shall we call this "Molecular Otosclerosis" [34]or should the term otosclerosis be abandoned in favor of a classification of otic capsule disorders? And should the term otosclerosis only refer to the clinical conductive hearing loss?

It appears that the time has come for a worldwide consortium of centers with expertise on oval window and otic capsule disorders to come together to create and coordinate research objectives and a classification of otic capsule disorders both in human and the genetically modified mice.

Bibliography

1. Valsalva, A., ed. *De aure humana tractatus*. 1704: Bononiae: Pisarii.
2. Joseph Toynbee, F., ed. *Descriptive Catalogue of Preparations Illustrative of Diseases of the Ear*. 1857, John Churchill: London.
3. Politzer, A., ed. *A Text-Book of the Diseases of the Ear*. Fourth Edition ed. 1902, Lea Borthers and Company: Phildelphia-New York. 906.
4. Gray, A.A., ed. *Otosclerosis (Idiopathic Degenerative Deafness)*. 1917, Lewis: London.
5. Pierce, N., McKernon, JF, Crockett, EA, Wilsonn, JG, Duel, AB, ed. *Otosclerosis: A Resume of the Literature to July 1928*. Vol. 1 and 2. 1929, Paul B Hoeber Inc: New York. 663.
6. Guild, S., *Histologic otosclerosis.* Ann. Otol. Rhinol, Laryng, 1944: p. 246-266.
7. Guild, S.R., *Does otosclerosis cause cochlear nerve degeneration?* Transactions - American Academy of Ophthalmology and Otolaryngology. American Academy of Ophthalmology and Otolaryngology, 1953. **57**(3): p. 356-65.
8. Shambaugh, G.E., Jr., *Clinical diagnosis of cochlear (labyrinthine) otosclerosis.* The Laryngoscope, 1965. **75**(10): p. 1558-62.
9. Sando, I., et al., *Cochlear otosclerosis: a human temporal bone report.* The Annals of otology, rhinology, and laryngology, 1968. **77**(1): p. 23-36.
10. Appaix, A. and A. Pech, *Cochlear otospongiosis.* Annales d'oto-laryngologie et de chirurgie cervico faciale : bulletin de la Societe d'oto-laryngologie des hopitaux de Paris, 1969. **86**(3): p. 175-84.
11. Britton, B.H., Jr. and F.H. Linthicum, Jr., *Otosclerosis: histologic confirmation of radiologic findings.* The Annals of otology, rhinology, and laryngology, 1970. **79**(1): p. 5-11.
12. Wright, J.W., Jr., *Multidirectional tomography in otospongiosis.* The American journal of otology, 1985. **6**(6): p. 472-5.
13. Valvassori, G.E., *Imaging of otosclerosis.* Otolaryngologic clinics of North America, 1993. **26**(3): p. 359-71.
14. Palacios, E. and G. Valvassori, *Cochlear and fenestral otosclerosis.* Ear, nose, & throat journal, 2001. **80**(7): p. 440.
15. Purohit, B., R. Hermans, and K. Op de Beeck, *Imaging in otosclerosis: A pictorial review.* Insights into imaging, 2014.
16. Harcourt, J.P., et al., *CT screening for temporal bone abnormalities in idiopathic bilateral sensorineural hearing loss.* The Journal of laryngology and otology, 1997. **111**(2): p. 117-21.
17. Zonneveld, F.W., et al., *Feasibility of high resolution CT in the demonstration of the vestibular aqueduct (Meniere) and otospongiosis of the labyrinth.* Der Radiologe, 1984. **24**(11): p. 508-15.
18. Meehan, T., et al., *Dehiscence of the posterior and superior semicircular canal presenting in pregnancy.* B-ENT, 2013. **9**(2): p. 165-8.
19. Minor, L.B., *Labyrinthine fistulae: pathobiology and management.* Current opinion in otolaryngology & head and neck surgery, 2003. **11**(5): p. 340-6.
20. Hirvonen, T.P., et al., *High-resolution CT findings suggest a developmental abnormality underlying superior canal dehiscence syndrome.* Acta oto-laryngologica, 2003. **123**(4): p. 477-81.
21. Schuknecht, H.F. and J.C. Kirchner, *Cochlear otosclerosis: fact or fantasy.* The Laryngoscope, 1974. **84**(5): p. 766-82.
22. Schuknecht, H.F., *Cochlear otosclerosis. An intractable absurdity.* The Journal of laryngology and otology. Supplement, 1983. **8**: p. 81-3.
23. Brookes, G.B., *Vitamin D deficiency and otosclerosis.* Otolaryngology--head and neck surgery : official journal of American Academy of Otolaryngology-Head and Neck Surgery, 1985. **93**(3): p. 313-
24. Zehnder, A.F., et al., *Osteoprotegerin in the inner ear may inhibit bone remodeling in the otic capsule.* The Laryngoscope, 2005. **115**(1): p. 172-7.
25. Sorensen, M., Qvortrup, K, Friis, M, Fritsch, T, *Lacuno-canalicular pathways and barriers in perilabyrinthine bone.* The Registry, 2005/2006. **13**(2): p. 1-5.
26. Aminpour, S., S.P. Tinling, and H.A. Brodie, *Role of tumor necrosis factor-alpha in sensorineural hearing loss after bacterial meningitis.* Otology & neurotology 2005. **26**(4): p. 602-9.
27. Karosi, T., et al., *Detection of osteoprotegerin and TNF-alpha mRNA in ankylotic Stapes footplates in connection with measles virus positivity.* The Laryngoscope, 2006. **116**(8): p. 1427-33.
28. Karosi, T., et al., *Expression of measles virus receptors in otosclerotic, non-otosclerotic and in normal stapes footplates.* European archives of oto-rhino-laryngology 2007. **264**(6): p. 607-13.
29. Karosi, T., et al., *Codetection of measles virus and tumor necrosis factor-alpha mRNA in otosclerotic stapes footplates.* The Laryngoscope, 2005. **115**(7): p. 1291-7.
30. Karosi, T., et al., *Osteoprotegerin expression and sensitivity in otosclerosis with different histological activity.* European archives of oto-rhino-laryngology 2011. **268**(3): p. 357-65.

31. Zehnder, A.F., et al., *Osteoprotegrin knockout mice demonstrate abnormal remodeling of the otic capsule and progressive hearing loss.* The Laryngoscope, 2006. **116**(2): p. 201-6.
32. Csomor, P., et al., *Expression of bone morphogenetic protein 2, 4, 5, and 7 correlates with histological activity of otosclerotic foci.* Acta oto-laryngologica, 2012. **132**(6): p. 624-31.
33. Gronowicz, G., Richardson, Flynn, J, Kveton, J, Eisen, M, Leonard, Aronow, M, Rodner, C, Parham, K, *Differences in otosclerotic and normal human stapedial osteoblast properties are noramlized by alendrolate in vitro.* Submitted Otolaryngology-Head and Neck Surgery, 2014: p. 7.
34. McKenna, M.J. and A.G. Kristiansen, *Molecular biology of otosclerosis.* Advances in oto-rhino-laryngology, 2007. **65**: p. 68-74.

Some pioneers in early stapes surgery

Tange R.A.

Dept ORL UMCU University of Utrecht - PO box 85500 3508 GA Utrecht - The Netherlands
e-mail: r.a.tange@umcutrecht.nl

Abstract

In this presentation the early attempts to treat stapes fixation due to otosclerosis by surgical procedures performed in the second half of the 19th century are enumerated. Stapes mobilisation and stapes extraction were the treatments. Some of the pioneers in otology and middle ear surgery of that time claimed good results by their treatments but recurrent infections and the lack of antibiotics resulted in non lasting results and in some cases to a fatal end. At the turn of the 19th century the leading otologists condemned all surgical procedures to treat otosclerosis and it lasted more than 30 years thereafter that middle ear surgery for otosclerosis became an option again.

Keywords: Otosclerosis,stapes surgery,history

Otosclerosis is an affection of the middle and the inner ear. The stapes plays an important role in this affection. It was Giovanni Ingrassia (1510-1580) who by accident discovered the stapes in the middle ear during cadaver studies. The physiology of the hearing organ was described for the first time by Volcher Koyter (1534-1600). Hieronymus Capivacci († 1589) found a test to distinguish between perceptive and conducting hearing loss. It was Antonio Valsalva (1666-1723) who recognized the nature of the disease nowadays called otosclerosis. In his *De aure humana tractatus*, written while he was Professor of Anatomy at Bologna, he was the first to connect deafness during life with an ossified and fixed stapes found at autopsy. Valsalva gave the earliest description of the pathological otosclerotic process. Giovanni Morgagni (1761) refined the anatomical and pathological studies on the middle ear of his teacher Valsalva. Meckel (1771) and Domenico Cotugno demonstrated that there was fluid and no air inside the labyrinth. Joseph Toynbee of London (1815-1866) focused attention upon the connection between stapes ankylosis and deafness after his study of 1659 human temporal bones in 1841. He found that in no less than 136 out of 1,149 dissected temporal bones, stapes ankylosis could be demonstrated. He distinguished four pathologic variations of stapes fixation. One of the first observations of an otosclerotic focus at the stapes footplate was by Adam Politzer in 1862 (Fig. 1). He described a case of white bony formation at the place of an immobile stapes footplate in a cadaver.

Fig.1 . Adam Politzer

Politzer could not explain the etiology of the process leading to the new bone formation and fixation of the stapes in human temporal bones. Because of the lack of signs of infection around the stapes in his histological slides, he stated that the ankylosis of the stapes could not be regarded as a result of middle-ear infections. Von Troltsch (1881) used the term 'sclerosis' for stapes fixation. He thought that sclerosis of the tympanic cavity mucosa caused stapes fixation. It was in 1890 that L.Katz from Ludwighaven gave a first full pathological report together with the results of the clinical examination of the patient with otosclerosis and finally filled in the completed clinical picture of the disease. He found microscopic evidence of otosclerosis resulting in stapes fixation. In 1893, Politzer described otosclerosis as a clinical entity and further characterized its pathology as a 'Primary Disease of the Bony Labyrinthine Capsule', in which neoplastic bone gradually takes the place of normal bone, and produces ankylosis of the stapes. With the early recognition that the otosclerostic process was in fact an immobilizing of the stapes in the oval window, many treatments were used experimentally without a full clinical appreciation of the actual process.

Because of the failure of these conservative methods, surgical intervention for the problem became an option. Ernst Mach (1838-1916; Fig. 2) from Prague was the first to perform experimental mobilization of the stapes with Johannes Kessel.

Fig. 2. Ernst Mach

Fig. 3 Johannes Kessel.

After experimental operations on dog and pigeon, Kessel (1839-1907; Fig. 3) performed his stapes mobilization and extraction operation in 1876. The problem of the mobilization of the stapes was the approach straight through the tympanic membrane. Kessel decided to go directly to the stapes footplate after ablation of the membrane and the middle ear ossicles after an antroatticotomy. Because in many cases the results were not satisfactory, Kessel also extracted the stapes. Ear surgery at that time was not without danger. There were no microscopes or antibiotics available, which meant that there was a great risk of – often fatal – meningitis. Furthermore, a facial-nerve palsy and intensive bleeding could occur. In 1876 J.Michel from Grenoble also published his experience with the mobilization. E.Boucheron from Paris published in 1888 60 cases of stapes mobilization with a success lasting more then sixteen months. Unfortunely, there were frequent infections in most of the cases. Boucheron was some kind of prophet when he wrote about the stapes mobilization: 'C'est une opération d'avenir. Il faudra, comme l'opération de la cataracte, attendre cent ans pour en apprécier la valeur.' Miot from Paris reported in 1896 on 126 cases of mobilization of the stapes, 24 cases of which were otosclerosis. In 18 of the 24 cases a good result was demonstrated. In the USA, C.J.Blake (1843-1919)(fig.4) and F.L.Jack (1861-1951)(Fig. 5) from Boston and J.H. Burnett from Philadelphia reported remarkable improvement in hearing after removal of the stapes. The results were not long-lasting. In only a few cases the result remained stable after ten years after the surgery.

Fig. 4. Clarence Blake

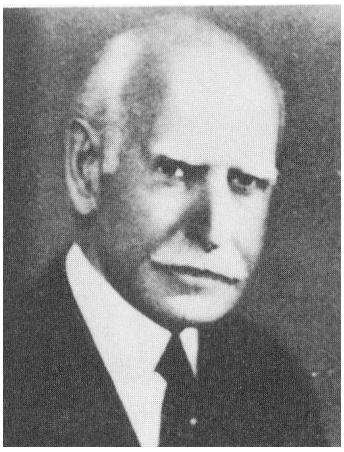


Fig. 5. Frederick Jack.

Table 1. The some of the well-known pioneers of otosclerosis surgery in the 19th century.

Surgeon	Year	Place	Result
Mach	1870	Prague	First experimental mobilization of the stapes
Kessel	1876	Jena	Mobilization of the stapes
Michel	1876	Grenoble	Mobilization of the stapes
Lucae	1884	Berlin	Repeated percussion of the chain
Boucheron	1888	Paris	60 cases, good results/frequent infections
Gellée	1888	Paris	Blind mobilization through membrane
Sexton	1889	New York	Removal of the stapes
Schwartze	1890	Halle	42% success
Blake	1890	Boston	Good results but not long-lasting
Jack	1892	Boston	Good results but not long-lasting
Miot	1896	Paris	24/18 = 75% success
Alderton	1896	New York	Footplate perforation with dental drill
Floderus	1899	Denmark	Fistula in semicircular canal
Faraci	1899	Palermo	Success in a few cases
Ballance	1900	London	Fistula in semicircular canal

The surgical approach to the fixated stapes in otosclerosis was unsuccessful due to recurrent infections and the risk of intracranial abscesses. To avoid the actual focus of disease, K.A.Passow (1859-1926) (Fig. 6) from Heidelberg made in 1897) an opening to the cochlea in the medial wall of the middle ear close to the oval window. The promontory was trephined and the opening covered with a mucoperiosteal flap. This can be considered as the first cochleostomy ever.No lasting improvement was obtained, and often the hearing was made worse.

Fig. 6 Carl Pasow

H.A. Alderton (1896) from New York tried to drill with a dental drill through the stapes footplate to create a fistula, but the results of this procedure were without lasting success. B. Floderus from Denmark in 1898 and C.A.Ballance (1856-1936) in 1900 were the first suggesting the creating of a fistula into the horizontal semicircular canal to stop vertigo problems and in some cases to improve hearing. By accident, Ballance opened the horizontal semicircular canal during mastoid surgery. He covered the fistula by a thin layer of squamous epithelium skin transplant and observed that the pre-operative vertigo was diminished and the hearing improved. Recurrent infections were an important reason that these fistulae operations for otosclerosis were unsuccessful.

Fig. 7. Emile Moure

In 1894, on an international congress on medicine in Rome, V.Cozzolino(1853-1911), E.Moure (1855-1914)(Fig. 7) and A.Politzer condemned the surgery on the stapes in cases of otosclerosis. F.Bacon reports in his *Manual of Otology* (1898) that operations on the stapes should not be considered for a moment. In 1900, two other leading authorities at that time, F.Siebenmann (1852-1928) and R.D.Botey(1855-1927), condemned the surgical treatment of otosclerosis at the International Congress of Otology again in Rome. In that pre-antibiotics era, an operation for hearing

loss with the possible side effect of often fatal meningitis was the reason not to perform the surgery for hearing improvement in otosclerosis anymore.Politzer stated, also in 1900, that the simple mobilization of the stapes only results in a temporary effect on the hearing and that operative extraction of the stapes was of no use and could even be dangerous. In 1904 A. Denker (1863-1941) from Munich declared that there was no future for the surgical treatment of deafness due to otosclerosis. Since these statements at the beginning of the twentieth century and the poor results at that time, stapes surgery was abandoned for almost more than two decades as treatment for otosclerosis.

Reference

Tange R.A. (2014) The History of Otosclerosis Treatment. Kugler Publications. Amsterdam

Audiometric results after stapedotomy with platinum prostheses

Skarzynski PH.[1,2,3,4], Krol B.[1,3], Pilka A.[1,3], Skarzynski H.[1,3]

1 – Institute of Physiology and Pathology of Hearing, Warsaw, Poland
2 – Institute of Sensory Organs, Kajetany, Poland
3 – World Hearing Center, Kajetany, Poland
4 – Medical University of Warsaw, Warsaw, Poland

Otosclerosis is a disease which can present with conductive or mixed hearing loss caused to stapes fixation [1]. The first definition of otosclerosis was formulated by Valsalva in XVI century, who discovered the stapes in middle ear and described the physiology of the middle ear. Later, other researchers published more accurate pathophysiology findings, and begun experimenting with different conservative methods of otosclerosis tratment. Lack of success with these therapies led to first attempts of surgical treatment in otosclerosis and the first case of stapes mobilization in XIX century. This experimental operation was the milestone in stapes surgery, which later experimenters would continue to develop in very dificult conditions, without microscopes or antibiotics and thus with high risk of complications, especially from intracranial infections [2].

The modern history of otosclerosis treatment begins in 1956, when John Shea developed the technique of stapedectomy [1]. Small-fenestra stapedotomy technique, popular from early 1970s, led to the development of first piston prostheses [3]. Such prostheses, despite many changes and modifications, application of different, more and more advanced materials and improvements of their construction, still remain a very efficatious and popular solution used commonly in stapes surgery [4].

Material of the otosurgeons from the Institute of Physiology and Patology of Hearing includes 14,423 ears after surgical otosclerosis treatment. For the present study were selected patients who underwent otosclerosis treatment between January and December 2011 in the World Hearing Center of the Institute. In the first stage of selection, only patients without any otological comorbidities were chosen for the study. Then, in the randomized qualification process, 330 patients were selected. The group included 228 women and 102 men, mean age 46.5 (range 6-76). All of them underwent stapedotomy with application of the teflon prosthesis with platinum wire (0,6mm diameter). Patients' data, including clinical histories, surgery protocols and hearing examination outcomes (in frequences 0.5, 1, 2 and 4 kHz) were analysed retrospectively. Mean observation time was 14 months after surgery.

The diagnosis of otosclerosis was based on medical histories, otoscopic evaluation and a type of hearing loss. All patients included in study material suffered progressive, conductive or mixed hearing loss, with 76% of them complaining about tinnitus before surgery and vertigo presenting in 12% preoperatively. Mean time from the first onset of disease symptoms to surgery was 6 years. In the vast majority of cases the etiology of otosclerosis was not clear. Only 12,73% declared incidents of otosclerosis in family. Among women: in 21.21% the onset was associated with menopause, in 9.21% with pregnancy, and in 7.46% with puberty. Among men, there were none who could confidently ascertain the occasion of the disease onset.

The most important observed effect of treatment, besides patient satisfaction, were the outsomes of pure tone audiometry (PTA) tests. In all patients PTA was performed during the qualification process and after surgery, at one month and about 12-16 months postop. PTA test was performed in frequencies 0.5, 1, 2 and 4 kHz. Preoperatively, in all subjects the air-bone gap was higher than 20dB, the cochleostapedial reflex was absent, and the tympanogram was type A or As.

The bone conduction thresholds before and after surgery were compared retrospectively for frequencies 0.5, 1, 2 and 4 kHz. Values pre-op and post-op for the each respective frequency were: 21.03-17.14dB, 28.88-23.41dB, 32.96-28.04dB, 34.19-34.07dB, difference of threshold values before and after treatment were respectively: 3,89dB (SD 9,63), 5,47dB (SD 9,28), 4,92dB (SD 9,84), 0,12dB (SD 11,32).

Results were statistically significant for frequencies 0.5, 1 and 2 kHz. The best improvement was observed at 1kHz, the worst was marked at 4kHz.

In the retrospective comparison of air conduction thresholds before and after surgery for frequencies 0.5, 1, 2, 4kHz, values before –after were respectively: 58,95-32,38dB, 58,86-33,42dB, 53,55-35,56dB, 56,91-45,03dB. Differences for the respective frequencies were: 26,63dB (SD 15,62), 25,43dB (SD 14,74), 17,98dB (SD13,96), 11,88dB (SD 16,79).

Results were statistically significant in all frequences. The best results were observed in 0,5kHz, the worst in 4kHz. It is reflected by the mean value of the air bone gap after surgery (Fig. 1).

The principal aim of otosclerosis surgery is to close the air-bone gap. The best expected effect of treatment is to reduce it to less than 10 dB, but there are studies where authors underline that closing the air-bone gap below 20 dB is a demanding goal [4].

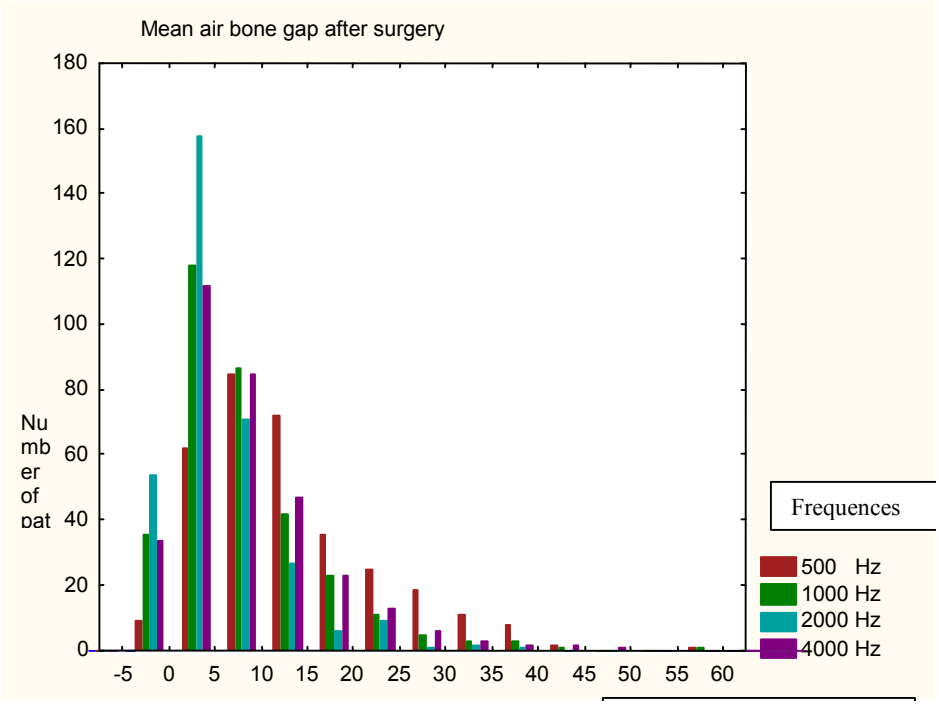


Fig. 1. The mean air bone gap [Hz] after surgery in patients included in the study at 4 frequencies (500 Hz, 1000 Hz, 2000 Hz, 4000 Hz)

Post-operative complications ocurred in only a few cases in present study. These complications included: temporary horda tymphani irritation (13 patients, 3,9%), subjective tinnitus intensification (13, 3,9%), vertigo (7, 2,1%), nervus VII paresis (1, 0,3%). All these symptoms were reported during the first follow-up visit at one week after surgery. In the full follow-up term (12-16 months) these symptoms resolved. Only in 2 cases it was necessary to perform the revision surgery.

Stapedotomy is good and effective method of treatment for otosclerosis. It should be recommened as the treatment of first choice in cases where there are no otological comorbidities and no contraindications. Complications of this treatment method are rare and the hearing effects are satisfactory both for patiens and surgeons. The key factor for the surgery success is the experience of the surgeon.

It should be noted that otosclerosis often afflicts young individuals, active in their professions. In males it often occurrs with fast deterioration of hearing threshold. Early detection and implementation of surgical treatment is necessary to avert the consequences of handicap and help patiens return to normal life. In cases with advanced pathology it is sometimes advisable to consider application of the the active middle ear implants and their combinations with different couplers, to help patients achieve acceptable functional results [5,6]. In the advanced cases of otosclerosis, if there is no contraindications, the CODACS device could be a proposed solution [7].

References

1. SHEA JJ JR. A personal history of stapedectomy. Am J Otol.,19(5), 2-12, 1998
2. LIVI W, RUOSI M, MOTTA G. Evolution of the operative technique. The rigid system stapedectomy. Acta Otorhinolaryngol Ital. 16(53), 13-8, 1996
3. SLATERRY WH 3rd, HOUSE JW. Prostheses of stapes surgery. Otolaryngol Clin North Am. 28(2), 253-64, 1995
4. GRISTWOOD RE, VENABLES WN. Analysis of long-term haring gains after stapes surgery with piston reconstruction for otosclerosis. Ann Otol Rhinol Laryngol. 122(8), 500-10, 2013
5. SKARZYNSKI H, OLSZEWSKI L, SKARZYNSKI PH, LORENS A, PIOTROWSKA A, POROWSKI M, MROWKA M, PILKA A. Direct Round Window Stimulation with the Vibrant Soundbridge (Med-El): 5-year Experience using Technique without Fascia, Eur Arch Otorhinolaryngol 271 (3), 477-482, 2014
6. OLSZEWSKI L, RATUSZNIAK A, OBRYCKA A, POROWSKI M, SKARZYNSKI PH, PASTUSZAK A, SKARZYNSKI H. Middle ear implant – a chance for elimination of certain restrictions on sound reception tied with the hearing aids in partial deafness treatment, International Proceedings Division, Monduzzi editore, 10th European Symposium on Pediatric Cochlear Implantation, 25-28, 2011
7. LENARZ T, VERHAERT N, DESLOOVERE C, DESMET J, D'HONDT C, GONZALES JC, KLUDT E, MACIAS AR, SKARZYNSKI H, VAN DE HEYNING P, VYNCKE C, WASOWSKI A. A comparative study on speech in noise understanding with a Direct Acoustic Cochlear Implant in subjects with severe to profound mixed hearing loss. Audio Neurootol. 18:19(3), 164-174, 2014

Soils and Food Security Under Global Change

Blum W.E.H.

Institute of Soil Research, Department of Forest and Soil Sciences, University of Natural Resources and Life Sciences (BOKU) Vienna, Austria
E-mail: winfried.blum@boku.ac.at

Abstract

Global change is caused by the increase of world population and change in spatial distribution, loss of fertile soil through urbanisation, industrialisation and further human impacts, changes in lifestyle

and demands for food, increasing demands for bioenergy, changes in world economy, climate change and decrease in fresh water supply. We describe the influence of these changes on global food security, and the delivery of further goods and services to humankind and the environment by soils , based on their six main functions.

Keywords: world soils, soil functions, global change, food security

Introduction

Soils are providing important goods and services to humankind and the environment, through the production of biomass, especially food, fodder, renewable energy and raw materials, but also through filtering, buffering and transformation between the atmosphere, the ground water and the plant cover, protecting the environment, especially the ground water and drinking water resources. – Moreover, soil is the largest biological habitat and gene reserve on earth.

In contrast to this, soil is also used as a physical base for technical, industrial and socio-economic structures and their development, e.g. industry, housing, transport, sports, dumping of refuse and others. In order to build such structures, soil is providing materials, e.g. clay, sand and gravel for construction or other uses. – Finally soils are a geogenic and cultural heritage, forming an essential part of the landscape and protecting palaeontological and archaeological remnants.

The problem of sustainable soil use is the competition in the use of these six main functions in space and time [1]. This is of special importance for food production under sustainable conditions.

Looking into the overall threats to soil at a world-wide level, it becomes evident that sealing through urbanization and industrialization is one of the most important impacts [2]. Moreover, local and diffuse contamination, erosion by water and wind, compaction and other forms of physical degradation, as well as decline in soil organic matter, loss of biodiversity, salinization and alcalinization, and floods and landslides are additionally impacting food security.

Soils and food security in the past

When looking into the historical development of food security, the most critical time span was between 1950 and 2000, when world population increased from 2.5 billions to 6 billions. Even under these conditions soils were able to satisfy the needs of this rapidly increasing population, by raising food production by more than 250%, thus nurturing most of the people, with the exception of those who had either no access to food or no money to buy it.

The global map of land quality [3], see Fig.1, shows that based on the land quality classes only 12% of the land surface are suitable for food and fiber production(classes I-III), 24% can be used for grazing and 31% produce forests. 33% of the land surfaces are unsuitable for any kind of sustainable use.

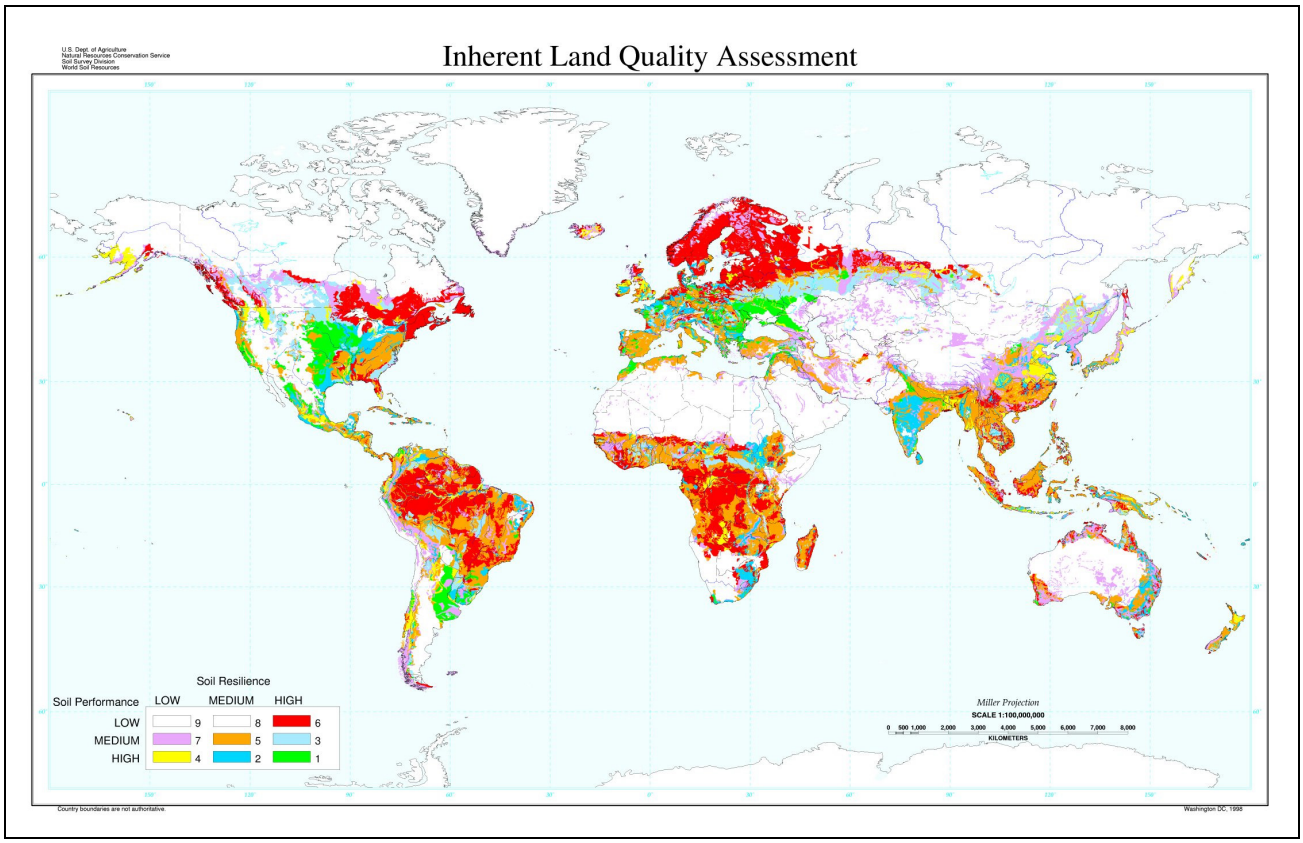


Figure 1: Global map of land quality (Blum and Eswaran, 2004)

Table 1: Percent of land area in major biomes as a function of land quality (Blum and Eswaran)

BIOMES	LAND QUALITY CLASS (Percent of ice-free land surface)									
	I	II	III	IV	V	VI	VII	VIII	IX	Total
Tundra								15.62		15.62
Boreal			2.03	0.67	0.50	3.05	2.63	1.08	0.07	10.02
Temperate	2.14	2.55	0.70	1.31	4.76	1.66	2.01		0.15	15.29
Mediterranean			0.30	0.15	1.35	0.08	0.65		0.03	2.56
Desert							1.42		28.19	29.61
Tropical	0.25	2.43	1.51	1.83	9.90	8.53	2.31		0.16	26.90
Total	2.38	4.98	4.55	3.95	16.51	13.32	9.01	16.69	28.59	100.00

Two thirds of these 12% are in the northern hemisphere, see Tab.1, and only one third in the southern hemisphere, which explains partly the actual problems in world nutrition.

On these 12% of the total land surface, 25% of the world population are producing all traded food or valuable fiber material, compare Tab.2. - This is the basis for discussing food security under global change.

Table 2: Global land quality with regard to land surface and population distribution (Blum and Eswaran, 2004)

Land Quality Class	Total Land Surface	World Population
I	2,4 %	6,1 %
II, III	9,5 %	19,0 %
IV, V, VI	33,8 %	53,6 %
VII	9,0 %	11,5 %
VIII, IX	45,3 %	13,1 %

Food security and global change

Global change is much more than climate change, and is manifest in 7 main problem areas:

1. The increase of world population and changes in its spatial distribution.

   Every year, about 80-85 million more people need more food, more space and more energy. Even more important is that every year, between 100 and 150 million people move from rural to urban areas, abandoning subsistence farming and increasing pressure on the local and regional food markets.

2. The best agricultural soils, compare Fig.2, are increasingly lost through urbanization, industrialization and further human impacts, because our ancestors were looking for the best soils, when they started to settle, and these initial settlements have grown in the meantime into urban agglomerations of great extension, see Fig.3.. Therefore, at a world-wide level, we are losing every day about 200-300 km² of fertile land by urbanization and industrialization, without counting further soil losses through erosion, contamination, salinization and other adverse impacts.

3. With increasing social and economic wealth, the life style of people is changing and demand for food is increasing.

   Besides the demand for more individual living space, in most of the industrialized countries people are wasting food and eat excessively, which leads to obesity. – For combating obesity, billions of US$ are annually spent, which could nurture to a great extent the hungry people world-wide, at the moment, close to 1 billion. Moreover, the increase in the consumption of animal protein (meat)

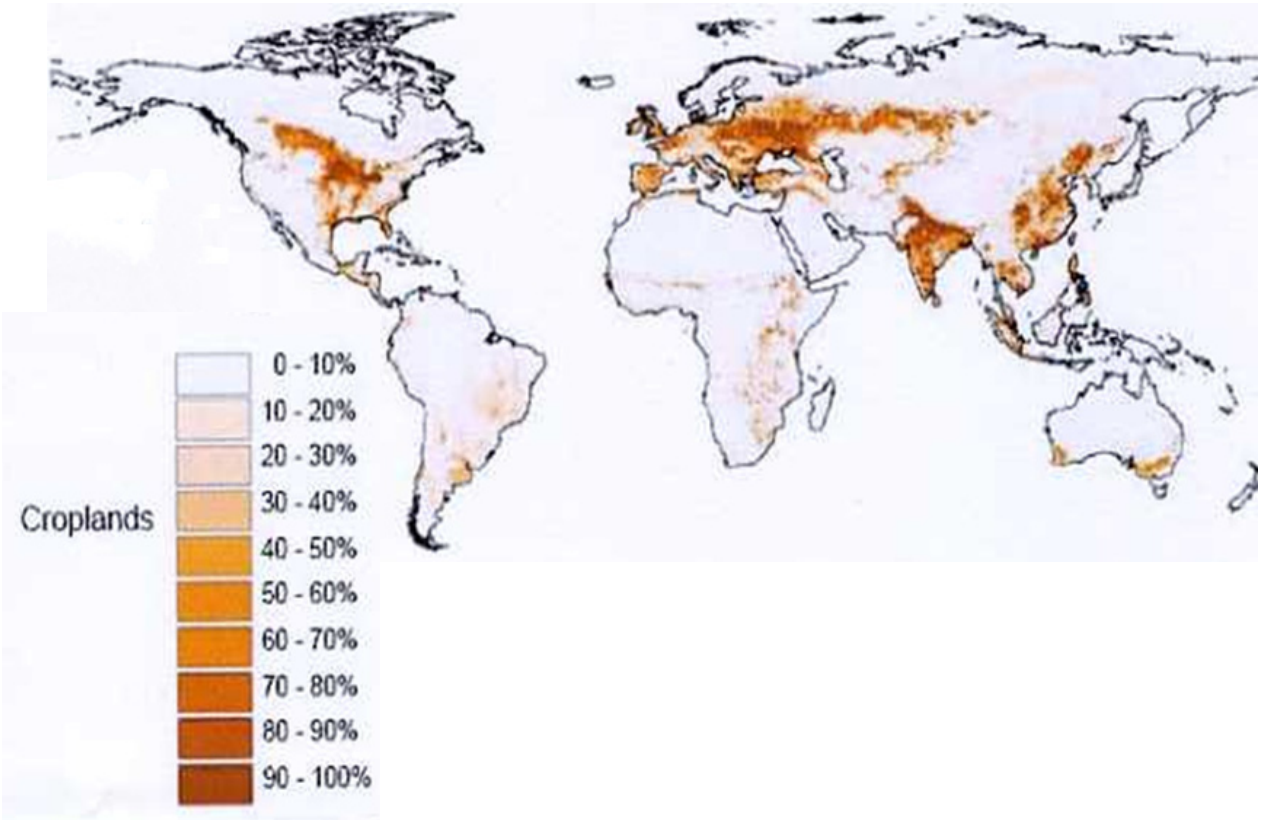


Figure 2: Global distribution of croplands (Foley et al., 2005)

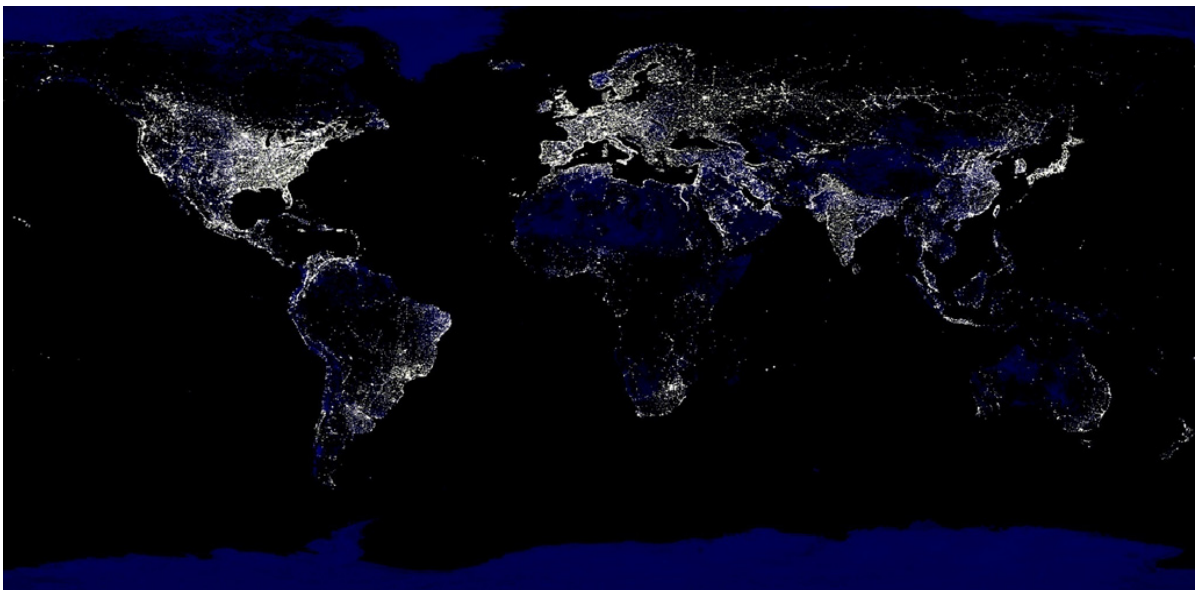


Figure 3: Sealing of land trough urbanisation and industrialisation by night lights (NASA)

increases demand for grain. For the production of 1 kg of chicken meat, about 2-3 kg of grain are needed, for 1 kg of pork, 4-5 kg, and for 1 kg of beef, 7-10 kg of grain.

In compensation of all these demands, the average yields of cereals on a world-wide level should be raised from 2.64 Mg/ha in the year 2000 to about 4.3 Mg/ha in the year 2050, without taking into consideration other foodstuff [4]. - In view of the already existing ecological limitations to industrial food production, it will be extremely difficult to reach those targets in the future.

4. Increasing demands for bioenergy, biogas, biofuel or solid material can be observed on a world-wide level.
   The production of ethanol from agricultural plants, e.g. grain or sugar cane, or biodiesel from oil plants (e.g. rape, soy beans or oil palms) shows exponentially increasing trends. In 2011, according to FAO and OECD, worldwide 13% of all grain and 35% of all sugar cane were used for ethanol production, 16% of all vegetable oil for biodiesel.
   In many parts of the world, the competition for water in the production of food or bioenergy is increasing and in those areas where food production is already limited by a lack of water, bioenergy production is no longer possible.
5. Changes in world economy and emerging economic trends in food production and marketing are further impacting food security production at a world-wide level, especially through increasing production costs (energy, fertilizers, pesticides, agricultural machinery etc.) But also new financial instruments in the marketing of agricultural products, mostly speculative performances, like e.g. derivates, are volatilizing the prices of agricultural commodities and are raising the price for food.
   A clear signal for the increasing demands is the land take in foreign countries for agricultural food and bioenergy production, called "land grabbing". Until 2011 about 700,000 km² of productive agricultural land were bought or rented by countries, bank consortia, large insurance companies and other interest groups, about 70% of these surfaces in Africa (FAO/OECD).
6. Moreover,, one of the most important global changes derives from climate change, which means the change in temperature, precipitation intensity and variability influencing soil and land management.
   Climate change is not only causing global warming, partly exceeding temperature thresholds for agricultural production, but also increased crop water requirements, and increased incidences of pests and diseases. The alteration in precipitation patterns is changing soil moisture conditions and surface runoff and the increased occurrence of extreme weather incidents are also causing soil erosion and other adverse impacts. This means that we are confronted with increased climate variability, which is not uniform at a world-wide level but very different in different regions of the world and predominantly negative for food security of countries in development.
7. Shortage in freshwater reserves is an increasing threat to food and fiber production, because more than 70 % of all fresh water is used in worldwide agricultural production.

References

[1] Blum W.E.H.(2005). Functions of Soil for Society and the Environment. – Reviews in Environmental Science and Biotechnology, **4**, 75-79.
[2] Blum, W.E.H.(1998). Soil Degradation Caused by Industrialization and Urbanization. In: Blume H.-P., H. Eger, E. Fleischhauer, A. Hebel, C. Reij, K.G. Steiner (Eds.): Towards Sustainable Land Use, Vol. I, 755-766, Advances in Geoecology 31, Catena Verlag, Reiskirchen.
[3] Blum W.E.H. and H. Eswaran (2004). Soils for sustaining global food production. Journal of Food Science 69, 37-42.
[4] Lal R.(2009). Soils and food sufficiency. A review. Agron. Sustain. Dev. 29, 113-133.

Seasonal Transpiration And Biomass Production

Novák V.

Institute of Hydrology, Slovak Academy of Sciences, Račianska 75, 831 02 Bratislava 3, Slovakia.
e-mail: novak@uh.savba.sk

Abstract

A method to estimate biomass production as a function of the seasonal transpiration totals is presented. This approach is based on frequently published linear empirical relationships between seasonal transpiration rates of particular canopy and biomass production (yield). Application of this approach leads to relatively simple method of yield evaluation with acceptable accuracy. Transpiration rates were calculated retrospectively with the HYDRUS-ET software package. The cumulative frequency distribution curve of seasonal transpiration was chosen as the basic characteristic of the soil water regime. This approach allows one to estimate cumulative frequency curves of actual and potential yields. The difference between these two curves is the cumulative frequency distribution of yield to be optimized by the irrigation system. Presented method permits a better cost-benefit analysis by comparing expected yield increases with the investment and operational expenses of the newly designed irrigation system, or of newly invoked water management practices.

Keywords: soil water regime, transpiration, biomass production, mathematical modeling.

Introduction

Soil water is only one of many preconditions to influence biomass production. It is known, that irrigation as one of the methods of soil water regime optimisation is contributing to the biomass production significantly. About 20 percent of irrigated soils of the world is producing more than 40 percent of plant production. Those soils are located mainly in arid or semiarid zones. A key step in design and implementation of irrigation or drainage system is to diagnose the existing (natural) soil water regime and possible influence of its optimisation on biomass production increase.

It is not easy to relate directly the soil water content to biomass production, therefore it is necessary to look for another ways of expression the relation between biomass production and soil water influence on it. Plant production can be evaluated by so called "crop growth models", calculating assimilation rate as a complex function of environmental parameters, which is difficult to estimate; those models are usually canopy oriented: WOFOST [1], MACROS [2], DAISY [3]. The soil water influence on plant production is expressed roughly there and they are not suitable to evaluate soil water regime influence on yields. Because direct and unambiguous relationships soil water content (soil water potential) – growth rate (biomass production) were not found, researchers tried to find another ways of expressing quantitatively the role of soil water in biomass production. To evaluate the influence of soil water on plant production using transpiration as an integral part of production process was used too.

Results of numerous measurement in vitro conditions demonstrated the low variability ratio of assimilation and transpiration intensity under given conditions [4]. From it follows linear relation between photosynthesis and biomass production rate. In reality, results of field measurements has shown linear relationship between plant production and transpiration total during vegetation period of particular plant.

A quantitative assesment of the influence of soil water in the soil root zone on biomass production can be made using well-known and widely accepted empirical relationships between biomass production (yield) and transpiration total during the growing season of a given crop [5], [6], [7], [8]. These relationships, are valid for a particular plant (canopy) and site subject to standard tillage and nutrition conditions. The only transient characteristic is the transpiration rate as influenced by

local meteorological conditions and soil water. The relationship between biomass production (yield) and the seasonal transpiration rate can be expressed by the linear equation.

The aim of this work was to evaluate the possible increase of the three important crops yield (maize, winter wheat, spring barley) and their variability by optimizing soil water regime in South Slovakia environment.

Method

Thirty one seasons totals of potential and actual transpiration were calculated, assuming stable properties of soil and plant; meteorological characteristics were changed only and they were measured at meteorological stations.

Simulation model HYDRUS – ET - version 1- [9] was used. It is modification of well – known one dimensional model HYDRUS (version 6.1) and HYDRUS1D with interactive graphical interface. This programme is based on governing Richards equation describing transport of water in variably saturated porous media and convective – dispersion equation for transport of solute and heat as well. Richards equation involves the term to calculate, water extraction by roots. Subroutine describing rain and irrigation water interception as well as evapotranspiration and its components calculation is a part of the model HYDRUS – ET. Modified version of the Penman – Monteith and Budagovskij method for calculation of evapotranspiration was incorporated in the model used [10].

Soil

Tab.1. Soil charakteristics used in simulation procedure. Sandy loam (Haplic Chernozem) at Most pri Bratislave, South Slovakia (Experimental field of Hydromeliorácie, s.e., Bratislava).

θ_v	θ_{la}	θ_{fc}	θ_s	K	α	n
0.18	0.28	0.35	0.4	$5.6.\ 10^{-7}$	0.0577	1.299

θ_v – volumetric soil water content corresponding to the wilting point [cm^3cm^{-3}], θ_{fc} – soil water content corresponding to the „field capacity“ [cm^3cm^{-3}], θ_s – water content of the saturated soil [cm^3cm^{-3}], θ_{la} - volumetric soil water content corresponding to the “limited availability” of soil water to plants [cm^3cm^{-3}], K_s – hydraulic conductivity of the soil saturated with water (saturated hydraulic conductivity) [$m.s^{-1}$], α [cm^{-1}] and n [-] – van Genuchten’s equation coefficients [11].

Canopies

Three types of plants (canopies) were chosen for analysis: maize, winter wheat and spring barley. The only source of water were precipitation, no irrigation was used. Duration of growth seasons of particular plants (Tab.2) were different; different were seasonal transpiration totals too. Actual growth period of winter wheat is longer than it is noted in the table, which does not include autumn and winter period of growth. It is assumed transpiration during winter period and plant production is not significant, the most influential is „hot“ period.

Table 2. Seasons of crops duration.

Plant	Growth period	Number of days
Maize	May 5 - September 16	134
Winter wheat	April 1 - June 25	86
Spring barley	april 7 - June 25	79

Results and discussion

Transpiration totals E_t of maize, winter wheat and spring barley were calculated retrospectively for 31 seasons (Fig.1). They are presented as empirical curves of exceedance for years 1971–2000 and 2003, the last was extraordinary hot. Length of vegetation periods of winter wheat and spring barley are close, but transpiration totals are quite different (Tab.3). Reasons are natural; meteorological conditions during their vegetation periods are different. Precipitation totals and air temperature are the most important factors. Winter wheat stage of ontogenesis during early part of spring vegetation period allowed quite different –higher - transpiration and growth rate.

The average characteristics of seasonal transpiration of the three canopies under study in seasons of years 1971 –2000 and 2003 are in Tab.3. Minimum transpiration totals were calculated for all the three canopies in year 1988, maximum transpiration totals were calculated for cereals in 1996, but yield of maize was the lowest (as well as transpiration total) in the season 1985. The reason of it was high precipitation total during the second part of the year 1996. It confirms quantitatively well known empirical information: particular vegetation period is of different suitability for different canopies.

Table 3.The average transpiration characteristics of three canopies during their vegetation period. Average values vere calculated for 31seasons. (E_t is seasonal average transpiration total, E_{tp} seasonal average potential transpiration total, $E_{t,d}$ daily average transpiration total, $E_{t,max}$, $E_{t,min}$ are daily averages transpiration total in season with maximum and minimum seasonal transpiration totals.

Canopy	E_t mm/year	E_{tp} mm/year	E_t / E_{tp}	$E_{t,d}$ mm/year	$E_{t,max}$ mm/day	$E_{t,min}$ mm/day
Maize	144	161	0.88	1.07	1.27	0.64
Winter wheat	113	148	0.78	1.13	1.68	0.85
Spring barley	68.9	82	0.83	0.87	1.1	0.55

Empirical curve of exceedance of dry grain yields Y of maize canopy (1), winter wheat (2) and spring barley (3) during the seasons in years 1971-2000 and 2003, Most pri Bratislave site are shown in Fig.2. Curves of exceedance in Fig. 2. were calculated using the type of relationship presented in Fig. 3. This empirical relationship is relating weight of dry maize grains yield *Y* and seasonal transpiration totals of maize, during its vegetation period E_t. This empirical relationship represents 5 seasons within the time interval 1971-2000 and 2003. Such type of relationships were estimated using field data even for other two canopies (not shown here).

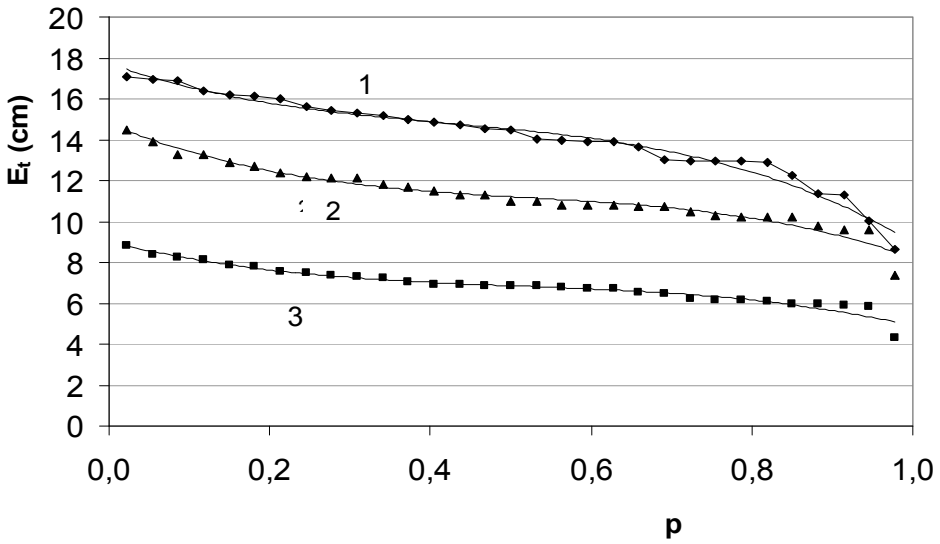


Fig. 1. Exceedance curve of seasonal transpiration totals E_t of maize (1), winter wheat (2) and spring barley (3) canopy in years 1971-2000 and 2003, Most pri Bratislave site, South Slovakia.

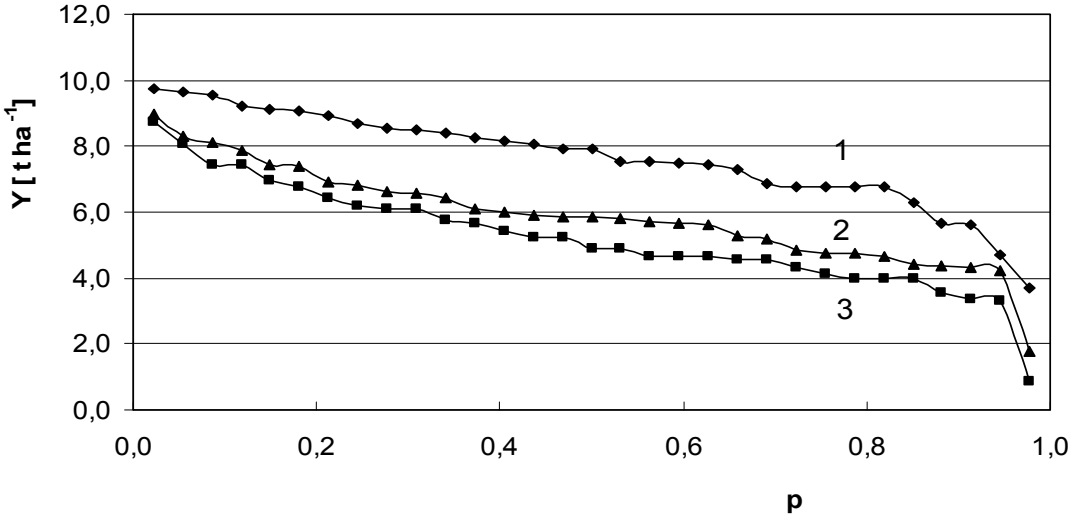


Fig.2. Exceedance curve of dry grain yields Y, of maize (1), winter wheat (2) and spring barley (3) during the seasons of years 1971-2000 and 2003, Most pri Bratislave site, Slovakia.

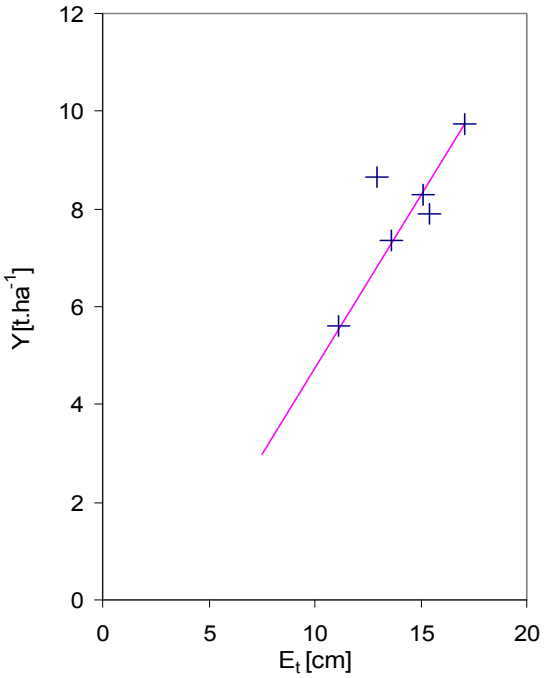


Fig.3. Dry grain yield Y and seasonal transpiration totals of maize canopy E_t. Empirical relationship represents 5 seasons within the time interval 1971-2000 and 2003. Most pri Bratislave site, South Slovakia.

The optimal soil water regime for plant growth allows potential transpiration,i.e. soil water content is not limiting transpiration. Exceedance curves of the corn grain yield (Y), the calculated potential yield (Y_p), and the difference (ΔY), for the 1971-2000 and 2003 growing seasons at Most pri Bratislave calculated from corresponding exceedance curve of potential transpiration totals E_{tp}, (Fig.4.) demonstrate relatively low capacity of soil water regime optimisation for the corn grain yield increase. The average corn grain yield (Y) was estimated to be 7.64 t ha^{-1}, and the average potential yield (Y_p) 9.03 t ha^{-1}. This means that the difference was ΔY =1.4 t ha^{-1}, which represents 18% of the average yield. The question now arises whether or not it would be reasonable (cost-effective) to design and operate an irrigation or drainage system to optimize the soil water regime to increase dry grain yield by 1.4 t ha^{-1}.

The described relationships are valid when extreme drought, heat and nutrition stresses are avoided and basic plant physiological functions are preserved [12].

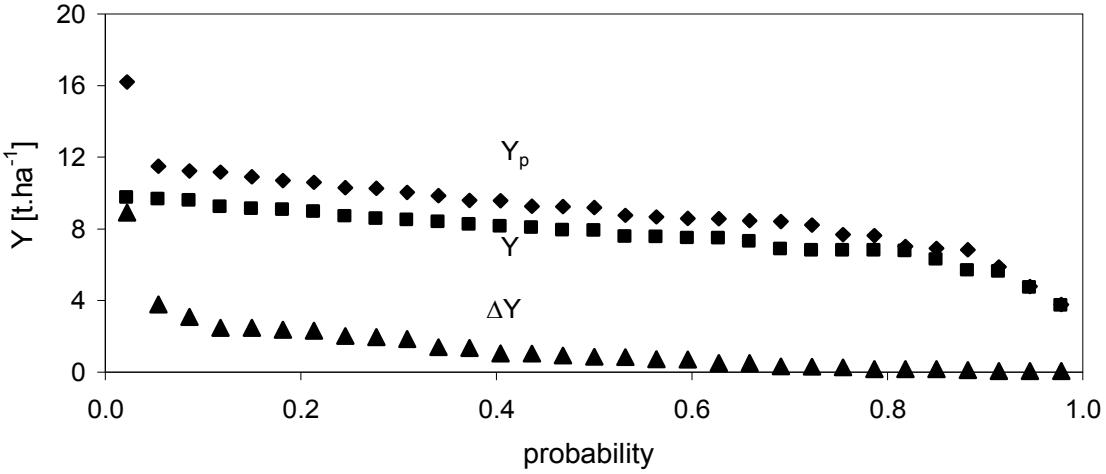


Fig. 4. Exceedance curves of the maize grain yield (Y), the calculated potential yield (Y_p), and their difference (ΔY), for the 1971-2000 and 2003 growing seasons, Most pri Bratislave site, Slovakia.

Conclusions

Mathematical model HYDRUS – ET with incorporated method of evapotranspiration and its components calculation using Penman– Monteith method modified by Budagovskij and Novák, was applied to calculate seasonal transpiration totals of three canopies (maize, spring barley and winter wheat) for 31 seasons in Southern Slovakia site.

Empirical curves of exceedance of dry grain yields of the three canopies (maize, spring barley and winter wheat) were estimated, using empirical relationship between grain yield (Y) and seasonal transpiration totals (E_t) - Fig.3. Relatively homogeneous field of grain yields (as an exception is the season 2003) demonstrates favorable conditions of South Slovakia for growth of cereals without irrigation.

Exceedance curves of the maize grain yield (Y), calculated potential yield (Y_p), and their difference (ΔY), for the 1971-2000 and 2003 growing seasons at Most pri Bratislave calculated from corresponding exceedance curve of potential transpiration totals E_{tp}, (Fig.4) demonstrate relatively low capacity of soil water regime optimization for the maize grain yield increase. The average maize grain yield (Y) was estimated to be 7.64 t ha^{-1}, and the average potential yield (Y_p) 9.03 t ha-1. This means that the difference was ΔY =1.4 t ha^{-1}, which represents 18% of the average yield. The question now arises whether or not it would be cost-effective to design and operate an irrigation or drainage system that will optimize the soil water regime to increase the dry grain yield in average by the 1.4 t ha^{-1}.

Acknowledgenment

Author appreciate the support of the Slovak Grant Agency VEGA (Project 2/0032/13), ITMS26240120004 Center of Excellence and the support of the OP Research and Development founded by the European Fund of Regional Development. .

References

[1] van Diepen, C.A., Wolf, J.,van Keulen, H., Rappoldt,C. 1989. WOFOST a simulation model of crop production. Soil Use and Management. 5, 16 – 24.

[2] Penning de Vries, F.W.T., Jansen, D.M., Pen Berge, H.F.M., Balkema, A. 1989. Simulation of ecophysiological processes of growth in several annual crops in several annual crops. IRRI Los Banos, Pudoc, Wageningen, pp.271.

[3] Hansen, S., Jensen, H.E., Nielsen, N.E., Svensen, H. 1990. DAISY: A soil Plant System Model. Danish simulation model for transformation and transport of energy and matter in the soil plant atmosphere system. The national Agency for Environmental Protection, Copenhagen, pp. 369.

[4] Hsiao, T.C. 1973. Effect of drought and elevated CO2 on plant water use efficiency and productivity.NATO ASI Series, Vol.I 16. Interacting Stresses on Plants in a Changing Climate. (M.B. Jackson & C.R. Black, Eds.). Springer Verlag, Berlin, Heidelberg.
[5] Hanks, R.J., Hill, R.W. 1980. Modeling crop responses to irrigation in relation to soils, climate and salinity. Inter. Irrig. Inform. Center, Publ. No. 6, Bet Dagan, Israel, pp. 57.
[6] Vidovič, J., Novák, V., 1987.The relation between maize yield and canopy evapotranspiration. Rostlinní výroba, 33, 6, pp. 663-670, (In Slovak with English summary).
[7] Feddes, R.A., R.W.R. Koopmans, and J.C. van Dam. 1999. Agrohydrology. Wageningen Univ., Dept. Water Resources, pp.205p.
[8] Kirkham, M.B. 2005. Principles of Soil and Plant Water Relations. Elsevier Academic Press, New York.
[9] Šimůnek, J., K. Huang., M. Šejna, Th. M. Van Genuchten, J. Majerčák, V. Novák, J. Šútor., 1997. The HYDRUS -ET software package for simulating the one - dimensional movement of water, heat and multiple solutes in variably - Saturated media. Version 1.1. Institute of Hydrology, Slovak Academy of Sciences, Bratislava.
[10] Novák, V. 2012. Evapotranspiration in the Soil – Plant – Atmosphere System. Springer Science + Bussiness Media, Dordrecht, pp.253.
[11] van Genuchten, M.Th.1980. A closed form equation for predicting the hydraulic conductivity of unsaturated soils. Soil Sci.Soc. Am.J. 44, 892 – 898.
[12] Lipiec, J., Doussan, C., Nosalewicz, A., Kondracka, K. 2013. Effect of drought and heat stresses on plant growth and yield: a review. Int. Agrophys. v. 27, 463 – 477, doi: 10.2478/intag-2013-0017.

Infiltration into stony soil: what are the differences between non-stony and stony soils?

Hlaváčiková H., Novák V., Rodný M.

Institute of Hydrology, Slovak Academy of Sciences, Račianska 75, 831 02 Bratislava 3, Slovakia.
e-mail: hlavacikova@uh.savba.sk

Abstract

Stony soils are composed of fractions (rock and fine soil) with different hydrophysical properties. Fine soil fraction is conductive for water, but rock fragments are usually of very low conductivity. Therefore, water movement in stony soil differs from non – stony soil. This contribution is presenting results of numerical simulation of ponded and rainfall infiltration, using HYDRUS–1D simulation model. As input data are used hydrophysical characteristics of stony soil and fine soil fraction alone (hydraulic conductivity, retention curve). Numerical simulation allows evaluating the influence of particular stoniness and initial soil water content on infiltration and percolation of water in soil. Under ponded condition, the faster movement of infiltration front and infiltration rate was identified in the soil profile without rock fragments. On the contrary, during rainfall rate less than the stony soil hydraulic conductivity, infiltration front penetration was faster in the stony soil profile. The reasons of such differences are discussed.

Keywords: infiltration, mathematical modeling, soil water, stony soil

Introduction

Soils containing rock fragments, generally assigned as stony soils are located mainly in mountainous areas. According to *Poesen and Lavee* [10] about 30 % of soils in Western Europe and about 60% of soils in the Mediterranean region are stony soils. According to *Šály* [12], the major portion of Slovak forest soils (up to 80 %) contain stones, with the stone content generally increasing with depth. Shape, size, degree of weathering and geological origin of rock fragments can strongly influence the soil hydrophysical properties, mainly the retention capacity and hydraulic conductivity. Therefore, it is important to characterize rock fragments properties and their distribution in the soil profile. It could be expected that rock fragments content in the soil will reduce the effective cross – section area through which water flow is realized. Furthermore, increase in stoniness results in higher soil water paths curvature what consequently leads to decreased hydraulic conductivity of the stony soil [1], [11], [2], [7], [9]. On the other hand, the shrinking – swelling phenomena could lead to the presence of temporal lacunar pores (voids along soil/stone interface), what could consequently cause presence of preferential flow, and thus increase in saturated hydraulic conductivity [13], [14], [18], [19].

The aim of this study is to explain and quantify the influence of stoniness and initial soil water content on water infiltration into stony soil and soil water content profiles formation under two qualitatively different boundary conditions: ponded and rainfall infiltration.

Materials and Methods

Site description

Effective soil hydraulic properties were determined for Leptosol Rendzic (LPrz) [4], with soil texture – sandy loam from study site Červenec - meadow (1500 m a.s.l.) located in Western High Tatra Mts. The study site is situated in the Jalovecky creek catchment. The area of high mountainous part of this catchment is 22.2 km^2, average elevation is 1500 m a.s.l. (range 820 – 2178 m a.s.l). The

average slope gradient is 30°. Rock fragments content of all soils is high (with stoniness 0.4 to 0.7). Crystalline rocks, granodiorites and limestones which together form 76 % of the catchment area, were found with low water retention capacity (in average 5 % of the volumetric water content). Therefore, water retention capacity of rock fragments was during simulations neglected. Further catchment characteristics can be found in publication *Holko et al.* [5].

Saturated hydraulic conductivity of a stony soil

Saturated hydraulic conductivity of a stony soil (characterizing stony soil as a whole denoted as bulk saturated hydraulic conductivity) needs to be estimated for quantitative description of soil water movement in the stony soils. Representative elementary volume (REV) used for its determination depends on the rock fragments size. For example, for soil containing stones with average diameter of 10 cm a REV of 1 m^3 is needed for its representative determination. It is technically extremely difficult to perform physical experiments with this size of soil sample. Therefore, solutions based on virtualization of classical laboratory experiments are taking place. In this study results of classical Darcy´s numerical experiment from the HYDRUS–2D model [15] performed by *Novák et al.* [9] were used. In their study impermeable stones were approximated in the cross-sectional area as circles, (in the full three-dimensional shape were representing by cylinders) and were equally distributed in the virtual soil sample of $1m^3$ volume. According to results of the numerical experiments *Novák et al.* [9] suggest to adjust the formula of *Ravina and Magier* [11] as follows:

$$K_s^{\,b} = (1 - aR_v)K_s^{\,f} \qquad (1)$$

where K_s^b is the bulk saturated hydraulic conductivity [cm h^{-1}], K_s^f is the saturated hydraulic conductivity of a fine soil fraction [cm h^{-1}], a is a parameter that incorporates the hydraulic resistance of the rock fragments to water flow as a function of shape, size and orientation of rock fragments [–] (e.g. a = 1.1 was estimated for sandy loam and of spherical rock fragments of 10 cm diameter), R_v stands for relative volume fraction of rock fragments [cm^3cm^{-3}] (stoniness).

Water retention curve of a stony soil

Relationship between water content and pressure head for stony soils was calculated using retention curve of the fine soil fraction. The volumetric water content of the stony soil (bulk volumetric water content) is expressed from the volumetric water content of a fine soil fraction alone, according to the formula *Bouwer and Rice* [1] given below. Retention capacity of the rock fragments was neglected.

$$\theta^b = (1 - R_v)\theta^f \qquad (2)$$

where θ^b is the bulk volumetric water content of the stony soil [cm^3cm^{-3}], θ^f is the volumetric water content of the fine soil fraction alone [cm^3cm^{-3}].

Bulk soil water retention curves (SWRC) and corresponding hydraulic conductivities as well as SWRC and hydraulic conductivity of the fine soil fraction (R_v=0) are presented in Fig.1. Infiltration was modeled using standard description of the hydrophysical properties [17], [8]. Parameters of the bulk retention curves and saturated hydraulic conductivities reflecting different stoniness, are presented in Tab.1.

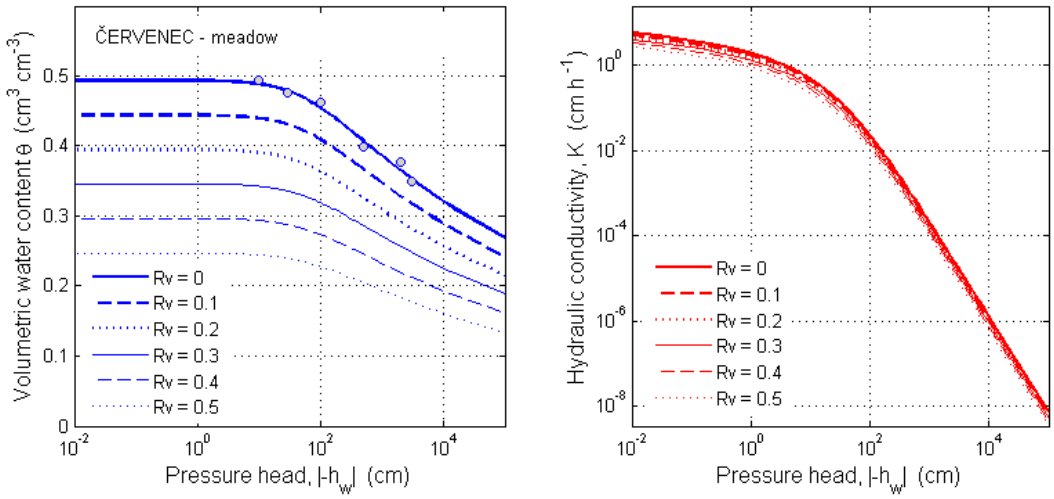


Fig. 1: Soil water retention curves (SWRC), (a) derived for the stony soils with different stoniness R_v from measured SWRC (R_v = 0), and (b) corresponding hydraulic conductivities. Site Červenec – meadow, depth 53.5–57 cm. Circles represent measured water retention data of the fine soil fraction.

Tab.1: Van Genuchten parameters and the saturated hydraulic conductivities of the fine soil fraction (R_v=0, Site Červenec – meadow, depth 53.5–57 cm) and of the stony soils with different stoniness R_v.

R_v (cm^3 cm^{-3})	**0**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**
θ_r (cm^3 cm^{-3})	0.05	0.045	0.04	0.035	0.03	0.025
θ_s (cm^3 cm^{-3})	0.495	0.4455	0.396	0.3465	0.297	0.2475
α (cm^{-1})	0.01809	0.0181	0.0181	0.0181	0.0181	0.0181
n (-)	1.09439	1.09439	1.09439	1.09439	1.09439	1.09439
K_s^b (cm h^{-1})	19.79*	17.61	15.44	13.26	11.08	8.91

*measured value

Infiltration into soils with different stoniness and initial soil water content

Influence of different stoniness on the water movement in the stony soil was evaluated using the HYDRUS−1D simulation model [16] in one domain environment. Complicated and unknown exact internal structure of the stony soil evokes mathematical modelling as a useful tool for the quantification of water movement. It allows to model water movement in the stony soil of defined structure, specified initial and boundary conditions.

Simplification of this type of modeling assumes no macropores between rock fragments and fine soil fraction (lacunar pores). Therefore, preferential flow is neglected and water transport is assumed to be performed through the fine soil matrix only. Water retention of the rock fragments and evaporation from the soil surface was neglected. It can be noted, that soil water content changes in soil matrix only.

Two qualitatively different upper boundary conditions were used to evaluate the stony soil behavior during infiltration. First, a Dirichlet boundary condition with constant 2 cm of water on the soil surface was used for simulation of the ponded infiltration. The second case was infiltration simulation from an observed precipitation event. The real precipitation of 80 mm h^{-1} intensity of one hour duration was used. Rainfall intensity was lower than the saturated hydraulic conductivity of the stony soil K_s^b, of the stoniness R_v = 0.5. Under this condition, surface runoff did not occur, rainfall has completely infiltrated into the soil profile.

Infiltration into virtual soil profiles of 95 cm deep, and of the two different stoniness values (R_v = 0 and R_v = 0.5) was modeled. For modeled soil profiles, the initial conditions were set as vertically uniform (h = -1 000 cm, and -15 000 cm). Bottom boundary condition was defined as a free drainage. Spatial distribution of rock fragments in the soil profile was assumed to be uniform.

Results and Discussion

Results of numerical simulation can be seen in Figs. 2–5. It is obvious, that movement of the infiltration front is faster under both, ponded and rainfall infiltration condition under higher initial soil water content (SWC), in comparison to the lower initial SWC. However, rate of the water movement in soil depends on rainfall intensity, and soil hydraulic conductivity, which depends on the stoniness itself. Infiltration rate in the field conditions follows precipitation intensity until it reaches the saturated hydraulic conductivity. Then the ponding starts, and infiltration continues as the ponding case. Ponding time depends upon the rainfall intensity, hydraulic conductivity, and actual infiltration capacity of the soil.

Rock fragments are reducing the effective cross – section area through which water flows. The saturated hydraulic conductivity of the stony soil is always smaller than that for a soil without stones (fine soil fraction). Under ponded condition on the soil surface, rate of water infiltration is finally approaching K_s^f (in a soil without rock fragments) and K_s^b in a soil with rock fragments. The result is, that infiltration front in the stony soil is advancing at a lower rate in comparison to the soil without rock fragments. How big this delay for the same initial condition is, depends on the stoniness reflecting the difference between K_s^f and K_s^b of the particular soils. Results of ponded infiltration modeling in stony soils presented by *Ma and Shao* [6] confirm our findings. In their paper this effect is weakened by the involved rock fragments retention capacity. Rock fragments in their study were calcium concretions from calcium sediments with quite high water retention capacity (up to 0.246 cm^3 cm^{-3}). They used therefore dual porosity model for presenting the exchange rate between the two domains (rock fragments, and the fine soil fraction).

Results of ponded infiltration simulation have shown higher rate of infiltration into the homogeneous soil, in comparison to the stony soil. The higher the stoniness, the lower rate of infiltration can be expected. Differences in infiltration front penetration are usually small (less than 10 % of the infiltration front depth). They depend on stoniness and initial soil water content (Fig. 2, 3). Higher initial soil water content increases infiltration front penetration rate significantly (up to 30 % of the infiltration depth). Influence of initial SWC upon the infiltration front penetration rate is even more distinctive in comparison to the stoniness influence.

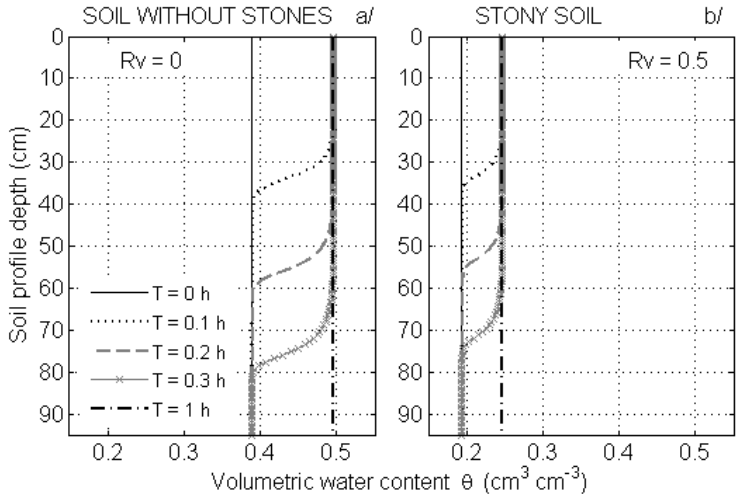


Fig. 2. Soil water content profiles during ponded infiltration with initial soil water potential h = -1 000 cm, θ_i = 0.38. a/ soil without rock fragments (fine soil fraction), b/ stony soil with stoniness R_v = 0.5, average SWC. Site Červenec – meadow, depth 53.5–57 cm.

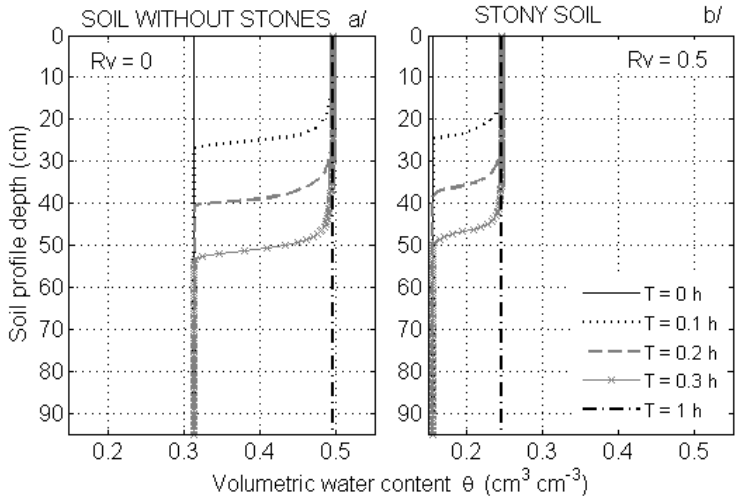


Fig. 3. Soil water content profiles during ponded infiltration with initial soil water potential h = -15 000 cm, θ_i = 0.31. a/ soil without rock fragments (fine soil fraction), b/ stony soil with stoniness R_v = 0.5, average SWC. Site Červenec – meadow, depth 53.5–57 cm.

If the precipitation rate is lower than K_s^b, infiltration is performed under unsaturated condition. In this case, infiltration rate at the soil surface is the same for the whole range of stoniness used. Faster infiltration front movement in the stony soil was observed (up to 37 %) in comparison with the soil without rock fragments (Fig. 4, 5). This phenomenon could be explained by the smaller water retention capacity of the stony soil, in comparison with the non – stony soil. Stony soil water capacity is lower, and therefore the infiltration front is advancing faster. Stones as a part of a stony soil are limiting the volume of fine soil fraction, therefore smaller amount of infiltrating water is needed to increase the soil matrix water content, and faster infiltration front movement was observed. Under such conditions, stones as a part of a stony soil profile could enable faster penetration of the dangerous substances like pollutants or others, into deeper zones. On the contrary, under ponding conditions, rock fragments as a part of the stony soil can slow down the movement of the infiltration front in a stony soil profile. It will depend mainly upon the size, shape, and position of the rock fragments in a soil profile, and on the stony soil fine soil fraction properties. Higher initial soil water content increases infiltration front penetration rate (up to 40 % of the infiltration depth) (Fig. 4, 5). Therefore, influence of the initial SWC upon the infiltration front penetration rate is similar to the stoniness influence.

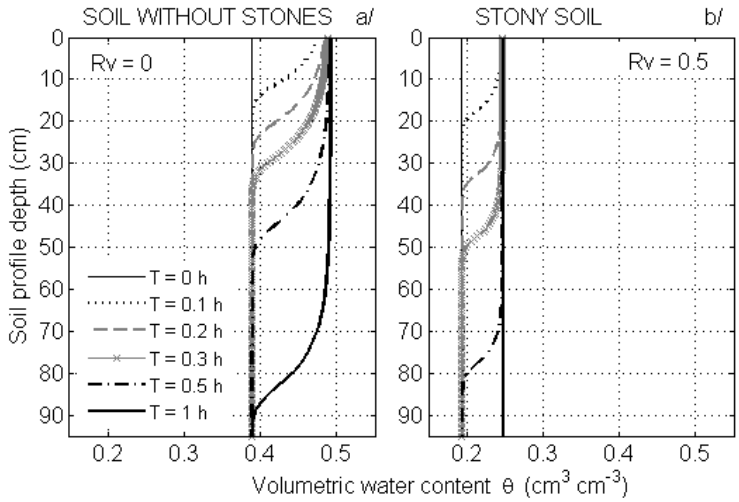


Fig. 4. Soil water content profiles during rainfall infiltration. Rain intensity was 80 mm h^{-1}, rain duration 1 h, initial soil water potential h = -1 000 cm, θ_i = 0.38. a/ soil without rock fragments (fine soil fraction), b/ stony soil with stoniness R_v = 0.5, average SWC. Site Červenec – meadow, depth 53.5–57 cm.

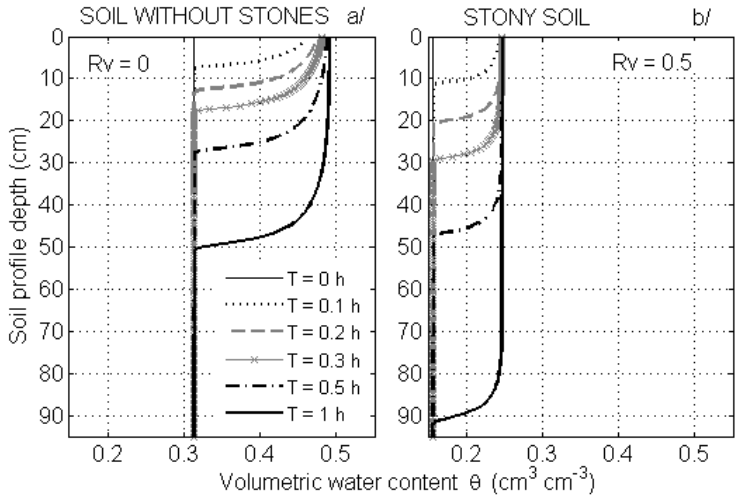


Fig. 5. Soil water content profiles during rainfall infiltration. Rain intensity was 80 mm h^{-1}, rain duration 1 h, initial soil water potential h = -15 000 cm, θ_i = 0.31. a/ soil without rock fragments (fine soil fraction), b/ stony soil with stoniness R_v = 0.5, average SWC. Site Červenec – meadow, depth 53.5– 57 cm.

Authors did not find information relating to the SWC profiles formation during infiltration to the stony soils from precipitation. The reason is probably because of difficulties in stony soil water profiles observation and measurements in the field as well as in the laboratory. Problem is with installing tensiometers and water sensors, as well as interpreting of the results. Moreover, sampling of the undisturbed soil cores containing rock fragments remains difficult, as well as measurement of the bulk stony soil characteristics needed as input data to the deterministic soil water dynamic model.

In comparison to our results of rainfall infiltration modeling, *Cousin et al.* [3] used reservoir model STICS for evaluation of water retention and percolation in calcareous stony soils during two seasons. *Cousin et al.* [3] have shown that percolation at base of the stony soil with inert rock fragments he used, appeared earlier in the stony soil than in the fine soil fraction of the same stony soil.

On the catchment scale, the rock fragments can contribute to faster runoff formation (together with other impacts like network of the macropores, hillslope, bedrock material depth, etc). Surface runoff occurrence in the mountainous catchments in temperate climatic zone is observed rarely. Even in the case of a heavy rain. Reasons are the high hydraulic conductivities of mountainous soils fine soil fraction. This phenomenon was already confirmed by *Holko et al.* [5], by measurements in the catchment under the study. In mountainous part of that catchment, the rainfall infiltrates mostly without ponding.

Conclusions

Results of numerical simulations indicate that the infiltration front penetration rate during ponded infiltration is faster in non – stony soil, than in the stony one. On the contrary, the infiltration front penetration is faster in the stony soil than in the soil without rock fragments during rainfall infiltration at rainfall rate lower than the saturated hydraulic conductivity of the stony soil.

The higher is the initial soil water content of the stony soil as well as the soil without stones, the faster is the infiltration front penetration rate. This influence is comparable in the stony and in the non-stony soil.

Surface as well as subsurface runoff, can therefore appear earlier in the stony soil than in the one without stones. Catchment with the stony soil can be more sensitive to runoff formation than that containing soils without rock fragments.

Acknowledgements

This contribution was partially supported by grant agency of the Slovak Academy of Sciences VEGA, project No. 2/0032/13 and 2/0167/12 and is the result of the project implementation ITMS 26240120004 Centre of excellence for integrated flood protection of land supported by the Research & Development Operational Program funded by the ERDF.

References

[1] Bouwer, H. and Rice R.C. (1984). Hydraulic Properties of Stony Vadose Zones. Ground Water. 22(6), pp. 696–705.
[2] Childs, S.W. and Flint, A.L. (1990). Physical properties of forest soils containing rock fragments. In Gessel, S.P. et al. (eds.) Sustained productivity of forest soils. Proceedings of the 7th North American forest soils conference. University of British Columbia, Faculty of Forestry Publication, Vancouver, Canada. pp. 95–121.
[3] Cousin, I., Nicollaud, B. and Coutadeur, C. (2003). Influence of rock fragments on the water retention and water percolation in a calcareous soil. Catena 53, pp. 97–114.
[4] FAO (2006). World reference base for soil resources 2006. World soil resources reports No. 103, ISSN 0532-0488, Rome, pp. 128.
[5] Holko, L., Kostka, Z. and Šanda, M. (2011). Assessment of Frequency and Areal Extent of Overland Flow Generation in a Forested Mountain Catchment. Soil&Water Res. 6(1), pp. 43–53.
[6] Ma, D.H. and Shao, M., (2008). Simulating infiltration into stony soils with a dual-porosity model. Eur. J. Soil Sci. 59, pp. 950–959.
[7] Ma, D. H., Shao, M.A., Zhang, J.B. and Wang, Q.J. (2010). Validation of an analytical method for determining soil hydraulic properties of stony soils using experimental data. Geoderma 159, pp. 262–269.
[8] Mualem, Y. (1976). A new model for predicting the hydraulic conductivity of unsaturated porous media. Wat. Resour. Res. 12(3), pp. 513–522.
[9] Novák, V., Kňava, K. and Šimůnek, J. (2011). Determining the influence of stones on hydraulic conductivity of saturated soils using numerical method. Geoderma 161, pp. 177–181.
[10] Poesen, J. and Lavee, H. (1994). Rock fragments in top soils: significance and processes. Catena 23, pp. 1–28.
[11] Ravina, I. and Magier, J. (1984). Hydraulic conductivity and water retention of clay soils containing coarse fragments. Soil Sci. Soc. Am. J. 48, pp. 736–740.
[12] Šály, R. (1978). Soil – basic component of forest production (in Slovak). Priroda Publ. House, Bratislava. pp. 235.
[13] Sauer, T. J. and Logsdon, S.D. (2002). Hydraulic and Physical Properties of Stony Soils in a Small Watershed. Soil. Sci. Soc. Am. J. 66, pp. 1947–1956.
[14] Shi, Z., Wang, Y., Yu, P., Xu, L., Xiong, W. and Guo, H. 2008. Effect of rock fragments on the percolation and evaporation of forest soil in Liupan Mountains, China. Acta Ecologica Sinica, 28(12), pp. 6090–6098.
[15] Šimůnek, J. and Šejna, M. (2007). HYDRUS (2D/3D), Software Package for Simulating Two- and Three-Dimensional Movement of Water, Heat, and Multiple Solutes in Variably-Saturated Media, User Manual, Version 1.02. PC Progress, Prague. pp. 203.
[16] Šimůnek, J., Šejna, M., Saito, H., Sakai, M. and van Genuchten, M. Th. (2008). The HYDRUS – 1D software package for simulating the one-dimensional movement of water, heat, and multiple solutes in variably-saturated media, Version 4.0, Hydrus Series 3. Department of Environmental Sciences, University of California Riverside, Riverside, CA, USA. pp. 296.
[17] van Genuchten, M. Th. (1980). A closed-form equation for predicting the hydraulic conductivity of unsaturated soils. Soil Sci. Soc. Am. J. 44, pp. 987–996.
[18] Verbist, K., Baetens, J., Cornelis, W.M., Gabriels, D., Torres, C. and Soto, G. (2009). Hydraulic Conductivity as Influenced by Stoniness in Degraded Drylands of Chile. Soil. Sci. Soc. Am. J. 73(2), pp. 471–484.
[19] Zhou, B., Shao, M. and Shao, H. (2009). Effects of rock fragments on water movement and solute transport in a Loess Plateau soil. C.R. Geoscience 341, pp. 462–472.

The correlation of human paraoxonase 1 (PON 1) serum levels with the cardiovascular risk for a group of hypertensive patients with associated chronic diseases

Popa C.[1], Popescu C.[1], Pilat L.[1,2], Puschita M.[1,2]

[1] Faculty of General Medicine, Pharmacy and Dental Medicine from „Vasile Goldis" Western University of Arad (Romania); [2]Emergency County Hospital of Arad (Romania).
Email: calinpopa@msn.com.

Abstract

Cardiovascular diseases are multifactorial diseases with an important genetic component, but from these illnesses the most important are hypertension and atherosclerosis. Identification of risk factors for the construction and implementation of effective therapeutic strategies is one of the primarily objective of researchers in the field.

Our study included hypertensive patients with associated chronic diseases (obesity, diabetes, kidney disease). For these patients we evaluated the lipid profile, further we calculated the atherogenic index of plasma as an indicator of atherosclerosis and the serum levels of human paraoxonase 1 for a better quantification of cardiovascular risk. Our results indicated strong correlations between these two markers for cardiovascular diseases risk.

Keywords: human paraoxonase 1, PON1, atherosclerosis, hypertension, metabolic syndrome, diabetes mellitus, kidney failure.

Introduction

Cardiovascular diseases are multifactorial diseases with an important genetic component, but from these illnesses the most important are hypertension and atherosclerosis [1, 2]. Some standardized studies showed that in 2008 more than 17 million people died from cardiovascular disease across the globe and hypertension alone affects 25% of the adult population and it is responsible for approximately 13% of all deaths worldwide [3, 4].

The latest genetic researches has shown that from the paraoxonase family (PON1, PON2 and PON3) the human paraoxonase 1 (PON1) plays an important role in the protection of LDL cholesterol and HDL cholesterol molecules to be oxidised by the free radicals [5]. Therefore, PON1 is directly involved in the process of atherosclerosis and in the risk of developing cardiovascular diseases. A low serum activity of PON1 is linked with an increased risk of coronary heart disease, myocardial infarction, and carotid atherosclerosis [6]. Atherogenesis process began in childhood, so early identification of atherosclerotic process can help prevent or delay the development of cardiovascular disease [7].

Identification of risk factors for the construction and implementation of effective therapeutic strategies is one of the primarily objective of researchers in the field. Therapeutic management is very poorly supported, drug therapy and high treatment costs limit the area of intervention of a medical specialist and appropriate patient tracking. Finding predictive markers that can be translational in preventive measures will substantially reduce the number of newly diagnosed hypertensive patients who are currently on an upward trend.

Methodology

We included in our research 120 patients (55 males, 65 females) diagnosed with hypertension. After collecting the anamnestic data from the patients, we split our study group in four different study groups as follows: first group (H1) included 30 patients (14 males, 16 females) with hypertension 1st degree without treatment, age between 35-45 years; the second group (H2) with 30 patients (17 males, 13 females) with metabolic syndrome (hypertension grade I/II, decreased glucose tolerance, obesity, sedentary), age between 45-55 years; third group (H3) also with 30 patients (11 males, 19 females) included hypertensive patients with diabetes mellitus type 2, age between 35-60 years; the fourth group (H4) with 30 hypertensive patients (13 males, 17 females) associated with type 2 diabetes and chronic kidney disease at different stages, age between 45-70 years. The fifth group, control group (C) had 25 healthy individuals (15 males, 10 females), age between 35-45 years.

Inclusion criteria for the study was hypertensive subjects over 35 years and under 70 years of age, without major cardiovascular events (history of bypass, stenting, cardiac arrest, stroke or heart failure) and without recent consumption of tobacco, alcohol or lowering lipids drugs.

Clinical data of hypertensive patients and control group were selected from anamnestic and clinical exams made to each patient at the moment of inclusion in our study. Blood pressure measurements were performed according to the guidelines of the European Society of hypertension (ESH) [8].

Blood was taken from vein into vacutainers and after centrifugation (10 min at 1500×g) serum was immediately separated and stored in aliquots at –80°C until use. Next step was to use the serum on ELISA kit PON1 for the quantitative determination of human serum paraoxonase 1 concentration. We also included the determination of total cholesterol (CT) from venous blood, triglycerides (TGR), low-density lipoprotein (LDL) and high-density lipoprotein (HDL).

After obtaining the PON1 data and lipid fractions levels we used the AIP (atherogenic index of plasma), which is based on a logarithmic formula that includes values of TG and HDL levels.

All results were expressed as mean and standard deviation (SD). The data were analysed for statistical significance using a *t-test* calculator. For values of $p<0.05$ was considered significant, for values of $p<0.01$ it was considered distinctive statistically significant, for values of $p<0.001$ was considered very significant statistically and for p values >0.05 were considered non-significant.

Results

Our results obtained from the history and clinical examination specific for each patient from the study groups are shown in Table I. The blood pressure (BP), body mass index (BMI) were very different for the study groups compared with the control group, so we can say that these parameters along with the age represent important risk factors for each hypertensive patient.

Higher values for BP were found in the H4 group of study (hypertensive patients with diabetes and kidney disease), also higher values had the H2 group (hypertensive patients with cardio-metabolic syndrome) for the BMI parameter, in this group were the most numerous patients with obesity.

Table I – **Anamnestic data for the hypertensive patients from the study groups by gender**

GROUP	Parameters*					
	Age[1]		BMI[1]		BP[1]	
	♂	♀	♂	♀	♂	♀
Control	38,5±2,3	37±4,2	24,3±3,2	20,4±1,6	124±2,1 / 78±1,7	118±1,2 /74±1,7
H1	42±5,7	39±4,5	25,9±2,3	25,1±1,2	148±1,7 / 96±1,8	146±1,9 / 94±2,4
H2	46,5±3,6	44.5±4,8	31,5±4,6	29,5±5,3	150±3,2 / 92±2,4	148±2,8 / 98±2,3
H3	47±5,1	46±7,2	25,8±1,7	24,9±1,4	168±2,2 / 106±2,4	156±2,6 / 104±2,8
H4	52±4,2	55±3,1	25,3±3,8	25,5±4,7	176±2,8 / 104±2,9	172±3,5 /108±4,1

Legend:
[1] *average (mean) and standard deviation; BMI = body mass index, BP = blood pressure;*

The lipid fractions for all the study groups were modified, higher values were observed for the study group H2 (hypertensive patients with metabolic sindrome) also the values for the AIP were very high for this group compared with the control group (p=0.0025 for the male subjects in study group H2) or even with all other study groups.

Table II – **Biochemical data for the study groups, compared with the control group**

Parameters for study groups	Control		H1		*p*	
	♂	♀	♂	♀	♂	♀
TC, mg/dl (SD)*	188,1 (4,2)	186,1 (1,2)	**212,4 (2,6)**	**205,3 (1,4)**	0,0257	0,0275
TG, mg/dl (SD)*	138,3 (5,4)	122,8 (4,2)	**198,4 (2,4)**	**194,2 (2,2)**	0,0227	0,0214
HDL, mg/dl (SD)*	52,1 (4,4)	58,1 (2,8)	**48,5 (2,9)**	**54,8 (3,4)**	0,0224	0,0189
AIP, (SD)*	0,078 (0,23)	0,054 (0,8)	**0,19 (1,4)****	**0,14 (1,2)****	0,0158	0,0147
	Control		**H2**		***p***	
TC, mg/dl (SD)*	188,1 (4,2)	186,1 (1,2)	**322,2 (1,7)**	**288,4 (1,5)**	0,0038	0,0026
TG, mg/dl (SD)*	138,3 (5,4)	122,8 (4,2)	**424,3 (4,7)**	**365,4 (5,1)**	0,0023	0,0068
HDL, mg/dl (SD)*	52,1 (4,4)	58,1 (2,8)	**38,5 (1,5)**	**40,2 (2,4)**	0,0224	0,0189
AIP, (SD)*	0,078 (0,23)	0,054 (0,8)	**0,68 (1,4)*****	**0,60 (1,2)*****	0,0025	0,0032
	Control		**H3**		***p***	
TC, mg/dl (SD)*	188,1 (4,2)	186,1 (1,2)	**234,4 (2,8)**	**220,7 (2,5)**	0,0096	0,0124
TG, mg/dl (SD)*	138,3 (5,4)	122,8 (4,2)	**260,3 (3,7)**	**256,2 (4,3)**	0,0142	0,0156
HDL, mg/dl (SD)*	52,1 (4,4)	58,1 (2,8)	**41,2 (1,6)**	**46,4 (1,8)**	0,0224	0,0189
AIP, (SD)*	0,078 (0,23)	0,054 (0,8)	**0,47 (1,9)*****	**0,38 (1,8)*****	0,0118	0,0221
	Control		**H4**		***p***	
TC, mg/dl (SD)*	188,1 (4,2)	186,1 (1,2)	**246,8 (6,2)**	**238,5 (4,2)**	0,0124	0,0148
TG, mg/dl (SD)*	138,3 (5,4)	122,8 (4,2)	**278,4 (2,3)**	**262,5 (3,5)**	0,0214	0,0262
HDL, mg/dl (SD)*	52,1 (4,4)	58,1 (2,8)	**36,4 (1,3)**	**38,5 (2,9)**	0,0259	0,0192
AIP, (SD)*	0,078 (0,23)	0,054 (0,8)	**0,53 (3,2)*****	**0,48 (2,8)*****	0,0025	0,0018

Legend:
*TC – total cholesterol, TG – tryglicerydes, HDL – high density lipoprotein, LDL – low density lipoprotein; AIP – atherogenic index of plasma: range of AIP values are: <0.11(low risk)**, 0.11 (intermediate risk), >0.21 (high risk)*** indicates high risk for atheroslerosis; * values expressed as mean (±standard deviation); the value of p<0.05 was considered significant, values of p>0.05 considered non-significant;*

The results obtained for human paraoxonase 1 (PON1) for all the study groups (H1-H4) were presented graphically in Fig. 1 for male patients and in Fig.2 for female patients. The normal range for PON1 is between 70-100 ng/dl.

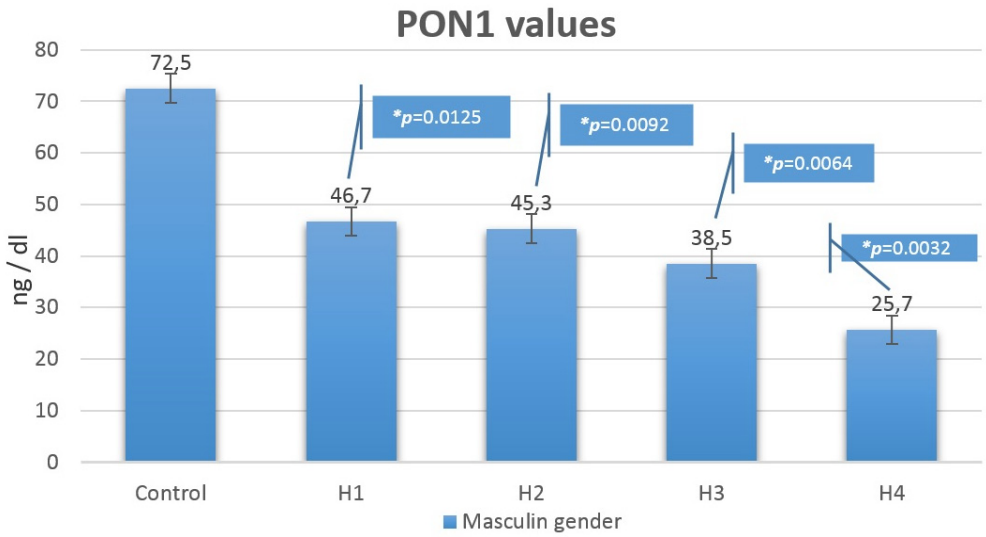


Fig. 1. **Human paraoxonase 1 values for the male patients**

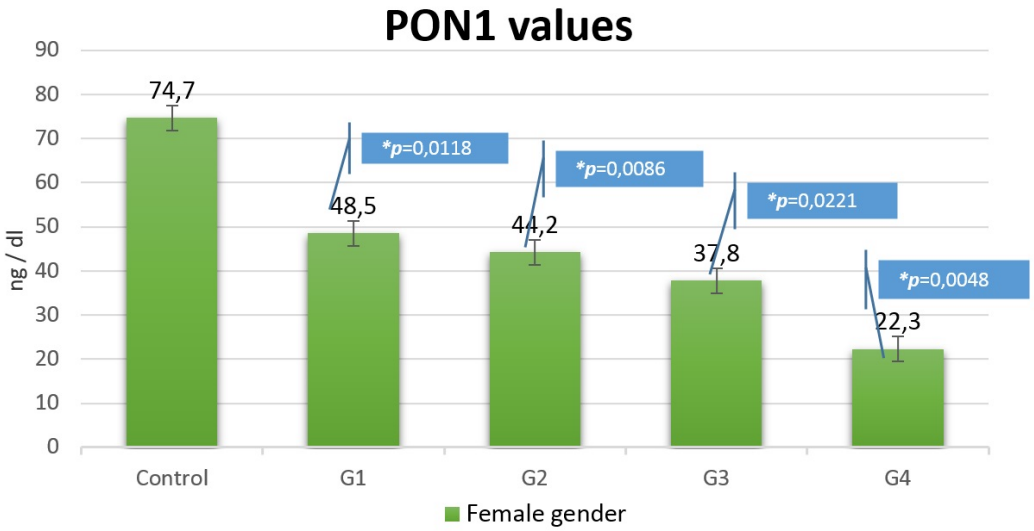


Fig. 1. **Human paraoxonase 1 values for the female patients**

For H1 study group (newly discovered patients with hypertension) PON1 values were on average 46,7 ng/dl for male individuals (compared to control group 72,5 ng/dl) and 48,5 ng/dl for female individuals (compared to control group 74,7 ng/dl).

Even if for the H2 group (hypertensive patients with metabolic syndrome) all the above presented parameters were significant modified compared with the control group and the others study groups, the PON1 values were on average 45,3 ng/dl for male persons (compared to control group 72,5 ng/dl) and 44,2 ng/dl for female individuals, which were low levels but not the lowest for this parameter.

H3 group (patients with hypertension and type II diabetes) PON1 values were on average 38,5 ng/dl for male patients (compared to control group to 72,5 ng/dl) and 37,8 ng/dl for the female patients (compared to control group 74,7 ng/dl).

The lowest serum levels for PON1 was for H4 group (patients with hypertension, type II diabetes and renal disease) which were on average 25,7 ng/dl compared to the control group 72,5 ng/dl for male individuals, and 22,3 ng/dl for female individuals compared to control group 74,7 ng/dl (Fig.1 and Fig.2).

As particular results, some subjects from the control group (3 females and 6 males) were having low serum levels of PON1 ranged between 55-68 ng/dl without having known risk factor from the anamnesis.

PON1 values can be easily modified by diet, exercise, stress, medication and lifestyle. Considering these conditions, our results were obtained for two different PON1 serum levels dosing at an interval of 2-3 weeks. The resulting values were close and we made an average of the two determinations.

Discussion

To estimate the atherogenic risk for patients with chronic diseases, currently are several methods, depending on which study it is based on, such as TC/HDL ratio or HDL/LDL ratio, computers that are using the Friedewald formula or the Iranian formula and more others [9]. Atherogenic index of plasma (AIP) is an atherogenic risk assessment tool and it is based on a formula that uses the TG and HDL levels [10, 11].

Recent studies have indicated that the logarithmically transformed ratio of TG/HDL can estimate the atherogenic risk better than all others atherogenic indexes used before [12]. TG and HDL reflects the perfect balance between atherogenic lipoproteins and protective lipoproteins. Atherogenic index of plasma (AIP) can predict cardiovascular risk and it can be very sensitive to pharmacological treatment, as a barometer of therapeutic success [13].

Our studies indicated high AIP values for all hypertensive patients with elevated lipid fractions. The highest values for AIP were for male patients as can it can be seen in Table II, the values for all lipid parameters and AIP (marked in red) were significantly higher.

Regardind the human paraoxonase 1 serum levels, Aviram *et al* [14] showed that this enzime has a major role in the endothelium, protecting HDL and LDL molecules to be oxidized by free radicals, and when its values in plasma are high it interferes even with atheromatous plaques already installed, reduces its size and causes local inflammation by peroxidase and lactonase activity (alleviating the cholesterol macrophages, the oxidized lipid accumulation and formation of foamy cells).

These facts are confirmed by Gugliuci and his team [15], as well as by other scientists, who also admitted that from the family of paraoxonase (PON1, PON2 and PON3), the most active and important in the atherogenesis process is PON1 with all its polymorphism variants in different populations. Also some other researchers showed that in hypertensive patients PON1 activity decreases, also for the patients with diabetes the lipid protection against oxidation by PON1 may be reduced because of lower enzyme activity [16, 17].

Our results confirm the medical literature in that the values of PON1 for each study group of hypertensive patients were indeed at a low level. Even more, PON1 values were significant lower for the hypertensive patients with diabetes mellitus and kidney disease, taking in consideration that these subjects' age was higher than the rest of the study groups and that these patients were having associated diseases compared with the other study groups. Values of *p* for each study groups indicate a statistically significant difference for the results of serum levels of PON1 compared with the control group.

Further in the AIP results we observed for the group H2 with hypertensive patients with metabolic syndrome, the lowest values for both male and female patients (0,68 for ♂ and 0,60 for ♀), with higher values for male patients. But for the same group the PON1 levels indicated low serum levels for the enzyme but not the lowest as it is for the H4 study group with hypertensive patients and with associated chronic diseases (diabetes and kidney failure) which had the lowest levels of PON1 (22,3 ng/dl for female patients and 25,7 ng/dl for male patients). Also we observed a difference between the risk indicated by the AIP results for the groups H3 and H4 and the atherogenic and cardiovascular risk indicated by PON1 values for the same hypertensive patients. The risk was more increased for the male patients than the female patients indicated by the AIP in these groups, but the PON1 values indicated a higher risk for the female patients than the male patients in the H3 and H4 groups of hypertensive patients.

Conclusions

Atherogenic risk can easily be estimated only by using the values of the lipid fractions from which can be calculated the cardiovascular risk indexes. From our study we determine that the AIP is

most sensitive to the values of lipid fraction. If we associate this index with determining PON1 values than we have a higher accuracy in estimation of developing cardiovascular disease.

Determining the PON1 values may be a useful and effective tool for quantifying the risk of atherogenesis and cardiovascular disease development over time.

PON1 is more sensitive for determining atherogenic and risk of future cardiovascular events even in patients with fewer risk factors and it is more accurate than cardiovascular risk indiated by AIP.

Even if PON1 values can be influenced by many factors (which can change its values by a few percents), it indicates accurately the cardiovascular risk.

Through evaluation of the data obtained and by linking to the two most useful new medical instruments, AIP and PON1 levels, we can estimate the patient's risk for possible CV events in the next years, and it can be also a personalized investigation. As it can be seen PON1 is more accurate than AIP for cardiovascular risk, for example, low levels of plasma PON1 for young patients in our study who were without any cardiovascular risk.

References

[1] Tiwari H. et al, (2007). Multifactorial inheritance and complex diseases. Rimoin's principles and practice of medical genetics, pp. 299–306.

[2] King R., Rotter J., Motulsky, A. (2002) The genetic basis of common diseases, Oxford University Press.

[3] Alwan A. et al. (2010). Monitoring and surveillance of chronic noncommunicable diseases: progress and capacity in high-burden countries. The Lancet (376), pp. 1861–1868.

[4] World Health Organization (2008). The global burden of disease: 2004 update. Geneva.

[5] Bin Ali A., Zhang Q, Lim YK, Fang D, Retnam L, Lim SK (2003). Expression of major HDL-associated antioxidant PON-1 is gender dependent and regulated during inflammation. Free Radic Biol Med (34), pp.824–829.

[6] Mackness M., Arrol S., Durrington P. (1991) Paraoxonase prevents accumulation of lipoperoxides in low-density lipoprotein. FEBS Lett (286), pp. 152–154.

[7] Hong Y. (2010). Atherosclerotic Cardiovascular Disease Beginning in Childhood. The Korean Society of Cardiology, Korean Circ J (40) pp.1-9.

[8] ESH and ESC Guidelines (2013). The Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC).

[9] MacMahon S., Robson R., Johnsona R. (1997). Use of the Friedewald Formula to Estimate LDL-Cholesterol in Patients with Chronic Renal Failure on Dialysis. Clinical Chemistry, 43 (11), pp.2183-2184.

[10] Dobiasova M, Frohlich J. (2001). The plasma parameter log (TG/HDL-C- C) as an atherogenic index. Clin Biochem 34, pp. 583-588.

[11] Nigeria U., Nwagha E., Ikekpeazu F., Ejezie E., Neboh I. (2010). Atherogenic index of plasma as useful predictor of cardiovascular risk among postmenopausal women in Enugu. Afr Health Sci. 10(3), pp. 248–252.

[12] Onat A., Can G., Kaya H., Hergenç A. (2010). Atherogenic index of plasma (log10 triglyceride/high-density lipoprotein−cholesterol) predicts high blood pressure, diabetes, and vascular events. Journal of Clinical Lipidology, 4(2), pp. 89–98.

[13] Arumalla V., Vani N., Dose R. (2011). Impacts on the Diagnostic Efficacies of Atherogenic Lipids in Adult Indian Smokers. Journal of Clinical and Diagnostic Research, 5 (7), pp. 1352-1355.

[14] Aviram M., Rosenblat M. (2004). Paraoxonases 1, 2, and 3, oxidative stress, and macrophage foam cell during atherosclerosis development. Free Radic Biol Med 37, pp.1304–1316.

[15] Gugliucci A., Kotani K., Kimura S. (2012). Paraoxonase 1 in Chronic Kidney Failure. Journal of Lipids Volume 2012, Article ID 726048, 10 pages.

[16] Bakhit M., Al-Najai M., Vigilla M., Al-Boudari O., Muiya P., Meyer B., AL-Mohanna F., Alshahid A., Dzimiri N. (2010). The PON1 is a susceptibility gene for hypertension. The FASEB Journal 24, pp.524-525.

[17] Flekač M., Skrha J., Zídková K., Lacinová Z., Hilgertová J. (2008). Paraoxonase 1 Gene Polymorphisms and Enzyme Activities in Diabetes. Physiol. Res. 57, pp. 717-726.

Prognostic Value of Proliferation Index in Patients with Breast Cancer

Trifonova I.

National Oncology Medical Center, 6 Plovdivsko Pole Str., 1756 Sofia, Bulgaria
E-mail: itrifa@abv.bg

Summary

The prognostic value of the proliferation index PCNA (proliferating cell nuclear antigen) in patients with breast cancer is evaluated in the paper. A tumor marker in soft matter has been derived, based on PCNA. It has been found that this marker is a virtual equivalent of a genomic marker, which evaluates breast cancer as a plasmid DNA.

Introduction

Soft matter is a matter between the nano and micron scales, where the new characteristics occur, due to the collective behavior in it and as well the complex flow there [1]. The scale of this matter is the same as the scale of life.

The aim of this work is to find the prognostic value of the proliferation index PCNA (proliferating cell nuclear antigen) in patients with breast cancer. Therefore it will be looking for a surrogate endpoint for the breast cancer survival in relation to the index of proliferation by the dual view ('network'/'system' medicine) of cancer via the intensity of the tumor growth in soft matter and the tumor motif in a soft matter and by chemotherapy in the soft matter, according to the resources of the soft matter.

Tumor marker in soft matter

The tumor marker in soft matter is composed of metric tumor marker and structural tumor marker.

Let PI is the proliferation index in percentages, divided by 10. The tumor marker in soft matter is computed from the proliferation index as follows: 1/ $\beta=\pi(PI^2-0.36)/(10PI)$, $\omega=4/\beta$; 2/ $K(\omega)/\pi=1.063889771190501 + 2.643153894293523\omega - 3.112863789173286\omega^2 + 1.853349991880126\omega^3 - 5.023123875855986D\text{-}01\omega^4 + 5.211379178766142D\text{-}02\omega^5$; 3/ $k^*=(K(\omega)/\pi-2)/10$, $\mu=\left| 10k^*/\beta-5 \right|^{1/2}$, $c_1=3.56(\mu-1)$, $c_2=-5\beta$; 4/ The value of the metric tumor marker is m_s = {2.2, if $100k^*<2.5$; 2.9, if $2.5\leq 100k^*<3.5$; 4.1, if $3.5\leq 100k^*< 5.7$; 7.3, if $5.7\leq 100k^*$}; 5/ The mode of the structural tumor marker is

Mode	c_2	c_1
1.1/ ring	$c_2<-8.26$	$16.34<c_1$
1.2/ ring	[-13.22 , -14.36)	$c_1<0.25$
2/ two stacked rings	[-9.08 , -8.56)	[12.38 , 14.7)
3/ knot	[-9.38 , -9.08)	[11.04 , 12.38)
4.1/ link	[-12.43 , -9.38)	[1.9 , 11.04)
4.2/ link	$c_2<-15.24$	$1.97<c_1$
5.1/ decahedral	[-12.56 , -12.43)	[1.53 , 1.9)
5.2/ decahedral	[-15.24 , -15.14)	[1.78 , 1.97)
6.1/ relaxation	[-13.22 , -12.56)	[0.25 , 1.53)
6.2/ relaxation	[-15.14 , -14.36)	[0.25 , 1.78)
6.3/ relaxation	[-8.56 , -8.28)	[14.7 , 16.34)

Here β is the inverse temperature, whereas k* is the intensity of tumor development in soft matter.

The metric tumor marker values are the grades of a tumor kicked impact. The mode of the structural tumor marker is specified by the parameters c_1 and c_2 of the type of its corresponding attractor of a dissipative system.

Tumor as plasmid DNA

Tumor energy in soft matter is energy of a turbulent flow. Then the tumor energy in soft matter E is obtained as the energy of the turbulent flow for time t with a wave number λ_w. In this case the time corresponds to the tumor development intensity k*, t=10k*, and the wave number corresponds to the inverse temperature β, λ_w=10β/ 3+5.

The state of plasmid DNA is determined by writhe/excess of the contact-free configurations of closed rods [2]. Then 'Plasmid DNA' state of the tumor in soft matter is obtained as the state of plasmid DNA with writhe W_{rJ}, set by the inverse temperature, W_{rJ}=10β/3.56, and excess ΔL_{kJ}, set by the tumor energy E, ΔL_{kJ}=191.415E.

Chemotherapy intensity

Let the cancer 'cycle' is a tumor development with a measured intensity ms, followed by immunoreactivity with a unit intensity. In this case the measured tumor intensity development m_s is discretization of the value 100k*, where k* is the tumor development intensity. Let the treatment consists of three successful identical chemotherapy cycles. Each cycle is a chemotherapy course with intensity k_s and a restoration period with single intensity.

The cancer cycle, followed by the three chemotherapy cycles, determines a cyclic system from four species with four resources. Chemotherapy intensity k_s is determined for this system when the tumor development changes the excess ΔL_{kJ} of plasmid DNA.

Tumor socio-thermodynamic state

Let a tumor and soft matter are two proteins that are either flexible or rigid. Binding of these proteins is a hawk-dove game. Socio-thermodynamic tumor state in soft matter before the cyclic chemotherapy corresponds to this game strategy with the hawk fraction inversely proportional to the tumor intensity development k* and with the price, equals to the inverse temperature β. Socio-thermodynamic tumor state in soft matter after the cyclic chemotherapy corresponds to this game strategy with the hawk fraction inversely proportional to the chemotherapy intensity k_s and with the price, equals to the inverse temperature β.

Flow in tumor development

Let the tumor in soft matter acts as stretching. The stretching force changes the internal soft matter energy. This change in internal energy is heard in ultrasonography.

Plasmid DNA tumor state in soft matter, determined by ultrasonography, is found as plasmid DNA state with writhe W_{rJ}, set by the inverse temperature β, W_{rJ}=10β/3.56, and excess ΔL_{kJ}, set by the transferred ultrasound energy E_n, ΔL_{kJ}=E_n.

Resource restoration of the soft matter with a tumor is determined by the stretching force and by the cyclic chemotherapy intensity k_s.

Plasmid DNA tumor state in soft matter, after the cyclic chemotherapy with the above resource restoration, is obtained as plasmid DNA state with a writhe W_{rJ}, set by the inverse temperature β, W_{rJ}=10β/3.56, and excess ΔL_{kJ}, set by the transferred chemical energy E_a, ΔL_{kJ}=E_a.

Success of cyclic chemotherapy

Success of the cyclic chemotherapy in soft matter is determined according to Bayes' formula by the transferred chemical energy in the soft matter E_a and by the entropy in the soft matter for cyclic chemotherapy intensity k_s. Herein the transferred chemical energy presents the toxicity of chemotherapy, at given efficacy, and the entropy presents the toxicity of chemotherapy.

Disscusion

The metric tumor marker in soft matter is a virtual equivalent of the quantum dot marker CdSe for breast cancer diagnosis. The structural tumor marker in soft matter is a virtual equivalent of a genomic marker, which evaluates the breast carcinoma as a plasmid DNA. Therefore the built tumor marker in soft matter evaluates the breast carcinoma as a plasmid DNA.

The tumor chemotherapy in soft matter is introduced as a treatment regimen with three cycles. This treatment is a social thermodynamics, which homogenizes the soft matter with a tumor in it, annuls the metastatic potential of the tumor, and exhausts the soft matter potential to affect the tumor. Then the built tumor marker in soft matter is a surrogate endpoint in the nanomedicine for heterogeneity of tumor tissue (tumor response), for the change of the system 'tumor organism' energy and for the recovery time after doing the chemotherapy course.

Bayes estimate of the probability for success of cyclic chemotherapy in soft matter is derived from the transferred chemical energy and from the soft matter entropy. Survival probability [3] of a patient with a tumor is juxtaposed to the time scaling probability of success of cyclic chemotherapy in soft matter. Then the built tumor marker in soft matter is a surrogate endpoint in the personalized medicine for the cancer survival, considered as plasmid DNA.

Conclusion

The prognostic value of the proliferation index PCNA in patients with breast cancer is found in this paper: 1/ Tumor marker is built in a soft matter, which evaluates the breast carcinoma as a plasmid DNA; 2/ It is shown that in nanomedicine this marker is a surrogate endpoint for the heterogeneity of the tissue with a tumor, for the change of the system 'tumor organism' energy and for the recovery time of course performed chemotherapy; 3/ It is shown that in personalized medicine this marker is a surrogate endpoint for the tumor survival, regarded as a plasmid DNA.

References

[1] DE GENNES PG, Soft matter, Angewandte Chemie International Edition in English, 842-845, 1992.
[2] COLEMAN BD, SWIGON D, Theory of self-contact in Kirchhoff rods with applications to supercoiling of knotted and unknotted DNA plasmids, Phil Trans R Soc Lond A 362, 1281-129, 2004.
[3] BIANCHINI G, IWAMOTO T, QI Y, COUTANT C, SHIANG CY, WANG B et al, Prognostic and therapeutic implications of distinct kinase expression patterns in different subtypes of breast cancer, Cancer Res 70, 8852-8862, 2010.

Numerical determinations of the resistance structures of the facial massive through CT histograms

Cioban G.C.*

* Vasile Goldiş" Western University Arad, Faculty of Medicine, Pharmacy and Dentistry, Romania

Abstract

The histogram is a technique that can be performed by CT densitometry measurements of body segments and graphically represented.

Histograms performed on areas of maximum strength and low strength of the architectural structures of the massive facial on 4 groups of 20 cases between 2-20 years, 20-45 years, 45-75 years and over 75 years have shown that the average density of segments of areas of maximum resistance the average values are 700 UH compared with bone structures in areas of low resistance with medium density below 300 UH.

CT densitometry measurements through histograms add arguments through numeric values in Housfield scale concerning the architecture of the head skeleton.

Introduction

Important component of the whole skeleton head strength functional structures were divided neurocranium and viscerocranium teaching architecture, but in reality integrable wearing phylogenetic fingerprints of cephalization.

In time functional structures of the skull resistance were analyzed by representative researchers as: SICHER, FELIZET, TANDLER, DUBECQ, BENNINGOFF, TESTUT, BRAUS and Romanian authors RAINER and GRIGORE T. POPA.

Through the studies of Niculescu V. and M. Niculescu, Romanian researchers, have developed new interpretations regarding the resistance of the skull base complex, and as well new names for some structures.

The Functional Structures of Resistance

The concept of the authors NICULESCU V. and M. NICULESCU compares neurocranium with a tight parcel surrounded by belts of resistance. These are composed by the vaults springs , and the rafters base which are continuous with each other through the resistor junctions, which replaces the traditional concept of columns or pillars. Are designed terms: central skull base resistance and resistance belts incorporating arches and rafters, beams composed of compact bone unevenly distributed in vertical and horizontal columns of different thicknesses.

Maximum resistance areas:

A. Sicher and Weimmdi (1941)vertical pillars of strength described by Richer (1873) are composed of 6 compact bone bays, three on each side of the massive facial. Are generated by functional pressure in the vertical maxillary jaw during mastication act. Bone bays that arise at the base jaws are held in bundles of compact bone, thickness ranging from 3-5 mm leading the forces to the base of the skull to disperse them bilaterally and symmetrically in this massive complex bone.

B. Vertical pillars are:

- Anterior pillar (naso maxillary) with path: bottom edge portion alveolar jaw joins the frontal.
- Lateral pillar (or zigomatico malar malar) alveolar region, pyramidal apophysis of malaria.

- Ilierul posterior or pterigoridian - maxillary tuberosity base and apophyses pterigoid.

C. Beams the facial massive, three on each side of the median line are composed of compact bone and very little cancellous bone.

- Lower beam formed by alveolar bone and palatine apophyses of the jaw.
- Middle beam constitute lower horizontal ledge.
- Upper beam forming upper orbital rim.

D. Floors or horizontal platforms are two on each side of the midline. Only the lower floor is an area of strength.

- lower floor (hard palate palatine consists of the union of apophysis of the jaw.

E. The facial massive cavities are multiple, symmetrical shape, volume and different functions, lined with a mucous adherent to the periosteum. Some cavities are pneumatic (nasal passages, sinuses, ethmoid cells) other contain organs (oral cavity, orbital and nasal cavities).

F. Interosseous sutures of the facial massive and between them and the base of the skull dampens shocks and creates a special resistance at this level.

Areas of low resistance:

Between the vertical pillars, horizontal beams and floors made of hard compact bone, is situated the great lines of low resistance located in thin lamellar bone of walls. There were class by La Fort in 1901 in three horizontal zones:

- First area intercranial – maxillary
- The second zone runs across the middle of the massive facial passing through orbital floor under the middle third of malar pterigoid apophysis.
- The third area is above the palatine platform up to the lower third of pterigoid apophyses.

Besides those three areas there are:

- Middle floor (orbital ceiling or orbital floor) with low resistance.
- Upper floor (of the facial massive) in the third lateral orbital form the upper wall and in the middle third complex naso-etmoido-maxillary, common element with the base of the skull, the skull bounded by the ethmoid riddled blade.

Aim of the Thesis

By using normal bone densitometry determinations of viscerocranium structures resistance provide arguments numerical endpoints that complement the modern concepts on the functional architecture of the facial massive.

The use of computerized tomography technical possibility of achieving histograms, creates the possibility of presenting quantitative maximum and minimum resistance structures of the skull, which represents its strength lines.

Material

We have studied 80 patients, who conducted computer scans of the facial massif, at CT compartment of Euromedic Center Arad

Method

The 80 cases proposed for research have been clinically examined and were performed to required X-ray on standard position and incidence useful to select a group for the study of 80 cases, which did not present the pathological changes to the skull.

Computer-tomographic examinations were performed with multislice unit at Euromedic Center Arad.

Were carried out centered histograms on viscerocranium bony structures, and the histograms results were evaluated correct numerical density of these bone structures. The histogram is a technique that allows the delimitation of a massive facial segment of interest which determines the average density.

Densitometry results, digital evaluated, are shown numerically and graphically.

Viewing the image in the axial plane is obtained after analog digital conversion that allows a numerical value correspond to to a gray level on the monitor. the optimal Adapted window is 1000 - 1500 UH.

The 80 patients scheduled for the study were selected in four age groups: 2-20 years 20-45 years 45-75 years over 75 years.

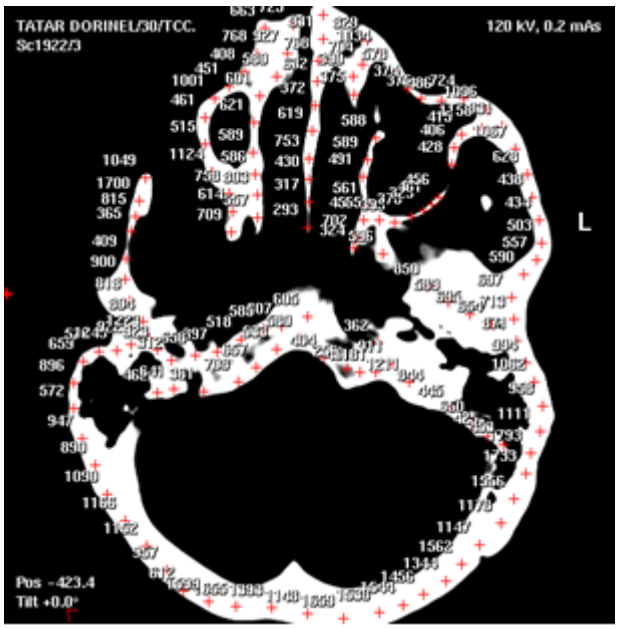
By using this method of research, the numerical measurements of bone density of resistance structure of the facial massive, we set numerical structures, of the maximum strength and weakness and differences of their bone density in the four age groups settled.

Results

Of the total of 80 patients who were performed computer-tomography in axial and coronal sections contiguous to 5mm and bilaterally histograms were selected 20 patients for age groups 2-20years, 20-45ani, 45-75ani and over 70.

CT densitometry determinations on the histograms made revealed the following:

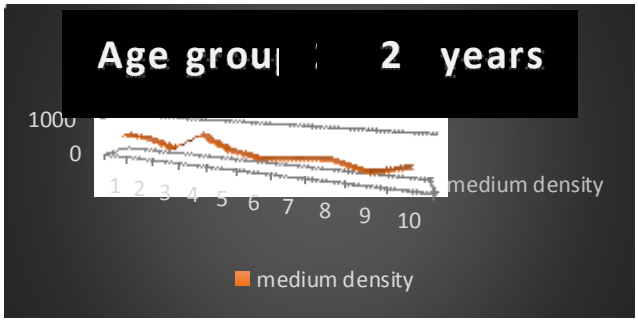
CT HISTOGRAM OF MASSIVE FACIAL STRUCTURES ARCHITECTURAL



CT INCIDENTAL AXIAL OF MASSIVE FACIAL: skull CT, coronal incidence. The average density is 750 U.H. with variations between 1306 U.H. the anterior extremity and 355 UH in the middle third**.**

<u>Densitometry results for the age group 2-20 years.</u>

	segment examined	medium density
1	Superior orbital wall	520
2	Lateral orbital wall	470
3	Median orbital wall	250
4	Inferior orbital wall	702
5	Ethmoid	354
6	nasal septum	224
7	Anterior maxillary wall	320
8	Lateral maxillary wall	370
9	Median maxillary wall	172
10	Inferior maxillary wall	370

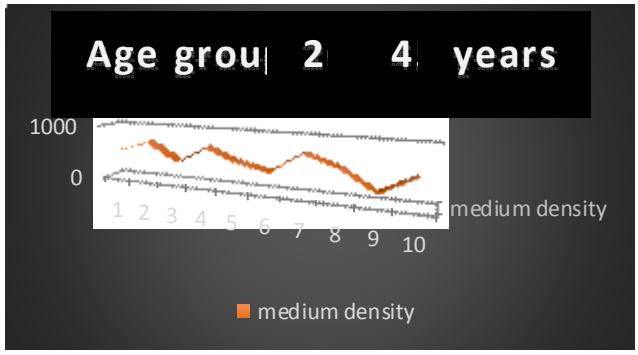


Segments with high densities over 500 UH which constitute the maximum resistance structures are superior orbital wall, lateral orbital wall, inferior orbital wall, lateral maxillary wall, lower jaw wall.

Segments with low densities below 300 UH are orbital medial wall, anterior maxillary wall, median maxillary wall, nasal septum, ethmoid are part of the weak resistance structures.

Densitometry results for the age group 20-45 years.

	segment examined	medium density
1	Superior orbital wall	530
2	Lateral orbital wall	724
3	Median orbital wall	395
4	Inferior orbital wall	706
5	Ethmoid	480
6	nasal septum	366
7	Anterior maxillary wall	733
8	Lateral maxillary wall	540
9	Median maxillary wall	184
10	Inferior maxillary wall	512

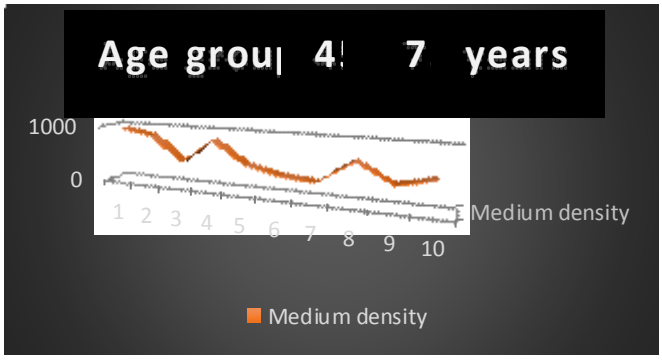


Segments with increased densities which constitutes the maximum resistance structures: superior orbital wall, lateral orbital wall, inferior orbital wall, maxillary lateral wall, anterior maxillary wall.

Segments with lower density: median maxillary wall, nasal septum, medial orbital wall, are part of the structures of low resistance.

Densitometry results for the age group 45 - 75 years.

	segment examined	medium density
1	Superior orbital wall	960
2	Lateral orbital wall	860
3	Median orbital wall	440
4	Inferior orbital wall	856
5	Ethmoid	480
6	nasal septum	350
7	Anterior maxillary wall	299
8	Lateral maxillary wall	696
9	Median maxillary wall	379
10	Inferior maxillary wall	520

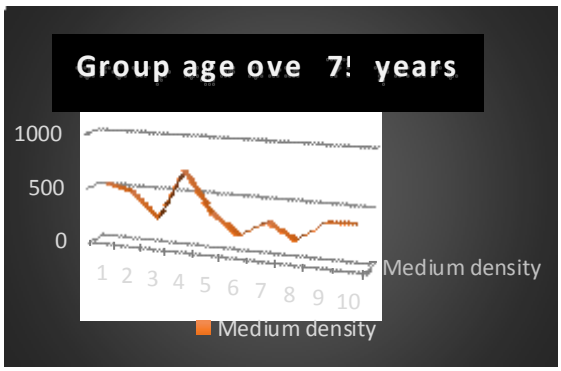


Segments with increased densities which constitutes the maximum resistance structures: lateral orbital wall, inferior orbital wall, maxillary lateral wall, inferior maxillary wall.

Segments with lower density: median maxillary wall, anterior maxillary wall, nasal septum, are part of the structures of low resistance.

Densitometry results for the age group over 75 years.

	segment examined	medium density
1	Superior orbital wall	535
2	Lateral orbital wall	480
3	Median orbital wall	250
4	Inferior orbital wall	714
5	Ethmoid	350
6	nasal septum	170
7	Anterior maxillary wall	320
8	Lateral maxillary wall	182
9	Median maxillary wall	370
10	Inferior maxillary wall	380



Segments with increased densities which constitutes the maximum resistance structures: lateral orbital wall, superior orbital wall, anterior maxillary wall, median maxillary wall.

Segments with lower density: median orbital wall, lateral maxillary wall, nasal septum, are part of the structures of low resistance.

Maximum resistance structures with high average densities are superior orbital wall, lateral orbital wall, lateral maxillary wall, pillar structure formations anterior (naso-maxillary) into the medium beam structure.

Inferior orbital wall is part of the lateral pillar (zigomatico-maxillary) is lower beam of the facial massive

Medial orbital wall, anterior maxillarywall, medial maxillary wall, ethmoid and nasal septum with densities under 300UH are part of the structures of low resistance.

Discussions

This paper aims to bring new arguments to support recent researches on the architectural structure of viscerocranium through computer tomography examination with digital assessing histograms, number densities of massive facial bone structure.

Recent research (Niculescu et al) based on sections of bone, radiographic imaging and computer tomography, confirmed by our research conducted through histograms, we have outlined new concepts on functional structures of resistance of the upper floor of viscerocranium.

Personal study through computer-tomography histograms on facial massive architectural structures, brought scientific elements embodied in Housfield units, which explains the importance of different bone densities structures viscerocranium resistance.

The study conducted by explaining the computer tomography, through histograms on bone mineral density, of a total of 80 healthy volunteers, divided into four groups by age: 2-20 years old, 20-45, 45-75 and over 75 years reveals the following important observations: the densities strength structures of viscerocranium are different by age.

- At age 2-20 years between 702 U.H. and 172 U.H.
- Between 20-45 years, 724 U.H. - 184 U.H.
- Between 45-75 years 856 values U.H. - 379 U.H.
- Over 75 years, values between 714 and 182 UH UH

Peak bone density is considered over 700 UH and densities below 300 UH are minimum density bone.

The average minimum densities are increased in the groups 45-75 years and age groups between 20-45 years and above 75 years are similar

Conclusions

After Niculescu et al are eight lamellar structure of resistance of facial massive, five vertical and three horizontal. Cylinders and cones of resistance of the facial massive have a structural and elasticity role. Knowing the functional structures of resistance of viscerocranium shows anatomic and practical interest through the diagnosis and prognosis of fractures, useful to many specialties.

Knowing the normal densities of viscerocranium resistance structures, enables an accurate, quantitative assessment, of the normal density of the pillars and the facial massive resistance groups.

Histograms performed separately of viscerocranium bony structures and graphical representation of their density is an objective method by using the imaging techniques, by using multislice computer tomography and densitometry measurements reformatting.

The segments of maximum strength, with bone density over 700 UH are inferior, superior and lateral orbital wall, lateral and inferior wall of maxilla.

Least resistance segments, with density below 300UH are median orbital wall, the anterior and median wall of the maxillary, nasal septum and ethmoid.

Fractures of the facial massif is in the ratio of 70% in the areas of least resistance.

Through the study we conducted regarding CT histograms raised objective arguments by determining proper densities of facial massive segments, new concepts on the functional architecture problems of the facial massive.

Refereces

1. Buthian D. TDM et IRM Cliniques Frison –Roche Paris 1991
2. Călin Gheorghe Ciobanu, Virgiliu Niculescu, Examinarea Computer tomografică a masivului facial. Referat pentru teză de doctorat 2003
3. Călin Gheorghe Ciobanu, Virgiliu Niculescu. Arhitectura funcţională a neurocraniului. Referat pentru teză de doctorat 2003
4. Călin Gheorghe Ciobanu, Virgiliu Niculescu. Particularităţi anatomice ale fracturilor masivului facial. Referat pentru teză de doctorat 2004.
5. Czerwinski M, Lee C: The rationale and technique of endoscopic approach to the zygomatic arch in facial trauma. Facial Plast Surg Clin North Am 2006 Feb; 14(1): 37-43[Medline].
6. DeMarino DP, Steiner E, Poster RB, et al: Three-dimensional computed tomography in maxillofacial trauma. Arch Otolaryngol Head Neck Surg 1986 Feb; 112(2): 146-50[Medline].
7. Egbert JE, May K, Kersten RC, Kulwin DR: Pediatric orbital floor fracture : direct extraocular muscle involvement. Ophthalmology 2000 Oct; 107(10): 1875-9[Medline].
8. Gerbino G, Roccia F, Benech A, Caldarelli C: Analysis of 158 frontal sinus fractures: current surgical management and complications. J Craniomaxillofac Surg 2000 Jun; 28(3): 133-9[Medline].
9. Gheorghe Ciobanu, Călin Gheorghe Ciobanu, Radiologie dentară, Editura Guttenberg Arad, 1999
10. Gheorghe Ciobanu, Liliana Anca Mihailovici. Ghid practic de tehnică radiologiocă cranio-facială Editura Facla 1986.
11. Harris L, Marano GD, McCorkle D: Nasofrontal duct: CT in frontal sinus trauma. Radiology 1987 Oct; 165(1): 195-8[Medline].
12. Hendler BH: Maxillofacial trauma. In: Rosen P, ed. Emergency Medicine Concepts and Clinical Practice. Mosby-Year Book; 1998:1093-1103.
13. Honig JF, Merten HA: Classification system and treatment of zygomatic arch fractures in the clinical setting. J Craniofac Surg. 2004 Nov;15(6):986-9.[Medline].
14. Johnson DH Jr: CT of maxillofacial trauma. Radiol Clin North Am 1984 Mar; 22(1): 131-44[Medline].
15. McRae M, Frodel J: Midface fractures. Facial Plast Surg 2000; 16(2): 107-13[Medline].
16. Schatz CJ, Becker TS: Normal CT anatomy of the paranasal sinuses. Radiol Clin North Am 1984 Mar; 22(1): 107-18[Medline].
17. Sicher H, DeBrul EL: Oral Anatomy. 5th ed. St. Louis: Mosby; 1970: 78.
18. Thomas, SH, Sheperd, SM: Maxillofacial injuries. In: Harwood- Nuss, ed. The Clinical Practice of Emergency Medicine. Lippincott, Williams & Wilkins; 1996:408-18.

Preparedness for a Mass Disaster during the 2010 FIFA World Cup Soccer in South Africa

Singh N.

University of Kwa-Zulu Natal

Introduction

Previous studies have alluded to the fact that International mass sports gatherings like the Fédération Internationale de Football Association (FIFA) World Cup Soccer and Olympics impose great challenges to healthcare systems and emergency medical services. History has proven that disasters do occur during these events, whether on a small or large scale. Disaster Management Practitioners from the public health perspective recognize that poor planning creates conditions of vulnerability. These result in poor measures to reduce the potential hazards. The International Committee of the Red Cross (ICRC) states that disaster preparedness provides a platform for the design of effective, realistic and coordinated planning. [1]

Thus there existed a need for eThekwini District to be prepared for a mass disaster. [2] The question being investigated was what were the levels of hospital's preparedness at the eThekwini District Hospitals in KwaZulu-Natal, South Africa, for the 2010 FIFA World Cup Soccer? A baseline survey was undertaken at all eleven of the public sector hospitals to assess their state of readiness. In order to undertake this study, a general assessment tool was formulated along with walk through visits with a checklist.

The study was quite important as no previous study had been undertaken at the eThekwini District to assess their preparedness for a world class event. It still remains quite imperative as eThekwini has become an international destination hosting many more international events namely the International Olympics Conference and Conference of Parties (COP17). The Hyogo framework for disaster management was adopted as it is based on a public health approach. It incorporates principles of disaster policy and legislation, education and awareness, training and facilitation, corporate governance and resource availability. [3] This framework was modified to a generalised user-friendly tool for eThekwini.

At a national level, South Africa has experienced a major sporting disaster. One was the Elis Park Stadium Disaster in 2001 were 40 00 people lost their lives due to a crowd stampede. The above incident led to the Disaster Management Act in South Africa being passed under the umbrella of the National Health Act. [4] This was to address disasters at all levels however, we still did not have any retrospective studies on international events in South Africa to compare to.

Methods

The study is an observational descriptive heath systems study. The study was undertaken in all nine state hospitals and the two state-aided hospitals located within the eThekwini District of the KwaZulu-Natal Province. A research tool was designed that consisted of a questionnaire and checklist. The questionnaire was formulated using the Yokohama Strategy. Since South Africa is a developing country, this was further modified to include a list of public health related criteria which would have an impact on a disaster. One of the key areas of the questionnaire was to determine if there were any disaster plans, policies, alerting systems and communications in operation. It was also devised to identify the types of medical services available and to assess the structural capacity of the hospital. This included the availability of emergency drugs and equipment necessary for resuscitation. There was also a focus on the availability of human resources and skills. Water, sanitation and infectious disease control was given paramount importance.

A perceived minimum criterion for the clinical aspect was obtained by the Provincial Task Team who had incorporated MIMMS into their standards. [5] The questionnaire was submitted to all the hospital and medical managers to be completed. The researcher used the additional checklist to increase the validity of the study. The data was then collected from the hospitals and entered onto Microsoft Xcel Spread sheet. Descriptive statistics were used in order to collate the data. The data was then analysed and the clinical criteria were compared against the perceived minimum requirements. The rest of the data was analysed into a flow of information for recommendations.

Results

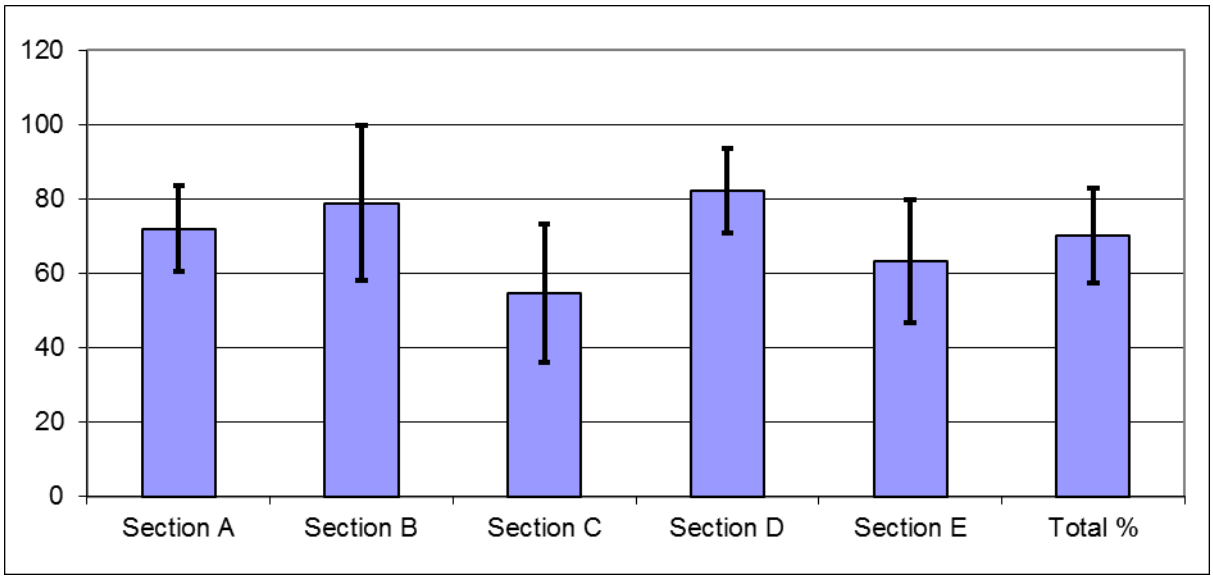


Figure 1: The overview of the total percentage response to all variables assessed in the 11 hospitals in the eThekwini District in terms of standard deviation, the upper and lower mean in 2009

All eleven hospitals in the study were analysed in terms of sections namely disaster plans, policies and communication; medical services and emergency facilities; drugs and equipment; human resources and water and sanitation. The hospitals were categorised as tertiary, regional, small or large district hospital according to their levels of care. The results of the disaster identified the gaps and challenges of the hospitals.

The total percentage response to each of the variables from the five sections was calculated summating all eleven hospitals (Figure 1). In Section A (Disaster Plans, Policies and Communication), the hospitals scored a total mean of 72%, with the upper mean (83%), standard deviation (11%). In Section B (Medical Services and Emergency Facilities), the hospitals scored a total mean of 79%, standard deviation (21%). In Section C (Emergency, Theatre and Isolation Areas), the hospitals scored a total mean of 54%, standard deviation (19%). In Section D (Drugs and Equipment), the hospitals scored a total mean of 82%, standard deviation. In Section E (Human Resources), the hospitals scored a total mean of 63%, with the upper mean (80%), standard deviation (16%).

Discussion

The study looked at the various categories for levels of preparedness in the hospitals and there appears to be many gaps and deficiencies in the healthcare system which is a recipe for disaster. The most important factor in the preparation for any mass event is a well-designed, tried and tested disaster plan and policy. The hospitals met a compliancy of 72% with a standard deviation of 11.5%. Most of the components of the plan were evident as recommended by the Department of Health.

The main evacuation procedure needed to be included for every unit in the regional hospitals and it was essential to include disaster exercises at least twice before the 2010 FIFA World Cup Soccer. Exercise reports or scheduled exercises needed to be planned and documented. Some of the

disaster guidelines mentions certain stages of alert but did not explain the definitions clearly for mass casualty plan. Mass casualty patient flow maps had to be included indicating all areas involved in disaster. Some of the hospitals had to include evacuation maps for all units indicating exits and fire extinguishers. All hospitals showed the presence of a corporate governing body.

In terms of communication, a strong inter-collaborative partnership needed to be fostered between the public and private hospitals, between each public hospital, and between each department. Good communication and understanding can help promote a strong teamwork which is imperative in the managing of a mass disaster. Formal methods (standing committees, joint teams), and informal methods (newsletter, posters) had to be developed to promote communication among services and individual staff members. Relevant community members had to form part of the communication network.

One of the constraints in resource poor health systems is providing access to emergency care to those who would otherwise suffer significant consequences, without being overwhelmed by those who can wait for treatment. The hospitals' disaster plan had to address staffing of the triage area, clinical evaluation and treatment areas, the admission or observation area and the resuscitation area. There was a clear need for applicable laws and regulations especially those of the Department of Health to be incorporated into the planning. The staffing plan therefore addressed the minimum level of emergency medicine/intensive care trained staffing acceptable in the emergency centre and contingency plans for use of staff from other departments in the event of high emergency caseloads.

Clinical managers needed to ensure that training and education needs for clinical issues were identified, that appropriate training and education was provided, and that staff showed proof of competence in terms of all emergency and first aid training. Staff also needed training in health and safety matters, infection control; and new equipment and procedures. There should be documented evidence that each staff member who has attended training has gained the required competencies. The hospital and medical managers needed to support on-going staff in-service education by making available space, equipment and time for education and training programmes.

Water quality can change suddenly due to many causes, some of which can be outside the organisation, such as a break in the supply line to, or contamination of the water source. Plans needed to be available to guide staff in the use and conservation of water if an emergency water source was used. An increased monitoring and testing of the water was important on the hospitals functioning to avoid the spread of disease and especially during bioterrorism or a cholera outbreak. Utility systems (electrical, water, oxygen, ventilation, vacuum and other utility systems) should be maintained regularly to minimise the risks of operating failures. Gas cylinders and medical regulators needed to be checked weekly, especially in units of casualty, trauma and theatres.

An occupational health and safety programme is important to include within all hospitals. Fire safety protocol and procedures of the hospitals had to form part of the integral plan in the disaster plan. Hazardous materials, its handling and storage of flammable and other materials were hazardous to employers and patients and needed to be highlighted. Staff should be educated on how to reduce risks, and how to monitor and report situations that pose risk.

Conclusion

The key for any successful mastering of a crisis is to be well prepared. The public health approach in disaster management is thus imperative as it focuses on the preventative aspects, rather than a full curative plan of action. The study has included many principals of public health that are important in the strengthening of disaster management. To begin with, having a well-integrated health policy framework for planning and programming at all levels are imperative in the preparedness and vulnerability reduction of disasters. Integrated corporate governance planning helps develop and strengthen the district and its hospitals. An inter-collaborative sectoral partnership must be created with all stakeholders such as non-governmental organisations, national and international aide.

Ethics permission was obtained both from the Biomedical Research Ethics Committee at the University of Kwa-Zulu Natal (UKZN) and from the Provincial Department of Health. The study was also commissioned by the Department of Health, eThekwini District Office. Permission to conduct the study was granted by all the hospitals' hospital managers and medical managers.

References

1. INTERNATIONAL FEDERATION OF RED CROSS AND RED CRESCENT SOCIETIES. IFRC. Disaster Management. Principles and rules for Red Cross Red Crescent Disaster Management and Relief. 2000 [cited 16 September 2010]; Available from: http://www.ifrc.org/what/disasters/responding/ethics.asp.
2. NATIONAL DEPARTMENT OF HEALTH. Preparations for the 2010 FIFA World Cup. 2008 [cited 15/01/09]; Available from: http://www.info.gov.za/issues/world_cup/background.htm.
3. UNITED NATIONS. World Conference on Disaster Management. Hyogo Framework for Action. 2008 [cited 20/02/2009]; Available from: http://www.unisdr.org/eng/hfa/hfa.htm.
4. NATIONAL DEPARTMENT OF HEALTH. Disaster Management Policy. Department of Health. Pretoria. Government Gazette. 2004.
5. KWAZULU-NATAL PROVINCIAL DEPRATMENT OF HEALTH. Integrated Major incident Management Philosophy. Durban. Department of Health. 2009.

Author Index

AUTHOR GUIDELINES

General outline:

Authors submitting to the International Reviw are requested to email full paper in English. Pictures, if imported, should be placed separately in the zip file in TIFF, JPG, GIF or CDR format in resolution of 300 dpi.

Manuscripts are blind reviewed with the understanding that they are substantially new and have not been previously published in whole. Publisher has the copyright to all published articles.

Practical guidelines

1)The length of the manuscript should not exceed 8 pages (including notes, references, appendices, tables, figures, charts, etc.). A paper must be written in English in text processor Microsoft Word, using font Palatino Linotype (size 11), in Latin alphabet, single spacing.

2) Page setup:**B5, margins: top 3 cm; bottom 3 cm; left 2,5 cm; right 2,5 cm**.

3) Paragraph setup: **first line indentation 0,6 cm, paragraph spacing: before 2 pt and after 2 pt**.

4) Title: **right, bold, size 16**

5) The author's name: last, middle and first name (bold, size 11) without titles. The author's affiliation name of institution, city, state, and country should be together with the author's name. Behind the name of the first author, a footnote should be inserted containing address and email of the first author. The maximum number of authors of one paper is three.

6) Abstract: italic, size 11, 3 line behind the author's name, the maximum length of 250 words. Key words should be 1 line behind the abstract up to ten key words.

7) Avoid endnotes.

8) Tables and figures should be numbered (1,2,3 etc.), in italics centred. All tables and figures (drawings) must be black & white.

9) Structure of the manuscript (only two levels). First heading: left, bold, size 12, before 18 after 9 pt. Second heading: left indention 0,6 cm, bold, size 12, before 12 after 6 pt.

10) Reference to articles and books in the text: Give full name (first name, middle initial, and last name) of author(s) and year of publication in the first citation, with page number(s) where appropriate. For example: Glenn Firebaugh (1999) [first reference]; Firebaugh (1999) [subsequently]; Andrea Boltho and Gianni Toniolo (1999) [first reference], Boltho and Toniolo (1999) *subsequently+; Albert Berry, François Bourguignon, and Christian Morrisson (1983) [first reference], Berry, Bourguignon, and Morrisson (1983) [subsequently]. When more than one work by the same author is cited, give the last name of author and year of publication in parentheses for each subsequent citation. When listing a string of references within the text, arrange first in chronological order, then alphabetically within years. If there are four or more authors, refer to the first author, followed by et al. and the year; for example: Stefan Fölster et al. (1998). If there is more than one publication referred to in the same year by the author(s), use the year and a, b, etc. (example: 1997a, b). References to authors in the text must exactly match those in the Reference section.

11) List of references (in alphabetical order by author' last names) should be at the end of the manu. JR uses AEA rules for references (Harward style).