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Garyounis Medical Journal (GMJ)

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Acknowledgements

Acknowledgements should he made only to those who have made a substantial contribution to the study. Authors are responsible for obtaining written permission from people acknowledged by name in case readers infer their endorsement of data and conclusions.

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3. *No author given*
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4. Article not in English
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4. *Conference proceedings*
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GMJ V01 21 N0 (2), PP01-14

Leucine Zipper, FYVE-finger protein Interacts with activating transcription factor 2 (ATF-2) and regulates its transcriptional activity.

Hany Abdel-Hafiz[1] ; Mahmoud El-Rehany[1] ; Jeffrey C. Dunkelberg[2].

Department of Biochemistry[1], School of Medicine, El-Minia University, Egypt, Department of Endocrinology[2], School of Medicine, UCHSC, Denver, Colorado, USA.

الملخص

وجد أن معامل النسخ الجيني ATF2 الذي يُخَلَّقُ في أنسجة الجرذان البالغة، ضروري لنمو العضلات وخلايا الجهاز العصبي المركزي. أثارت هذه النتائج تساؤلات عن احتمال وجود بروتينات خاصة بهذه الأنسجة تتفاعل مع ATF2 خاصّةً في مراحل تطور ونمو الأجنة. استخدمنا في هذه الدراسة نوعين مختلفين من البروتينات المكتشفة حديثاً التي تُخَلَّقُ فقط في خلايا الأجنة، وهما البروتين ذو الوزن الجزيئي 52 كيلو دالتون (Zn-52) والذي يحتوى على منطقة ارتباط بذرات الزنك (أصابع الزنك) والبروتين ذي الوزن الجزيئي 67 كيلو دالتون (67-LZ) والذي يحتوى على منطقة لولب الليوسين، وذلك لدراسة ارتباط وتأثير كل منهما على معامل النسخ الجيني ATF2. أظهرت نتائج البحث أن قدرة البروتين 67 على التفاعل مع ATF2 في الخلايا الحية تعتمد على نشاط مجموعة MEKK-1 مما يدل على أن فسفرة معامل النسخ الجيني ATF2 بإنزيم P38 كينيز لازمة لتفاعل ATF2 وهذا البروتين في الخلايا الحية. من هذه النتائج يتضح لنا أن وظيفة ATF2 تُنَظِّمُ في مراحل نمو الأجنة وذلك بالعديد من التفاعلات مع مختلف البروتينات الخاصة بهذه الأنسجة.

Abstract

Background & objectives: ATF-2 is ubiquitously expressed in adult mouse tissues, and yet the ATF-2 knockout mouse demonstrated that ATF-2 is specifically required for normal skeletal and central nervous system development. These findings suggest that developmental stage and/or tissue-specific proteins interacting with ATF-2 could dictate cell-specific functions of ATF-2.**Materials and Methods:** Technique used included protein gel electrophoresis, fat westron blot analyzer, elctrophoresis mobility shift assay, Immunoprecipitation and protein dimerization. **Results:** We have identified two novel proteins, 52-Zn and 67-LZ, that interact with ATF-2 in yeast two-hybrid assay and we showed that their mRNA expression is restricted to the embryonic stage of mouse development. 52-Zn contains a putative zinc finger domain and interacts only with full-length ATF-2 in vivo. By contrast, 67-LZ contains a leucine zipper-like domain followed by a phosphatidylinositol 3-phosphate (PI3P)-binding FYVE-finger domain, is able to bind to the DNA-binding/leucine zipper domain of ATF-2 in vivo and in vitro and markedly stimulates in vitro DNA-binding by ATF-2. The ability of 67-LZ to functionally interact with ATF-2 in HeLa cell transfection assay is dependent on MEKK-1 activity, suggesting that ATF-2 phosphorylation by p38 kinase and/or Jun kinase is required for in vivo interaction between ATF-2 and this protein. **Conclusions:** Together, these data provide important insights into how ATF-2 function may be regulated in an embryonic stage-dependent manner, and indicate that distinct ATF-2 structural domains dictate different protein-protein interactions, thus increasing the versatility of ATF-2 function.

Key words: Leucine Zipper, ATF-2, Transcription, FYVE-finger, Protein.

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Introduction

The transcriptional mechanisms that regulate cellular proliferation and differentiation are influenced by hormone- and growth factor-stimulated signal transduction

Pathways. The initiation of transcription mediated by cellular signaling pathways is dependent on the coordinated expression and/or activation of specific transcription factor proteins that bind to control regions of eukaryotic genes. Activating transcription factor-2 (ATF-2), a protein that is required for normal skeletal and nervous system development [1,2] is involved in protein-protein interactions that are necessary for the transcriptional regulation of a variety of genes [3-17]. ATF-2 is a member of the cyclic AMP response element binding protein (CREB)/ATF family of transcription factors [18]. It binds the cyclic AMP response element (CRE, 5'-TGACGTCA-3') as a homodimer, and binds DNA elements containing CRE half-sites or non-consensus CREs as a heterodimer with cJun [4,19,20]. ATF-2 is a 505 amino acid polypeptide with an amino-terminal zinc finger domain and a carboxyl-terminal basic DNA-binding/ leucine zipper (bZIP) domain [21]. Sequences within zinc finger motifs of certain transcription factors are involved in both DNA-binding specificity and protein-protein interactions [22,23]. The leucine zipper motif, originally described as a series of four or five leucine residues each separated by six amino acids, allows for positioning of hydrophobic leucine residues on one face of an idealized alpha-helix [24]. Proteins containing this motif selectively homo- and heterodimerize via the leucine zipper by forming a coiled-coil structure [25]. Homo-or heterodimerization is required for DNA-binding by CREB/ ATF proteins [18,19,26]. The leucine zipper domain of ATF-2 mediates homodimerization and heterodimerization with c-Jun, another bZIP protein. ATF-2/c-Jun heterodimers regulate the expression of the c-Jun [4,15], urokinase [16], interferon β [8,9] E-selectin [17,27,28] and T-cell tumor necrosis factor alpha [13,14] genes.

Although ATF-2 typically heterodimerizes with the c-Jun bZIP protein, several cellular factors and viral onco-proteins that lack a bZIP motif have been shown to interact with ATF-2 to regulate gene expression. DNA-bound NFkappa B [8] and DNA-bound HMG I (Y) [8,29] proteins interact directly with ATF-2 homodimers or ATF-2/c-Jun heterodimers, that are bound in close proximity at a CRE site in the promoter region of the human interferon beta gene, to maximally effect interferon beta gene transcription. Additionally, HMG I (Y) protein that is not bound to DNA is capable of interacting with and enhancing DNA-binding of ATF-2 by stimulating ATF-2 homodimerization [29]. Cellular Rb protein interacts and functionally cooperates with ATF-2 to activate TGFβ2 gene transcription [10,11] The adenovirus E1a protein physically interacts with the bZIP domain of ATF-2 [30,31], however, the amino-terminal zinc finger domain of ATF-2 is required for the E1a enhancement of the ATF-2 response in vivo [3,30,32-34]. ATF-2 also interacts with the human T-cell leukemia virus type I (HTLV I) Tax protein [6,35,36] and with the hepatitis B virus (HBV) X protein [7]. Tax interacts with the bZIP domain of ATF-2 to enhance ATF-2 homodimerization, thereby stimulating DNA-binding by ATF-2 [35,37]. The interaction of ATF-2 with HBV X protein alters the DNA-binding specificity of ATF-2 to enable

binding of ATF-2 to a non-consensus CRE in the HBV enhancer [7]. Cellular homologues of E1a, Tax and HBV X have not been described.

ATF-2 is a weak transcriptional activator when overexpressed in mammalian cells together with a reporter gene that is regulated by ATF-2 or by a GAL4 DNA-binding domain (DBD)-ATF-2 fusion protein [3,38,39]. However, when specific ATF-2 interacting proteins, such as Rb or E-1a, are co-expressed GAL4DBD-ATF-2, enhancement of reporter gene expression occurs [39], indicating that the additional transcriptional effects of ATF-2 are dependent on the ability of ATF-2 to interact with other proteins. It has been demonstrated that the amino-terminus of ATF-2 contains a regulated transcriptional activation domain which is unmasked by protein-protein interactions at the leucine zipper domain [40], and/or by the reversible phosphorylation of ATF-2 at two amino-terminal threonine residues (Thr 69 and Thr 71) by c-Jun N-terminal protein kinase (JNK) [41,42,43]. Indeed, the combined effects of JNK activation and E1a or Rb co-transfection results in maximal ATF-2 –mediated transcriptional response [38].

Finally, recent experimental evidence indicates that ATF-2 has an important role in cellular differentiation. Specifically, the phenotype of the ATF-2 knockout mouse demonstrates that ATF-2 is absolutely required for normal skeletal and central nervous system development [1,2] Although ATF-2 is ubiquitously expressed in all mammalian cell lines and tissues [21] the demonstration that ATF-2 has a critical role in the regulation of embryonic skeletal and central nervous system development suggests that ATF-2 may interact with novel cellular proteins to form tissue-specific transcriptional complexes that regulate developmental stage gene expression.

Here we report the biochemical and functional mechanisms of interaction of ATF-2 with two novel, embryo-specific proteins that contain putative motifs characteristic of transcription factor proteins.

Experimental procedures

Mammalian expression plasmids

The mammalian GAL4DBD ATF-2 full-length (FL) expression vector was created by inserting the ATF-2 FL coding sequence in-frame with the GAL4 DBD coding sequence in the pSG424 plasmid [3]. The mammalian VP16 transactivation domain (TA)-67-LZ and VP16 (TA)-52-Zn expression vectors were created by subcloning the coding sequences into pCDNA3 (Invitogen, Carlsbad, CA). A plasmid that expresses constitutively active mitogen activated protein kinase kinase (MEKK-1) [44], pCMV-MEKK-1-COOH, was a generous gift from Dr. Gary Johnson (National Jewish Center for Immunology and Respiratory Medicine, Denver, CO). The reporter plasmid was p5x UASTKluc.

Protein gel electrophoresis and Far-Western blot analysis

Expression and purification of glutathione S-transferase (GST) fusion proteins was performed as described, except that the HB101 Escherichia coli strain was used [45,46] Overexpression and purification of full-length and truncated ATF-2 proteins was carried out as previously described [47]. Radio-iodination of ATF-2 bZIP (amino acids 350-505) and GST-67-LZ proteins (10μg) with ^{125}I was performed in reaction vessels coated with Iodo-gen iodination reagent (Pierce, Rockford, IL) as directed by the manufacturer. Protein gel electrophoresis was conducted using a discontinuous buffer

system [48] Far-Western blot analysis was performed as described previously [50].Purified proteins were separated on 10% SDS-PAGE and transferred to a nitrocellulose membrane, nonspecific sites were blocked with 5% nonfat milk in TNE-50 buffer (10mM Tris-HCl pH 7.5, 50 mM NaCl, 1 mM EDTA, 1 mM DTT). After blocking, filters were incubated for 16 h at 25°C with 1 X 106 cpm/ml ^{125}I-labeled ATF-2-bZIP protein diluted in TNE-50 + 5% nonfat milk. Filters were washed with several changes of TNE-50 for 30 min, and protein-protein interactions were assessed after autoradiography of dried blot. Western blot analysis was performed as described previously [50]

Electrophoretic mobility shift assay (EMSA)

Fifty nanograms of bacterially-expressed ATF-2 bZIP protein (amino acids 350-505) [47,49] with or without increasing concentrations of GST-fusion proteins (0.01-1 μg), in buffer D (100 mM KCl, 20 mM HEPES pH 7.9, 12.5 mM MgCl2, 1 mM EDTA, 17% glycerol), were mixed with 1μg plydI-dC and 20,000 cpm ^{32}P-labled COL-8 duplex oligonucleotide [47] (COL-8, sense-strand,5'GATCCAGCTTGATGACGTCAG CCG-3') in gel shift buffer (10mM HEPES pH 7.9, 5% Ficoll, 50 mM KCl, 20 mM EDTA). After a 20 min incubation at 25°C, loading buffer was added and the protein-DNA complexes were separated by electrophoresis through a 4% polyacrylamide gel with 0.5X TBE buffer. The gel was dried and radio-labeled bands were visualized by autoradiography.

Immunoprecipitation

Polyclonal antiserum to ATF-2, was incubated with protein A-Sepharose beads (Sigma Chemical, St. Louis, MO) at 4°C overnight. GST-67-LZ protein was labeled with ^{125}I, and equal amounts of the labeled protein were incubated alone or with increasing amounts of ATF-2 protein (25 ng-1 μg) for 2 h. Washed antibody-beads complexes were added to each mixture and incubated for another 2h. All incubations were performed at 4°C in IP buffer (20 mM HEPES pH 7.9, 100 mM KCl, 12.5 mM $MgCl_2$, 10 mM $ZnCl_2$, 0.1 mM EDTA, 17%glycerol). After incubation, the immobilized recombinant protein complexes were washed three times with IP buffer plus 0.1 mg/ml BSA and once with IP buffer only. The immune complexes were pelted by centrifugation at 12,000 rpm in a refrigerated microfuge for 5 min, re-suspended in SDS loading buffer and boiled for 2 min. The labeled immunoprecipitated proteins were visualized by autoradiography after electrophoresis on a 10% polyacrylamide-SDS gel.

Protein dimerization assay

Purified GSt-67-LZ peptide (500 ng) was incubated for 30 min in TNE buffer at 25 °C. One-half volume of 3x non-reducing gel sample buffer (1x: 62 mM Tris-HCl, pH 6.8, 0.1 % SDS, 10% Glycerol) was then added and proteins were loaded onto SDS-PAGE. The chemical stability of 67-LZ dimmer formation was assessed by including DTT (0- 20 mM) in the initial 30-min incubation. Two control samples were incubated in standard Laemmli sample buffer; one was boiled for 5 min prior to loading while the other was not boiled. Proteins were visualized by staining the gel with Coomassie brilliant blue.

Results

67-LZ protein interacts directly with ATF-2 in vitro: In order to determine if 52-Zn and 67-LZ proteins interact directly with

ATF-2 bZIP (amino acids 350-505) in vitro Far-Western analysis [49] was performed (figure1). GST fusion of 67-LZ, 52-ZN and 50-Zn proteins (Figure 2), and control bZIP proteins were bacterially-expressed, purified and separated by SDS-PAGE.

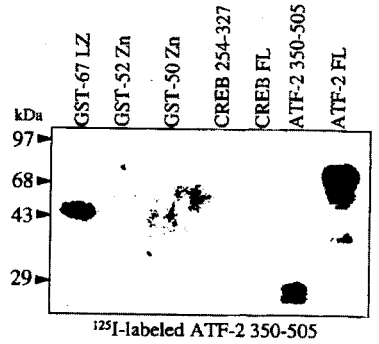

Figure 1: Detection of protein-protein interaction between GST-67-LZ and ATF-2 by Far Western assay. Five µg of the indicated proteins were separated by electrophoresis on a 10% SDS-PAGE. Proteins were transferred to nitrocellulose membrane, which was then probed with ^{125}I-labeled recombinant ATF-2 fragment encompassing the bZIP domain (amino acids 350- 505).

The proteins were transferred to a nitrocellulose membrane, which was then incubated with ^{125}I-labeled ATF-2 bZIP protein. ATF-2 bZIP interacted specifically with GST-67-LZ (figure 1, lane 1), but not GST-52-Zn (lane 2) or GST-50-Zn (lane 3), both of which were expressed as intact GST fusion proteins by Coomassie blue staining (figure 2).

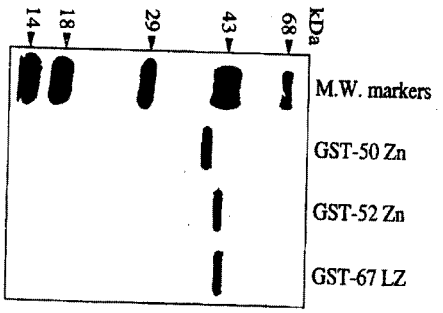

Figure 2: 50-Zn, 52-Zn and 67-LZ expressed as GST fusion proteins. 500 ng of the indicated GST fusion proteins were separated by SDS-polyacrylamide gel electrophoresis and were visualized by Coomassie Brilliant Blue staining. Molecular weight standards were separated in the first lane.

As expected, ATF-2 bZIP homodimerized with ATF-2 bZIP and ATF-2 full-length protein, but did not interact with CREB binding region or full-length CREB protein [39]. 67-LZ protein also interacts directly with full-length ATF-2 protein in vitro (Figure 3). Increasing concentrations of ^{125}I-GST-67-LZ co-immunoprecipitated with ATF-2 when constant amounts of ^{125}I-GST-67-LZ were incubated with increasing concentration of bacterially expressed full-length ATF-2 (lanes 2-5), followed by incubation of these proteins with protein A-Sepharose beads coated with polyclonal antibody to ATF-2. In lane 1, nonspecific binding of ^{125}I-GST-67-LZ to protein A/anti-ATF-2 beads is shown.

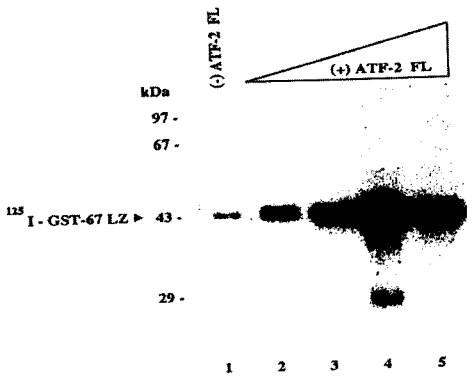

Figure 3: GST-67-LZ protein co-immunoprecipitates with full length ATF-2 protein. Increasing amounts of purified, bacterially expressed unlabeled ATF-2 FL protein (25 ng- 1 μg) were mixed with constant amounts of ^{125}I labeled GST-67-LZ protein and incubated for 2 h. Protein A-Sepharose beads coated with polyclonal antibody to ATF-2 were added to the mixtures, incubated for another 2 h and pelted. Labeled GST-67-LZ protein bound to ATF-2 FL was visualized by autoradiography after separation on a 10% SDS-PAGE.

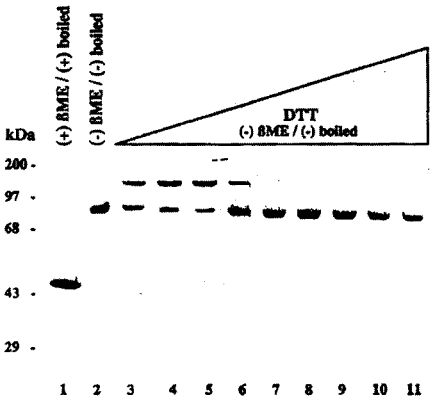

Figure 4: GST-67-LZ protein is capable of homodimerization. Purified GST-67-LZ protein (500 ng) was incubated for 30 min in TNE buffer at room temperature. Non-reducing sample buffer was added and proteins were separated on a conventional SDS-PAGE. The chemical stability of 67-LZ dimmer formation was assessed by including DDT (0-20 mM) in the initial 30-min incubation (lane3-11). Two control samples were incubated in standard Laemmli sample buffer (lane 1 and 2); one was boiled 5 min prior to loading (lane 1) while the other was not (lane 2). Proteins were visualized by staining the gel with Coomassie brilliant blue.

GST-67-LZ protein is capable of homodimerization

The potential for the leucine zipper domain of 67-LZ to dimerize in solution was assessed by separating the GST-67-LZ protein on a non-reducing SDS-PAGE (Figure 4). Purified protein was incubated for 30 min in TNE buffer and then mixed with non-reducing gel sample buffer treated as described in the methods section. The chemical stability of 67-LZ dimmer formation was also assessed by incubation with DDT (0-20 mM) in the initial 30 min incubation.

Under standard denaturing reducing conditions, in the presence of 2% SDS and 5% BME (β Mercaptoethanol) with boiling (lane 1), GST-67-LZ migrates as a monomer. In contrast, GST-67-LZ migrates as a higher molecular weight complex, possibly a dimmer, under the same condition without boiling (lane 2).

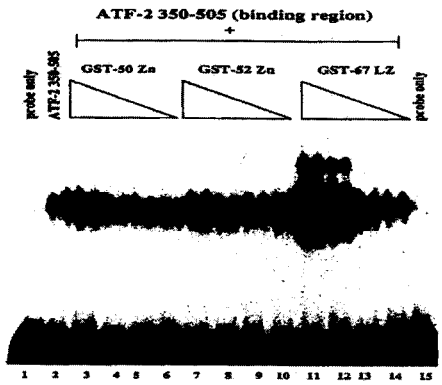

Figure 5: GST-67-LZ protein stimulates DNA binding by ATF-2 bZIP (350-505) protein. The effect of GST-50-Zn, GST-52-Zn and GST-67-LZ proteins on DNA binding by ATF-2 bZIP protein were analyzed by electrophoretic mobility shift assay using ^{32}P-labeled CRE oligonucleotide probe. The CRE probe was incubated with a constant amount of ATF-2 bZIP protein (50 ng) alone, and with increasing concentrations of each GST-fusion protein (0.01-1 µg). Protein-DNA complexes were separated by electrophoresis and an autoradiogram was obtained. Lane 1 and 15 show the migration of the CRE probe alone, lane 2 shows the CRE/ATF-2 complex in the absence of GST fusion proteins.

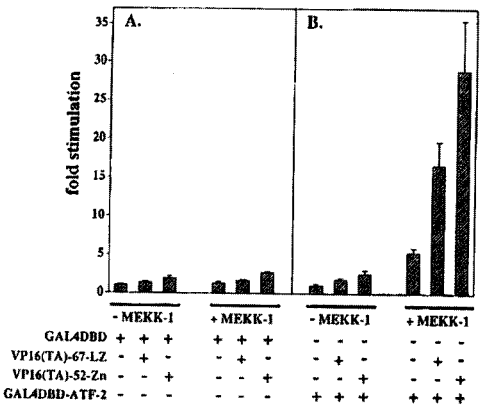

Figure 6: 76-LZ and 52-ZN interactions with ATF-2 in HeLa cells are MEKK-1 Dependent. HeLa cells were transfected with 3 µg of 5x UASTKluc reporter plasmid with or without 2.5 ug of plasmids encoding GAL4DBD, GAL4DBD-ATF-2, pCDNA3 empty vector, VP16-52-Zn or VP16-67-LZ and 9 µg of plasmid encoding constitutively active MEKK-1, as indicated. Cells were harvested after 24 ı and assayed for luciferase and β-galactosidase activity. Results are expressed as for stimulation over basal level (first lane in each panel) and are the mean +/- standard deviation on nine transfections. results of transcfections with plasmid encoding GAL4DBD are shown in panel A.

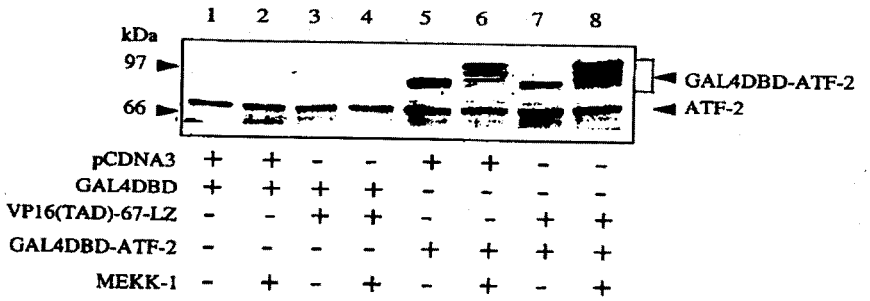

Figure 7: Retardation of migration of GAL4DBD-ATF-2 FL protein in response to expression of MEKK-1. HeLa cells were transfected with or without 2.5 ug of pCDNA3 empty vector or plasmids encoding GAL4DBD, VP16-67-LZ,or GAL4DBD-ATF-2 and 9 ug of plasmid encoding constitutively active MEKK-1 as indicated. After 24 h cells were harvested and proteins were separated by SDS-PAGE. Western blot was performed using polycolonal anti-ATF-2 antibody.

This result suggests that the putative dimeric form of 67-LZ is stable in 2% SDS, and the resistance to 5% BME in the absence of complete heat denaturation suggests that disulfide bonds are not mediating dimerization. Under non-denaturing conditions, lane 3-11, 67-LZ migrates as a dimmer. The presence of a complex migrating between 97- and 200-kDa at lower DTT concentrations (Figure 4 ,lanes 3-6), which is larger than the putative dimeric complex and is absent at higher DDT concentrations (Figure 4, lanes 7-11), suggests that protein aggregation, or possibly intramolecular disulfide bonds between cysteine residues, can lead to higher order homodimeric 67-LZ complexes. Finally, at higher DTT concentrations (Figure 4, Lanes 7-11), 67-LZ migrates primarily as a dimmer. The persistence of the dimeric at DTT concentrations to 20 mM suggests that the homodimeric 67-LZ protein-protein interaction is mediated by factors other than disulfide bonding. These data suggest that this leucine zipper domain of 67-LZ is capable of homodimerization via leucine zipper interactions.

67-LZ protein enhances DNA binding by ATF-2 bZIP protein

Electrophoretic mobility shift assays (EMSA) were performed in order to determine if 52-Zn or 67-LZ proteins affected DNA binding of the bacterially expressed bZIP domain of ATF-2 protein (Figure 5). The ATF-2 bZIP domain was used in these experiments because bacterially expressed full length ATF-2 protein is unable to bind DNA, because it is not phosphorylated in bacteria and is, therefore, unable to assume the conformation required for homodimerization [39,47] In contrast, bacterially expressed ATF-2 bZIP protein is able to homodimerize and bind DNA [49] IN the EMSA experiment, ATF-2 bZIP bound to radio-labeled DNA containing the CRE [47] (figure 5, lane 2), and this protein-DNA complex appeared to be unaffected by incubation with increasing concentrations of GST-50Zn (lanes 3-6) or GST-52-Zn (lanes 7-10) proteins, consistent with the finding that 52-Zn only binds to full length ATF-2. In contrast, incubation of GST-67-LZ together with ATF-2 bZIP and labeled DNA markedly enhanced DNA binding by ATF-2 bZIP (lane 11-14). Also, a slower migrating protein DNA complex occurred when increased concentrations of GST-67-LZ were present.

52-Zn and 67-LZ functionally interact with ATF-2 in mammalian cells

Although 52-Zn and 67-LZ interacted with ATF-2 in the yeast two hybrid system, and 67-LZ could bind ATF-2 full length and bZIP proteins directly in vitro, 52-ZN did not show direct physical interaction with ATF-2 in vitro. This suggests that either a post-translational modification of 52-Zn or ATF-2 is required and/or that a bridging protein mediates the in vivo interaction between 52-Zn and ATF-2.

To further address the biological relevance of the interactions of ATF-2 with these novel embryo-specific proteins in mammalian cells, the ability of 67-LZ and 52-Zn to interact with ATF-2 in a transcription function assay was determined in HeLa cells. The full-length ATF-2 cDNA was subcloned into a mammalian expression vector, pSG424 [3], in-frame with the GAL4DBD coding sequence such that a GAL4DBD-ATF-2 fusion protein was expressed. The reporter gene, 5xUASTK-luc, consisted of five GAL4 DNA binding

sites upstream of a minimal thymidine kinase promoter fused to a luciferase reporter gene. In order to determine whether the interaction of ATF-2 with 52-Zn or 67-LZ in HeLa cells was regulated by the amino-terminal phosphorylation of ATF-2 by JKK, or p38, a plasmid encoding constitutively active mitogen activated protein (MAP) kinase kinase 1 (MEKK-1) (44)
was used in these experiments.
As shown in figure (6A), when either the VP16(TA)-67-LZ or VP16(TA)-52-Zn fusion proteins were co-expressed into HeLa cells with the GAL4DBD protein (without ATF-2), there was minimal enhancement of the luciferase gene expression compared to that which was observed when GAL4DBD alone was expressed, in the absence or presence of MEKK-1. Similarly, when analyzing the transcriptional response to GAL4DBD-ATF-2 (figure 6B, minimal enhancement of luciferase gene expression occurred when the 67-LZ or 52-Zn fusion proteins were co-expressed with GAL4DBD-ATF-2 in the absence of MEKK-1, compared to that observed when GAL4DBD-ATF-2 alone was expressed. The interaction between ATF-2 and 67-LZ or 52-Zn was markedly enhanced in HeLa cells that expressed MEKK-1 (Figure 6B).When GAL4DBD-ATF-2 was expressed with consitiutively active MEKK-1 in the absence of 67-LZ or 52-Zn a 5.3-fold increase in luciferase activity was demonstrated compared to the activity measured with GAL4DBD-ATF-2 in the absence of transfected MEKK-1. In addition, when 67-LZ or 52-Zn fusion proteins were co-expressed with GAL4DBD-ATF-2, in the presence of MEKK-1, a 16.6 and 28.8-fold increase in luciferase activity, respectively, was observed compared to the level obtained when GAL2DBD-ATF-2 was expressed alone in the absence of MEKK-1 (figure 6B). Finally, if the 5.3 fold induction observed with GAL4DBD-ATF-2 alone in the presence of MEKK-1 is set to 1, then the co-expression of 67-LZ or 52-Zn fusion proteins with GAL44DBD-ATF-2 + MEKK-1 led to a 3.1 and 5.4 fold induction, respectively, of luciferase activity above that of GAL4DBD-ATF-2 alone (figure 6B). No enhancement of reporter gene expression occurred when VP16 (TA)-50-ZN was used in these experiments, indicating that the protein-protein interactions are specific to 67-LZ and 52-Zn and are not mediated by the VP16 (TA) domain.

Western blot analysis of transfected HeLa cells indicates that MEKK-1 expression is associated with the presence of higher molecular weight species of GAL4DBD-ATF-2 (Figure7), suggesting that phosphorylated forms of GAL4DBD-ATF-2 may result from MEKK-1 expression in the transfected cell population, whereas endogenous ATF-2 may fail to show up shift since this is derived from larger population of non transfected cells. Thus, the interaction of GAL4DBD-ATF-2 with 67-LZ and 52-Zn may require phosphorylation of GAL4DBD-ATF-2, possibly resulting in alteration of the conformation of ATF-2.

Discussion

ATF-2 is a ubiquitously expressed bZIP transcription factor whose optimal function is dependent upon both protein-protein interactions with specific co-activators and phosphorylation [47] The tissue specific defects evident in the ATF-2 knockout mouse [1] suggest that stage-and/or tissue specific proteins that interact with ATF-2 dictate cell-specific embryonic

functions of ATF-2. We have identified two novel proteins, 67-LZ and 52-Zn, that interact with ATF-2, and we show that the mRNA expression of these two factors is restricted to the embryonic stage of mouse development [2]. Several lines of evidence, including biochemical and functional approaches, show that 67-LZ interacts both physically and functionally with ATF-2. By contrast, we have been unable to demonstrate any direct physical interaction of 52-Zn with ATF-2, despite evidence that this protein can interact with ATF-2 in yeast and HeLa cells. Also, 67-LZ enhances DNA-binding of the bZIP domain of ATF-2, whereas, 52-Zn does not. These data suggest that the precise molecular mechanism of action of 67-LZ and 52-Zn are distinct, and raise the important possibility that factors interacting with ATF-2 may do so by different mechanisms, requiring distinct ATF-2 structural domains, thus increasing the versatility of ATF-2 function.52-Zn, containing a putative zinc finger structure, and 67-LZ, containing a putative leucine zipper-like motif, bind to ATF-2 via different ATF-2 structural motifs. Specifically, the protein encoded by clone 52-Zn only interacts with the full-length form of ATF-2 protein in the yeast two hybrid functional assay [2] 52-Zn also interacts with full-length ATF-2 in a mammalian cell functional assay (figure 6). The lack of detectable in vitro interactions between 52-Zn and ATF-2 when bacterially expressed recombinant proteins are used suggest that post-translational modifications of either, or both, of the protein partners is required for protein-protein interactions to occur. The ability of 52-Zn to interact only with full-length ATF-2 in yeast, and in a MEKK-1 – dependent manner in HeLa cells, might indicate that a higher order structure of ATF-2 is required for the interaction, and that this structure cannot be recapitulated by individual ATF-2 domains or by unphosphorylated ATF-2 protein. Alternatively, the interaction between 52-Zn and ATF-2 might be mediated by intramolecular bridging factors present in yeast and HeLa cells.The deduced amino acid sequence for 67-LZ contains multiple leucine and isoleucine residues in heptad repeats consistent with a leucine zipper, coil-coil structure, 67-LZ also contains a glutamine-rich domain. Similar glutamine-rich domains are described in the transcription factors CREB [52], Oct-2 (2) and Sp-1 [51], where they are required for maximal transactivation capability, indicating that certain glutamine-rich regions may constitute transactivation domains. In yeast, 67-LZ protein interacted with full-length ATF-2 protein and with a truncated form of ATF-2 consisting of the carboxyl-terminal bZIP region, as would be expected if the interaction is mediated by leucine zipper domains. Far-western analysis and co-immunoprecipitation experiments demonstrated direct physical interactions between GST-67-LZ and the bZIP or full-length version of ATF-2 protein, further supporting the notion that the interactions are mediated by the leucine zipper-like domain. The precise mechanisms by which 67-LZ, or other co-activators, might effect ATF-2 function remain unknown. However, the data reported here for 67-LZ suggest several possibilities. Current evidence suggests that ATF-2 exists in a 'closed' form (Figure 8), whereby the amino-terminal half interacts with the carboxyl-terminal half, in cis, resulting in an inactive effector [39,40]. Phosphorylation of the amino-terminus of ATF-2 by JNK results in activation of ATF-2 transcription potency and induces the

ability of ATF-2 to functionally interact with co-activators such as E1a and Rb [31,38,47] The hypothesis previously presented based on these data was that phosphorylation of amino-terminal ATF-2 sites (Thr69 and Thr71) results in a dissociation of the interamolecular interaction, thus generating an 'open' ATF-2 molecule that is competent for homo-or heterodimeriztion [39,40]. Here, we present similar data whereby activated MEKK-1 (44), which activates JNK and p38 [53,54,55], is required in order for 67-LZ to interact functionally with full-length ATF-2 (figure 6). Indeed, we show that the GAL4DBD-ATF-2 fusion protein displays multiple forms with reduced mobility on SDS-PAGE only when MEKK-1 is co-transfected (figure7) consistent with MEKK-1 induced phosphorylation of the ATF-2 fusion protein. These results suggest that 67-LZ either: 1) stabilizes the phosphorylation-induced 'open' conformation of ATF-2; 2) stabilizes ATF-2 homo- or heterodimers and induces DNA-binding; and/or 3) function as a bridging factor between ATF-2 homo-or heterodimers and the basal transcription machinery.

With regard to the possibility, if 67-LZ were to induce or stabilize the 'open'ATF-2 conformation, then 67-LZ should have no effect on the DNA-binding activity of an ATF-2 molecule that is designed to be in the 'open' state. By deleting the amino-terminus and expressing the bZIP domain alone, we produced an ATF-2 molecule that is capable of being DNA independent of any kinase-mediated activation, and here we show that 67-LZ significantly enhances the DNA-binding activity of this amino-terminally deleted ATF-2 (figure 5). These results argue against a key role of 67-LZ in inducing or stabilizing the 'open' conformation. By contrast, since ATF-2 dimerization is required for DNA-binding, we infer that these same DNA-binding data support the second possibility; that 67-LZ stabilizes ATF-2 dimerization and thus enhances DNA-binding. What is less clear at this point is whether 67-LZ acts stoichiometrically and remains in the ATF-2/DNA complex, or if it acts catalytically and simply induces dimerization. Although we have not been able to detect 67-LZ in the ATF-2 bZIP/DNA complex, the possibility remains that 67-LZ is present in this complex, but is present in a conformation or complex that cannot be recognized by the monoclonal antibody. Interestingly, similar to our findings here, HTLV I Tax has not been detected in ATF-2 bZIP protein/CRE DNA complexes in EMSA analysis, despite the ability of Tax to enhance DNA-binding by ATF-2 [36,37]. However, immuno-depletion studies have shown that Tax can form a stable ternary complex with ATF-2 bZIP protein and its DNA-binding site in solution [35]. Moreover, the transfection data, in which eliminate the effects of 67-LZ on ATF-2 DNA-binding by using a GAL4 DBD-ATF-2 fusion and a UAS-driven reporter gene, revealed that 67-LZ can also act as a co-activator and that this effect is MEKK-1 dependent (figure 6). Of note, this co-activation is not due to 67-LZ induction of GAL4DBD DNA-binding activity, since 67-LZ had no effect on other GAL4DBD fusion constructs, such as GAL4DBD-Elk-1 and GAL4DBD-Myc ± MEKK-1. The effects of 67-LZ in the transfection studies were MEKK-1 dependent (figure 6), further supporting the notion that phosphorylation and induction of the 'open' ATF-2 conformation is required for 67-LZ interaction.The description of the ATF-2 knockout mouse demonstrates that ATF-2 is critically important for normal skeletal and

central nervous system development [1]. Given the ubiquitous expression of ATF-2 in adult tissues [21], and its interaction with other widely expressed cellular factors, such as cJun [4,5], RB [10,38,56,57], NFkappa B [8], HMG I(Y) [8,29] and several virus encoded factors, such as adenovirus E1a [3,34], HTLV-1 Tax [6,35,36] and HBV X protein [7], these tissue specific effects of ATF-2 were not anticipated [1]. However, possible explanation for these results are that cell-and/or stage specific co-activators that interact with ATF-2 are required for ATF-2 functions, or that ATF-2 is differentially expressed in a cell-and/or stage specific manner during embryogenesis, but ubiquitously expressed in the adult. In this regard, our observation that 52-Zn and 67-LZ mRNAs are not detected in the adult mouse tissues analyzed, but were present in mouse embryos [2], suggests that these putative co-activators serve to direct embryo-specific functions of ATF-2. Additionally, 67-LZ display a tissue restricted nuclear distribution in mouse embryos [2], further suggesting that this co-activator may mediate certain tissue specific effects of ATF-2. Thus, the identification of two novel, embryo-specific gene products that contain putative zinc finger and leucine zipper motifs characteristic of transcription factor proteins, and the documentation that physically and functionally interact with ATF-2 by two distinct mechanisms, provide important information that has significantly advanced our understanding of the functions of ATF-2 as a transcription regulatory protein.

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GMJ. Vol 21(2), PP15-19

Hypotension during Spinal Anesthesia: Bupivacaine Versus Bupivacaine-Fentanyl Intrathecal Injection

Masoud Ali Lfeituri ; Jaber Rashid EL Madani.

Department of Surgery, Unit of Anaesthesia and IntensiveCare, Faculty of Medicine, Garyounis University, Benghazi, Libya.

الملخص

في مجموعتين من المرضى شملت هذه الدراسة أربعين مريضا تعرضوا لعمليات جراحية مختلفة في الجزء السفلى من الجسم تحت التخدير النصفي، وقورنت شدةُ حدوث هبوط الضغط الشرياني ونسبته في المجموعتين خلال أول ساعتين من التخدير. المجموعة الأولى حُقنَتْ بمادة 5 % بوبيفاكين (15مج) والمجموعة الثانية حقنت بمزيج من مادة 5 % بوبيفاكين (10 مج) والفنتانيل (25 ميكروجرام).بفروق إحصائية هامة كان الضغط الشرياني الانقباضي والانبساطي والمتوسطي في المجموعة الأولى أقل منه في المجموعة الثانية. في المجموعة الأولى كانت النسبة بين اقل ضغط انقباضي وانبساطي ومتوسطي وبين القيم الأساسية الأولى لهذه الضغوط هي 79% و 80% و 79% على التوالي، ويقابلها بالمجموعة الثانية 87 % و 88 % و 86 % على التوالي. نسبة حدوث هبوط الضغط الشرياني في المجموعة الأولى كان 70 % وفي المجموعة الثانية 40 %.لعلاج هبوط الضغط الشرياني احتاج مرضى المجموعة الأولى لجرعات أعلى من مادة الأيفدرين (30.4 مج ± 24.5) منه في المجموعة الثانية (12.6 مج ± 17.4) وكان الفارق بينهما مهم إحصائيا.

Abstract

Forty adult patients of two groups scheduled for surgical procedures involving the lower part of the body were enrolled in a study comparing the incidence and severity of hypotension during the first two hours of spinal anesthesia (SA). SA was induced either by 15mg of 0.5% hyperbaric bupivacaine (B group, 20 patients) or 0.5% hyperbaric bupivacaine (10mg) + 25 microgram fentanyl (FB group, 20 patients). Systolic (SBP), diastolic (DBP) and mean (MAP) arterial blood pressures were significantly lower in group B than in group FB. In group B, the lowest percentages of SBP, DBP and MAP pressure were 79%, 80% and 79% respectively, versus 87%, 88% and 86% for group FB. 70% of patients in group B and 40% in group FB developed hypotension. Total doses of ephedrine therapy were significantly higher in group B (30.4 mg ± 24.5) than group FB (12.6 mg ± 17.4).

Key words: Bupivacaine- intrathecal fentanyl - hypotension.

Introduction

Hypotension is a common serious and well-documented complication of spinal anaesthesia (SA).[1,6] Large surveillance studies of SA reported an incidence of hypotension around 33%.[2,3] It occurs from decreases in systemic vascular resistance and central venous pressure caused by sympathetic block with vasodilatation and redistribution of central blood volume to lower extremities and splanchnic beds.[4] Shift in cardiac autonomic balance toward the parasympathetic system may result in sudden bradycardia that may exaggerate hypotension.[5] Risk factors for hypotension include spinal puncture above L3-L4, high block level (≥ T5), age ≥ 40 years, hypovolaemia, and baseline systolic blood pressure (SBP) <120 mmHg.[2] Hypotension during SA is commonly prevented and treated by vasopressors and/ or intravenous (IV) fluids (IVF).[3,4,6] Unfortunately, these methods are not always suitable especially

in cardiac and elderly patients.[2] A more recent prophylactic approach to limit hypotension during SA suggested manipulation of the type and dose of intrathecally administered drugs.[1,7] Usage of smaller doses of local anaesthetics (LA) alone can be useful in short cases, but may be inadequate for longer procedures.[7,9] When administered together with lower doses of LA, a potent synergistic analgesic effect of intrathecal opioids may be obtained. Thus, a reliable SA can be achieved with otherwise inadequate doses of LA. With such optimization the of hypotension may be minimized. [9, 10.]

The goal of this study was to compare the incidence and severity of hypotension during two different techniques of SA induced by either hyperbaric bupivacaine or hyperbaric bupivacaine and fentanyl.

Table: Demographic data in bupivacaine (B) and fentanyl bupivacaine (FB) groups. Values are represented as total numbers or mean ± (SD).

	Group (B)	Group (FB)	P values
Number of patients	20	20	
ASA (I / II)	13 / 7	12 / 8	
Sex (M / F)	9 / 11	10 / 10	
Age (years)	44.3 ± (8.95)	41.05 ± (6.04)	0.186
Weight (kg)	69.15 ± (13.42)	71.25 ± (7.89)	0.686
Height (cm)	165.9 ± (7.27)	166.8 ± (6.67)	0.549

Methods

Forty adult patients scheduled for surgical procedures involving the lower part of the body were enrolled in a study comparing the incidence and severity of hypotension during the first two hours of SA.

Demographic data obtained in each patient included name, age, sex, body weight, height, and American Society of Anesthesiologist (ASA) physical status. Subjects had no history of hypertension, endocrine or neurologic diseases. All patients received approximately 10 ml/kg of IV 0.9% isotonic saline or Ringer's lactate solution just before start of anaesthesia.

A set for a single-space combined spinal-epidural anaesthesia (BD adjustable durasafe-plus, Madrid, Spain) was used to start SA (G27 spinal needle, G18 epidural needle and G19 epidural catheter). Midline lumbar puncture was performed at the level of L3 / L4 in the sitting position. After skin infiltration with 1% lidocaine, the epidural needle was inserted and a test dose of 40-50 mg of 1% lidocaine was given epidurally. After few minutes, the spinal needle then inserted through the epidural needle. According to the type of intrathecal injecate, patients were divided into two groups, each consist of 20 patients: group (B) received hyperbaric 0.5% bupivacaine (15mg), and group (FB) were injected with intrathecal hyperbaric 0.5% bupivacaine (10mg / 2ml) + fentanyl (25 microgram + 0.5 ml water for injection). In both groups the total volume of the intrathecal injecate was 3 ml. An epidural catheter was left in the epidural space and then patient was immediately kept supine thereafter. Sensory block height to pinprick was determined at the midclavicular line bilaterally.

Patients were not premedicated, but repeated small doses (2-4mg) of IV midazolam were used for sedation intraoperatively. Patient's monitoring (Diascope-Traveller, Artema MEC, Albertslund, Denmark) included continuous electrocardiogram, heart rate, non-invasive blood pressure, and peripheral arterial oxygen saturation. Data were recorded preoperatively just before induction of SA (baseline values), ten and twenty minutes after induction of anaaesthesia and then at twenty-minute intervals for the first two hours. Hypotension was defined as a SBP of less than 90 mmHg or 20% less than baseline value. Such hypotension was treated with repeated bolus doses of IV ephedrine (6-12 mg). All patients received approximately 4-6 ml /kg/ hour of either 0.9% isotonic saline or Ringer's lactate solution for intraoperative fluid maintenance, and to treat hypotension when necessary. The total ephedrine used for each patient and intraoperative complications

such as pain, itching, respiratory difficulty, nausea and vomiting were recorded.

Within-group statistical differences were determined using ANOVA test with Dunnett's multiple comparison test or paired Student's t-test when suitable. The unpaired Student's t-test was applied for assessment of significant inter-group differences. Results are expressed as means ± SD, and a statistical significance was considered when $P < 0.05$.

Table 2: Study data in bupivacaine (FB) groups SBP, and MAP are systolic and mean arterial blood pressures respectively. Vaues are represented as total numbers or mean ± (SD).

	Group (B)	Group (FB)	P value
Lowest SBP: [Range]	96.9 ± (6.15) [86-106]	103.1 ± (11.01) [87-124]	0.035
Lowest DBP: [Range]	58.1 ± (6.45) [45-68]	64.1 ± (5.66) [53-74]	0.0036
Lowest MAP: [Range]	70.7 ± (6.13) [58-78]	75.5 ± (6.29) [64-78]	0.0193
Lowest / baseline SBP: [Range]	0.79 ± (0.06) [0.68-0.88]	0.87 ± (0.08) [0.68-0.96]	0.006
Lowest / baseline DBP: [Range]	0.80 ± (0.06) [0.71-0.92]	0.88 ± (0.07) [0.67-0.97]	0.0009
Lowest / baseline MAP: [Range]	0.79 ± (0.05) [0.69-0.88]	0.86 ± (0.06) [0.73-0.95]	0.0027
Number of patients received ephedrine:	14 / 20	8 / 20	
Total ephedrine (mg):	30.4 ± (24.5)	12.6 ± (17.4)	0.0117
IV fluids (ml):	1820 ± (405)	1733 ± (382)	0.489

Results

Demographic data of patients were comparable in both groups (table-1). Peak sensory block height in both groups was similar and ranged from T4 to T6. Study results are summarized in table-2. Systolic, diastolic (DBP) and mean (MAP) arterial blood pressures were significantly lower in group B than in group FB (table-2). Severity of the hypotension was determined by calculating the percentages of lowest / baseline blood pressures. In group B, the lowest percentages of SBP, DBP and MAP pressure were respectively 79%, 80% and 79%, versus 87%, 88% and 86% for group FB. The mean of differences between baseline and SBP, DBP and MAP pressures in both groups are plotted in figure-1.

Fourteen patients (70%) in group B and eight patients (40%) in group FB developed hypotension and treated with IV bolus doses of ephedrine for 59 and 26 occasions respectively. Total doses of ephedrine therapy were significantly higher in group B (30.4 mg ± 24.5) than group FB (12.6 mg ± 17.4).

Four patients in group B and three patients in group FB complained of nausea. Two patients complained of mild itching over the abdomen in group FB. SA was successful and adequate for two hours in all patients, and no more complications were observed during the study period.

Discussion

Our result showed that more severity and higher incidence of hypotension occurred in group B than group FB. Group B required higher doses of total ephedrine than group FB, and this may also be used as an indicator for severity of hypotension in each group.

Injection of LA into cerebrospinal fluid (CSF) may result in a reduction in blood pressure that is directly related to the peak level of SA.[1, 2, 4] In our studied groups the level of the block was similar and ranged from T4 to T6.

Twenty two (55%) of our studied patients developed hypotension but the incidence still higher in group B (70%) than in group FB (40%). The lower incidence of hypotension in FB group of our patients is in agreement with Ben-David et al.'s study[9] who demonstrated similar results in elderly patients. They used lesser doses of intrathecal hyperbaric bupivacaine and fentanyl (20 microgram fentanyl with 4mg bupivacaine versus 10mg bupivacaine

GMJ. Vol 21(2), PP15-19

alone). Their results showed higher incidence (90% and 10%) and severity of hypotension in the bupivacaine group than in fentanyl-treated group respectively.

Because addition of intrathecal fentanyl to LA produces synergistic analgesia for somatic and visceral pain without increased sympathetic block, [4,7,10,11] and as the spread of hyperbaric bupivacaine is minimally affected by the addition of fentanyl,[12] one would expected that fentanyl will not increase the incidence of hypotension. Although, Singh et al.[13] reported a higher incidence of hypotension when fentanyl is added to bupivacaine (43%) than when bupivacaine is used alone (14%). In their study they used 25 microgram of fentanyl mixed with 13.5mg of 0.75% hyperbaric bupivacaine (3ml mixture) and compared it with 13.5mg of 0.75% hyperbaric bupivacaine alone (3ml mixture) in adult male patients. This discrepancy of results may be because they used different doses and concentrations of LA in patients with different demographics. Also the inter-individual response to LA and opioids is known to be variable and could participate.[1,7, 10,14, 15] Recently, volume and density of CSF have been classified as important factors responsible for the variability in patient's response to SA.[16, 17] An inverse relationship between the CSF volume and peak sensory block was described by Carpenter and colleagues[16] who demonstrated the variability in lumbosacral CSF volume as an important contributing factor responsible for the variability in the duration and spread of sensory blockade during SA. Higuchi et al.[17] represented similar results and described also a positive correlation between CSF density and sensory block level. Since CSF density and volume vary between individuals, variable dilutions and responses to intrathecal LA could be expected. In fact, many factors have been identified and may interact and influence the extent of spread of LA solutions in CSF, and thus, the dermatomal block level, and blood pressure. Some important related factors may include type, baricity, concentration, dose and volume of the injecate, site of injection, and patient age.[1,2, 9,12,13,15, 18]

Intrathecal fentanyl may produce dose-limiting side effects including pruritus, nausea, vomiting, respiratory depression, etc.[7, 9-11] The incidence of these complications is usually low, and may be related to the low-dose requirement of spinal fentanyl. Also, intrathecal fentanyl redistributes rapidly from the CSF into the epidural space and fat resulting in minimal systemic absorption.[18] In our fentanyl-treated group, the incidence of itching and nausea was 10% and 15% respectively. In group B, a 20% incidence of nausea was reported, and could be due to the hypotension itself. Boucher et al.[19] reported higher incidences of itching and nausea of 23% and 15.4% versus 11.5% and 19.2% in their fentanyl-treated and control (procaine) groups, respectively. The authors gave no explanation for the itching occurred in their procaine control group. Anaphylactoid reactions are more common with ester type of LA, including procaine, than amide LA that we used, and this could be an explanation.[1, 4]

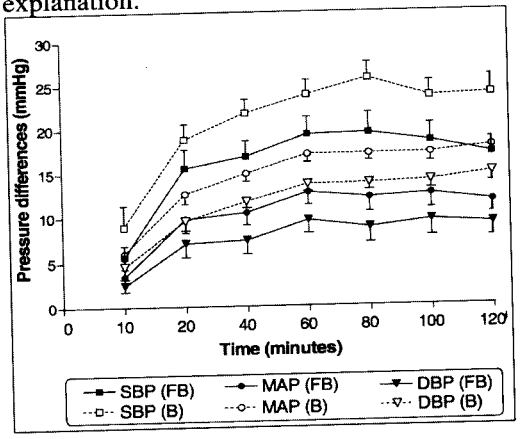

Figure-1. Mean of differences between baseline and systolic (SBP), diastolic (DBP) and mean (MAP) pressures in bupivacaine (B) and fentanyl-Bupivacaine (FB) groups. Vertical bars are standard errors.

Conclusion

Although both techniques result in an adequate spinal block for at least two hours, intrathecal injection of 10mg of 5% hyperbaric bupivacaine added to fentanyl (25 microgram) produces lesser incidence and severity of hypotension than 15mg of 5% hyperbaric bupivacaine alone.

Correspondence
Masoud Ali Lfeituri: P.O.Box:2856 Benghazi-Libya.
Tel:061-2227963,84340.
Tel-Fax: 061-2230207
e-mail: melfeituri@yahoo.com

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GMJ. Vol. 21 No (2), PP20-36

Clinical Features of 690 UK Patients With Oral Lichen Planus

Mohamed S Ingafou[1]; Stephen R Porter[2]; Crispian Scully[2].

[1]Department of Oral Medicine Faculty of Dentistry. Garyounis University. Benghazi, Libya. [2]Department of Oral Medicine and Special Needs Dentistry, Eastman Dental Institute for Oral Dental Health Care Sciences. University of London. 256 Gray's Inn Road. London WC1X 8LD UK.[1]

الملخص

الأهداف: هذه الدراسة المستفيضة، تعتبر الأكبر على الإطلاق التي تجرى على مجموعة كبيرة من المرضى من المملكة المتحدة أجريت لوصف الخصائص السريرية و الإحصائية لمرض الحزاز المنبسط الفموي عند هذه المجموعة الضخمة من المرضى، و مقارنة النتائج بتلك المتحصل عليها من دراسات لأجناس أخرى.**المواد و الطريقة:** أخذت البيانات من ملفات مرضى أحيلوا للمعالجة أو الاسترشاد إلى مجموعة محددة من اختصاصي طب الفم في الفترة من فبراير 1974 إلى نوفمبر 1997 ، والذين ثبت أن لديهم العلامات السريرية وغالبا ما لديهم التغيرات النسيجية للحزاز المنبسط الفموي.**النتائج و الاستنتاجات:** مرضى الحزاز المنبسط الفموي البريطانيون من ذوي الأعمار المتوسطة أو متقدمي العمر يعانون من درجات متفاوتة من الإحساس بعدم الارتياح بالفم الناتج عن أذيات عادة ما تظهر على كلا الجانبين من الفم، وتنتشر في الغالب على الغشاء المخاطي المبطن للشدقين وظهر اللسان واللثة، ولكن قلما تظهر على سقف الحلق أو قاع الفم. عادة ما تتخذ هذه الاذيات أشكالا متعددة مثل الشكل الشبكي و الشكل شبه الصفيحي و النوع الأكال، مع إن كثيرا من المرضى يتزامن لديهم ظهور أكثر من شكل واحد من تلك الاذيات في آن واحد. أقلية هم المرضى الذين تظهر لديهم أذيات المرض على مناطق أخرى من أجسامهم، وبشكل خاص على الأطراف. من الممكن في أغلب الحالات تشخيص هذا المرض بناءً على أسس سريرية فقط شريطة أن يؤخذ تاريخ مضبوط للحالة ويجرى فحصها سريريا فحصاً كافياً. بيد أنه يتحتم إجراء فحص للمتغيرات النسيجية أو إجراء بعض البحوث المصولية لتشخيص المرض في الحالات التي قد يحيد مظهرها عن الشكل النموذجي الذي يظهر عليه المرض عادة. يستمر المرض في أغلب الأحيان لعقود مع إمكانية الشفاء منه في حالات قليلة فقط. سجلت في هذه الدراسة تغيرات سرطانية لدى 1.9% من الحالات.

Abstract

Objectives: This largest ever detailed study of a UK patient group with oral lichen planus (OLP) was carried out to describe the demographic and clinical characteristics of OLP in a considerably large group of affected patients for comparison of its findings with those of other populations. **Materials and methods:** Data available from the medical records of 690 patients referred to one group of Oral Medicine specialists between February 1974 and January 1997, subsequently found to have clinical and usually histopathological confirmatory features of OLP. **Results:** UK patients with OLP are typically middle-aged or elderly persons complain of variable degrees of oral discomfort and usually have bilateral lesions affecting the buccal mucosa, dorsum of tongue and/or gingivae and rarely the palate or floor of the mouth. Lesions are typically reticular, plaque-like and/or erosive, although patients often have more than one type of oral lesions simultaneously. **Conclusions:** A minority of patients may have lichen planus affecting other mucocutaneous regions, typically the skin of the extremities. Provided that proper history and adequate clinical examination had been carried out, majority of the cases can be diagnosed on clinical bases, however histopathological and serological studies may be necessary for diagnosis of the cases with otherwise atypical presentation. Oral lesions can last for decades with few cases of remission. Malignant transformation was recorded in 1.9% of the cases.

Key Words: Oral Lichen Planus, Clinical Study, Oral mucosal diseases, UK.

GMJ. Vol. 21 No (2), PP20-36

Introduction

Oral lichen planus (OLP) is a relatively common mucocutaneous disorder of middle aged and elderly persons with prevalence of 0.1% to 2.4 % in general population and probably have no racial or gender preponderance.[1-3] Intraorally, the lesions may manifest in a variety of clinical forms including white keratotic forms and red erosive lesions. This disease seems to represent spectrum of conditions that share common background with slightly different clinical presentations.[4] Patient's symptoms can range from mild, painless white keratotic lesions, to severe and painful disease with multiple erosions and ulceration.[5]

To date, most of the more detailed epidemiological and clinical studies of OLP have been undertaken in the United States and Scandinavia,[6-10] while studies on possible disease-associations and immunopathogenesis often been on UK and other European patients.[11-13] Furthermore some of the earlier studies of the demographic and clinical presentation of OLP included relatively small numbers of affected patients.[14-16] Thus in view of the paucity of data concerning the demographic and clinical features of European and UK patients with OLP, the aim of this study was to examine the general features and clinical presentation of a large cohort of patients ultimately found to have the clinical features of OLP and followed for variable periods of time.

Materials and Methods

Patient group

The study group comprised 690 patients referred to (one group) of Oral Medicine specialists in Southern England and followed up in the period between February 1974 and November 1996. All patients were interviewed in regard to their chief symptom, history of current illness, medical history, medication intake and dental history. Histological studies and haematological investigations were ordered in some cases as indicated by the history and clinical examination findings. All such patients were subsequently found to have clinical and usually histopathological features of OLP. All the included patients had been clinically monitored for at least 3 months after diagnosis of their OLP.

Data analysis

Case records of all 690 patients were reviewed and relevant retrospective data extracted and loaded on a clinical epidemiological statistical spreadsheet package (Epi Info version 6) provided by the Centre for Disease Control and Prevention (CDC) Atlanta, Georgia, USA. Descriptive statistical analysis was used to summarise the demographic and clinical features of the study group. Chi square test and Fissure Exact test were used for the comparisons of non-parametric data.

Clinical diagnostic criteria of OLP used in this study

It is appreciated that different diagnostic criteria may have been applied with time by the attending clinicians due to increased knowledge about OLP and changes occurred in diagnostic criteria. However the clinical presentation of the lesions and their configuration formed the bases of assigning the lesions to their clinical types. In general, the diagnostic criteria for OLP used in this study included:

GMJ. Vol. 21 No (2), PP20-36

1. The presence on the oral mucosa of keratotic, pinhead sized, white, slightly elevated papules (papular lichen planus), which may be discrete or arranged in straight lines, annular fashion or network (reticular lichen planus) or patchy (plaque-like lichen planus) configurations.[10,17,18]
2. Atrophic lichen planus when there was a thinning of epithelium leading to the appearance of atrophic red areas within the white lesions.[19] These lesions when involving gingiva gave rise to desquamative gingivitis.[16]
3. Ulcerative lichen planus when there were presence or development of areas of well defined ulceration not due to local trauma within the above mentioned lesions.[20]
4. Bullous lichen planus when there were presence or development of bullous areas within the above mentioned lesions.[21]

It is not uncommon in previous studies, to encounter some degree of confusion in classifying lesions of oral lichen planus, particularly atrophic, erosive and ulcerative types.[22] These lesions had been reported differently by various studies as separate or jointly (Table 1). To avoid such confusion, in this study much attention has been made to designate lesion type according to its actual clinical description by the attending clinician. As example the description of red areas with thinning of epithelium should be denoted as "atrophic lesion" and whenever there is frank ulceration the lesions should be classified as "ulcerative lesion". Likewise the lesions in proximity to restorative materials or those lesions attributed to the use of specific drug reported to cause (lichenoid reaction), such lesions can only be best described as "lichenoid lesions".[23]

Results

Patient gender

Four hundred and thirty nine patients (63.6%) were females and 251 (36.4%) were males; with a female to male ratio of 1.75 to 1. There were no differences in the medical status or ethnicity between the two genders ($p < 0.05$).

Age of onset

The median age of likely onset of initial symptoms or signs of OLP (as determined from patient interview) was 52 years (53 years for females and 48 years for males) with an age range of 16 to 83 years. The majority of patients had an onset of disease at their 5th or 6th decade of life; less than 1% having developed OLP when they were under 20 years of age (Table 2). There has been a tendency of all types of OLP to occur in male patients at an earlier age than in female patients (Figure 1).

Ethnic origin

The majority of affected patients in this study were Caucasian (68.7%), while (15%) were from Indian subcontinent and about (8%) were either blacks of Afro Caribbean origin, Chinese or Orientals. The ethnic groupings of (7.4%) of the patients could not be traced.

Source of referral of patients

About 82% of the patients had been referred to Oral Medicine specialists by general dental practitioners (GDP), Seventy five patients (10.9%) were referred from other dental specialities. Twenty nine (4.2%) were referred by their general medical practitioners (GP), 14 (2%) by dermatologists and only 8 (1.2%) patients presented themselves to Oral Medicine clinics without prior referral.

Socioeconomic status

In order to have an idea about the employment status, patients were grouped according to the criteria of the "Central Statistical Office for Social Trends" (1985). According to these criteria there was an unequal distribution of the patients across the socio-economic groups ranging from 2.3% of patients being employers or managers to 20% being semi-skilled manuals. Retired persons with unknown pre-retirement occupation and those who were unemployed were classified separately. Almost 25% of the patients could not be classified accurately as their details were unavailable or inappropriate. The employment status of the patients is indicated in (Table 3).

Data about social status revealed that about 54.2% of the patients were married or living with partner, 5.4% were divorced or separated, 7.8% were widowed and 10% were single, while the social status of 22.6% was not recorded. About 70% of patients had children, (38% of them had 2 children, 23% had one child, 23% had 3 children and less than 4% had 4 children or more).

Common symptoms of OLP

Patients' symptoms ranged from asymptomatic intraoral white patches or roughness, to severe oral soreness. This soreness (of variable degrees of severity) was the main reason for about 62.5% of the patients to seek specialist advice. Buccal mucosa, tongue and gingiva were the main sites of discomfort. It is unsurprisingly noticed that oral soreness was commonly associating with atrophic-erosive lesions more than white keratotic lesions (Table 5). Only 11 (1.6%) patients reported gingival soreness and bleeding as their main oral complaint. One hundred and thirteen (16.4%) patients had self-reported painless white patches of the oral mucosa, while seventy-six patients (11%) had asymptomatic white patches that had been discovered by clinicians on routine oral examination; 59 patients (8.6%) complained of roughness at different oral mucosal sites (mainly buccal mucosa) with no pain symptoms (Table 4).

Duration of symptoms prior to referral to Oral Medicine clinic

Just over 65% of patients had some relevant oral complaints for less than 12 months prior to their attendance in the Oral Medicine unit. Further 14% had oral problems for up to 24 months and about 7% of patients had relevant oral complaints for more than 60 months. Symptoms of some of these patients had supposedly been controlled by their attending clinicians for many years before being referred to Oral Medicine specialists for more specialised care or consultation (Table 6).

Clinical types of OLP

The main recoded types were reticular, papular, plaque-like, atrophic, bullous and ulcerative lesions. About 95% of the patients had bilaterally symmetrical distribution of oral lesions. Apparently, the frequency of different types of OLP lesions was not influenced by the patient's gender or age. Reticular lesions were the most common type as being present in 651 (94.3%) patients. Sixty percent of these reticular lesions were in combination with other types of OLP. Atrophic lichen planus was the next most common type, occurring in almost 37% of patients, followed by plaque-like type in 32% and papular in only 11%. The least common types were the bullous in 4% and ulcerative in only 2%. All these types usually presented in various combinations and were rarely present alone as detailed in (Table 7). Only 281 patients had only one type of OLP lesions at the time of initial examination. Four hundred and nine

(59.3%) patients had more than one type of lesions, of those 268 (38.8%) had 2 types, 133 (19.3%) had 3 types and 8 (1.2%) had more than 3 types of lesions.

There was little variation in the pattern of involvement of intraoral sites by different types of OLP lesions (Table 8), as the buccal mucosa was being the most common site of involvement by all types of OLP, followed by the lateral borders of tongue and gingiva respectively. No single type of OLP had an exclusive preferred site of predilection (i.e. any type of OLP could arise anywhere on the oral mucosa).

Natural course and complications

Eighty five patients (13%) had complete resolution of both OLP symptoms and clinical signs. Such lesions disappeared within 12 months to 22 years. Interestingly, the medical status and social habits of this group of patients did not differ from the remaining patients. Of note, 26 of these patients did not use any medication for treatment of OLP lesions. On the hand, 87% of the patients had no resolution of their symptoms despite the use of different regimens of therapeutic agents and the majority of them are still being under regular review visits.

Malignant changes occurred in 13 (1.9%) patients (8 males and 5 females, with median age of 65 years). Twelve lesions were squamous cell carcinoma and one case was carcinoma in situ. There was no obvious correlation of malignant potentiality of the lesion and the sex or race of the patient or the clinical presentation of their initial lesions. The mean time interval from initial presentation until the malignant development was 7 years.

Status of dentition and periodontium

Unfortunately no precise records of the status of dentition and periodontium were satisfactorily recorded in substantial number of patients. The status of dental restorations has not been clearly indicated and only few patients had a replacement of restorations adjacent to OLP lesions in an attempt to relief OLP symptoms, but none of them had total disappearance of OLP lesions from the mouth.

Associated systemic diseases

No single associating disease condition in this study emerged as a major causative factor of OLP when the data compared with control subjects of similar age and gender (data not shown). On the other hand there has been an increased intake of some drugs in the OLP group than that in the control subjects. These medications included non steroidal anti-inflammatory agents, oral hypoglycaemic drugs and some cardiovascular drugs (mainly calcium channel blockers and Angiotensin converting enzyme ACE-inhibitors). These drugs were taken by patients to control their systemic problems such as arthritis, diabetes mellitus and hypertension.

Role of laboratory investigation in the diagnosis of OLP

Histopathological examination of intraoral incisional biopsy performed in 546 (79%) cases usually to confirm the clinical diagnosis of OLP, or whenever there is atypical presentation. However, it was possible in 21% of the patients to be confidant about the diagnosis of OLP based on clinical grounds alone. Results of direct immunofluorescence studies in 161 patients were non-specific and in most instances only showed fibrinogen deposition at BMZ and colloid bodies. Blood investigations that had been carried out for many patients were of little help in the diagnostic process of OLP per se, but they were invaluable in assessment of the patient's general health.

Extraoral lichen planus lesions

Eighty five patients (12.6% of total study group; 56 females and 30 males with median age 52 years and age range of 24 to 81 years) had or had had lichen planus affecting the skin or other mucous membranes. The age and gender distribution of the OLP patients with these lesions did not differ from those of patients without non-oral involvement.

The majority of patients developed mucocutaneous involvement after the onset of oral disease, only ten patients had skin lesions prior the onset of OLP (Table 10). No patient had simultaneous development of both oral and non-oral mucocutaneous lesions. Cutaneous involvement was almost always symmetrically bilateral and the lesions typically occurred on the flexor surfaces of the wrists (60%), arms (40%), or extensor surfaces of the legs (30.6%). Eleven (12.9%) patients with non-oral mucocutaneous lesions had lichen planus of the genitalia, nine of whom were females (Table 11). Five patients had vertical ridges of finger nails, other 5 had regional lymphadenopathy, possibly due to chronic local orofacial infection and another 4 patients had cervical lymphadenopathy of unknown cause (Table 12).

Table 1: Distribution of clinical variants of OLP according to seven surveys

Type of OLP *	Percentage in various studies						
	Andreasen *1968* (n=115)	Kovesi & Banoczy *1973* (n=326)	Neumann-Jensen et al, *1977* (n=611)	Silverman et al,, *1985* (n=570)	Axell & Rundquist *1987* (n=410)	Thorn et al,, *1988* (n=611)	Silverman et al,, *1991* (n=214)
Reticular	43	52	86	32	77	92	29
Atrophic	30	-	55	22	33	44	30
Plaque	12	13	51	-	43	37	-
Papular	3	-	7	-	10	11	-
Ulcerative	20	28	6	46	8	9	41
Bullous	2	8	0	-	1	1	-

- Many patients had two or more variants together

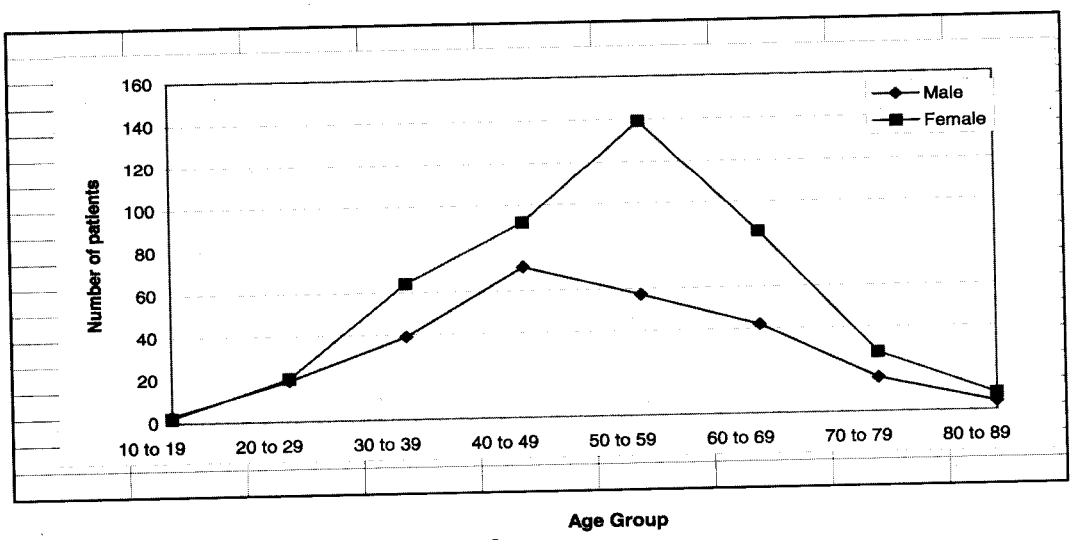

Figure 1: Age of initial symptoms of both genders

Table 2: Relationship between type of OLP and patient gender and age

Age Group (Years)	Number of patients			Type of oral lichen planus lesion and patients' gender											
				Reticular		Papular		Plaque-like		Atrophic		Bullous		Ulcerative	
	M	*f*	*Total*	*F*	*m*	*f*	*M*	*F*	*M*	*f*	*m*	*f*	*m*	*f*	*m*
< 20	3	2	5	2	3	0	1	1	1	-	-	-	-	-	-
20 to 29	19	20	39	19	16	1	1	5	8	6	6	1	1	1	0
30 to 39	39	64	103	60	37	3	2	25	7	20	10	3	0	1	1
40 to 49	71	92	163	87	66	12	9	31	24	30	24	6	2	3	2
50 to 59	57	139	196	134	55	13	6	40	21	54	16	4	3	3	1
60 to 69	42	86	128	81	39	11	7	29	13	37	21	1	1	2	0
70 to 79	16	28	44	28	15	4	3	13	6	15	8	0	1	1	1
> 79	4	8	12	6	3	1	0	1	0	7	2	3	1	-	-
Total	251	439	690	417	234	45	29	145	80	169	87	18	9	11	5

***m* = Male.** ***f* = Female**

Table 3: Employment status of patients with OLP

Employment status	Number of patients	Percent of patients
Semi-skilled manual	138	20.0
Retired	106	15.4
Unskilled manual	82	11.9
Intermediate and junior non-manuals	62	9.0
Skilled manual	52	7.5
Unemployed	32	4.6
Professional	30	4.3
Employers and managers	16	2.3
Unclassified	172	24.9
Total	690	100.0

Table 4: Complaints of 690 patients with OLP at the time of initial clinical presentation

Main oral complaints	Number of patients	Percent of patients
Generalised oral discomfort and soreness	431	62.5%
Symptomless white oral mucosal patches	113	16.4%
Dentists discovered asymptomatic intraoral lesions	76	11.0%
Mucosal roughness	59	8.5%
Gingival soreness and bleeding	11	1.6%
Total	690	100%

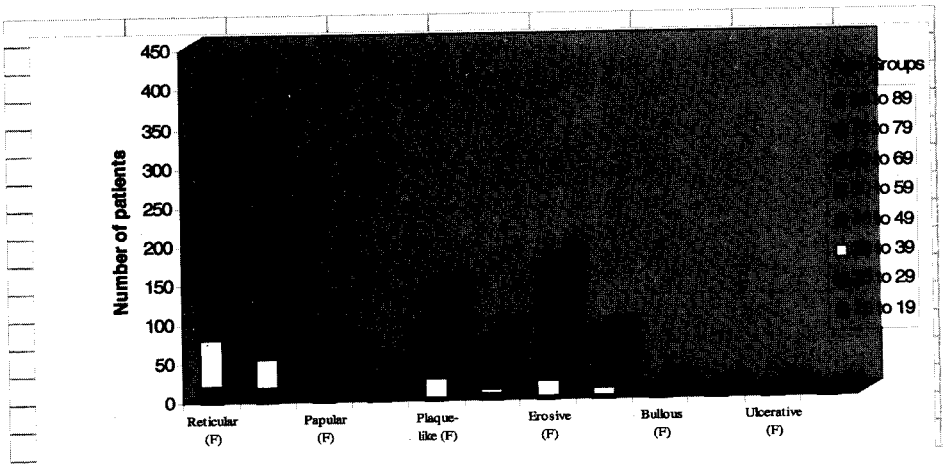

Figure 2: Relationship between the age, gender and clinical type of oral lichen planus

Table 5: Association of oral soreness with clinical types of OLP

Clinical type of OLP	Total number of patients with lesions	Patients with soreness	Percent of Symptomatic cases
Ulcerative	16	16	100.0
Atrophic	256	243	94.9
Bullous	27	17	62.9
Reticular	651	391	60.1
Plaque-like	225	121	53.7
Popular	71	18	23.0

Table 6: Duration of patients' symptoms prior to referral to Oral Medicine

Duration (months)	Number of patients	Percent of patients
Less than 12	448	64.9
12 to 23	99	14.3
24 to 35	50	7.2
36 to 47	25	3.6
48 to 59	17	2.5
60 or more	51	7.4
Total	690	100.0

Table 7: combinations of various types of OLP in 690 patients

Type of lesion	Percent of this type from 690 patients with OLP	Percent of patients with single type of lesions
Reticular	94.3	39.6
Atrophic	37.1	6.75
Plaque	32.6	2.7
Papular	11.3	2.8
Bullous	3.9	0.0
Ulcerative	2.3	0.0

Table 8: Intraoral distribution of different types of OLP in 690 patients

Site	Reticular lesions (n=651)	Plaque-like lesions n=(225)	Papular lesion (n=78)	Atrophic lesions (n=256)	Ulcerative lesions (n=16)	Bullous lesions (n=27)
Buccal mucosa	622 (95.5)	150 (66.7)	55 (70.5)	206 (80.5)	11 (68.8)	21 (77.8)
Tongue (lateral border)	184 (28.3)	83 (36.9)	8 (10.3)	69 (27.0)	1 (6.3)	9 (33.3)
Alveolar ridge mucosa	106 (16.3)	19(8.4)	6 (7.7)	6 (2.3)	0 (0.0)	0 (0.0)
Dorsum of tongue	100 (15.4)	50 (22.2)	2 (2.6)	37 (14.5)	1 (6.3)	2 (7.4)
Gingiva	85 (13.1)	16 (7.1)	2 (2.6)	39 (15.2)	3 (18.8)	0 (0.0)
Lower labial mucosa	39 (6.0)	5 (2.2)	1 (1.3)	14 (5.5)	1 (6.3)	0 (0.0)
Upper labial mucosa	39 (6.0)	4 (1.8)	0 (0.0)	13 (5.1)	1 (6.3)	1 (3.7)
Palatal mucosa	30 (4.6)	10 (4.4)	1 (1.3)	18 (7.0)	1 (6.3)	6 (22.2)
Buccal vestibule	28 (4.3)	2 (0.9)	1 (1.3)	10 (3.9)	1 (6.3)	3 (11.1)
Tongue (ventral surface)	21 (3.2)	17 (7.6)	1 (1.3)	16 (6.3)	0 (0.0)	1 (3.7)
Floor of the mouth	19 (2.9)	5 (2.2)	0 (0.0)	4 (1.6)	0 (0.0)	0 (0.0)

Table 9: Reported site distribution of OLP by different studies

Authors	Number of patients	Percentage of involvement of different sites *						
		Buccal Mucosa	Tongue	Gingiva	Hard palate	Soft palate	Labial mucosa	Floor of mouth
Cooke, 1954	50	94	36	16	12	8	8	-
Shklar & McCarthy, 1961	100	80	65	<10	<10	-	20	<10
Altman & Perry, 1961	50	90	60	4	-	-	-	-
Andreasen, 1968	115	99	73	71	20	7	14	8
Sklavounou& Laskaris, 1983	50	88	28	26	-	-	6	4
Silverman etal, 1985a	570	87	45	60	16	-	14	8
Silverman et al,, 1991	214	86	46	68	14	-	13	12
Present study, 1998	690							

* Patients could have lesions at more than one site

Table 10: Time of onset (of non-oral) mucocutaneous LP in relation to development of OLP

Onset of involvement	Number of patients	Percent of patients with mucocutaneous lesions
Extraoral lesions prior to oral lesions	10	11.8
Oral lesions prior to extraoral lesions	75	88.2
Oral lesions erupt simultaneously with extaoral lesions	0	0
Total number with extraoral lesions	85	100.0

Table 11: Distribution of extra-oral lesions of lichen planus in 85 patients

Site of extra-OLP lesions	Number of affected Patients	Percent of patients with cutaneous lesions
Wrists	51	60.0
Arms	34	40.0
Legs	26	30.6
Genitalia	11	12.9
Trunk	10	11.8
Back	7	8.2
Neck	6	7.1
Ankle	6	7.1
Scalp	6	7.1
Feet	5	5.9
Abdomen	5	5.9
Knee	4	4.7
Hands	2	2.4
Palms and soles	2	2.4
Nails	2	2.4

Table 12: Other extraoral lesions in 17 patients with OLP

Lesion	Number of patients	Percent of group (N=17)
Vertical ridging of finger nails	5	29.4
Submandibular lymphadenopathy	5	29.4
Cervical lymphadenopathy	4	23.5
Blepharitis	2	11.8
Conjunctivitis	1	5.9
Total	17	100.0

Discussion

Retrospective and observational studies have several inherited limitations and usually confronted with many difficulties. An example is the data that usually obtained from hospital records may suffer from incompleteness due to missing information or missing patient records. Furthermore, there are wide differences in clinical judgement among clinicians, partly due to ever changing diagnostic criteria due to the increased knowledge and awareness about various aspects of the condition under investigation. However, the retrospective studies are useful in evaluating demographic characteristics of the populations [24] and can serve as a basis for deeper research undertaken by other study designs. For this study, the data were collected from many patients over twenty years during which period there were changing criteria for the diagnosis of lichenoid lesions and emergence of new concepts and variants of OLP [13], but the clinical presentation of the lesions and their description were both undertaken by the same group of clinicians may give more homogeneity to the clinical judgement.

Another problem faced by many previous studies and this study also is the type of included population. As mentioned before most of the patients were referred from their GDPs or other specialities; this may have some impact on the types of oral lesions they commonly presented with. Arguably, the referred patients should have larger fraction of lesions associated with pain symptoms (e.g., ulcerative, atrophic and bullous lesions, including desquamative gingival lesions) than could be expected to be found in general population. Because many cases of asymptomatic lesions would have not been referred for specialists and even some symptomatic lesions would had supposedly been treated by their attending clinicians for many years before being referred to Oral Medicine specialists (Table 6).

This is the first detailed study of a large UK patient group with OLP despite this being common oral mucosal disorder often requiring some clinical intervention. In general, the results of the present UK study confirm observations from studies in the USA and Scandinavia (Tables 1 & 9). In agreement with studies of other predominantly Caucasian patient groups, OLP of the present group of patients seemed to develop in middle to late life, but perhaps surprisingly could arise in young adults as young as 16 years. However no child was observed in this group, perhaps reflecting its rarity in childhood. [25-27] In agreement with other similar studies, OLP developed at similar ages in both genders, although there was a tendency for all types of OLP to occur in male patients at an earlier age than females, the precise reasons for this occurrence are unknown and probably not of clinical or aetiological significance. [28]

OLP has previously been reported to be more frequent in females than males [9, 29], the same finding was shown by the present study, and however, some epidemiological population studies have shown that men and women are affected almost equally by the disease. [30,31]

Oral lichen planus (OLP) is a disorder that affects all ethnic groups. Results of previous European studies suggested that up to 2.4% of Caucasians may have lichen

planus [32-35], while rates of 0.5 to 1.5% have been reported in an Iranian and Indian population. [30,36] In the present study OLP was reported in patients of several ethnic backgrounds and is thus in agreement with results of population studies that suggested the occurrence of OLP in all racial groups. [37,38] Although not detailed in the present study there was no significant differences in the demographical or clinical features between patients of different ethnic backgrounds.

As there was no significant systemic disease association despite the plenty of data reporting association between lichen planus and spectrum of systemic diseases, few of these occur in UK patients with OLP. [39] Furthermore the large number of patients included in this study are a powerful indication that at least some of these associations are overestimated. The lack of link between OLP and systemic disease would suggest that OLP is likely to reflect local immunological changes, possibly combined with subtle, and unidentified systemic upset rather than an oral response to profound systemic disease. [40]

There is increasing evidence that social deprivation may be important in the aetiology of significant number of oral mucosal diseases including malignancy. [2] In this study the recorded information in this context were not enough to conclude any possible effects of socio-economic status on OLP, because the proposal of such relationship is only recent and was not evident when most of the present records were made. Data also were insufficient to examine the employment status of many patients. Of note, only few patients with OLP were professional contradicting the notion that OLP afflicting professionals and higher social groups and the majority of the patients were manual workers or semiskilled workers. Unsurprisingly a considerable number of patients (15%) were retired, as this disorder usually affects middle aged and elderly individuals and further it lasts for long periods of time. The present data are insufficient however to determine any association between socio-economic status and propensity to OLP, as again results could reflect the attendance pattern of patients to Oral Medicine units. Although several studies reported high anxiety scores in patients with OLP, it is unlikely that the observed psychologic alterations are a direct etiologic factor or are a direct consequence of OLP. [41,42]

From the available data it is doubtful if the maternal or parental status or number of children in any way influenced the development of OLP of the present group of patients.

Majority of patients had experienced some degree of oral soreness. Typically there was generalised oral discomfort (Table 4), but, as in other studies. [28, 43] patients with non-atrophic and non-ulcerative OLP often still complained of oral discomfort. Indeed over 60% of the present group of patients with reticular OLP had some degree of oral soreness (Table 5). Oral pain was the main reason for patients being referred to Oral Medicine units, however the pain was likely to be generally mild unlike the pain of a periapical abscess or trigeminal neuralgia, as patients had usually had oral symptoms several months prior to referral to an Oral Medicine unit (Table 6). As about 86% of the patients were referred by their general practitioners, this reflects the importance of motivation and encouragement of general public to have regular check up visits to their dentists and general practitioners.

As with studies of other cohorts of patients (Table 1) reticular and atrophic lichen planus were the most common

types of OLP in the present group of patients, although in contrast only 2.8% of the patients had ulcerative lesions.[44] In this study the lesions only considered as ulcerative when there is frank oral ulceration, whereas the lesions considered atrophic when they were red atrophic with erosions confined to epithelium with no ulceration. It is evident that the majority of patients could have more than one type of OLP (Table 7), often there being combinations of both white and atrophic lesions.[8,17] Albeit their rarity, ulcerative lesions were described in 16 patients in this group of patients, may be a form of chronic ulcerative stomatitis (CUS), as most of such lesions were amenable to most available therapy.[45,46]

As expected (e.g. Andreasen, 1968) the oral lesions of LP were typically symmetrical and in agreement with previous studies the buccal mucosa and tongue were the most commonly affected sites (Table 9). Patients often had lesions affecting several oral mucosal surfaces but there were no notable differences in the frequencies of different types of OLP at different oral sites. As the palate was rarely affected, accurate diagnosis of OLP may be possible based upon clinical grounds alone and hence differentiation between most cases of typical OLP and lupus erythematosus may often be possible without detailed histopathological investigation.[24, 47] Perhaps reassuringly all the present group of patients with clinical features suggestive of OLP who had biopsy of lesional tissue were found to have histological features of lichen planus or lichenoid reactions.

The status of dentition and periodontal condition were not precisely recorded or estimated in the majority of the present patient group. Similarly, lesions due to the use of drugs reported to cause lichenoid eruptions had not been separated on clinical ground from idiopathic lichen planus lesions. In fact the present group of patients included all variants of OLP.

The most important complication of oral lichen planus (OLP) is the development of oral squamous cell carcinoma[8], particularly in cases with atypical presentation.[49] In the present study there was a 1.9% rate of malignant change of OLP lesions, with no obvious correlation with age, gender, and tobacco or alcohol usage. This is in accordance with other reports.[50, 51] Erosive OLP was the most common type adjacent to sites of malignant changes at the tongue, buccal mucosa or gingiva. A constant close follow up is thus mandatory in all OLP-like lesions that show dysplasia.[52] Only universally accepted criteria of inclusion in different studies could be helpful in the correct estimation of the true rate of malignant transformation of OLP.[53]

Cutaneous and genital involvement of lichen planus can precede; arise concurrently; or after the development of OLP[54]. It is estimated that 20% to 34% of patients with OLP will have cutaneous or other mucosal lesions of LP.[8] In the present study 85 (12.3%) patients had possible features of cutaneous or other mucosal LP; the majority developed these affections after the onset of oral disease (Table 10). While these lesions were diagnosed by attending consultant dermatologists few were formally biopsied. Therefore this could be an overestimation of the frequency of non-OLP in the patients with oral disease. Nevertheless it is evident that cutaneous per se involvement is not an uncommon feature of patients with principal symptoms and signs of OLP, as these lesions typically occur on the forearms and legs and that genital involvement is

GMJ. Vol. 21 No (2), PP20-36

rare (Table 11). There is thus good reason to carefully examine the skin of the hands, feet and legs of patients attending oral medicine clinics with possible OLP. Nevertheless in view of the similarities between genital lichen planus and disorders such as lichen sclerosus et atrophicus, [55] detailed mucocutaneous assessment should only be undertaken by appropriate specialists. [56]

In summary, the results of this study revealed that UK patients with OLP are typically middle-aged, complain of variable degrees of oral discomfort and usually have bilateral lesions affecting the buccal mucosa, tongue and gingivae. The lesions are usually reticular plaque-like and/or atrophic, although patients often have more than one type of OLP. A minority of patients may have lichen planus affecting other mucocutaneous regions, typically the skin of the extremities.

Correspondence to

Dr M Ingafou. Department of Oral Medicine Oral Pathology Diagnosis and Radiology. Faculty of Dentistry, Garyounis University. B.O. Box 9504. Benghazi – Libya Tel +218 (61) 9096045. Fax +218 (61) 9093771.

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GMJ. VOL 21 NO.(2), PP37-40

Evaluation of some common temperature Monitoring sites during general anaesthesia

Masoud Ali Lfeituri; Fadwa Saleh El Tarhoni.

Department of Surgery, Unit of Anaesthesia and Intensive Care, Faculty of Medicine, Garyounis University, Benghazi,Libya.

الملخص

شملت هذه الدراسة 20 مريضاً بالغاً أجريت لهم عمليات جراحية اختيارية تحت التخدير العام، إذ قِيسَتْ درجات حرارة الجسم خلال أول ساعتين من الجراحة وذلك في ثلاثة أماكن مختلفة من الجسم وهي البلعوم الأنفي، والمريء والإبط وأُوجِدَتْ العلاقة (معامل الارتباط) والفروق (مدى الدقة) بينها وبين درجات حرارة طبلة الأذن التي قِيسَتْ بواسطة جهاز الثيرموسكان والذي يعمل بالأشعة دون الحمراء. وقد كان معامل الارتباط بين كل من درجات حرارة البلعوم الأنفي والمريء والإبط وبين درجات حرارة طبلة الأذن يتراوح على التوالي ما بين 0.88 إلى 0.96 و 0.85 إلى 0.94 و 0.63 إلى 0.84 أما متوسط الفروق بينها وبين درجات حرارة طبلة الأذن فكانت (المتوسط ± الانحراف المعياري) 0.118 (±0.019) و 0.205 (±0.030) و 0.489 (±0.040) درجة مئوية على التوالي.

Abstract

Twenty adult patients underwent elective surgical procedures under general anaesthesia were enrolled in a study comparing the correlation (relationship) and the differences (accuracy) between three different temperature measurment sites, namely nasopharyngeal, oesophageal, and axillary, and that obtained from tympanic membrane (TM) taken with infrared ThermoScan thermometer during the first 120 minutes of surgery. The correlation coefficients between tympanic membrane measurements and nasopharyngeal, oesophageal, and axillary measurements respectively ranged from 0.88-0.96, 0.85-0.94 and 0.63-0.84, and the mean differences were (mean ± SD) 0.118 °C ± 0.019 (P> 0.05), 0.205 °C ± 0.030 (P> 0.05), and 0.489 °C ± 0.040 (P< 0.01) respectively.

Key words: General anaesthesia; temperature; monitoring sites.

Introduction

Hypothermia is common during anaesthesia and surgery.[1,3] It has been shown to be associated with serious adverse outcomes including coagulopathy with increased blood loss, decreased drug metabolism and clearance with delayed postanaesthetic recovery, postoperative shivering and myocardial ischaemia, and wound infection with delayed discharge from hospital.[2,4] Thus, the hazards of perioperative alteration in body temperature clearly justify the importance of temperature measurement which nowadays become a routine part of monitoring. Temperature monitoring sites are many and each has advantages and disadvantages.[1, 5-7] In order to avoid obtaining false readings and thereby inappropriate management, determination of the most accurate and safest site and method for monitoring of body temperature is desirable. Tympanic membrane (TM) have been used in several studies as a conventional standard for core temperature because it shares the same vascular supply that perfuses the hypothalamus.[3,5,8-10] TM injury was reported when tympanic temperature measured by ear probes.[6] In our study we used an infrared thermometer to measure tympanic temperature as it has been proven to be

GMJ. VOL 21 NO.(2), PP37-40

accurate and safe both in adults and children.[7,8,11] This device is a portable instant thermometer measures the body temperature by detecting the thermal infrared energy that is normally emitted from the TM without any direct contact with the membrane. The aim of this study was to evaluate the relationship and the differences between the temperature that obtained from TM and that of three commonly used temperature monitoring sites, namely nasopharyngeal, oesophageal and axillary during the first 2 hours of surgery.

Patients and Methods

Body temperature of twenty adult patients (9 males and 11 females) scheduled for elective general and gynaecological surgical procedures was monitored at four sites namely TM, nasopharynx, lower esophagus, and axilla. Demographic data obtained in each patient included name, age, sex, body weight, height, and ASA physical status. Subjects had no history of diabetes, fever, thyroid disease or problems with the TM or middle ear. Anaesthesia was induced with IV fentanyl (100 microgram), 2.5% thiopentone (4-5 mg/kg) and atracurium (0.5 mg/kg). Throughout the operation 66% N_2O in oxygen and 0.5-1 volume% of halothane were used. Preoperative tympanic and axillary temperatures were measured just before induction of anesthesia (interval P). The axillary probe sensor was placed high in the axilla and the arm secured in an adduction position thereafter. Immediately after induction, oesophageal and nasopharyngeal probes were placed in the lower third of oesophagus and posterior nasopharynx respectively, and taped in place. Temperatures from the four measurement sites were recorded simultaneously immediately after induction of anaesthesia (interval A) and then at intervals of fifteen minutes for the first two hours of surgery. Nasopharyngeal, oesophageal and axillary temperatures were measured using Yellow Springs 400 thermistor probes (Yellow Springs, Ohio) and all were allowed to equilibrate at least four minutes prior to the first reading. Tympanic temperature was recorded from both ears using ThermoScan thermometer to detect the maximum tympanic temperature that was used in this study as a reference of core body temperature. Heart rate, blood pressure, and arterial oxygen saturation were monitored throughout the study period. The relationship of temperature measurements at each site was analysed by Pearson correlation coefficient between the measured and the tympanic temperatures at the same time. Accuracy was calculated as the differences in temperatures between each probe and the tympanic temperature measured simultaneously at each interval, and analysed by one-way analysis of variance and Dunnett's multiple comparison test. Data are displayed as mean ± SD and differences considered statistically significant when $P<0.05$.

Results

Mean (and range) of age, body weight and height were 53.1 years (21-74), 66.3 kg (52-97), and 168.1 cm (160-178) respectively. 5 patients had ASA I, 11 patients had ASA II, and 4 patients had ASA III. The differences between the right and left tympanic membrane temperatures were 0.0 ± 0.1°C and these were not significant but indicated an excellent reproducibility.

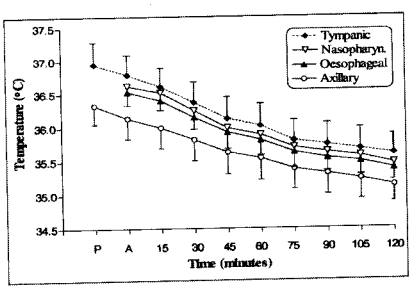

Figure 1: Mean body temperatures as measured at tympanic membrane, nasopharynx, lower third of esophagus and axilla. (P) is preoprerative and (A) is anaesthesia induction time. Vertical bars are SD. For esophageal temperature SD is omitted for clarity (ranged from 0.25 to 0.32)

Mean body temperatures at each of the sites monitored are plotted in figure-1 as a function of time; tympanic, nasopharyngeal, and oesophageal temperatures were clustered together while axillary temperature was significantly lower than TM temperature ($P<0.01$).

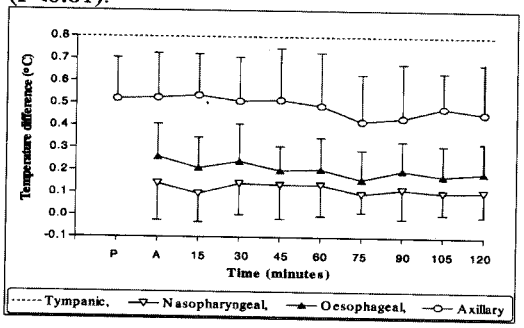

Figure 2: accuracy of different temperature measurement sites as calculated by comparing the difference between each measurement site and tympanic temperature simultaneously. (p) is preoperative and (a) is anaesthesia induction time. Vertical bars are the sd.

Figure-2 shows the accuracy of different probes that was defined as the mean differences between tympanic measurements and those of the other sites measured simultaneously. Nasopharyngeal and oesophageal measurements did not differ significantly from tympanic temperature at any time ($P>0.05$), whereas the axillary measurements did ($P<0.01$). The mean of differences (mean ± SD) between TM temperature and nasopharyngeal, oesophageal and axillary recordings were 0.118 °C ± 0.019 ($P> 0.05$), 0.205 °C ± 0.030 ($P> 0.05$), and 0.489 °C ± 0.040 ($P< 0.01$) respectively.

Figure-3 illustrates the relationship between each measurement site and that of the TM which established by calculating the correlation coefficients at each time interval.

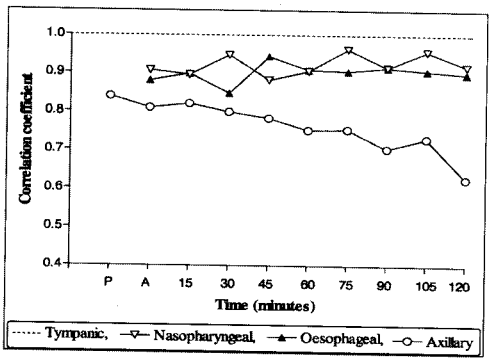

Figure 3: Correlation of nasopharyngeal, oesophageal and axillary temperatures with tympanic membrane temperature. (P) is preoperative and (A) is anaesthesia induction time.

Nasopharyngeal and oesophageal temperature measurements were closely correlated to TM temperature, and had correlation coefficients ranged respectively from 0.88 to 0.96 and 0.85 to 0.94 throughout the course of anaesthesia studied. On the other hand, correlation of axillary temperature measurements was poor both preoperatively and intraoperatively, and ranged from 0.63 to 0.84.

Discussion

Temperature monitoring sites have been considered precise and accurate when they correlate and compare favourably with tympanic temperature.[5, 7,10] As reported by others,[5,7] we found that tympanic, nasopharyngeal, and oesophageal temperatures were similar in anaesthetised adults, while axillary temperatures were poorly correlated to, and significantly lower than the tympanic temperatures.

Conventional TM thermometers used thermistor or thermocouple probes in direct contact with the eardrums, and may put the anaesthetised patient at the risk of TM perforation.[6] Infrared thermometers are superior to thermistor and thermocouple probes in that they use an infrared sensor probe that oriented toward, but not in direct contact with the eardrum, therefore do not predispose patients to any possible trauma.

GMJ. VOL 21 NO.(2), PP37-40

Obtaining an accurate measurement is dependent on orienting the probe correctly toward the TM. The use of the maximum tympanic temperature in our study is reasonable since falsely low readings may be obtained by misdirection of the probe tip toward the canal wall. A study by Shinozaki *et al.*[8] demonstrated that infrared thermometer to be accurate both in vivo and in vitro over the temperature range of 34.0 °C to 39.5 °C. In vivo measurements were made on patients undergoing open heart surgery and were correlated with pulmonary artery catheter thermistor by 0.98. Our study demonstrates a good correlation and a favorable comparison between tympanic temperature obtained by ThermoScan thermometer and nasopharyngeal and oesophageal temperatures; this may also indicate reliability of the infrared tympanic measurements and allowed us to conclude that the infrared ThermoScan thermometer can safely and confidently be used as an index of core body temperature.

Conclusion

In conclusion, during general anaesthesia the nasopharyngeal and oesophageal temperature measurements are accurate and correlate closely with tympanic temperature measurements obtained by ThermoScan thermometer. In contrast, axillary temperature measurements are significantly different, and correlate poorly with tympanic temperature measurements. Therefore, axillary temperature can not be considered as a substitute for determining central body temperature.

Correspondence
Masoud Ali Lfeituri: P.O.Box:2856 Benghazi-Libya.
Tel: 061-2227963,84340. Tel-Fax: 061-2230207
e-mail: melfeituri@yahoo.com

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GMJ. Vol 21 No (2), PP41-49

Serotypes and Frequency of Multi-drug Resistant Salmonella Isolated from Diarrheal Children In Benghazi, Libya.

Aisha Y. Gerged[1], Saleh H. Baiu[1], Adel I. Ahmaida[2].

Faculties of Science [1] and Medicine [2], Garyounis University, Benghazi, Libya

الملخص

الخلفية: تشيع السالمونيلا في الدول النامية مسببة الإسهال و حمج الدم و الحميات المعوية. الهدف: معرفة أنواعها في مرضى الإسهال داخل مستشفي الفاتح لطب و جراحة الأطفال و ما إذا كانت مقاومتها للبكتيريا قد تغيرت.**طرق البحث:**شملت الدراسة 1017 مريضا و 80 حالة للمقارنة في الفترة من شهر 7/ 2000 و حتى 2001/6 و ذلك باستعمال طرق العزل المتعارف عليها. **النتائج:** وجدت نسبة عالية من المقاومة ضد المضادات الحيوية في وجود تعرض سابق للمضادات الحيوية خاصة. تنتمي أغلب الفصائل متعددة المقاومة إلى المجموعة ب. أما أكثر الأنواع المصلية التي عزلت فكانت كالآتي: س.تايفيموريوم (22%) ، س. مينشن (17%)، س. باراتايفي (17%). **الإستنتاج:** رغم أن معدلات المقاومة في تناقص فإن المعدل ما يزال عاليا جدا كما لم تُعْزَلْ السالمونيلا من النوع أ رغم أن هذا النوع كان أكثر الأنواع المعزولة في دراسة مشابهة سابقة.

Abstract

Background: salmonella infection is known to be common in developing countries leading to gastroenteritis, septicemia and enteric fevers. **Objectives:** to know the contribution of salmonella species to acute diarrheal illness and the prevalent sero-groups and types, and to assess wither there is a change of the known antibiotic resistance patterns. **Setting:** A prospective open study done in Al-fateh Children's Hospital, Benghazi, Libya during one year from July 2000-June 2001.**Patients and Methods:** 1017 patients with acute diarrhea and 80 controls were included. Clinical characteristics were taken and stool samples were processed by the usual well known microbiological methods. **Results:** salmonella was isolated in 60 patients (5.9%), with 11 cases nosocomially acquired. More than 85% of the cases were in under 2 years of age. The serogroups isolated included B, C and D with B and C representing the majority (38.3 and 33% respectively). Seogroup A reported in a study done recently in the same locality was not shown by us. The main serotypes identified were S. typhimurium (22%) and S. muenchen and S. paratyphi (17% each). 83% of the isolates were resistant to one antibiotic or another with multiple antimicrobial resistance in 80% of isolates. High resistance rates were found for amoxicillin/clavulenic acid (45%), ampicillin (40%) amoxicillin (36.7%) and gentamicin (35%). Only 8.3% and 15% were resistant to piperacillin and ciprofloxacin respectively. Isolation of multiresistanct strains of Salmonella was significantly associated with prior exposure to antibiotics (p=0.028). The majority of multiresistant strains were of group B, which was significantly different from other groups (p= 0.005). The resistance rate declined significantly for Amoxicillin/clavulenic acid (p=0.04), ampicillin (p=0.004) and tetracyclines (p=0.019) **Conclusions:** Acute diarrhea is still a major burden on health services. Serogroups B,C and D largely replaced group A. Though resistance rates for some antibiotics have declined, the overall resistance is still high.

Key words: Acute Gastroenteritis, Salmonella, Antimicrobial Resistance, Benghazi, Libya.

GMJ. Vol 21 No (2), PP41-49

Introduction

Salmonella is a genus belonging to the enterobacteriaceae family. They are gram negative motile rods which lead to a variety of clinical illnesses in humans and animals. These illnesses include acute gastroenteritis, enteric fever, septicaemia, asymptomatic carrier state and invasive disease such as osteomylitis and meningitis [1,2]. They are facultative anaerobes and are relatively easy to identify in the laboratory. They are oxidase negative and virtually all are lactose negative (white on MacConkey agar plates); most Salmonellae produce hydrogen sulfide which is easily detected on selective indicator plates such as the Salmonella-Shigella medium[3]. They are widespread epidemiologically and their contribution to acute diarreahal illness is on the increase in some developed countries. The prevalent serotypes vary in different geographical areas and from time to time in the same area. Salmonellae enteritidis, typhimurium and heidelberg are the serotypes most frequently isolated in the United States in recent years.[4] Although serogrouping may provide a clue as to the specific organism but this may not be useful clinically since salmonellae belonging to the same group may produce quite different illnesses. As an example, both for S. enteritidis and S.typhi belong to serogroup D though they cause totally different illnesses.

Nontyphoidal Salmonellae are associated with animal reservoirs and therefore with agricultural products, especially eggs and poultry.[5] Nontyphoidal Salmonellae have also been associated with meats, and milk [6]. Since 1989, multidrug resistant salmonella (MDR) became a world wide problem with high potential risks[7].Many isolates with MDR were Salmonella typhimurium definitive phage type 104 (DT104), a virulent epidemic strain which had earlier become widespread in the U.K.[8]. A group in France found that a single clone with S. typhimurium with a chromosomal integron carrying a PSE-1 beta lactamase gene accounted for the majority of animal and human MDR strains in that country. This clone was indistinguishable from the DT104 isolates from the United Kingdom implying spread of this organism through animal and human ecosystems in these countries. Resistance of S.typhimurium to ampicillin, chloramphenicol, sulfonamides, tetracyclines and even the recently introduced fluoroquinolones is well documented. Some of these antibiotics are not used in children except in narrow examples. Locally, multi-drug resistant salmonella contributed to a number of outbreaks particularly within hospital settings [9,10] .The aim of this study is to know the contribution of salmonella species to acute diarrheal illness and the prevalent sero-groups and types, and to assess wither there is a change of the known antibiotic resistance patterns.

Materials and Methods

A total of 1097 specimens were collected from children aged from 3 days to 12 years between July 2000-June 2001. Eighty of these samples were taken from age and sex-matched controls recruited from El-Fowehat MCH clinic, Al-Jamahiriya kindergarten and Baraem Al-Tahadi primary school. The rest (1017) were collected from in-patients at Al-fateh Children's' Hospital (AFCH) with acute diarrheal illness. Entry of patients in the study was allowed only once. Stool flecks or

rectal swabs were inoculated directly on several selective and differential media including: MacConky agar (Oxoid , LTD , UK) , Salmonella – Shigella agar (SSA , Oxoid) , Xylose lysin desoxycolate agar (XLD , Oxoid) and selenite broth (Oxoid) . Inoculated media were incubated aerobically at 37°C for 24 hours. After overnight incubation, 3 – 5 loopfuls of selenite broth were subcultured onto another set of MacConky agar, XLD agar and SS agar and incubated for 24 h. The non lactose fermenting colonies were subjected to API 20E (BioMerieux, France). Isolates confirmed biochemically as members of the genus Salmonella were tested to identify the serogroups by slide agglutination using O and H antisera (MAST ASSURE). Strains were described to serogroups by their reaction to group specific antisera according to the Scheme of Kaufmann and White. All Salmonella isolates were tested for their sensitivity to antibiotics using the disk diffusion method of Bauer and Kirby. The control strain used was Escherichia coli NCTC. We assumed differences to be significant if P value was less than 0.05. SPSS version 8 was used in the analysis. Data are expressed as numbers, percentages, ranges, standard deviations and P values as appropriate.

Results

Mean age was 22.4 months (3 days-12 years, SD± 34.4 months). Males accounted for 57.6% of cases. Three quarters of patients (75.6%) were under 2 years, 616 (60%) under 1 year and 334 (32.4%) under 6 months. There were no statistically significant age and gender differences between patients and controls. None of the controls was positive for salmonella. Salmonella was isolated from 60 patients (group I). Patients with non- salmonella caused diarrhea constituted group II (957 patients). There were no statistically significant age and gender differences between these 2 groups and the control. The isolation rate of salmonella cases peaked during October and November (38.4%) while the non-salmonella showed a relatively more uniform distribution throughout the year of the study. Positive family history of recent diarrheal illness was noticed in 13 cases (21.7%) in group I and in 102 cases of group II (10.7%). The difference was highly significant (P=0.009). One hundred and sixty eight cases (16.5%) developed acute gastroenteritis 2-26 days after admission to the hospital, 11 of whom proved to be due to salmonella with a mean pre-illness (i.e. acute gastroenteritis) hospitalization period of 6.9 days (SD±5.7). Patients were at no particular risk of acquiring nosocomial salmonella gastroenteritis when compared with those acquiring nosocomial non-salmonella gastroenteritis (P=0.696). Neonatal unit showed the highest number of nosocomial cases. Forty nine salmonella cases acquired the infection in the community with a pre-hospitalization duration of illness of 1-21 days (mean= 3.9, SD±3.5).

Forty seven percent of those under 2 years had an access to breast milk but only 105 (13.6%) were on exclusive breast feeding. However, there were no statistically significant differences between group I, group II and the control group regarding breast feeding and other feeding practices. There were no significant differences between groups I and II in the clinical features except for tenesmus (P=0.01) and visible blood (P=0.034). Presence of occult blood in the stool did not help in differentiating patients with salmonella from

others. Table I shows the pattern of use of antibiotics in our patients. Thirty four of Group I were exposed to antibiotics (56.7% of salmonella cases) while the corresponding figure in group II was 307 (32.1%).

Table I: pattern of use of antibiotics

	Prior use of antibiotics*	Already on antibiotics	No exposure to antibiotic	Total
GroupI	7	27	26	60
Group II	91	216	650	957

*(within 30 days of onset of illness)

The serogroups found were B (n=23; 38.3%), C (n= 20; 33.3%), D (n=10; 16.7%), G (n=5; 8.3%) and F (n=2; 3.3%). Serotyping was done only for 41 isolates. The commonest serotypes were S.typhimurium, S. paratyphi B, and S.muenchen accounting for 9, 7, 7 cases respectively. All paratyphi B were nosocomially acquired. Table II shows the serotypes in detail.

Table II: salmonella serotypes in 41 isolates.

Salmonella Serotype	No.
Typhimurium	9
Paratyphi B	7
Muenchen	7
Typhi	5
Bovis morbificans	4
Enteritidis	3
Newport	2
Bareilly	2
Derby	1
heidelberg	1

Only 10 isolates were sensitive to all antibiotics tested giving a resistance rate of 83.3%, meanwhile all isolates were sensitive to colistin sulfate. High resistance rates were shown for amoxicillin/clavulenic acid (45%), ampicillin (40%) and amoxicillin (36.7%). Only 8.3% and 15% were resistant to piperacillin and ciprofloxacin respectively. Out of the 50 antimicrobial resistant isolates, 10 were resistant to a single agent whereas the remainder showed resistance to 2 or more agents. Table III shows distribution of resistance among serogroups whereas table IV shows the number of antibiotics to which serogroups are resistant. Table V shows the number of antimicrobials to which each serotype is resistant. Results showed that there was significant differences in the MDR pattern among different groups (P=0.005) and serotypes (P=0.025). Twenty of the forty MDR isolates were in group B (50%), followed by group C (32.5%). Within group B, all isolates of S.paratyphi B were multiresistant to 4-8 antimicrobials.

Table III: No. of resistant isolates from each serogroup to individual antibiotics

Group	AMC	AMP	AMX	GM	SXT	C	TE	K	S	CIP	PIP	CS
B	12	10	12	13	9	11	10	10	5	4	3	0
C	10	9	7	7	7	5	4	2	4	3	2	0
D	2	3	1	0	1	0	0	0	0	2	0	0
G	2	2	1	1	2	0	0	0	2	0	0	0
F	1	0	1	0	1	1	0	1	0	0	0	0

AMC:amoxicillin, AMP:ampicillin, AMX:amoxicillin,GM:gintamicin, SXT:sulfamethoxazole-trimethoprim, C:chloramphnicol, TE:tetracyclin, K:kanamycin, CIP:ciprofloxacin, PIP:piperacillin, CS:colistin sulfate,S:streptomycin.

Table IV: No. of resistant isolates in relation to the No. of antimicrobials.

	No. of Antibiotics	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**≥8**
Group	**MDR %**									
B	20(50%)	1	2	5	2	2	2	1	2	4
C	13(32.5%)	3	4	3	2	1	4	1	2	-
G	4(10%)	1	-	3	-	1	-	-	-	-
D	2(7.5%)	5	3	-	2	-	-	-	-	-
F	1(2.5%)	-	1	-	-	1	-	-	-	-

Table V: No. of resistant isolates of serotypes to individual antibiotic.s

Serotype	**AMC**	**AMP**	**AMX**	**GM**	**SXT**	**C**	**TE**	**K**	**S**	**CIP**	**PIP**	**CS**
typhimurium	3	4	5	5	2	4	3	4	1	2	2	0
paratyphi b	5	3	4	5	4	6	6	5	2	1	1	0
muenchen	5	4	3	3	2	2	0	0	2	1	0	0
typhi	1	2	1	0	1	0	0	0	0	2	0	0
bovismorbificans	1	1	1	0	2	1	0	0	0	2	0	0
enteritidis	1	0	0	0	0	0	0	0	0	0	0	0
newport	1	1	0	0	1	0	0	0	0	0	0	0
bareilly	1	1	0	0	0	0	0	0	0	0	0	0
derby	1	0	1	1	0	1	1	1	0	0	0	0
Heidelberg	0	1	1	0	1	0	0	0	1	1	0	0

Discussion

Salmonella is one of the predominant bacteria causing gastroenteritis in infants and children all over the world, especially in developing areas such as south – east Asia[11], India [12] and Africa [13]. However, the differences in methods of subjects selection, collection and storage of specimen, isolation and identification of Salmonellae make the comparison between isolation rates in different studies less than satisfactory.

Similar to what had been reported before[13-15] more males than females were encountered in this study and children less than two years old were disproportionately more likely to be affected [16]. The majority of children positive

for Salmonella were less than 12 months of age and most of them were less than 6 months. This could be due to the immaturity of immune system and food practices [17,18] .Regarding nosocomial infection , Salmonella gasrroenteritis occurred after a period of hospitalization of 2 – 19 days which exceeded the usual incubation period (6 – 48 h) .Most of these cases became infected while they are on treatment with antibiotics , Nosocomial infection is an alerting problem in many hospitals worldwide especially in pediatric hospital and nurseries [19]. Transmission commonly occurs by contact between patients and staff, or contaminated equipment or through food, milk or water [20, 21].

In neonatal ward, many procedures such as giving medications, feeding and changing napkins are preformed by nursing staff and the organisms can spread by way of hands. The way of delivering feeding bottles to patients in this hospital may be one of the routes of acquisition of infection [9, 22]. It has been noticed that feeders are kept for long time before being distributed which may enhanced microbial content of milk. A separate study of this situation is needed to determine the real contribution of feeders handling to the problem of infection. Most of Salmonella infected patients (45 %) have received antimicrobial agents for other illnesses. This has shown a highly significant association with Salmonella infection (p = .000). Use of antibiotics cause alteration in intestinal normal flora [23] which favors the persistence of bacterial pathogens [24, 25] .Undisturbed intestinal flora was noted to be distinctly antagonistic to the presence of Salmonella [26]. The peak incidence of Salmonella gastroenteritis was in October and November. This was different from some of previous studies where the peak incidence was seen in the hot months [17,22]. The majority of isolates in this study belong to serogroup B followed by C and D . This is in accordance with the results of similar studies from Saudi Arabia [27] and Kuwait [28].

On the other hand , our results are different from the report of a similar study from Spain [29] where the majority of isotates were serogroup D followed by B and C. As it has been suggested by others, these differences may be due to geographical variation in the distribution of Salmonella serogroups around the world [27, 28]. What is of more relevance here is that our results are also different from the results reported by Ghanim[15] who carried out his study in Al – fateh children hospital and revealed group A as the most prevalent Salmonella isolates followed by group C , F and B . The reason for this difference is probably the different source of infection in the two periods of studies, or change of the prevalent Salmonella serogroups from time to time. The resistance frequencies reported in this study probably reflects the widespread use of antibiotics both in hospital and within the community. The preponderance of isolates resistant to Amoxicillin/Clavulenic acid and Amoxicillin may be due to the more frequent use of these antibiotics for treating respiratory tract infection, diarrhoea and other diseases. Resistance to Ampicillin and Gentamicin was at very high level (40%and 35 % respectively), especially in organisms isolated from patients who acquired Salmonella in the hospital. These antibiotics are widely used in the hospital. When the resistance of Salmonella isolates to different antimicrobial agents was compared with that of previous studies, some differences were noticed especially in resistance to the three important anti-Salmonella agents (Ampicillin, Chloramphenicol and Trimethoprim / Sulfamethoxazol). The 40 %

resistance to Ampicillin of our isolates was comparable with findings reported by others [28, 29, 30]. Resistance rates of 13-16% were quoted by Kambal and Ling [27, 31]. The percentage of chloramphenicol resistant isolates (28.3 %) compared with that reported by Ling et al (23.5 %) [31] from Hong Kong but was different from the 11% reported by both Kambal et al27 and Munoz et al 29.In our study 33.3 % of isolates were resistant to Trimthoprim / Sulfamethoxazol. These differences might reflect the different practices in the use of antibiotics in these countries [32].

These differences in resistance may be affected by the type of patients involved, local policies for the treatment and isolation of infected patients, the use of antibiotics, and the addition of antibiotics to animal food. Resistance may be engendered not only to the antibiotic used but also to other antibiotics [33]. A markedly high rate of resistance was noted in our isolates when compared with that from previous studies. This may be due to the fact that our subjects were all hospitalized patients and many of our patients were exposed to antibiotics before developing acute diarrhoea. This is in agreement with Kambal et al [27] and Lee et al [34] who reported that most resistant isolates were from inpatients and attributed this to over use of antibiotics . Previous studies in the same hospital reported Salmonella to be resistant to several antibiotics such as Ampicillin, Chloramphenicol. Kanamycin, Gentamicin, Augmantin and Tetracyclin [9,22]. Comparing our present Salmonella isolates resistance patterns with results done few years ago in this hospital [15] , the frequency of resistance for all tested antibiotics declined and this was only significant for Amoxicillin/Clavulenic acid (p=0.041) , Ampicillin (p= 0.004) and Tetracyclin (p = 0.019) . This may be due to the difference in the groups of Salmonella isolated. However, this could not be confirmed because these studies did not mention the resistance pattern of serogroups to different antibiotics. In our study all of Salmonella isolates were susceptible to Colistin sulfate which compared well with Ghanim [15] and Salih [22]. Eighty five percent of isolates were sensitive to Ciprofloxacin ; the antibiotic which is currently a recommended drug for the treatment of S . typhi and invasive nontyphoidal Salmonella spp. and used for prophylaxis in recurrent cases of salmonellosis [28,35,36]. However, colistin is no longer used because of its side effects and availability of safer drugs. On the other hand, ciprofloxacin is not of general use in children because of potential adverse effects on cartilage though it seems that this concern was over stretched [37, 38].

The lower figure of resistance to ciprofloxacin in our study and the changing view on its use in children might open the door for its use locally in the future. Further studies on this issue are needed. Rates of multiple drug resistance in our isolates were 80 %, a much higher figure than that reported by many others where it ranged from 16-53% [27, 29, 31].

In our study , isolation of multiresistant strains of Salmonella was significantly associated with exposure to antibiotics during isolation of Salmonella (p=0.028) , and this may be represent acquisition of resistance by sensitive strains during antimicrobial therapy. The majority of multiresistant strains were group B, which was significantly different from other groups (p= 0.005). This is similar to reports by Yamamato and Ashton [16], Kambal [27] and Jamal et al [28].

In conclusion, this work shows that Groups B,C and D replaced group A as the most prevalent groups and that antibiotic resistance; though declining; is still a major problem. It also shows that MDR is commoner than single drug resistance. Regular follow up studies are needed to see wither this pattern is a consistent one or changing.

In conclusion, acute diarrhea is still a major burden on health services. Serogroups B,C and D largely replaced group A. Though resistance rates for some antibiotics have declined, the overall resistance is still high.

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GMJ. Vol 21 No (2), PP50-54

Mesiodistal Tooth Width in Libyans with Normal Occlusion

Mohamed S. H Ingafou; Hussein E Elfaituri.

Faculty of Dentistry; Garyounis University. Benghazi Libya

الملخص

الهدف: أخذت قياسات السطح الإنسي ـــالوحشي للأسنان الدائمة من عينة عشوائية تكونت من 85 ليبياً من ذوي الإطباق الطبيعي وذلك من أجل تحديد القياسات الطبيعية لعرض أسطح أسنانهم. . **النتائج:** بدا نمط الازدواج الجنسي واضحاً إذ إنّ الذكور عموماً لديهم أسناناً أكبر من الإناث، مع وجود فوارق ضئيلة في حجم الأسنان بين الجهتين اليمنى واليسرى من الفكين. وجد بأن النسبة بين العرض المركب لكل الأسنان العلوية إلى العرض المركب لكل الأسنان السفلية يساوي 94.7:87.6، أما عرض الأسنان الستة الأمامية العلوية إلى السفلية فكان 74.7: 100. **الاستنتاج:** يمكن استخدام النتائج المتحصل عليها في هذه الدراسة قاعدةَ بيانات لأية دراسات مستقبلية عن عدم تر اصف الأسنان وكذلك لتحليل المساحة المتاحة عند مرحلة الأسنان المختلطة.

Abstract

Objective: The mesiodistal crown widths of permanent teeth in a randomly selected 85 Libyans with normal occlusion were measured on dental casts in order to establish their norms. **Setting**: Dental faculty of Garyuonis University in Benghazi. **Results:** Sexual dimorphism was obvious as males were having overall larger teeth than females, however there were only minor variations in teeth size between the right and left sides. The combined arch width ratio between all maxillary and all mandibular teeth was 94.7: 87.6, and the widths of the 6 maxillary anterior teeth ratio to the 6 mandibular anterior teeth was 100: 74.7. **Conclusion**: The results obtained from this study can be used as a database for future studies of crowding as well as in space assessment analysis in mixed dentition.

Key words: Tooth size, Libyan Orthodontics, Dental Norms.

Introduction

The normal values of various components of dentofacial complex can provide clues for the ultimate result the clinician tries to achieve by implanting various preventive and interceptive orthodontic measures.[1] Crown size determination is one of the most important factors determining normal occlusion. The normal tooth size for a given ethnic group may be determined by both genetic and environmental factors, and controlled by multiple genes and different mechanisms.[2-4]Tooth size measurements (especially the mesiodistal width) is significant factor in many aspects of research in dentistry, such as the determination of normal occlusion, analysis of space requirements for mixed dentition, the design of direct composite restorations.Disturbances of teeth size may associate with some syndromes.[5,6]Studies to find out the tooth crown size of Libyans are scanty, so this study is designed to provide data about the crown size for both sexes. Furthermore, it reports the extent of variability in sizes of dental arches as well as between males and females.

Materials and Methods

This study comprised 85 Libyans (35 males and 50 females; with age range of 12 to 21 years) living in Benghazi city and its surroundings. Individuals included

in this study were selected at the department of Oral Medicine Oral Pathology Diagnosis and Radiology of the Dental faculty, Garyounis University, and referred to the department of orthodontics, where the study models and measurements were undertaken.

Subjects included in this study must be Libyan descendent with both parents of Libyan origin, with no history of previous orthodontic treatment and having normal occlusion with straight profile and competent lips. Dental casts from chosen subjects should have all permanent dentition (with the exception of third molars) with no crowding, normal overjet and overbite, and normal anterior-posterior and molar relationships.

The mesiodistal width of a tooth was recorded in accordance with the procedure described by Hunter and Priest.[7], by measuring the maximum distance between the tooth sides (proximal) on a line parallel to the occlusal and buccal surfaces using pointed calipers. All measurements were rounded to the nearest 0.1 mm. Descriptive statistical analysis was used to find out the algorithmic mean, standard deviation, minimum and maximum values computed for each tooth. The results of this study were compared to previously reported results of two studies from North America as shown in (Table 3).

Results

Values of the measured mesiodistal width of permanent incisors, canines, cupids and the first molars of both dental arches are shown in (Table 1). Comparison of the right and left sides of corresponding teeth shows little variation (Table 2). But the summed maxillary arch width was greater in males than females by 4.04 mm; likewise the summed mandibular arch width of males was 3.28 mm greater than that of females (Table 3).

The front 6 maxillary teeth width was 31.5 mm for males and 22.94 mm for females and the width of 6 front mandibular teeth was 30.88 mm for males and for females in a ratio of 100:74.4, and the ratio of all maxillary teeth to all mandibular teeth was 100 : 92.3. In comparison to two previous studies from North America, Libyans have larger anterior teeth than the Americans and both have similar size of posterior teeth (Table 4).

Discussion

Many definitions, methods and tools have been used to identify and measure tooth width.[8] Measurements on study models may be more convenient and accessible than direct measurements on actual teeth in the mouth (especially for the posterior segment). As the observed difference between these methods is not statistically significant[9] so all measurements in this study were done on study models.

Comparison between the right and left sides exhibited little variation, whereas male teeth were consistently larger than female teeth, in consistent with the findings of other studies.[10,11] The summed total maxillary arch width was 4.04 mm and the mandibular arch width of males were 4.04 mm and 3.28 mm greater than those of females.

It is well known fact in orthodontics that the crown sizes of human teeth are sexually dimorphic, with male having larger teeth than females[12] This holds for most human groups, though the extent of dimorphism varies among populations.[13] It is believed that the sexual dimorphism in mesiodistal tooth sizes is probably due to differences in dentin thickness and not enamel thickness[14] and influenced by many factors including the differential

GMJ. Vol 21 No (2), PP50-54

effects of the X and Y chromosomes on the growth of tooth enamel and dentin.[3,15] Posterior teeth were generally less variable than anterior teeth and constantly male teeth were larger in this study than those of females, similar to the findings of previous studies.[10]

Table 1: Mesiodistal dimensions of maxillary teeth of Libyans with normal occlusion

Tooth	Sex	Maxillary teeth			Mandibular teeth			Difference of maxillary & mandibular
		Min	*Medium*	*Max*	*Min*	*Medium*	*Max*	
Right central incisor	M	8.3	8.93	10.2	5.00	5.68	5.60	3.25
	F	7.5	8.67	9.60	4.50	5.44	6.00	3.23
Left central incisor	M	8.3	8.94	10.2	5.00	5.71	6.80	3.23
	F	7.3	8.71	9.80	4.50	5.45	6.50	3.26
Right Lateral incisor	M	6.6	6.84	7.5	5.60	6.16	7.40	0.68
	F	5.2	6.69	9.00	5.00	6.03	6.80	0.66
Left Lateral incisor	M	6.6	6.84	7.5	5.70	6.17	7.40	0.67
	F	5.6	6.81	8.40	5.00	6.02	7.00	0.79
Right Canine	M	7.0	8.06	9.00	6.20	7.08	8.00	0.98
	F	7.0	7.59	8.60	5.70	6.71	7.80	0.88
Left Canine	M	7.0	7.57	9.00	6.20	7.08	8.00	0.49
	F	6.2	6.89	8.00	7.70	6.64	7.50	0.25
Right 1st Premolar	M	6.4	7.14	8.20	6.40	7.14	8.00	0.00
	F	6.6	6.85	8.00	5.80	6.89	8.00	-0.04
Left 1st premolar	M	7.0	8.07	9.20	6.40	7.16	8.20	0.91
	F	6.0	7.13	8.20	6.00	6.87	8.00	0.26
Right 2nd Premolar	M	6.0	6.68	7.40	6.40	7.19	8.00	-0.51
	F	6.0	6.59	7.50	6.00	7.00	8.00	-0.41
Left 2nd Premolar	M	6.0	6.70	7.40	6.40	7.18	8.40	-0.48
	F	6.0	6.65	7.40	6.30	7.02	8.40	-0.37
Right Molar	M	9.0	10.54	11.70	10.50	11.35	12.80	-0.81
	F	9.2	10.11	12.00	9.70	10.94	12.20	-0.83
Left Molar	M	9.0	10.52	11.50	10.50	11.30	12.50	-0.78
	F	9.0	10.10	11.50	9.60	10.91	12.50	-0.81

Table 2: differences in tooth size between right and left sides for both sexes

Tooth	Sex	Maxillary teeth			Mandibular teeth		
		Right	*Left*	Difference	*Right*	*Left*	Difference
central incisor	M	8.93	8.94	0.01	5.68	5.71	0.03
	F	8.67	8.71	0.04	5.44	5.45	0.01
Lateral incisor	M	6.84	6.84	0.00	6.16	6.17	0.01
	F	6.69	6.81	0.12	6.03	6.02	0.01
Canine	M	8.06	7.57	0.49	7.08	7.08	0.00
	F	7.59	6.89	0.70	6.71	6.64	0.07
1st Premolar	M	7.14	8.07	0.93	7.14	7.16	0.02
	F	6.85	7.13	0.28	6.89	6.87	0.02
2nd Premolar	M	6.68	6.70	0.02	7.19	7.18	0.01
	F	6.59	6.65	0.06	7.00	7.02	0.02
1st Molar	M	10.54	10.52	0.02	11.35	11.3	0.05
	F	10.11	10.1	0.01	10.94	10.91	0.03

Table 3: Differences between lower and upper arches

	Maxillary	Mandibular
Sum of all the arch males	96.83	89.2
Sum of all the arch females	92.79	85.92
Sum of all the arch both sexes	94.69	87.61
Difference between genders in the sum of all the arch	4.040	3.280
Width of males anterior 6 teeth	31.55	23.72
Width of females anterior 6 teeth	30.88	22.94
Difference between anterior 6	0.67	0.78

Table 4: comparative study of tooth sizes of different populations

Tooth	Sex	Maxillary teeth			Mandibular teeth		
		Libyan	American	Mexican	Libyan	Americans	Mexican
Right central incisor	*M*	8.93	8.61	8.45	5.68	5.34	5.53
	F	8.67	8.61	8.15	5.44	5.29	5.45
Left central incisor	*M*	8.94	8.61	8.42	5.71	5.38	5.52
	F	8.71	8.61	8.20	5.45	5.25	5.40
Right Lateral incisor	*M*	6.89	6.72	6.55	6.17	5.86	6.02
	F	6.69	6.58	6.52	6.02	5.74	5.81
Left Lateral incisor	*M*	6.84	6.67	6.60	6.16	5.87	6.03
	F	6.81	6.43	6.47	6.03	5.84	5.91
Right Canine	*M*	8.07	7.82	7.94	7.08	6.78	6.87
	F	7.59	7.49	7.56	6.64	6.41	6.39
Left Canine	*M*	8.06	7.82	7.96	7.08	6.80	7.00
	F	7.57	7.43	7.31	6.71	6.42	6.46
Right 1st Premolar	*M*	7.13	6.95	7.06	7.16	6.90	6.02
	F	6.96	6.77	6.65	6.87	6.85	6.71
Left 1st premolar	*M*	6.74	6.93	6.94	7.14	6.97	7.05
	F	6.85	6.72	6.63	6.89	6.83	6.73
Right 2nd Premolar	*M*	6.70	6.72	6.85	7.18	7.05	7.27
	F	6.65	6.50	6.60	7.02	6.82	6.95
Left 2nd Premolar	*M*	6.68	6.66	6.97	7.19	7.11	7.39
	F	6.59	6.51	6.64	7.00	6.91	7.00
Right Molar	*M*	10.52	10.35	10.59	11.30	10.96	10.90
	F	10.10	10.00	10.26	10.91	10.45	10.48
Left Molar	*M*	10.54	10.44	10.54	10.35	10.99	10.67
	F	10.11	10.08	10.23	10.94	10.64	10.61

According to Bolton analysis, the present values are indicating that in comparison to other ethnic groups, Libyans posses slightly more tooth material (especially in the anterior maxillary segment) than the other ethnic groups. Their teeth sizes were, in general, larger than those of the Caucasians, comparable with Northern Chinese, but smaller than those of Australian Aboriginals.

As this study included only the subjects with normal occlusion, further studies are needed to study the tooth size in the persons with classes of malocclusion. It is hoped that this study can provide reliable data for orthodontists so they can accurately apply their preventive or interceptive measures based upon data of the same population.

Correspondence to

Dr M Ingafou. Department of Oral Medicine Oral Pathology Diagnosis and Radiology. Faculty of Dentistry, Garyounis University. B.O. Box 9504. Benghazi – Libya Tel +218 (61) 9096045. Fax +218 (61) 9093771.

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GMJ .Vol 21, No. (2), PP 55-63

Histological study on the effect of histamine antagonists (ranitidine and promethazine) on the Distribution of collagen fibers in the uterus of the Pregnant rat on days 7 and 10 of pregnancy

Akram Y.Yasear [1] ; Abdel-Raoof A. Khatal [2]; Ismeal A. Feitus [2]

[1] Department of oral Biology, Faculty of Dentistry, Garyounis University
[2] Department of anatomy and Histology, Faculty of Medicine, Garyounis University, Benghazi, Libya.

الملخص

الهدف: معرفةُ تأثير مضادات الهستامين والرانتيدين على انتشار الكولاجين في رحم الجرذ الحامل خلال فترة الغرس في اليوم السابع والعاشر بعد الجماع. **الطريقة والمواد:** 60 أنثى فأر تأكد حملهن قُسِّمنَ إلى مجموعتين علاجيتين (العدد= 48) و حقنت إما بالبروميثازين أوالرانتيدين على التوالي داخل الصفاق ومجموعة تحكيم واحدة شملت اثنا عشر جرذاً حُقِّنتَ بسائل ملحي طبيعي بنفس الطريقة. طُبِّقَ التكنيك النسيجي على مقاطع من أماكن الغرس في اليوم السابع والعاشر، إذ صبغت العينات بـــ هيماتوكسيلين وايوسين وصبغة كومورس. **النتائج:** تمكنت الأكياس الأريمية من الغرس و تكوين الغشاء الساقط في أرحام كل الجرذان. و نتيجة طبيعية فأن ألياف الكولاجين لم تكن موجودة في كل من مناطق الغشاء الساقط الأولي و الثانوي بينما كانت واسعة الانتشار في المنطقة غير المتميزة. في اليوم العاشر كثر وجود الألياف الكولاجينية في منطقة الغشاء الساقط المسراق-رحمية. الألياف الكولاجينية كانت داعماً مهماً للأوعية الدموية في هذه المساحة التي تغذى الفائض المشيمي في الجرذ. بينت هذه الدراسة أن الحقن داخل الصفاق بمضادات الهستامين للمستقبلات هـــ1 و هـــ 2 لم يتعارض مع العملية الطبيعية لغرس الكيسة الأريمية في الجرذان.

Abstract

Objective: To determine the effect of histamine antagonists promethazine (H1 blocker) and ranitidine (H2 blocker) on distribution of collagen fibers in the pregnant rat uterus during the implantation period on days 7 and 10 dpc (day post coitum). **Material & methods**: Sixty female rats of confirmed pregnancy have been divided in to two treated groups (48 rats) received intraperitoneally (i/p) promethazine and ranitidine respectively and one control group (12 rats) received normal saline via the same route. Histological technique was applied for sections taken from implantation sites at days 7 and 10dpc.the sections were stained in hematoxylin and eosin and Gomoriś one step trichrome stains. **Results;** Blastocysts were able to implant successfully and have initiated the implantation reaction and decidualization in the uteri of all rats used. As a normal reaction on day 7 dpc collagen fibers was found to be virtually absent in the decidual tissue of primary decidual zone (PDZ) and secondary decidual zone (SDZ)in contrast to undifferentiated zone(UDZ) in which the fibers was widely distributed. On day 10dpc collagen fibers were abundant in the mesometrial decidual zone (MDZ). Collagen fibers were an important support to the blood vessels in this area which represents a prime route for establishment of nutrient supply through maternal blood vessels supplying the chorioallantoic placenta of rat. **Conclusion:** The results have revealed that i/p injection of histamine antagonists for H1 receptors (promethazine) and H2 receptor (ranitidine) did not interfere with the normal process of implantation of rat blastocyst.

Key Words: Histamine antagonists, implantation, rat, decidualization, collagen fibers

GMJ .Vol 21, No. (2), PP 55-63

Introduction

Histamine is among many factors which have been proposed as mediators for increasing permeability, and are essential for initiation of implantation of blastocysts into the endometrium of various mammals viz: rat[1]; rabbit [2, 3]; mouse[4]; human[5] .

The question which has been repeatedly been asked: is the use of histamine antagonist for varieties of clinical considerations, during pregnancy, associated with an increasing risk for interfering with implantation and subsequent success of pregnancy and a cause of congenital malformation or not?. Histamine antagonist like ranitidine (H 2) blocker and promethazine (H1 blocker) are commonly used for gastrointestinal distress and antiemisis respectively. Many researchers [6; 7] have stated that H2 blockers ranitidine used during the first trimester does not represent a major teratogenic risk. Systemic administration of combination of H1 and H2 receptors antagonist at the time of implantation was associated with retarded fetal development [8].Normally the process of implantation is marked by development of decidual tissue[9,10]. As a consequences to implantation changes in the distribution of fibrillar components of the extracelullar matrix have been noted in morphological studies of the rat uterus during pregnancy [11; 12; 13]

Aniline blue staining suggested the absence of collagen bundles throughout the decidual tissue [11, 12, 14]. On day 6-8 of pregnancy, decidual tissue was found to contain very little collagen when compared with nonimplantation sites [15].The aim of the present work was to assess the nature of changes in the distribution of collagen fibers in the uterus of the pregnant rat treated with histamine antagonist H1 and H 2 blockers ,and compare that with normal pregnancy.

Materials and Methods

Sixty three month old virgin female rats were used in this experiment. They were maintained under light program of LD 12:12 and fed ad libitum. All experimental rats taken were weighed 175-225 gram. The animals were mated and the day on which spermatozoa were found in the vaginal smear was designated day 1 of pregnancy.

The design of the experiment is outlined in table (1).All female rats were divided into two main groups: The G1 group was the rats sacrificed on day 7 of pregnancy (dpc), The G 2 group was the rats sacrificed on day 10 of pregnancy (dpc).

Each group was subdivided into five subgroups in accordance to administration of drug, as in table 1.

Administration of the drugs:

The promethaine (Phenegran®) H1 receptor antagonist was available as 2 ml ampoules, each containing 50 mg of the drug (Theraplix-Rhone-Polulance Rorer, France).

The Ranitidine(Zantac®) H2 receptor antagonist, was available as 2 ml ampoules, each containing 50 mg of the drug (Glaxo operation UK LTD).

Calculation of dose:

The dose for the rat was calculated according to Pagat and Barnas [16] equation:

A-Promethazine(Phenegran®):0.036ml/day was given for 200gm rat as therapeutic dose.

B- Ranitidine (Zantac®): 0.216 ml/day was

given for 200gm rat as therapeutic dose
The calculated dose(therapeutic and double therapeutic dose) of aqueous solution of histamine antagonist drugs were injected intraperitoneally one time daily for six days (G1group) to the animal sacrificed on the morning of day 7 dpc, and for nine days (G2 group) to the animals sacrificed on the morning of day 10 dpc. Normal saline was injected via the same route to the control animals of both groups(see table1). Parenteral rather than oral administration was used to ensure that the entire dosage was received by every animal every time.

Tissue sampling and processing:

Rats from all subgroups in each critical day were anaesthetized with ether, lapromatized. The uteri were then removed and divided into segments containing the implantation sites. These sites were fixed in 10% formal saline for 48 hours. The samples then dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin wax. The blocks were carefully oriented to have the cross sections to be cut from the implantation sites. Five µm thickness serial sections containing the implantation sites only were cut. The sections were deparaffinied and hydrated for hematoxylin and eosin (for general histological picture). For demonstrating the distribution of collagen fibers Gomoriś rapid one step trichrome staining [17] were followed It stain up collagen fibers green.

Results

Day seven dpc (G1 group):

The findings for hematoxylin and eosin stained sections taken from the implantation sites of control and treated rats at this day of pregnancy were showing the same results regarding the arrangement of decidual tissue. Similarly, the Gomoriś one step trichrome stained sections have revealed no significant differences in the distribution of collagen fibers.

The initial site of endometrial stromal cells modification for decidualiation which have been considered as an indication of successful implantation was in the antimesometrial side of endometrium. Subsequently decidualization has proceeded mesometrially. In the sections stained with hematoxylin and eosin four main zones could be identified (Fig. 1):

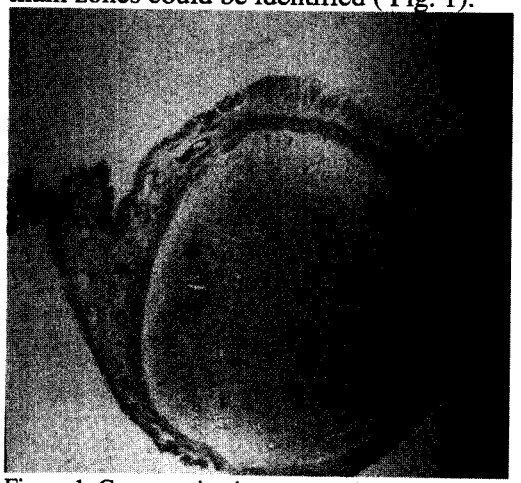

Figure 1 :Cross section in uterus at day 7 dpc from rat treated with double theraputic dose of ranitidine(D.th.H2 subgroup).Note the presence of decidual reaction.PDZ=Primary decidual zone;SDZ=secondary decidual zone;UBZ= undifferentiated basal zone, IZ=implantation zone; Ms=mesometrial side of endometrium; Am=antimesometrial side;My=myometrium. Hematoxylin and eosin stain .X 40.

1- The primary decidual (PDZ): of closely packed decidual cells that surround the blastocyst and luminal epithelium.
2- The secondary decidual zone (SDZ) situated between PDZ and Undifferentiated basal zone (UBZ) and occupying most of the area of endometrium forming a circle around the PDZ.
3- The implantation zone (IZ) was a small

zone located antimesometrial to the embryo where the epithelium was denudated.

4- UBZ: It was a narrow band of tissue extended about ¾ of the way around the circumference of the endometrium separating the decidual of SDZ from the inner circular layer of the myometrium.

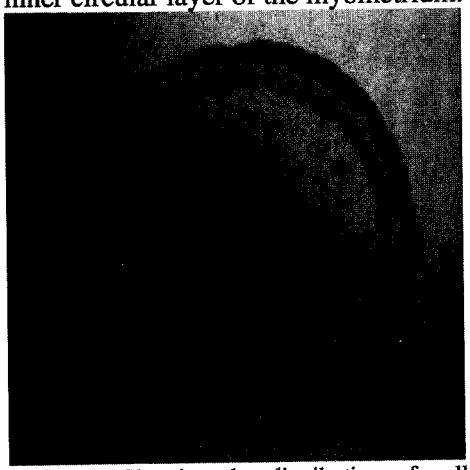

Figure 2: Showing the distribution of collagen fibers in the different zone of decidual tissue. The collagen fibers were absent from PDZ,present in minimal amount in SDZ,but the fibers were densely accumulated in theUBZ; Ms=mesometrial side of endometrium. Day 7 dpc; D.th.H1 subgroup. Gomori one step trichrome stain. X40.

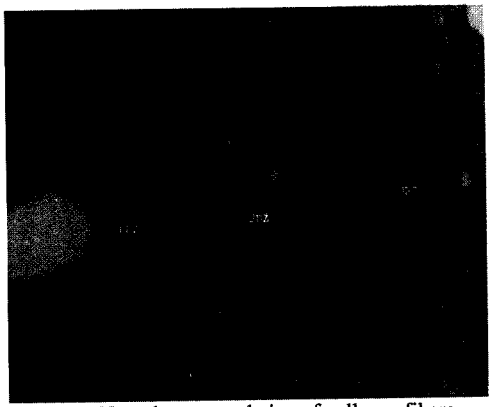

Figure 3 : Note the accumulation of collagen fibers within the UBZ,moderate Amount in SDZ,while no collagen fibers within PDZ. Day 7 dpc. Th.H1 subgroup. One step trichrome stain . X 200.

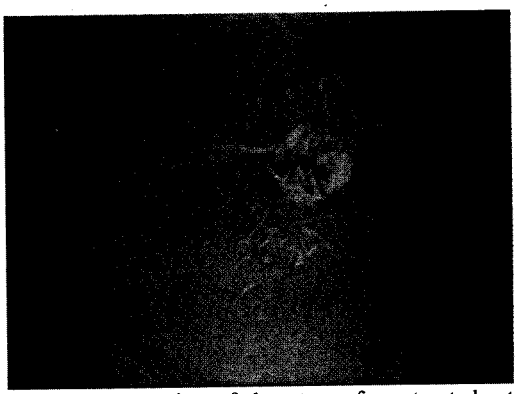

Figure 4: A section of the uterus from treated rat (D.th.H1 subgroup)at day 10 dpc, passing through the implantation site.Note the rotation of the embryo (E) mesometrially.Blood sinusoids(BS) radiatind toward the mesometrial side (Ms) where larger blood vessels there.MDZ=mesometrial decidual zone ; AMZ=Antimesometrial decidual zone;SSZ=spiny shaped decidual zone. Hematoxylin and eosin stain. X 40.

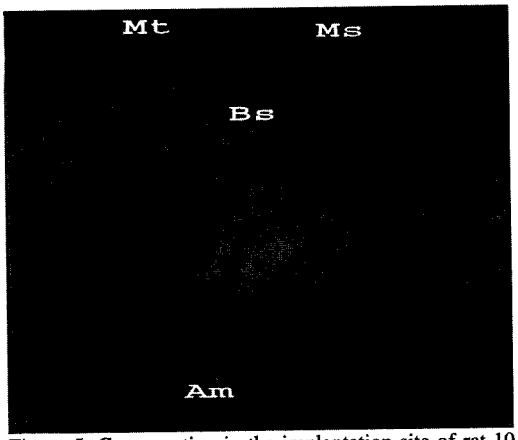

Figure 5: Cross section in the implantation site of rat 10 days pregnant ,from (D,th.H1 subgroup), Stained for collagen fibers(green color).Note the uneven distribution of the fibers.They are either absent or sparse on the antimesometrial side(Am),but widely distributed in between decidual tissue and extensively branched and tortuous blood sinusoids (Bs) on the mesom- metrial side (Ms)of endometrium.The blood sinusoids are continuous with larger blood vessels in the mesometrial triangle(MT) ;E=embryo. One step trichrome stain .X40.

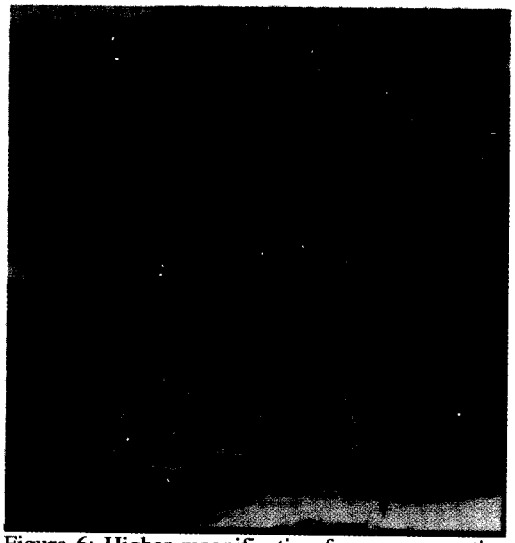

Figure 6: Higher magnification for a cross section taken from same area of figure 5. Note the wide distribution of collagen fibers (green color) in between the blood sinusoids (Bs) and decidual tissue of mesometrial side of the endometrium(Ms). Segment from the of the embryo (E) also shown. One step trichrome stain .X 200.

Table 1: Showing the arrangement of experimental design:

Group	Sub-groups				
	Co	Th. H1	D.th-H1	Th-H2	D.th-H2
G1(7)dpc	6	6	6	6	6
G2(10) dpc n=	6	6	6	6	6

G1 (7) = Groups of female rats that were scarified on day (7) dpc **2 (10)** = Groups of female rats that were scarified on day (10) dpc **n=** Number of rats in each subgroup. **Co** = Control rats injected with normal saline.**Th. H1** = female rats were treated with therapeutic dose of the promethazine (Phenegran) i.e., H1-receptor antagonist. **D.th.H1** = female rats were treated with therapeutic dose the ranitidine (Zantac) i.e., H2 receptor antagonist. **D.Th.H2**=female rats were treated with double therapeutic dose of the ranitidine (Zantac) i.e., H2 receptor antagonist.

The Gomoriś rapid one trichrome stain had revealed the distribution of collagen fibers in the endometrium and the decidual tissue. The embryo was first located in apposition to the antimesometrial luminal epithelium. This area correlates with the region in which decidualization has been initiated, then progressed mesometrially.

In the PDZ the extracelullar matrix was devoid of collagen fibers, mesometrially and antimesometrially (Fig.2). The area with minimal amount of collagen fibers corresponds to the differentiating tissue of SDZ.This zone extends around the mesometrial aspect of the uterine lumen and incorporates an area of marked edema antimesometrially (Fig.3). Dense accumulation of collagen fibers was seen in the stromal tissue of UBZ (Fig. 3).The collagen fibers of this zone were arranged in a form of whorl around the inactive endometrial glands.

Day 10 dpc(G2 group)

The implantation sites at day 10dpc were exhibiting very clear bead-like appearance in which decidualization was in the highest degree of growth and development.

In hematoxylin and eosin stained sections, the endometrium could be divided into five main zones (Fig. 4):

1. Decidual crypt zone (DCZ): Was located at the antimesometrial pole and in direct contact to the embryo. The decidual cells of this zone were closely packed to form the wall of the crypt.
2. Antimesometrial zone (AMZ): Constitute a zone of tightly packed decidual tissue in the antimesometrial side of endometrium. It was extending from the DCZ toward the nondecidulized tissue the undifferentiated basal zone (UBZ).
3. Undifferentiated basal zone (UBZ): A zone of nondecidulized, undifferentiated stromal cells, located between the myometrium and the fibrinoid capsule.

The cells of this zone resembled the fibroblast- like cells of the original endometrium. They have wide extracelullar space.

4. Mesometrial decidual zone (MDZ): The MDZ was occupying a triangular area of Endometrium located between the myometrium and the mesometrial pole of embryo .The decidual cells of this zone were not densely packed as in ADZ. There was large tortuous blood spaces associated with this zone radiating from the mesometrial pole of the embryo toward the mesometrial triangle (Fig. 5).

5. Spiny-shaped decidual cells zone (SSZ): It was located lateral to the MDZ, and characterized by smaller sized cells with long numerous cytoplasmic processes. The extracelullar spaces in this zone were wide

In Gomoriś one step trichrome stain the collagen fibers were unevenly distributed within the different zones of decidual tissue and the endometrium.The collagen fibers were not displayed in the DCZ zone and the AMZ zone (Fig.6). But they were abundantly seen in the mesometrial triangle and the peripheral part of MDZ zone nearest to the myometrium, especially around the blood vessels of this zone(Figs. 5 and 6).Modrate amount of collagen fibers were seen in the SSD zone. High condensation of collagen fibers within the UBZ zone were accumulated in between the undifferentiated stromal cells and the endometrial glands

Discussion

One of the prominent results seen in the control and treated animals of this work was the presence of decidual reaction. Welsh and Enders [18] have pointed out that further expansion and successful development of the conceptus was closely tied to changes in decidua.By comparing the results the results of day 7 and 10 dpc, it was apparent that the endometrium have undergone marked changes reflecting a normal sequence of events seen in normal pregnancy of the rat [3,18]

Among many features of PDZ demonstrated in the present work was the absence of capillaries, tightly packing of their decidual cells, and absence of collagen fibers. In addition to that several kinds of intercellular junctions between cells of this zone have been described viz: tight junctions [3]; gap junctions [19, 20, 21, 22]. Those findings suggested a barrier function of PDZ to the trophoblastic invasion during early stages of implantation [23].

Moreover, the cells of this zone could play a major role in maintaining coherence and structural support to the endometrium during early pregnancy as collagen fibers was absent in this zone.

Present observations regarding the presence of minimal amount of collagen fibers in SDZ was in agreement of [15]. The differing distribution of collagen fibers within PDZ and SDZ may reflect either the degree of decidualization within each area or distinct functional role. The cells of UBZ were not included in the process of decidualization[18]. Thus, by day 7 dpc The UBZ is a wide band of loosely packed tissue. Subsequent growth of the decidual tissue zone at day 10 dpc leads to compaction of the UBZ with regularly arranged bundles of collagen becoming its major constituent.

The present study had demonstrated that one of the demarcation criteria between the decidualiszed tissue and nondecidulized tissue was marked by the distribution of collagen fibers. The PDZ was devoid of collagen fibers, while SDZ contained only minimal amount of collagen fibers. On the other hand, the UBZ was rich in collagen fibers.

The removal of collagen fibers from around the embryo at implantation site on

day 7 dpc was closely related to the progressive steps of decidualization which was necessary for successful implantation and pregnancy. In the present work, no difference was noted between control and treated rats with regards to the distribution of collagen fibers.Tung etal[24]; and Clark,et al,[15]have reported the absence of collagen fibers from decidual tissue and suggested that cellular adhesion between decidual cells allow to form an immunological barrier protecting the embryo from the mother immune response. If such concept was correct the absence of collagen fibers in decidual tissue during implantation may allow for necessary remodeling needed for establishment of decidual tissue. However the presence of minimal amount of collagen fibers in SDZ may be a stimulus for vasculogenesis. It had been shown that collagen fibers were a stimulating factor for vascular tube formation in vitro [25]. The blood vessels have been noticed to be present in SDZ of the present work and previous studies [14, 26]

Collagen fibers are likely to be important for support of placental vessels which will be functioning at day 8 dpc in the rat [26].In this regards sections of day 10 dpc of this study displayed area with abundant amount of collagen fibers in the peripheral parts and around the blood vessels of MDZ nearest to the myometrium and in the mesometrial triangle. These two areas represent the prime route for establishment of nutrient supply via maternal blood vessels to supply the chorioallantoic placenta of the rat [23]. This correlates with many reports [13, 27, 28] that the mesometrial sinusoids radiating out from the mesometrial aspect of implantations chambers act as a venous system. Fainstat [12] had explored the disappearance of collagen fibers in the early stage of pregnancy in rat. He concluded that, these fibers broke up into finer filaments during implantation and suggested that local decidual collagenase action released by growing decidua was responsible. Recently, it has been shown that the process of degradation of collagenous as well as noncollagenous components of extracelullar matrix is involving a class of enzymes called matrix metalloproteinases (MMPs) [29, 30]. The MMPs enzyme has been localized in the cytotrophoblast cells which migrated to the endometrium during early pregnancy in human [31]; and Macaque [32]. The disappearance of collagen fibers from some parts of decidual tissue of the rats in both the control and treated groups raised the question of the involvement of MMPs enzymes released by migrating cytotrophoblast cells in this process. Although the migration of cytotrophoblast cells has been exhibited in the rat [23, 33], MMPs enzymes localization in the migrating cytotrophoblast in this animal has yet to be elucidated.

Our results have revealed that, the intraperitoneally administered antihistaminic drugs ,H1 blocker (promethazine) and H2 blocker (ranitidine), separately have not interfered with implantation, decidualization, and rearrangement of collagen fibers seen on days7 and 10 dpc,which are essential for successful early pregnancy not just in the rat but in all types of hemochorial placentae[23] .In contrast , a significant disruption of implantation reaction has been reported in rabbits following intrauterine administration of H1 blocker(mepyramine)or H2 blocker (burimamide)[2].

The present investigation also raised the question of involvement of other substances, as prostaglandin [34]; epidermal

growth factor [35]; insulin like growth factor [36].

However, the use of histamine antagonists in late pregnancy can cause teratogenic effect [37, 38].

In view of the results of the present work and previous studies, we can advise women in fertile period of life to use antihistamines with caution especially during ovulation. Further studies are required to elucidate the actual effects of long term use of antihistamines.

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GMJ. Vol 21 No (2), PP 64-71

The Effect of Vitamin K on Bone Homeostasis in Experimental Renal Failure

Abdussalam El-Shakmak[1]; Balázs Györffy[1]; Erika Heninger[1]; András Györffy[2]; Helmut Reichel[2]; András Szabó[1].

Semmelweis University, Department of Pediatrics I[1]Research Laboratory for Pediatrics and Nephrology, Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary. [2]Nephrology Center, Villingen-Schwenningen, Germany

الملخص

تدل الدراسات الحديثة على أن فيتامين ك يعزز تمعدن العظام. **الهدف:** تحديد تأثير فيتامين ك الذي حُقِنَ تحت الجلد على استقلاب العظم في مرضى الكلى. **الطريقة والمواد:** تمت دراسة ثلاث مجموعات من الجرذان كل مجموعة بها 10، المجموعة أ : المجموعة سليمة وأُجريت عليها عملية مزيفة. المجموعة ب : تم فيها إجراء استئصال غير كُلي للكلية. مجموعة ج تم فيها إجراء استئصال جزئي للكلية بالإضافة إلى معالجتها بفيتامين ك 1 ملغم/كجم من وزن الجسم مرة واحدة في اليوم لمدة أسبوعين. أُميتَتْ الحيوانات بعد 8 أسابيع من العملية الثانية. **النتائج:** تبين وجود فرط الدُريقات في المجموعتين ب و ج كما تبين زيادة في الإفراز البولي لكل من البريدونيلين وديوكسي بريدونيلين والكالسيوم في المجموعة أ مقارنة بالمجموعة ب مما يدل على زيادة تقلّب العظام بينما لم يكن هناك فرق يذكر في هذه المواد بين المجموعة الأولى و الثالثة مما يشير إلى أن المعالجة بفيتامين ك منع حدوث تغير في تقلب العظم المصاحب عادة للفشل الكلوي. أما محتوى العظم من الكالسيوم و الفوسفور فكان أقل في المجموعة الثانية منه في الأولى بينما لم يحدث نقص في محتوى هذين العنصرين في المجموعة المعالجة بفيتامين ك كما حدثت تغييرات موازية في كثافة العظام. **الاستنتاج:** نتائجنا تدل على أن المعالجة بفيتامين ك قد يعمل على تحوير عمليات الأيض للمعادن في عظام الحيوانات المصابة بمرض العظام الناتج عن الفشل الكلوي التجريبي و ذلك عن طريق إنقاص تقلّب العظام بطريقة تعتبر مجهولة حتى الآن.

Abstract

Recent studies indicated that vitamin K influences bone mineralization. **Objectives:** Our aim was to investigate the effect of exogenous vitamin K on bone metabolism in experimental renal failure. **Materials and Methods:** Three groups of rats (10 per each group) were studied: (Gr. I) sham-operated intact animals (Intact); (Gr.II) sub totally nephrectomized animals (sNX+V); (Gr.III) sub totally nephrectomized animals treated with vitamin K (Konakion MM®) at a dose of 1 mg/kg body weight (bw)/day subcutaneously for 2 weeks (sNX+K). The animals were sacrificed 8 weeks after the second operation. **Results and Discussion:** Hyperparathyroidism was present in sNX + V and sNX + K animals. Urinary pyridinoline, deoxypyridinoline and calcium excretion were elevated in sNX + V as compared to intact documenting increased bone turnover. In contrast, these parameters were comparable in sNX + K and Intact, suggesting that vitamin K treatment prevented the uremia-associated increase in bone turnover. Calcium and phosphorus content of bone were lower in sNX + V as compared to Intact. In sNX + K, vitamin K treatment prevented the decrease of calcium and phosphorus content of bone found in sNX + V. Bone mineral density decreased significantly in sNX + V vs. Intact, but not in sNX + K. **Conclusion:** Our results indicate that vitamin K treatment may modify bone mineral metabolism in experimental hyperparathyroid renal bone disease by attenuating the increase in bone turnover by an as yet unidentified mechanism.

Key words: vitamin K, bone homeostasis, chronic renal failure, renal osteodystrophy, rat.

GMJ. Vol 21 No (2), PP 64-71

Introduction

Vitamin K-dependent gamma carboxylated proteins play a role in several cellular functions. In addition to vitamin K-dependent blood coagulation factors (VII., IX., X.), other gamma-carboxyglutamic acid (Gla) containing proteins include osteocalcin in bone and the matrix Gla protein (MGP) in cartilage [1]. Osteocalcin is a 49 amino acid calcium binding protein found exclusively in bone and dentin. Glutamic acid residues 17, 21, and 24 are carboxylated, residue 17 showing only partial carboxylation [2]. The carboxyl groups of the Gla residues potentially facilitate the binding to hydroxyapatite [3]. Carboxylation of Gla residues occurs via post-translational modification [4], which is Vitamin K dependent, and which is similar to the carboxylation of clotting factors in the liver. Osteocalcin is involved in the recruitment and activation of osteoblasts and osteoclasts. The circulating levels of osteocalcin are regarded as a specific marker of osteoblastic activity [5,6,7,8]. The process of bone resorption is mediated by osteoclasts, which degrade the organic matrix and dissolve the mineral elements resulting in the release of Ca^{2+}, phosphate, enzymes, and a number of matrix degradation products. Pyridinoline (PYR) and deoxypyridinoline (DPD) crosslinks are among the degradation products released into the blood and excreted into the urine. PYR and DPD are specific parameters of bone resorption[7]. Matrix Gla protein (MGP), osteocalcin and other Gla containing proteins may act in combination with other signal peptides to influence bone metabolism [9,10] The presence of Gla containing proteins in bone suggests a possible role for vitamin K in bone metabolism. Bone metabolism and bone structure are affected upon administration of vitamin K in animal experiments and in clinical studies [11, 12, 13, 14]. The aim of our study was to investigate effects of Vitamin K treatment on bone homeostasis. The treatment and prevention of renal osteodystrophy is intesively investigated. One of the open clinical problems is the development of strategies to reduce bone loss associated with the various forms of renal bone disease. We hypothesized that administration of vitamin K in an animal model of renal failure and increased bone turnover might modify the course of renal bone disease.

Materials and Methods

Animals

Thirty male Sprague Dawley rats (Charles River, Germany) were housed in an environment with controlled light (12 h on 12 h off cycles), constant temperature (21°C) and humidity (75%). At the beginning of the experiments, animals were 8-9 weeks old; the mean body weights were 201±13 g. Animal experiments described in this study were undertaken in accord with accepted standards of animal care. The research animals were acquired in compliance with Hungarian laws and institutional regulations. Animals were maintained according to the NIH Guide for the Care and Use of Laboratory Animals (http://www.nap.edu/readingroom/books/labrats/). An independent institutional committee on animal care approved the study.

Experimental protocol

Animals were randomly divided into 3 groups (n=10 in each group): (Gr.I) Intact = sham operation + vehicle; (Gr.II) sNX+V = subtotal nephrectomy + vehicle; (Gr.III) sNX+K = subtotal nephrectomy + vitamin K. On the first day, sNX+V and sNX+K rats underwent a 2/3 right nephrectomy while the Intact group was sham-operated by decapsulation of the kidney. Five days later a total left nephrectomy was performed in

GMJ. Vol 21 No (2), PP 64-71

sNX+V and sNX+K (5/6 two-step subtotal nephrectomy). In Controls, a second sham operation was performed. All surgical procedures were performed under intraperitoneal pentobarbital (Nembutal) anesthesia. Six weeks later, the sNX + K group received vitamin K1 (1mg/kg/day s.c. Konakion MM) for 2 weeks. The sNX+V and the Control groups were treated with vehicle (0.1 ml glycocholic-acid and lecithin mixed micelles in H_2O solution) simultaneously. The animals had free access to water. Animals were pair-fed with standard rat pellets (Ca: 0.8%, P: 1.2%, Altromin 1324, Lage/Lippe, Germany). Body weight was measured weekly. Before the end of the experiment the animals were kept in metabolic cages for 24h urine collection. Eight weeks after the second operation the animals were sacrificed under anesthesia. Plasma, urine, and tibia samples were taken for analysis.

Laboratory measurements

Blood and 24h urine samples were stored at -20°C. Routine plasma and urine chemistry (creatinine, BUN, protein, albumin and alkaline phosphatase, calcium, phosphorus, sodium and potassium) was measured using autoanalyser technique (Hitachi 712 Automatic Analyzer). The serum intact (1-84) PTH was measured using the rat PTH Nichols assay (Nichols Institute Diagnostics, San Clemente, CA, USA) [15]. The serum osteocalcin was measured by enzyme immunoassay Rat osteocalcin EIA KIT (Biomedical Technologies Inc, Stroughton, MA, USA).

Pyridinoline (PYR) and deoxypyridinoline (DPD) were determined in the urine by a reverse phase HPLC after HCl acid hydrolysis at 150°C and CF-1 cellulose fractioning (Crosslink HPLC, BioRad GmbH, München, Germany).

Bone mineral density and content

The left tibia of the animals was removed and stored at -20°C. The tibia size (length and width) was measured and bone mineral density (BMD) was measured by radiodensitometry. The computer software GelPro Analyzer (MediaCybernetics, Leiden, the Netherlands) was used to determine optical density, which was adjusted to bone area determined by using the value of bone length (OD/cm^2). Finally the tibiae were crushed by microwave assisted digestion, and calcium and phosphorus contents were determined by ICP spectrometry (Jobin-Yvon JY24 sequential ICP spectrometer, France).

Statistical analyses

Data are given as mean ± SD. After testing for normality, Kruskal-Wallis test or one-way ANOVA were chosen for analysis of variance, followed by Duncan`s multiple range test to determine whether the differences between the groups were significant. Independent groups t-test or Wilcoxon Rank-Sum (Mann-Whitney U) test were also performed. The results were considered significant when the probability of error (p) was < 0.05.

Results

As shown in Table 1, food consumption, body weight and animal size were comparable in the different groups.

Table 1. Food consumption, body weight and length of animals.

	Intact	sNX + V	sNX + K
n=	10	10	10
food consumed (gr/d)	19.2±5.6	19.3±5.6	19.2±5.5
Animal weight (gr)	390.6±22.11	369.8±74.83	377.9±40.61
Animal length (cm)	47.1±0.6	46.6±2.2	46.2±2.0

Data are given as mean ± SD. Intact: sham-operated control animals; sNX + V: subtotal nephrectomy and vehicle treatment; sNX + K: subtotal nephrectomy and vitamin K treatment. n=10 per group. No significant differences between groups.

Biochemical measurements are summarized in Table 2. There were no differences in mean serum calcium and phosphorus concentrations between the three groups. After subtotal nephrectomy serum creatinine, BUN, iPTH, alkaline phosphatase, osteocalcin levels were significantly higher and serum albumin level was lower in Gr.II (sNX + V) as compared to Gr.I (Intact). Urinary calcium, PYR, and DPD excretion was also significantly higher in Gr.II(sNX + V) as compared to Gr.I(Intact). Mean serum creatinine, BUN and albumin concentrations did not differ between Gr.II (sNX + V) and Gr.III (sNX + K). In contrast, urinary calcium, PYR, and DPD rose significantly less in Gr.III (sNX + K) as compared to Gr.II (sNX + V).

Mean serum alkaline phosphatase, osteocalcin and intact PTH concentrations were also some what lower in Gr.III (sNX + K) as compared to Gr.II (sNX + V), but the differences missed statistical significance. The mean tibia size was comparable between the groups (Table 3). Bone mineral density (BMD) had decreased significantly ($p<0.05$) in Gr.II (sNX + V) vs. Gr.I (Intact). In Gr.III (sNX + K), there was no significant decrease of BMD vs. Gr.I (Intact). Subtotal nephrectomy led to a significant decrease in bone calcium and phosphorus contents in Gr.II (sNX + V) vs. Gr.I (Intact). Bone calcium and phosphorus contents remained unchanged during vitamin K administration to sub totally nephrectomized animals. There was also a significant difference ($p<0.01$) in bone calcium and phosphorus contents between Gr.II (sNX + V) vs. Gr.III (sNX + K) (Table 3).

Table 2. Effects of vitamin K treatment on serum and urinary parameters in experimental renal failure.

Treatment	Intact	sNX + V	sNX + K
Creatinine (µmol/l)	41.22± 6.52	106.18± 21.76*	117.70± 42.09*
BUN (µmol/l)	4.54± 2.07	21.56± 10.03*	24.70± 12.74 *
Calcium (mmol/l)	2.66± 0.08	2.45± 0.36	2.48± 0.28
Phosphorus (mmol/l)	2.87± 0.42	3.64± 1.01	2.50± 1.02
iPTH (ng/ml)	19.8± 11.7	200.2± 182.5*	150.5± 154.3*
albumin (g/l)	25.8± 1.2	23.6± 1.2*	22.9± 1.8*
Alkaline phosphatase (IU/l)	393.6± 101.6	612.6± 252.0*	489.8± 74.2*
Osteocalcin (ng/ml)	27.2± 7.3	80.1± 29.3*	63.0± 14.6*
Urinary Ca/creat.	0.6± .0.2	2.9± 2*	1.7± 1.2 *#
Urinary PYR/creat.	97.6± 32.5	242.5± 123.0*	167,3 ±47.5 *#
Urinary DPD/creat.	73.3± 22.4	210.9± 135.4*	142.7± 42.2 *#

Data are means ± SD. Intact: sham-operated control animals; sNX + V: subtotal nephrectomy and vehicle treatment; sNX + K: subtotal nephrectomy and vitamin K treatment. PYR: pyridinoline, DPD: deoxypyridinoline, BUN: blood urea nitrogen. n=10 per group.

*Significant difference vs. Intact ($p<0.05$)

Significant difference vs. sNX + V ($p<0.05$)

GMJ. Vol 21 No (2), PP 64-71

Table 3. Effects of vitamin K treatment on bone parameters in experimental renal failure.

Treatment	Intact	sNX + V	sNX + K
Tibia length (cm)	4.2±0.2	4.2±0.2	4.2±0.1
BMD (OD/cm^2)	305.4±70.5	250.1±35.9*	274.8±50.0
calcium content (gr/kg)	235.7±11.9	204.9±19.8*	229.8±16.4 #
Phosphorus content (gr/kg)	123.3±5.7	112.0±10.9*	128.2±8.7 #

Data are means ± SD. Intact: sham-operated control animals; sNX + V: subtotal nephrectomy and vehicle treatment; sNX + K: subtotal nephrectomy and vitamin K treatment. BMD=bone mineral density. n= 10 per group
* Significant difference vs. Intact ($p<0.05$).
# Significant different vs. sNX + V ($p<0.01$)

Discussion

Marked hyperparathyroidism developed in our model of experimental renal failure within eight weeks. The elevated parathyroid hormone resulted in a higher bone turnover, as documented by elevated serum ALP and osteocalcin concentrations. This is also supported by the elevated urinary PYD, DPD and calcium excretions. High ALP and osteocalcin levels suggest elevated bone formation, while urinary PYR and DPD suggest elevated bone resorption. The rate of bone formation to resorption determines the changes in bone mass. Since calcium content and BMD were decreased in Gr.II (sNX + V), we suspect that in our animal model bone resorption was more accelerated than bone formation; therefore bone resorption with consecutive bone loss predominated [16]. In our study, we have investigated the effect of vitamin K treatment on urinary and serum markers of bone metabolism, on bone mineral density and on bone mineral content in uremic animals. Most notably, administration of vitamin K attenuated some of the effects of PTH excess on bone. As compared to vehicle-treated uremic rats, vitamin K-treated uremic rats had significantly lower urinary calcium, PYR and DPD excretion. This indicated a possible inhibitory effect of vitamin K on PTH-induced bone resorption. Our findings are corroborated by the results of measurements of bone calcium and phosphorus contents, in that bone calcium and phosphorus were significantly higher in vitamin K-treated uremic rats than in vehicle-treated uremic rats. In human studies, radiodensitometry was able the detect significant differences between patients with mild and severe osteoporosis [17]. In our animal model we demonstrated a significant reduction of BMD in Gr.II (sNX + V) as compared to Gr.I (Intact). We were able to show, that administration of vitamin K prevented this significant reduction vs. Gr.I (Intact) in Gr.III (sNX + K). Direct measurement of calcium and phosphorus contents provide additional information on bone mineral content. Vitamin K had a pronounced effect in that it prevented the decrease of bone calcium and phosphorus content in experimental renal hyperparathyroidism. Our findings were further supported by the smaller urinary calcium excretion in Gr.III (sNX + K) vs. Gr.II (sNX + V). Our findings might be explained by differences in plasma PTH between vitamin K- and vehicle-treated animals. However, mean plasma PTH was comparable between the two groups suggesting an effect of vitamin K, which was independent of circulating PTH. Our results do not support the hypothesis, that vitamin K treatment enhanced osteocalcin production by osteoblasts as osteocalcin levels did not differ between vitamin K treated and untreated animals with chronic renal failure. This suggests that the effect of

vitamin K on bone turnover was not mediated by circulating osteocalcin under our experimental conditions. Other vitamin K-dependent proteins in bone, including MGP, Gas6 and Protein S, could theoretically mediate the effects of vitamin K on bone but data to support this hypothesis are not available. Taken together, we were able to demonstrate a beneficial effect of vitamin K on bone in our experimental model of renal hyperparathyroidism in that administration of vitamin K attenuated the PTH-associated increase in bone turnover. This may be indicative of a shift in bone formation/resorption towards formation, or an inhibition of bone resorption in hyperparathyroid bone disease during vitamin K therapy. As discussed above, our data do not favor the hypothesis of absolute or relative stimulation of bone formation by vitamin K. An interaction of Vitamin K with PTH-induced bone resorption seems more likely but the mechanism is as yet unidentified. Vitamin K could exert direct or indirect effects both on osteoblasts and/or on osteoclasts.. Osteoblasts and osteoclasts interact closely in bone metabolism [18, 19] therefore the inhibitory effect of vitamin K on bone resorption could also be osteoblast-mediated. Furthermore, in vitro studies have also demonstrated effects of vitamin K on osteoclast activity [20]. In renal failure the serum concentration of vitamin K is unchanged [21]. However, it is not clear, whether the need for vitamin K to maintain adequate bone turnover is altered in chronic renal failure. The current recommended daily allowance for Vitamin K [22] is based on the needs for normal coagulation status as assessed by simple clotting time. It does not take into consideration Vitamin K requirements in bone and vessel walls, which are higher than those in the liver [23].A number of studies have demonstrated the involvement of vitamin K in physiological and pathophysiologiacal bone metabolism. Vitamin K enhanced osteoblast function [24], and effects of vitamin K on bone homeostasis have been shown in several animal experiments. Vitamin K prevented bone loss in rats subjected to simulated weightlessness[12]. Menatetorenone (vitamin K2)-treatment prevented the reduction of BMD in growing rats with phenytoin induced osteopenia [13]. In another experiment, warfarin-induced vitamin K insufficiency elevated the level of circulating undercarboxylated osteocalcin in growing female rats [25]. Osteocalcin accumulates in the extracellular matrix of human osteoblasts grown in culture in the presence of vitamin K [24]. Evidence from observational studies and first intervention clinical trials indicated that vitamin K supplements, higher than the current recommendations, improved bone density and other biochemical markers of bone formation [26]. Elderly osteoporotic patients with femoral neck fractures had lower vitamin K levels as compared to osteoporotic patients without fractures [11]. Menatetrenone increased the BMD and reduced osteoporotic vertebral fractures in postmenopausal women with osteoporosis [27]. A correlation between carboxylation of osteocalcin and bone quality was also described in healthy prepubertal children [28]. Clinical data on the effect of vitamin K on bone metabolism of uremic patients are sparse. One study, which was reported as an abstract several years ago, showed a favorable effect of high dose vitamin K supplementation on bone mineral density in hemodialysis patients [29]. In a recent study in 68 hemodialysis patients, a low vitamin K concentration was associated with an increased fracture risk and a higher

prevalence of hyperparathyroidism [30]. Our study under experimental conditions would support a beneficial effect of vitamin K under clinical conditions in patients with end-stage renal disease. Formal proof of this hypothesis can only be obtained by a controlled clinical study.

Conclusion

We have found, that Vitamin K treatment has a beneficial effect on bone remodeling in experimental renal hyperparathyroidism in that vitamin K-treatment resulted in less marked bone resorption. The exact mechanism of vitamin K action on bone is still unclear. The effects of vitamin K do not appear to be mediated through the actions of osteocalcin in this experiment. Further investigation of the mechanism of action of vitamin K on bone metabolism with possible involvement of gamma-carboxylated protein other than osteocalcin is needed.

Correspondence

Abdussalam El-Shakmak. El Fateh Childrens' Hospital.Benghazi, Libya. E-mail: amfnn@hotmail.com.

Phone: +218612226971. Fax: +218612226957

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GMJ. Vol 21 No (2), PP72-76

Body temperature changes during Spinal anaesthesia

Masoud Ali Lfeituri; Fadwa Saleh El Tarhoni.

Department of Surgery, Unit of Anaesthesia and Intensive Care, Faculty of Medicine, Garyounis University, Benghazi, Libya.

الملخص

في مجموعتين من المرضى، شملت هذه الدراسة التغيرات التي تحدث في درجات حرارة الجسم أثناء تخدير تحت الأم العنكبوتية (المجموعة الأولى، 20 مريضاً) ومقارنتها بما يحدث أثناء التخدير العام (المجموعة الثانية، 20 مريضاً). أُخِذَ قياس درجات الحرارة من طبلة الأذن والإبط وذلك قبل التخدير وبعده مباشرة ثم كل 20 دقيقة خلال أول ساعتين من الجراحة. درجات الحرارة في المجموعتين تناقصت تدريجا بصورة متشابهة و لم يكن بينهما أي فارق إحصائي مهم. ضمن كل مجموعة من المرضى كانت درجات حرارة الإبط أقل منها في طبلة الأذن وذلك في جميع الفترات الزمنية المقاسة أثناء التخدير.متوسط الفروق بين قياسات درجات حرارة الإبط وطبلة الأذن كان (المتوسط ± الانحراف المعياري) 0.35 ± 0.05 و 0.29 ± 0.04 درجة مئوية في المجموعتين الأولى والثانية على التوالي، بينما تراوح معامل الارتباط على التوالي ما بين 0.50 إلى 0.85 و 0.58 إلى 0.91 .

Abstract

In this study we compared the changes in body temperature during spinal anaesthesia (SA, 20 patients) with that occurring under general anaesthesia (GA, 20 patients). Temperature recordings were obtained from tympanic membrane (TM) and axilla, and were collected preoperatively, immediately after induction of anesthesia and then at twenty-min intervals for the first two hours of surgery. Body temperatures at the two measurement sites decreased gradually throughout the studied period in a similar pattern in both groups of patients. Comparing the corresponding inter-group measurement sites, neither tympanic nor axillary temperatures differed significantly at any time period. Within each of the groups, axillary measurements were significantly lower than the tympanic measurements at all intervals. The mean differences between the two measurement sites were 0.35 °C ± 0.05 ($P<0.01$) and 0.29 °C ± 0.04 ($P<0.01$) in the SA and GA groups respectively, and the correlation coefficients respectively ranged from C.50-0.85 and from 0.58-0.91).

Key words: monitoring- tympanic- axillary temperature- spinal anesthesia.

Introduction

Despite evidence that preoperative hypothermia occurs commonly, monitoring of body temperature often is ignored, especially during regional anaesthesia (RA).[1,5] Main reasons of ignoring temperature monitoring during neuraxial anaesthesia are the- absence of risk for malignant hyperthermia and the lack of a convenient and reliable site for temperature-probe insertion. Inadequate monitoring during anaesthesia may also be related to the anaesthesiologist who restricts temperature monitoring to patients with an expected risk of hypothermia. This implies that he can predict patient thermal status without monitoring core temperature.[2, 3] It is well known that under neuraxial anaesthesia there is an apparent warm input from the portion of the body below the level of neural blockade making patients unlikely to complain of cold sensation even if they are awake.[3] Therefore, intraoperative hypothermia often is neither recognized nor treated [2,7].

Depending on the type and depth of general anaesthesia (GA) and the level of the block during RA, regulation of body temperature

is significantly impaired by interaction of three main factors: first, decreased metabolic heat production, second, reduced compensatory responses such as vasoconstriction and shivering, and third, increased heat loss to the environment from cutaneous vasodilatation, surgical exposure, inhaled dry respiratory gases, and cold transfused blood and intravenous (IV) fluids[5, 8]

Although it saves the upper parts of the body, RA can induce core hypothermia that may be as severe as during GA.[2,8,9] RA below the level of block induces sympathetic, sensory and motor blockade resulting in direct peripheral vasodilatation and increased cutaneous blood flow (core to peripheral heat redistribution), decreased thermal receptors input and abolished shivering.[8,10] As any substantial heat deficit will be compensated by the body to regain normothermia, the resulting fall in body temperature may be detrimental when patients, especially the elderly and those with limited cardiopulmonary reserves, reestablish their thermoregulatory mechanisms postoperatively, at which the threat of cardiac arrhythmia and ischaemia supervene.[5,10] The aim of this study was to compare the effects of spinal anaesthesia (SA) and GA on body temperature during the first two hours of surgery. At the same time, we evaluated the relationship between axillary and tympanic membrane (TM) temperature measurements.

Patients and Methods

Forty adult patients of two groups scheduled for surgical procedures involving the lower part of the body were enrolled in a study comparing body temperature changes during the first two hours of surgery under either SA (20 patients) or GA (20 patients). Body temperature was measured at the TM, using an infrared thermometer (ThermoScan Inc. San Diego, California), and at the axilla, using a 400 series thermal probe (Yellow Springs Instruments, Ohio). TM temperature was recorded simultaneously from both ears to detect the maximum tympanic temperature that was used to indicate the true TM temperature. The axillary probe sensor was placed high in the axilla at the side that was not used for administration of IV fluids or blood, and the arm secured in an adduction position thereafter. Temperature recordings were obtained preoperatively (P), immediately after induction of anaesthesia (A) and then at twenty-min intervals. Ambient temperatures were measured from probes positioned near the patients. Non-invasive blood pressure and heart rate were recorded at the same intervals. Demographic data obtained in each patient included name, age, sex, body weight, height, and ASA physical status. Subjects had no history of diabetes, fever, thyroid disease or problems with the TM or middle ear.

All patients received approximately 10 ml/kg of IV isotonic saline or Ringer's lactate solution just before induction anaesthesia. GA was induced with IV fentanyl (100 microgram), 2.5% thiopentone sodium (4-5mg/kg) and atracurium (0.5 mg/kg). Throughout the operation 66% N_2O in oxygen and 0.5-1 % of halothane were used.

A set for combined spinal-epidural anaesthesia (BD adjustable durasafe-plus, Madrid, Spain) was used to start SA (G27 spinal needle, G18 epidural needle and G19 epidural catheter). The epidural needle was inserted at the level of L3 / L4 and a test dose of 40-50 mg of 1 % lidocaine was given. After few minutes, the spinal needle then inserted through the epidural needle and 15mg of 0.5 % bupivacaine hydrochloride was injected intrathecally to maintain a sensory block level at T8-T10. An epidural catheter was left in the epidural space for postoperative analgesia. Repeated small doses (2-4 mg) of IV midazolam were used for sedation during SA. No body heat-conserving measures were performed before or during anaesthesia and surgery.

The unpaired Student's t-test was applied for assessment of significant inter-group differences. Within-group differences were determined using ANOVA test with Dunnett's multiple comparison test or paired Student's t-test when suitable. Results are expressed as means ± SD, and a statistical significance is considered when P< 0.05.

Results

Patient's clinical characteristics and perioperative data obtained were comparable in both groups (table-1). After induction of anaesthesia, body temperatures in both groups decreased gradually and followed a similar pattern (figure-1). Comparing the corresponding inter-group measurement sites, neither tympanic nor axillary temperatures were differed significantly at any time period. Within-group differences and coefficients of correlation between TM and axillary are shown in figure-2; axillary measurements within each group were significantly lower than the simultaneously recorded tympanic temperatures at all intervals.

The mean differences between the two measurement sites were 0.35 °C ± 0.05 (P<0.01) and 0.29 °C ± 0.04 (P<0.01) in the SA and GA groups respectively, and the correlation coefficients respectively ranged from 0.50-0.85 and 0.58-0.91.

Table 1: Perioperative data and clinical characteristics of spinal (SA) and general (GA) anaethesia groups. Values are represented as totoal numbers or mean ± (SD).

	SA	GA	*P* value
Number of patients	20	20	
ASA (I / II / III)	5 / 9 / 6	7 / 10 / 3	
Sex (M / F)	11 / 9	12 / 8	
Age (years)	52.0 ± (12.3)	53.3 ± (16.6)	0.780
Weight (kg)	74.3 ± (11.8)	73.4 ± (18.5)	0.855
Height (cm)	165 ± (6.55)	167 ± (6.35)	0.383
Room temperature (°C)	21.6 ± (0.34)	21.9 (0.8)	0.154
IV crystalloid fluids (ml)	1660 ± (403)	1440 ± (475)	0.118
Blood transfusion (ml)	303 ± (32.1)	402 ± (134)	0.269

Mean arterial pressure and heart rate did not differ significantly between the groups. No complications related to the study or surgeries were identified.

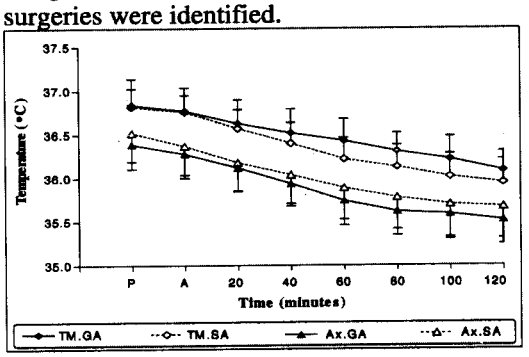

Figure 1: Mean tympanic (TM) and axillary (AX) temperatures in general (GA) and spinal (SA) anaesthesia groups during the first 2 hours of surgery. (P) is pre-anaestic control and (A) is immediately after induction of anaesthesia. Vertical bars are the SD. Changes in body temperature were similar during general and spinal anaesthesia, and the inter-group differences between the corresponding measurement sites were not statistically significant at any time period.

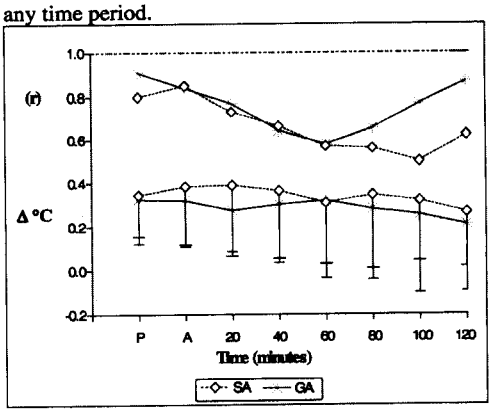

Figure 2: Within-group differences (Δ°C) and coefficient of correlation (r) between tympanic and axillary temperature measurements in spinal (SA) and general (GA) anaesthesia groups of patients during the first 2 hours of surgery. (P) is pre-anaesthetic control and (A) is immediately after induction of anaesthesia. Vertical bars are the SD. Although axillary measurements within each group followed the changes of tympanic measurements, they were significantly lower than the tympanic temperatures at all period intervals recorded stimulatneously.

Discussion

In both groups of patients, and shortly after induction of anaesthesia core temperature decreased in a similar fashion. The post-induction drop in body temperature was most probably due to body-heat redistribution from the central compartments to the periphery. The next continuous temperature drop may be explained by the fact that heat loss to the environment was exceeded body heat production.[8,10, 11] Our results showed no significant difference between SA and GA regarding intraoperative heat loss. This finding is in accordance with the results of some previous studies.[11-13] In the SA group of patients, approximately two thirds of the entire skin surface area were blocked and remained vasodilated throughout the surgery, body heat is therefore distributed over a larger mass and dissipated at the expense of core temperature. This may explain the similarity of effects of GA induced by attenuation of the central thermoregulatory mechanisms, and that of SA produced mainly by direct sympathetic block.[8] However, other investigators have found a greater heat loss with RA,[14] whereas others have shown a greater heat loss with GA.[15] Since the anaesthetic technique is not the only detrimental predictor of intraoperative heat loss, and many other factors, such as patient's age and physical status (ASA), duration of surgery, ambient room temperature, and level of the dermatomal block, can interact and influence body temperature, any discrepancy of the aforementioned results might be accepted. [1, 9, 11] The correlation between high-level spinal blockade and low core body temperature during SA is consistent with the known physiologic effects of RA.[9, 10] Both vasomotor tone and shivering are inhibited below the level of spinal block, so the greater the proportion of the body that is blocked, the greater the level of thermoregulatory dysfunction that can be expected. Frank et al.[9] defined a high dermatomal level of block and increasing age as the best predictors of hypothermia during SA. He demonstrated that for each additional increase in dermatomal block level, core temperature decreased by 0.15°C, and for each year of increasing age, core temperature decreased by 0.3°C. In the SA group of our study the level of the block was between T8-T10 which was not high, and the age factor was comparable between the two groups.

For tympanic thermometry, we preferred an infrared thermometer because it is more tolerable by conscious patients, safe, precise and accurate.[13,16,17] The use of the maximum tympanic temperature in our study was reasonable since falsely low readings by misdirection of the probe tip toward the wall of the auditory canal could be detected. Although axillary temperatures were significantly lower than the tympanic measurements, both preoperatively and intraoperatively, they satisfactorily followed the trend of changes of tympanic core temperatures (Fig.1). This is could be due to the fact that axilla is little far from the core compartments and lies in an intermediate zone between the core and periphery, and hence axillary recordings were neither accurate nor precise in detecting the exact core temperature.

Conclusion

Patients undergoing spinal anaesthesia are predisposed to the same risk of developing intraoperative hypothermia as during general anaesthesia. Axillary temperature measurement sites are not sufficiently accurate, but they might be used perioperatively to indicate the trend of changes in core temperature. Body temperature should be monitored properly during general as well as during spinal anaesthesia.

Correspondence
Masoud Ali Lfeituri: P.O.Box:2856 Benghazi-Libya.
Tel: 061-2227963,84340. Tel-Fax:061-2230207.
e-mail: melfeituri@yahoo.com

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Occurrence of significant fractures during one Year in Al-jala Hospital Benghazi- Libya

Sadek Makhlouf; Mohamed S.Ambarek; Salem El Mahdawi; Mustafa Elfadli; Idris Eltajori; Mabrok Bohalfaya

Orthopedic department, Al-Jala hospital, Benghazi, Libya

الملخص

الخلفية: من المعروف لدينا أن معالجة الكسور تستهلك الكثير من إمكانيات قطاع الصحة ولهذا فإن معرفة معدل الكسور وأنواعها السائدة محلياً قد يساعد في توجيه الخدمات الصحية وتوفير الإمكانيات. **الهدف:** تحديد حجم المشكلة و أهم أنواع الكسور محلياً. المكان: مستشفي الجلاء بنغازي في الفترة من يناير 2000- ديسمبر 2000. **الطريقة والمواد:** دراسة رجعية لوصف الأعراض السريرية للمرضى الزلاء بالقسم والبيانات التي حُلِّلَتْ هي التي جمعت من ملفات المرضى. 4380 نزيلاً (مريض) أُدخلوا لمستشفي الجلاء بقسم العظام خلال فترة الدراسة منهم 1193 مريضا (27.23%) يعانوا من كسور يعتد بها. **النتائج:** نسبة الذكور إلى الإناث 2.7: 1. إصابات الجهة اليمنى (58.7%) المكان الأكثر شيوعاً في الكسور المنطقة السفلى، الفخذ، الأيدي، الذراع والفترة التي يلاحظ فيها زيادة عدد الكسور هي الربيع والصيف. العلاج الجراحي . الأطراف العليا كانت متضمنة في 52% من المرضى، حوادث السير والسقوط كانت هي الأسباب الأكثر شيوعاً. أكثر الأماكن إصابةً كانت كالآتي (مرتبة تنازلياً): عظم الفخذ، الأيدي، كلا عظمى الذراع. أغلب الكسور كانت في موسمي الصيف والربيع. تم اللجوء إلى الجراحة في (3.17%) من كسور الفخذ،7% من كسور الذراع، 6.2% من كسور فوق اللقمة في الأطفال و (5.53%) كسور كلا عظمى الساق. بلغت نسبة الكسور في الأطراف العليا 52% . **الاستنتاج:** بعكس التقارير السابقة فإننا وجدنا أن كل أنواع الكسور كانت أكثر حدوثاً في الذكور باستثناء الكسور ما بين المدورين. ما عدا ذلك، فأن أنواع الكسور وتوزيعها كان مشابهاً في دراستنا للدراسات الأخرى.

Abstract

Background: It is felt that fracture management poses a major burden on health services. Knowing the frequency, type and fracture combinations prevalent locally might help in determining future health and training requirements. **Objectives:** to determine the magnitude of the problem as well as the frequency of significant fractures in our regional trauma centre. **Setting:** Al-Jala Hospital, Benghazi, Libya over one year period (Jan. 2000-Dec.2000). **Materials and methods:** a retrospective descriptive study of some clinicoepidemiologic features of inpatients. Only data of those with significant fractures were analysed. **Results:** 4380 patients were admitted to Al-Jalla hospital orthopedic department during the study period. Out of whom 1193 patients (27.23%) suffered from significant fractures. Males outnumbered females by a ratio of 2.7: 1. The right side was involved in 58.7 % of the times. The most common fracture sites were in descending order: shaft of femur, the hands and both forearm bones. The fractures were most commonly seen in Spring and Summer. Surgical treatment was resorted to in the following frequencies: shaft of femur (17.3%), both forearm bones (7.04%), suprachondylar fracture of the humerus in children (6.20%) and fracture of both leg bones (5.53%). The upper extremity was involved in 52% of patients. Road traffic accidents and fall were the most common underlying causes. Conclusions: Unlike other reports, we found that all fractures types were common in males except the intertrochanteric fracture. Otherwise , the fracture pattern in our study was similar to other reports.

Key words: fractures, trauma, Benghazi, Libya

Introduction

Symptomatic fractures represent a significant problem in terms of morbidity and financial cost. Markd variation in both total and site specific fracture incidence has been documented internationally [1-4].

Several authors has discussed the etiology and influence of age, gender and seasonal variation on the incidence of various type of fracture [4-6]

It has always been an impression of a high incidence of significant fractures that necessitate admission to our busy hospital. Al-Jala Hospital is the main trauma centre in the Eastern part of Libya. This impression was never translated into actual figures. We felt that it might be helpful to

GMJ. Vol 21, No(2), PP77-82

analyse the local experience on significant fractures hoping to shed a light on any peculiar features pertaining to the region. It is known that the level of adherence to regulations and safety measures varies between countries and even within the same country. We felt it is necessary to publish this data to enlighten the figures of the exact magnitude of the problem, to compare it with the international figures and to recommend preventive measure for reduction of fracture incidence, and to help health policy makers as well as researchers in this field.

Subject and Method

We reviewed retrospectively the records of the Operating Theatre's list and the record of Admission office statistical department in AL-Jalla Hospital. 4380 patients were registered during the year 2000. Patients who sustained undisplaced fracture (simple fracture), lacerated wound and cut tendon as well as those with polytrauma were excluded. The remaining 1193 had a significant fracture. We considered a fracture as significant if it required an operative intervention either by closed or open reduction. The records of these patients were reviewed to assess the age, gender, fracture number, site and mode of treatment as well as the seasonal variation of fractures. Results are expressed as numbers, averages and percentages.

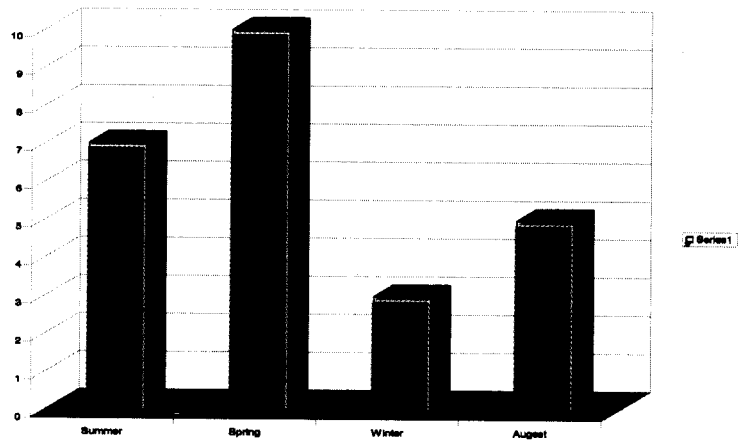
Results

Of the 1193 patients, 873 (73.2%) were males with a male to female ratio of 2.7:1. Fractures were more common on the right side (n=700, 58.7%). The average ages of fractures of shaft of femur, hand , both forearm bones, neck of femur, and intertrochanteric fracture were 27, 26, 25, 60, 69 years respectively (see table). Spring and summer were the seasons with highest fracture frequency (figure 1).

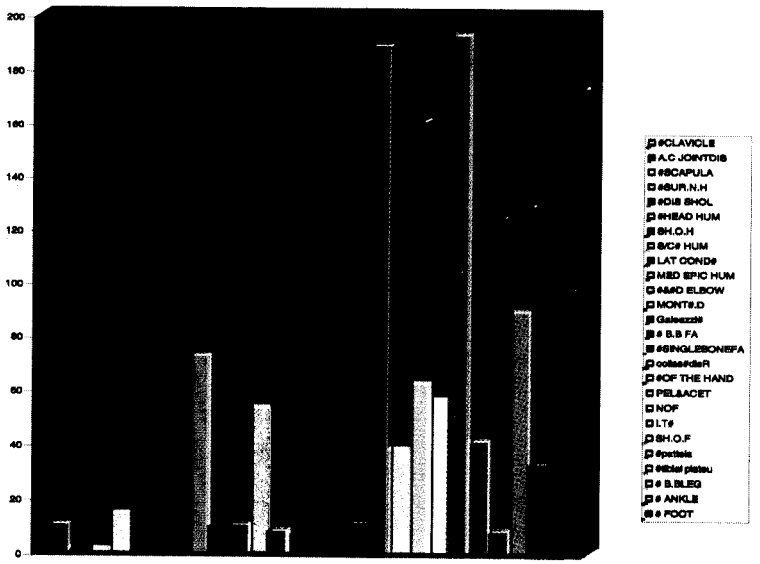
In 620 patients (52%), the upper extremity was afflicted compared to 573 (48%) involvement of the lower extremity.

The most common fracture sites were shaft of femur and hand followed by fractures of both bones of the leg and both forearm bones and shaft of humerus (figure 2). Only one case of fracture head of the humerus is recorded. Higher surgical treatment rate was seen in fracture shaft of femur (17.3%), fracture both forearm bones (7.04%), suprachondylar fracture of the humerus (6.2%) and fracture of the both leg bones (5.53%) (fgure 2). All patients with supracondylar fractures were children.

GMJ. Vol 21, No(2), PP77-82



Seasonal variation.Fig(1)



Site of the fracture. Fig (2)

GMJ. Vol 21, No(2), PP77-82

Table: Distribution and properties of the fractures.

Fracture site	Number of fracture	Male	Female	Average age	Peak season	Number treated surgically
Fracture clavicle	12	11	1	28	summer	4
A.C joint dislocation	3	3	0	24	Autumn	3
Fracture scapula	3	3	0	43	Spring	0
Fracture surgical neck humerus	16	11	5	20	Autumn	4
Fracture dislocation shoulder	20	13	7	45	Spring	2
Fracture head humerus	1	1	0	39	Spring	1
Fracture shaft of humerus	80	61	19	23	Summer	26
Suprachondylar fracture of humerus	74	38	36	8	Summer	74
Fracture of the lateral condyle humerus	12	9	3	9	Spring	12
Fracture of the medial epicondyle of the humerus	12	9	3	13	Spring	10
Fracture & fracture dislocation of the elbow	55	42	13	26	Summer	45
Monttaggia fracture dislocation	10	8	2	Data not available	Spring	10
Galeazzi fracture dislocation	9	7	2	36	Summer	9
Fracture both bones forearm	90	75	15	25	Autumn	84
Fracture single bones forearm	17	15	2	Data not available	Winter	17
Colles fracture	13	12	1	Data not available	Summer	13
Fracture of the hand	193	160	33	26	Winter	56
Fracture of the pelvis and acetabulum	40	18	22	29	Autumn	8
Fracture of neck of femure	64	36	28	60	Autumn	64
Interochanteric fracture	58	27	31	69	Spring& summer	58
Fracture shaft of femure	195	162	33	27	Spring	164
Fracture Pattella	43	31	12	39	Spring	37
Fracture Tibial plateu	10	8	2	40	Spring	10
Fracture both bones legs	91	66	25	28	Winter	66
Fracture ankle	35	17	18	33	Spring	35
Fracture Foot	39	31	8	24	Spring	16
Total	1193	873	320			952

Discussion

Sahlin[1] reported on 3060 fractures treated in their regional trauma center during one year and in his study forearm fracture was the most common fracture and fractures of the upper end of the femur occurred in 53%. In our study the most common fracture site was the shaft of femur. Donaldson et al [3] studied the incidence of fractures in a geographically defined population to describe the population based age and sex, specific incidence of fracture at different site in large English health district.

In their study and under the age of 55 years all fracture showed a higher incidence amongst males with a consistent fall in the male: female ratio in those over 55 years with some sites showing a striking female preponderance.

In our study all type of fractures were common in males except the intertrochanteric fracture which is more common in females. This pattern could be explained by the sociocultural differences between males and females. We reviewed the records of 1193 consecutive patients of different ages to demonstrate the age pattern of each fracture type and its relation to gender and side.

The male to female ratio in our study was (2.7:1), whereas Coole et al [4] demonstrated that the corresponding fracture incidence in males and females were 1248 and 1916 per 100000 person-year respectively.

Worlock and Stower[5] from Nottingham, UK encountered the fracture as more common during Summer in their study of childhood fractures. Reed[6] had similar result in his evaluation of childhood extremity fracture and dislocation.
In our study group, the fracture frequency in children was highest in Summer followed by spring.

In our study hip fracture showed similar pattern in both gender, being uncommon in young with an exponential increase after the age of 60 years,a finding similar to that of other observers[7]. There are three interacting factors: bone strength, the risk of falling, and the efficiency of neuromuscular responses which protect the skeleton .In the age group 50 to 74 years, Cooper et al[8] found that reduced bone mass was a strong independent risk factor for hip fracture, but over 75 years, osteoporosis may be less important than impairment of protective neuromuscular responses. We found that diaphyseal fractures of the femur, tibia, forearm and humerus had an average age in the range of 25 to 28 years. It has been reported that the incidence of forearm fracture[9] and humeral shaft fracture [10] did not increase with age, but an association between femoral shaft fracture and increasing age has been described [11].

Fracture both forearm bones and supracondylar fracture were the commonest fractures in children which is similar to the report of Aktas et al[12]. Surgical treatment rate in fracture shaft of femur (17.3%) was higher than any other fracture followed by fracture both forearm bones (8.9%) and fracture of both leg bones (7%). In children the highest surgical rate was seen in supracondylar fracture of the humerus. In our study road traffic accidents were responsible in 67%, fall in 25% and violence in 8% of the cases which is similar to other reports [13,14].

Conclusion

Our study revealed the pattern of fracture in a large catchment area of the eastern part of the country. This data could be used as a base for future studies related to the mechanism of injury, prevention as well as directing the available resources towards the needed services.
This will be helpful in planning training programs of emergency and orthopedic surgery as the most frequent fracture should take priority in training and should be taught more comprehensively. we found that all fractures types were common in males except the intertrochanteric fracture. Otherwise , the fracture pattern in our study was similar to other reports.

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GMJ. Vol 21 No (2), PP83-87

Detection of Bacteria in the Neonatal Intensive Care Units

Elyousfi, Masaoud A. ; Ramadan Abdoalbast ; Tbeny Nabil; Alalwany Narges and Almagerhy Reem.

Health Inspection Office-Tripoli Medical Center (TMC)

الملخص

الخلفية: العدوى البكتيرية في حديثي الولادة مشكلة أساسية بين الخدّج خاصة و أولئك المحتاجين للعناية الخاصة بسبب نقص الدفاعات ضد العدوى (سواء كانت منذ الولادة أو داخل المستشفيات). تتميز كثير من المُمرضات البكتيرية المكتسبة داخل المستشفيات بتعدد مقاومتها للمضادات الحيوية. **الهدف:** 1. عزل البكتيريا وتعريفها ومعرفة مصدرها في غرفة العناية الفائقة بقسم حديثي الولادة بمركز طرابلس الطبي 2. تقييم مقاومتها للمضادات 3. معرفة سبب زيادة معدل الوفيات بين حديثي الولادة. الدراسة تمت في الفترة من 1998 إلى 2003. **الطريقة والمواد:** أخذت العديد من العينات المزدوجة من 3 غرف عناية من قسم حديثي الولادة ودُرست باستخدام الوسائل المعملية المعتادة. **النتائج:** عُزِل 20 نوعاً من البكتيريا غالبيتها هوائية سالبة الجرام وبكتيريا لا هوائية ت من إجمالي 145 عينة خلال 11 زيارة إلى 3 أقسام عناية لحديثي الولادة. حُدِّدت جميع أنواع البكتيريا عملياً واغلب العينات الملوثة هي التي عُزِلَتْ من الحضّانات أو الماء المستخدم في الحضانات. أغلب البكتيريا التي عزلت كانت مقاومة لأغلب اختبارات المضادات.**الاستنتاج:** معظم البكتيريا تم تحديدها ومعظمها كانت مقاومة لغالبية مضادات الميكروبات كما بينت سجلات المستشفى ارتفاع معدل الوفيات بين حديثي الولادة للأسباب التالية: 1. مضاعفات الخدج (السبب الرئيسي للوفاة) 2. الاختناق. 3. التهابات الدم 4. الاختناق أثناء الولادة 5. التشوهات الخلقية 6. العدوى.

Abstract

Background: Bacterial infection in newborns is mainly a problem in premature babies, and those receiving intensive care due to reduced defenses against infection. Such an infection, may be acquired nosocomial or congenital. Many or most nosocomial bacterial pathogens in the hospital environment are resistant to several antimicrobial agents. **Objective:** This study was performed to: 1- isolate and identify bacterial isolates, and their sources in Neonatal Intensive Care Unit (NICU) at Tripoli Medical Center (TMC), Tripoli-Libya. 2- Asses their susceptibility to different antibiotics. 3-investigate the reasons for high mortality rate among newborns. The present study was conducted between 1998 and 2003 during several interval visits to the (NICU) at (TMC). **Materials and Methods:** Several duplicate samples were taken from 3 (NICU) within the (TMC) and examined using standard bacteriological procedures. **Results:** 20 bacterial isolates mostly gram negative aerobic, and facultative anaerobic bacteria were isolated from a total of 145 samples during 11 visits to 3 (NICU) within the (TMC), and identified, and all were considered to be clinically significant as a cause of serious nosocomial infections in this high risk group of patients. The most contaminated samples were those taken from the incubators and water used with the incubators. Most bacterial isolates were resistant to most antibiotics tested. **Conclusion:** several bacterial isolates were identified most of which were resistant to most antimicrobial drugs tested. Hospital records show high mortality rate within the (NICU) in (TMC) due to: 1-complication of pre-maturity (the leading cause of death among newborns in (NICU) 2-sepsis. 3-septicemia. 4-birth asphyxia. -5congenital anomalies. 6- Infections.

Key Words: Neonatal intensive care units, newborns, newborns incubators, nosocomial infection, mortality, antibiotics, antibiotics resistance, gram negative bacteria, S. aureus, S. epidermidis.

GMJ. Vol 21 No (2), PP83-87

Introduction

Infants in special –care nurseries often are sick, preterm, and underweight. They need many supportive invasive procedures and frequently receive antimicrobial therapy. The aim of neonatal intensive care is to provide life support and vital-signs monitoring in a controlled, stable environment, with minimal upset to the ill infant [1]. Nosocomial infection is an important cause of morbidity, and mortality in hospitalized newborns, and children. Bacterial infection in newborns is mainly a problem in premature babies especially male babies, and those receiving intensive care due to reduced defenses against infection. Such an infection may be acquired nosocomial , or congenital. [2, 3] Increased risk of the infections acquired during hospitalization is associated with prolonged hospitalization, intensive care, the use of invasive, prosthetic devices (such as intravenous cathers, urinary catheters, endotracheal tubes), and an immunocompromised host [4]. Among the most frequent pathogens of nosocomial infections are: 1- The enterococci (particularly in intensive care units). 2- Acientobacter species.3- Pseudomonas aeruginosa. 4- E. coli. 5- Staphylococcus aureus [5]. Several pathogenic bacteria have been isolated from different sources within the hospital environment such as hospital sinks most of which possess resistance to antibiotics [6, 7, 8].

The widespread use of antibiotics in hospitals results in the selection of antibiotic-resistant organisms in the hospital environment [9,10]. Many or most nosocomial bacterial pathogens in the hospital environment are resistant to several antimicrobial agents [5]. Antimicrobial resistance results in increased illness, deaths, and health-care costs [11, 12]. Special care must be taken when dealing with patients who are receiving antibiotics on a regular basis[13].

The hospital records between January 1998 and December 2001 showed that 3548 in-door, and outdo newborns (term, and preterm females, and males) were admitted to (NICU), with a duration of stay ranging from less than 24 hours to more than 28 days, 1641 newborns expired (843 males, and 798 females) in the intensive care unit because of the following main reasons: 1-complication of prematurity (the leading cause of death among newborns in (NICU) 2-sepsis. 3-septicemia. 4-birth asphyxia. 5-congenital anomalies. 6- Infections.

Materials and Methods

The samples were taken from the (NICU), from the following object:

1- Water sinks. 2- oxygen masks. 3- milk (from feeding bottles). 4- syringes for milk feeding. 5- equipments', and medicines' tables. 6- scale for measuring newborns' weight. 7- room's air, and air filters, floors, walls, and phones.

8- electrocardiograph machine , heart pulse machine, oxygen supply machine and ultra violet machine. 9- newborns' Incubators (beds linen, water, ventilation tubes, filters, fluid pumps, and aspirator machines associated with incubators).

All samples except for air samples were taken by a sterile cotton swab, which was then inoculated on MacConkey agar, and blood agar, and incubated at 35 C for 24-48 hours. After purification, and gram staining all gram (-) isolates were identified by the 20 E API system while gram (+) cocci were tested for by the coagulase, and catalase tests. For air samples, plates were left open for 15 minutes then processed as mentioned above. Antimicrobial susceptibility testing of the bacterial isolates was performed by the disk diffusion method using the following

antibiotics for gram negative bacteria : amoxicillin, agumentin, carbencillin, cephaloridine, cephalexin, erythromycin, doxycydin hydrochloride, fusidin, kanamycin, neomycin, and tetracycline. In addition to those used for gram negative bacteria the following antibiotics were also used for gram positive cocci bacteria: cloxacillin, lincomycin, methicillin.

Results

20 bacterial isolates mostly gram negative aerobic, and facultative anaerobic bacteria were isolated from several samples in 3 (NICU) within the (TMC), and identified, and all were considered to be clinically significant as a cause of serious nosocomial infections in this high risk group of patients. The isolates and their sources were Acinetobacter calco. Var lwoffi (newborns incubators, water used for respiratory machine, fluid pump, air, and the water sink where staff, and nurses wash their hands), Acinetobacter calco. Var. anitrat. (newborns incubators, water used for respiratory machine, and ultra violet machine), Cirtobacter freundii (Incubator water), Enterobacter agglomerans (floors, and milk feeding bottles), Enterobacter intermedium (milk from feeding bottles), Enterobacter sakazakii (floors), Enterobacter species (phone), Escherichia coli (incubators water), gram positive bacilli (air, and phone), gram negative dipliococci (incubator water), Klebsiella pneumoniae (scale, milk from feeding bottles, syringes for milk feeding, water sink, walls, rooms air filters, and aspirator machines), Klebsiella species (aspirator machine, and, electrocardiograph machine), Proteus mirabilis (syringes for milk feeding), Pseudomonas aeruginosa (water used for newborns Incubators, and respiratory machine, aspirator machines , and water sink where staff, and nurses wash their hands), Pseudomonas species (milk, newborns incubators, and the water sink), Serratia liqueficans (milk feeding bottles, and the water sink) Serratia marcescens (Incubator filters, and the water sink) Serratia rubidaea (water sink, milk feeding bottles, and aspirator machine), S. aureus (scale, newborns incubators, floors, air, rooms air filters, tables, fluid pump, and heart pulse machine), and S. epidermidis (air, water sink, tables, phone, oxygen mask, Scale, bed linen, and rooms air filters) .

It should be noted that the most contaminated samples were those taken from the incubators and water used with the incubators, water sinks, and milk feeding bottles respectively. Out of the 145 samples tested, 37.2% (isolated 54 times) were positive for S. epidermidis, 26.9% Acinetobacter species, 20.7% S. aureus, 9.6% K. pneumoniae, 7.6% P. aeruginosa, gram positive bacilli 6.2%, Serratia species 4.1% , Klebsiella species 3.4% Pseudomonas species 3.4%, Staphylococcus species 3.4% , Enterobacter species 2.8%, E. coli 2.1%, P. mirabilis 2.1%, and C. freundii 0.7%..

Discussion

Since most bacterial isolates were important causative agents of several nosocomial infections such as septicemia which is associated with significant mortality, thus, they might contribute to the high mortality rate among newborns in (NICU) within (TMC). S. epidermidis which is considered the most isolated bacterial isolate in this study, is now the most common cause of septicemia due to the increasing use of intravascular devices [16], keeping in mind that septicemia is one of the causes of death among newborns in (NICU) within the (TMC). In addition, several bacterial isolates were isolated from several newborns' incubators, water, and filters used with the

newborns' incubators which were considered the most contaminated samples in this study. This may reflect the overcrowding, incubator shortage, which in turn does not allow enough time for proper cleaning, disinfection and sterilization. Thus, such items might also contribute to a high mortality rate among newborns in (NICU). Furthermore, several bacterial isolates were found in milk, and milk feeding bottles through which several diseases can be transmitted. This may reflect improper handling of milk, and cleaning of milk feeding bottles. In fact, it was noticed during our visits to (NICU) that there was a shortage in milk feeding bottles which may explain the reason of contamination of such bottles in an overcrowded (NICU) with nurse staff shortage. Since several bacterial isolates were isolated from water sinks in (NICU), the results of this study are , therefore, in agreement with other studies that reported the ability of gram-negative bacilli to survive in wet environments such as water sinks for long periods of time.

Previous studies in these units in (TMC) indicates that Enterobacter cloacae, and P. aeruginosa were the main problematic bacteria, since newborns infected with such bacteria were dead; P. aeruginosa was isolated from all equipment in close contact with the newborns. The source of these bacteria seems to be the humidifier, and ventilator that is shared by many newborns. This finding shows the importance of proper ventilation with efficient filters against dust within the (NICU). In addition, E. cloacae was isolated from different samples such as an intravenous fluid bottles used for newborns, and from the blood of one of the dead patients.

The results of our study revealed the importance of following appropriate infection control measures and the development of neonatal intensive care programs to reduce cross infection within the (NICU) which might decrease the risk of infection and lower the immortality-morbidity of infection. The goals are to prevent transmission by hands, air, blood, and equipment. Common measures for prevention include proper hand washing, avoiding hand contact during medication, and sterilization methods such as sterilizing ventilators to full scale air filtering. All procedures, such as invasive techniques in (NICU) should be carried out under full aseptic conditions. All infant formula should be prepared, and stored under appropriate conditions. It was noticed during our visits to (NICU) that staff members occasionally left doors open, did not wear disposable gloves, or gowns while performing their duties.

Isolation of infected newborns from other newborns [which we did not observe during our visit to the (NICU)], is needed to limit the transmission of bacterial pathogens among newborns. Staff and visitors with infectious diseases should not be allowed to enter the (NICU). There should be one skilled experienced nurse for every three to four normal newborns, and one for every two should be available within the (NICU) to overcome nurse staff shortage in (NICU). Out door admission should be prohibited, to avoid over crowding which was noticed within the (NICU) in (TMC). Thus, hospital care at home is required for some neonatal disorders to overcome over crowding, hence, lowering nosocomial infections and mortality.

Antibiotic susceptibility testing showed high resistance rates to be widely spread among the bacterial isolates since most of them were resistant to most antibiotics tested. Bacteria belonging to the Klebsiella, Proteus, Pseudomonas, and Serratia genera were all resistant to most antibiotics tested, while all Acinetobacter species were sensitive to most antibiotics. Bacteria belonging to the genus Staphylococcus were resistant to carbenicillin, cephaloxin, cloxacillin,

fusidine, kanamycin, methicillin, and penicillin, and were sensitive to cephaloridin. About 20% of S. aureus solates were resistant to methicillin. Accordingly, adequate programs should be followed to prevent the development, and spread of antibiotic resistance within (TMC). This finding is similar to results reported by several studies.

Conclusion

From this study we conclude that the number of bacterial isolates identified during this study was high. However, this finding might be acceptable relatively to the large number of deliveries admitted to the delivery rooms and the large number of newborns admitted to the intensive care units in (TMC), in addition to the out door newborns. Also considering the few scattered intensive care units, equipment, materials, incubators, nursing staff shortage, and lack of nursing experience within the (NICU). These all lead to increased contamination and higher numbers of bacteria. There are several preventive measures for preventing nosocomial infections and spread of pathogens within the (NICU). Alternative antibiotics should be used, and specific control strategies that control the widespread use of antimicrobial agents are needed to overcome spread of multi-resistant bacteria within the (NICU), and for proper antimicrobial therapy of the infections caused by these bacteria that may play a role in the noticed increase in mortality rate among newborns.

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GMJ. Vol 21 No (2), PP88-92

Antibiotic Resistance in Bacterial Isolates From the Delivery Department and Delivery Rooms

Elyousfi Masaoud A.; Ramadan Abdoalbast; Tbeny Nabil; Alalwany Narges and Almagerhy Reem.

Department of laboratories, Faculty of Medical Technology
Al Fatah University-Tripoli, Libya

الملخص

الخلفية: المشكلة الرئيسية في المعالجة بالمضادات الحيوية هي ظهور البكتيريا المقاومة للمضاد وتكرر المقاومة يعتمدُ على البكتيريا والمضاد الحيوي المستخدم.بلغت الولادات في مستشفي طرابلس المركزي في الفترة من يناير 1998 إلى نهاية ديسمبر 2001 عددا و قدره 39827. **الهدف:** معرفة أنواع البكتيريا المعزولة وقابليتها للمضادات الحيوية **الفترة و المكان:**أجريت الدراسة في م.طرابلس المركزي في الفترة من أكتوبر 1999 إلى يونيو2001. **الطريقة والمواد:**تم إتباع وسائل العزل و تحديد النوع المعتاد في مثل هذه الدراسات. **النتائج:** عُزلت 17 جرثومة، غالبيتها هوائية سالبة الغرام ، وعصويات لاهوائية اختيارية ، مِنْ مجموع 90 عينة من القسم وغرف الولادة. تبين بأنها كَانَت مقاومة إلى أكثر مِنْ نوع من المضادات الحيوية، المستعملة عموماً لمُعَالَجَة الأمراض الجرثومية. الجراثيم المتكرّرة المهمة في المستشفي التي اكتسبتْ المقاومة هي المكورة العنقودية (44.4 %)، العنقودية البشروية (24.4 %)، الزائفة الزنجارية .(12.2 %) العينات الأكثر تلوثاً كَانتْ تلك المَأْخُوذة مِنْ أسرّة الولادة. **الخاتمة:** هذه الدراسة بَيّنتْ بأنّ عددَ الجراثيم المَعْزولَة كَانَ عالياً. علاوة على ذلك، غرف الولادة، وقسم الولادة يَجبُ أنْ يُطهّرَ بشكل دوري، حيث إن الجراثيم المعزولة كَانتْ مقاومة جداً إلى عدّة مضادات حيوية، لذا، يجب استعمال أنواع بديلة وسياسة تقييدية على الاستعمال الصحيح للمضادات لمَنْع انتشار مقاومة المضاد الحيوي في مركز طرابلس الطبي.

Abstract

Background: A major problem in antibiotic therapy is the emergence of drug resistant bacteria ; the frequency of resistance depends on the bacteria, and the antibiotic concerned. Delivery devices such as forceps, and vacuum extractors are well known devices that carry health risks during delivery. The total deliveries within the Tripoli Medical Center (TMC), between January 1998 and December 2001, were 39827 births (alive and dead ; stillbirths are not included). **Objective:** 1- to isolate and identify bacteria from different samples within the delivery rooms and delivery department, in order to evaluate the extent of contamination within such a department. 2- to determine the susceptibility of bacterial isolates to several antibiotics using the disk-diffusion method . This bacteriological study was conducted between October 1999 and June 2001 during 5 interval visits to the delivery rooms and delivery department at (TMC). **Materials and Methods:** Several duplicate samples were taken from the delivery department and examined using standard bacteriological procedures. **Results:** 17 bacterial isolates, mostly gram negative aerobic, and facultative anaerobic bacilli, were isolated from a total of 90 samples within the department and delivery rooms. Several bacterial isolates, were found to be resistant to more than one of the antibiotics, that are used commonly for treating bacterial diseases. The most frequent bacterial isolates, which are considered to be important bacteria in hospital acquired infections, were Staphylococcus aureus (44.4%), Staphylococcus epidermidis (24.4%), and Pseudomonas aeruginosa (12.2%). The most frequent contaminated samples, were those taken from the delivery beds. **Conclusion:** This study demonstrated that the number of bacterial isolates from the delivery rooms, and the delivery department was high. Furthermore, the delivery rooms, and the delivery department should be disinfected periodically. Since most bacterial isolates, were highly resistant to several antibiotics tested, therefore, alternative drugs must be used and a restrictive policy on their proper use is important to prevent the spread of antibiotic resistance within (TMC).

Key Words: Nosocomial infection , antibiotic resistance, gram negative bacteria, S. aureus, S. epidermidis .

GMJ. Vol 21 No (2), PP88-92

Introduction

A large percentage of infections are acquired during a hospital stay. Such an infection is called a nosocomial infection, and the microorganisms involved are called opportunists. Infections can affect the mother, the newborns or both in the perinatal period. Mean rate of nosocomial infection in obstetrics is about 4% over a three-year period in a large teaching hospital 1. Acquisition of nosocomial infections is determined by both factors; immunity of the patient and interventions performed that increase risk. Forceps, and vacuum extractors are well known delivery devices that carry risks during delivery. Infection control is becoming increasingly complex 2.The emergence of drug resistance is a major problem in antibiotic therapy, especially among pathogens causing hospital acquired infections. The frequency of this resistance depends on the bacteria, and antibiotic concerned. Some bacteria rapidly acquire resistance such as S. aureus and certain coliforms, while others rarely do so, for example Streptococcus pyogenes .

Several pathogenic bacteria have been isolated from different sources within the hospital environment, many of these bacteria possess resistance to antibiotics. Major nosocomial pathogens increasingly resistant to antimicrobial drugs include Escherichia coli, S. aureus, coagulase-negative staphylococci, Enterococcus species, and P. aeruginosa 3, 4. Infections from methicillin- resistant staphylococci, vancomycin-resistant enterococci (VRE), andaminoglycoside-resistant

Pseudomonas species are becoming common 5. The prevalence of resistance is directly proportional to the amount of the antibiotic used. This is illustrated by the increased antibiotic resistance found in countries with unrestricted use of antibiotics, and in hospitals when compared to the community6. Antimicrobial resistance results in increased illness, death, and health-care costs 7, 8. 70% of the infections are due to organisms resistant to at least one antimicrobial agent. Certain bacteria such as S. aureus readily appear in multiple resistant forms especially in hospitals 9.There are different mechanisms by which bacteria become resistant to antimicrobial agents, which can spread to other bacteria. This may be done by acquiring resistance coding- plasmids by transduction, and properly by conjugation 9, for example. Certain bacteria resist antibiotics due to enzyme production that breaks down antibiotics. About 80% of S. aureus produce pencillinases enzymes that break down beta lactam ring in penicillin. In addition most hospital Acientobacter species are resistant to penicillins, including ampicillins, while P. aeruginosa are resistant to several antibiotics.Since bacteria isolated from hospitals are the major cause of several infections in hospitalized patients, precise knowledge of their antimicrobial susceptibility is of vital importance. Several studies have reported increasing resistance to antibiotics by hospital bacterial isolates over the past years.

Materials and Methods

The samples were taken from the delivery department and delivery rooms, from the following objects:

1- Legs' holder.2-Patients' bed. 3-Delivery beds, and chairs. 4- Heart pulse machine. 5-Aspiratory machine.6-Electrocardiogram machine. 7-Length measuring machine. 8-Special machine for newborns. 9- Machine for measuring food content. 10- Newborns' beds,

newborns' incubators.11-Machine that provides oxygen to patient (Ventilator). 12-Table for measuring newborns length. 13-Equipment, and examining tables. 14-Face mask associated with the machine that provides oxygen to patient. 15-Rooms' air, floors, walls, and water sinks. All samples except for air samples, were taken by a sterile cotton swab, which was then inoculated on MacConkey agar, and blood agar, and incubated at 35C for 24-48 hours. After purification, and gram staining all gram (-) bacterial isolates were identified by the 20 E API (Enterobacteriacea Analytical profile index) system (bio-Merix, France) and oxidase test, while gram (+) cocci were tested for by the catalase, and coagulase tests. For air samples, plates were left open for 15 minutes then processed as mentioned above. Antimicrobial susceptibility testing of the bacterial isolates was performed by the disk diffusion method using the following antibiotics (Oxoid, UK) for gram negative bacteria : amikacin (30ug), amoxicillin (25ug), ampicillin (10ug), cephaloridine (30ug), cephaloexin (30ug), ciprofloxacin (10ug), erythromycin (15ug), doxycydin hydrochloride (30ug), kanamycin (30ug), oxytetracycline (30ug), penicillin (10ug), and ticarcillin (75ug). In addition to those used for gram negative bacteria, the following antibiotics were also used for gram positive cocci bacteria : cefotetan (30ug), ceftriaxone (30ug), chlormphenicol (30ug), lincomycin (2ug), trimethoprim ,(5ug) and vancomycin (30ug) .

Results

17 bacterial isolates mostly gram negative aerobic, and facultative anaerobic bacilli were isolated on both media from several samples within the delivery department and delivery rooms. The most frequent bacterial isolates, were S. epidermidis (44.4%) , which is a universal skin commensal opportunistic pathogen, S.aureus (24.4%) , which is by far the most important human pathogen in the staphylococci, and considered as a human nose, and skin commensal, and P. aeruginosa (12.2%), which is widely distributed in the hospital environment. The most frequent contaminated samples, were those taken from the delivery beds. The bacterial isolates, which were considered to be important bacteria in hospital acquired infections, were Acinetobacter calco. Var lwoffi (Delivery beds, patients' beds, legs holder, floors, and water sink where health staff wash their hands), Acinetobacter calco. Var. anitrat. (delivery beds), E. coli (water sink), Enterobacter agglomerans (delivery beds, equipment table, special machine for newborns, and water sink) , Enterobacter cloacae, (water sink) , Enterobacter species, (floors, and newborn's aspirator machines) , gram positive bacilli (delivery beds , and table for measuring newborns' length) , Klebsiella pneumoniae (newborn's aspirator machines), Proteus mirabilis (delivery beds, and newborns' aspirator machines), Providencia alcalifaciens (delivery beds), Providencia rettegeri (delivery beds) Providencia stuartii (delivery beds , and chairs), Providencia species (water sink, and delivery beds , and chairs), Pseudomonas aeruginosa (oxygen mask, newborns' aspirator machines, delivery beds, legs holder, heart pulse machine, machine for measuring food content, and water sink), Pseudomonas species (floors, aspirator machines , delivery beds , and chairs, medicine tables, and water sink), S. aureus (air, delivery beds, beds linen, legs holder, heart pulse machine, newborns' beds, balance, and medicine tables), and S. epidermidis (air, delivery beds , newborns' beds, newborns' incubator, legs holder,

heart pulse machine, electrocardiogram machine, length measuring machine, oxygen mask, floors, walls, and medicine tables) . It should be stated that of the 90 samples tested, 44.4% were positive for S. epidermidis (isolated 40 times), 24.4% S. aureus, 12.2% P. aeruginosa , 11.1% Acinetobacter species, 8.9% gram positive bacilli, 7.8% Pseudomonas species, 5.6% Enterobacter species, 4.4% Providencia species, 1.1% E. coli, 1.1% K. pneumoniae, and 1.1% P. mirabilis.

The results obtained showed significant differences among bacterial isolates in their antibiotic susceptibility. Several bacterial isolates, were found to be resistant to more than one of the antibiotics, that are commonly used as the drug of choice for treatment of hospitalized patients.

71.1% of Acinetobacter species, were resistant to impekin, 50.0% to trimethoprim, 40.0% to tetracycline, 22.0% to ticarbencillin,14.0% to kanamycin, 10.0% to chlormphenicol, 6.0% to amikacin, 6.0% to ampicillin, 6.0% to ciprofloxacin, and 6.0% to doxycycline-hydrochloride. 52% of P. aeruginosa, were resistant to ticarbencillin, 46% to doxycycline-hydrochloride, 35.0% to oxytetracycline, 33.0% to erythromycin, 33.0% to trimethoprim, 30.0% to tetracycline, 25.0 % to amikacin, 16.5% to chlormphenicol, 16.0% to kanamycin, 14.0% to ciprofloxacin, and12.5% to impekin,. 36.0% of Enterobacter species, were resistant to ticarbencillin, 25.0% to trimethoprim, 24.0% to ampicillin, 21.0% to kanamycin, 20.0% to tetracycline, 12.0% to amikacin, 10.0% to chlormphenicol, 6.5% to ciprofloxacin, 4.0% to tetracycline, and 4.0% to doxycycline-hydrochloride. 40.0% of Klebsiella pneumonia, were resistant to tetracycline, 25% to trimethoprim, 20% to kanamycin, 11.0% to ticarbencillin, 10.0% to chlormphenicol, 6.0% to ciprofloxacin, 4.0% to tetracycline, 6.0% to ampicillin, 6.0% to amikacin, and 3.0% to doxycycline-hydrochloride.In addition 80.0% of S. aureus , were resistant to trimethoprim, 50.0% to cefotetan , 30.0% to kanamycin , 30.0% to penicillin, 24.5% to erythromycin, 21.0% to oxytetracycline, 20.6% to ampicillin, 16.5% to ticarbencillin, 16.0% to chlormphenicol, 9.0% to lincomycin, 4.0% to vancomycin, 3.6% to ceftriaxone, and 2.0% to ciprofloxacin. Furthermore, 70.0% of S. epidermidis, were resistant to trimethoprim, 53.0% to cefotetan, 36.2% oxytetracycline, 32.0% to kanamycin, 29.0% to ceftriaxone , 22.0% erythromycin, 18.5% to ampecillin, 16.0% to ciprofloxacin, 16.0% to chlormphenicol, 14% to lincomycin,15% to ticarbencillin,12% to penicillin, 7.5% to lincomycin, and 1.7% to vancomycin. Keeping in mind , that such results may vary from one hospital to another and among different locations in the same hospital due to differences in bacterial strains tested, and changes of their distribution with time.

Discussion

This study demonstrates that the number of bacterial isolates from the delivery department and delivery rooms is high and that the delivery beds, were the most contaminated samples tested, which may be related to the large number of deliveries admitted to (TMC) with few delivery rooms, and equipment shortage, etc. This fact could be one of the reasons, that explains the large number of newborns admitted to the neonatal intensive care unit (NICU) in (TMC). All health personnel are required to follow standard precautions when caring for all patients. Also, sterilization of the delivery rooms and improvement in obstetric care and early neonatal care, should be done immediately since delivery rooms are the first source of infection to newborns, this may be the best effective measure to lower the number of bacterial contaminants,

and avoid acquisition of hospital acquired infections.Our results indicated that S. epidermidis , which is a coagulase negative Staphylococci, was the most frequent bacterial isolates, reflecting the wide spread of this bacteria within the (TMC) environment, thus, may be the cause of hospital acquired infection. Several bacterial isolate, such as Acientobacter species, and P. aeruginosa , were isolated from water sinks. This finding is in agreement with details in literature .Thus, all wet environments, such as water sinks, should be dried properly to avoid being a source of contamination within hospitals. The high prevalence of antibiotic resistance by several bacterial cultures tested in this study, may indicate that they possess one or more mechanisms for antibiotic resistance, emphasizing that a restrictive policy on the use of antibiotics is recommended to prevent the spread of antibiotic resistance among bacteria within the hospital environment, hence, effective medication. A lot of literature states that, Acientobacter species are usually susceptible to trimethoprim, carbencillin, and cefotaxime. Pseudomonas, especially P. aeruginosa , are resistant to many frequently used antibiotics. Most strains of S. aureus are penicillin resistant, mainly related to Beta-Laclamase production, and some are resistant to methicillin. S. epidermidis, which is carried by staff and patients, is more often resistant to antibiotics tested than is S. aureus, most of which are resistant to penicillins. Our results confirm such details. Drug resistance in clinical practice which is associated with antibiotic use (9), is common among bacterial isolates in hospitals, however, bacterial isolates vary greatly in antibiotic sensitivity.

Conclusion

Alternative antibiotics, avoiding the use of antibiotics without prescription, and proper antimicrobial policy are needed for successful medication in order to overcome the acquisition and spread of drug resistance among bacteria since most bacterial isolates tested were resistant to most antibiotics tested. The finding of this study might be acceptable corresponding to the large number of deliveries admitted to TMC with few delivery rooms, equipment, materials, and nursing shortage. However, antiseptic measures, adequate nursing staff training, and proper nurse patient ratio are recommended to help prevent nosocomial infection.

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GMJ. Vol 21 No (2), PP93-99
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Effect of Acetaldehyde on Post-natal Growth and Purkinje cell Count in Rats

Idris Barassi[1]; R. N. Sreenathan[1]; Salha Zoubi[1] and Osama Tashani[2].

[1]Faculty of Medicine and [2]Faculty of Science, University of Garyounis, Benghazi, Libya

الملخص

الخلفية: الاسيتالدهيد أول ناتج أيض للكحول والإفراط فيه له تأثير ماسخ يرى عادةً في مجموعة أعراض الكحول المميت (FAS) **الهدف:** التأكد من فرضية التقليل من حجم الدماغ وعدد خلايا بركنج في المخيخ. **الطريقة:** استخدم 40 فأراً حاملاً استخدم خمسة منها ضوابط وقُسِّم الباقي إلى 3 مجموعات وكل مجموعة أعطيت جرعات مختلفة من اسيتالدهيد في الصفاق في اليوم أل 14 من عمر الحمل، الجرعات كانت كالتالي 25جم/ كجم لوزن الجسم حقنت في 5 أمهات (مجموعة I)، 50جم/كجم لـــ 16 أم (مجموعة II) و 75 جم/كجم حقنت لـــ 8 أمهات (مجموعة III) . في الاختبار التمهيدي الجرعة 100 جم/كجم من وزن الجسم جربت على 6 أمهات نتج في 64% معدل وفاة (ن=25). أما بالنسبة للمجموعات الأخرى فإن موت الجراء فيها كالتالي: مجموعة I قتل 3 جراء من اصل 18 ، 88 في مجموعة II مات منها 15 عند الولادة و 21 ماتت بعد الولادة. مجموعة III (31) مات منها 22 بعد الولادة. في اليوم 10 بعد الولادة قُتلت الجرذان الباقية (عدد=98) و أُخذَت القياسات التالية بـــ مم كالتالي 1. من قمة الرأس إلى جذر الذيل 2. الساعد 3. الذراع 4. الساق 5. القدم 6. الذيل بالإضافة إلى قياسات الدماغ ككل. كما حُضِرتْ خلايا بركنج. **النتائج:** للاسيتالدهيد تأثير مؤذى عند 50جم/كجم أو أكثر على نمو الجرذان، الصلة بين زيادة أطوال الساعد، الذراع، الساق، القدم وجرعة الاسيتالدهيد كانت سالبة مع معامل صلة بمقدار 0.89 ، 0.84، 0.83، 0.7 على التوالي. نستنتج من ذلك أنه قد توجد علاقة بين الاسيتالدهيد ونشوء بعض الأمراض مثل (أعراض الكحول المميت) ولكن لم يكن ممكناً عزو بعض الاعراض العصبية إلى الاسيتالدهيد كما وان الوظيفة التي تجعل الايثانول قادراً على إنقاص حجم الدماغ تحتاج إلى بحوث أخرى.

Abstract

Background: The Acetaldehyde, the first metabolite of ethanol, was recently blamed for the teratogenic effects usually seen in Fatal Alcohol Syndrome. **Objectives**: To investigate the general effects of acetaldehyde injected to pregnant mothers on body development and Purkinje cells count of the cerebellum of newly born rats. **Materials and Methods**: 40 pregnant rats were used. With 5 controls, intraperitoneal doses of acetaldehyde were given on day 14 of the gestation period. The doses were 25 mg/kg of body weight injected into 5 mothers (group I), 50 injected into 16 mothers (group II) and 75 injected into 8 mothers (group III). Earlier, mothers subjected to 100mg/kg b.wt resulted in 64% mortality rate of pups (n=25). On day 10 Post delivery the survived pups (n=98) were sacrificed and different somatometric measurements were taken. Histological sections of the cerebellum were examined to count the number of purkinje cells. **Results:** In group II, the correlation between an increase in forearm, arm, leg and foot lengths and the dose of acetaldehyde were highly negative with a correlation coefficient of - 0.89, - 0.84, - 0.83 and - 0.7 respectively. Weight, length and breadth of brains of treated pups were slightly higher than controls, but these changes were highly significant ($P<0.05$). However, differences in Purkinje cells count were not verified statistically. **Conclusions:** acetaldehyde may be implicated in the general pathogenesis of FAS. But, its neurotoxicity has not been proven here. The mechanism by which ethanol reduce the size of fetal brain tissue needs further investigation.

Keywords: Acetaldehyde, Teratogenicity, Neurotoxicity, Alcoholism, FAS, Purkinje Cells, Rats

GMJ. Vol 21 No (2), PP93-99

Introduction

Clinical and experimental reports have proved beyond doubt that alcohol is teratogenic. The pattern of anomalies associated with maternal drinking is called Fetal Alcohol Syndrome (FAS). The initial recognition of multiple effects that alcohol can have on the developing fetus may be attributed to Lemoine et al, but Jones and Smith were the first to give a full definition of Fetal Alcohol Syndrome.[1,2] Since then it has become clear that prenatal alcohol exposure can be associated with a wide range of malformation in offspring.[3] More than 80% of children with FAS demonstrate prenatal and postnatal growth deficiency, mild to moderate mental retardation, microcephaly, infantile irritability and characteristic facial features. Fifty percent of affected individuals also have poor coordination, hypotonia, attention deficit disorders with hyperactivity, decreased adipose tissue, and other identifiable facial features. Additionally, 20% to 50% of affected children demonstrate a variety of other birth defects, including cardiac anomalies, hemiangiomas, and eye and ear anomalies. [4,5,6].

Acetaldehyde, the first metabolite of ethanol has been recently implicated in pathogenesis of FAS. The toxic property of acetaldehyde has become the subject of much interest; partially due to the increasing evidence starting in the seventies of last century that acetaldehyde is involved in many of the toxic effects of ethanol. [7,8] But the experimental studies on its role in FAS have not yielded convincing results. Therefore, the present experimental study was undertaken to investigate the teratogenic effect of acetaldehyde. It is a study of postnatal growth of pups following prenatal exposure to acetaldehyde includes Histological and stereological features of Purkinje cells of cerebellum.

Materials and Methods

A total of 40 female rats of approximately 100-120 days of age and weighing about 250g were used in this study. 5 animals were taken as ad-libitum controls. After mating with males pregnancy was confirmed by the presence of sperms in the vaginal smear examined on the following morning. Positive day was designated as day (1) of pregnancy and sperms positive females were weighed and housed individually in the departmental animal house, which is noise free and maintained on a light dark cycle of (14:10) hours. On day (13) following sperm positive day (1), the abdomen of the animal was examined for pregnancy and if rounded nodules of fetuses were palpated then they were separated and marked for injection. The animals were given intraperitoneal injection of Acetaldehyde on day (14). The following doses were used for four different groups:

Control 5 animals
Group (I) 5 animals 25mg/kg of b.wt
Group (II) 16 animals50 mg/kg b.wt
Group (III) 8 animals75 mg/kg b.wt
Group (IV) 6 animals100 mg/kg b. wt

All the animals were allowed to deliver. Careful recording was under taken to note opening of eyes, ear lid opening and hair appearance of newly born pups. The pups then were subjected to the following measurements using slide calipers. This standard method was adopted by Sreenathan et al.[9]

1-CR length is taken in (mm) from crown to the root of tail
2-Forearm length in (mm) of forelimbs
3-Arm length in (mm) of forelimbs
4-Length of leg was the patello calcaneal length in (mm)
5-Length of foot from the calcaneum to the distal point of the foot in (mm)

6-Tail length is measured in (mm) from to tip of the tail

The measurements were taken for (10) days after delivery. On the day (10) the pups were sacrificed using ether and then (10 %) of formalin was injected into right atrium while the left ventricle was open. The advantage of this perfusion method is that the preservative substance replaces all the fluid in the tissues including the brain. The sacrificed animals then transferred into jar contains (10%) of formalin and kept for one day. The head was dissected and brain was removed free. The volume of the brain was taken by water displacement of graduated tube. Then the brain was fixed in (10%)of formalin. After three days of fixation in formalin the hardened specimen of the brain including cerebellum was measured. The length, height and width of whole brain were measured using fine slide Vernier calipers. The volume of the brain was calculated by multiplying height, breadth and length. The volume thus computed is compared with the volume of the whole brain obtained by displacement method. The weight of the whole brain and cerebellum was taken and recorded. The whole brain fixed in (10%) of formalin and was processed for histological sectioning and normal (H & E) staining.

Purkinje Cells count

A square grid of (121) points of (5mm) sq .div. of American Optical Corporation was introduced into the eyepiece and the resulting square grid got superimposed over the section. The methodology used by Sreenathan *et al*, was applied to meet the specific arrangement of purkinje cells in the histological sections of cerebellum.[10] Fifty fields out of each cerebellum were examined and the purkinje cells in these fields were counted then the average was presented as number of cells per square millimeter.

Statistical analysis

Unless otherwise stated the numerical data are expressed as arithmetic mean ± standard deviation. Percentage increase in weight and other measurements was calculated by subtracting the final value (at day 10 post-delivery) from the initial value (at day one) and then dividing the result by the initial value and multiply it by 100.

Different tests for significance were used during this study according to the types of data analyzed. Ranked data were analyzed by Chi-square on a few occasions and by other nonparametric tests. Z-statistic was applied when comparison of two proportions or percentages was desired. One way Analysis of Variance was applied more frequently to compare between several groups and the test of choice for further analysis of variance was the Tukey Honestly Significant Difference of Unequal sample (THSD). This post-hoc comparison test was chosen after testing the data with more liberal alternatives and it proved to be suitable. T-test was also applied when comparison of means of only two groups was needed.

In all the tests P values < 0.05 were recorded as significant. Most analyses were computed by Windows environment using the Statistica program (statsoft).

Results

Injecting acetaldehyde into the mothers caused death of insignificant number of mothers but resulted in a considerable mortality of pups. As shown in table 1 the mortality of pups was high when the injected dose was 50 or 75 mg/kg b.wt, and it was extremely lethal when the dose was 100 mg/kg b.wt.

Table 1. Mortality of mothers and pups subjected to different doses of acetaldehyde.

Group (mg/kg b.wt) Acetaldehyde	Number of Mothers	Mortality of Mothers N (%)	Mortality of Pups		
			Total number of pups	At delivery N (%)	Post-delivery N (%)
Control	5	0 (0)	35	0 (0)*	2 (6)*
I (25)	5	0 (0)	18	3 (16.6)	0*
II (50)	16	2 (12.5)	88	26(29.5)	21 (33.9)
III (75)	8	2 (25)	31	12(38.7)	10 (32.3)
IV (100)	6	1 (16.7)	25	3 (12)	16 (72.7)

*Significantly different from other groups (z statistic).

The dead pups showed haematoma, oedema and emaciation and one case of anencephaly was seen in group IV. No significant difference was found between groups concerning the length of pregnancy. Day by day, any comparison of weight of pups of the control group with those pups injected by acetaldehyde failed to yield meaningful statistical differences in most instances. The weight of pups of group I and II showed a slight increase in weight over 10 day post-delivery period (Figure1). And these changes were significant. However, there was poor correlation of weight of pups with the dose of acetaldehyde when other groups were incorporated in the analysis.

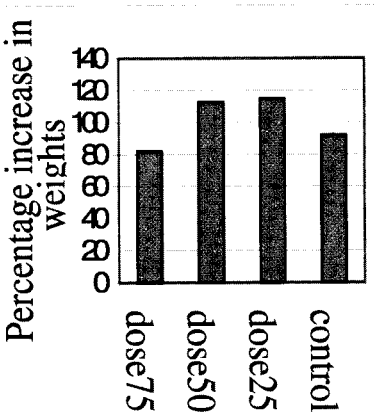

Figure (1): Percentage increase in weight of pups, at day 10 post delivery, injected with acetaldehyde compared to the control. The pups of those mothers that were injected by 25 and 50 mg/kg b.wt. showed a significant increase in body weight.

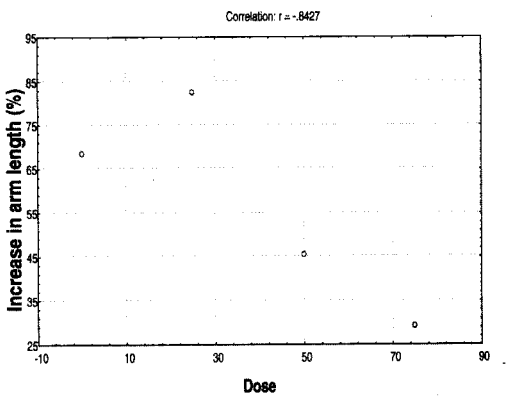

Figure (2): Correlation of dose of acetaldehyde and increase in arm length over 10 days post-delivery. This relation is highly negative, showing a decrease of arm length with an increase of dose.

Somatometric parameters

Differences of **crown rump length** between groups were only evident at day 9 of age. Crown rump length at day 9 of group III (75 mg/kg b.wt) was 43.9±1.8 mm and this was statistically lower than the measurements of controls, gruops I (25mg/kg b.wt) and II (50mg/kg b.wt) which were: 47.9±3.2, 48.2±1.9 and 47.4±4.4 mm respectively. The Correlation of Crown Rump length with dose proved to be a negative relation with r = -0.58.

Measurements of **forearm length** of pups of Group III were slightly lower than the control on days 8, 9 and 10 of age and measurement of day 10 only of group II was also lower than that of the control. Statistical analysis of these measurements revealed a significant effect of dose with F= 41.5 and P=0 (*ANOVA*). The correlation of percentage changes of the forearm length from day 1 to day 10 after delivery was negative with r = -0.9. This highly negative correlation indicates a strong effect of dose on this parameter.

The mean **arm length** of the pups of groups treated with (50mg/kg b.wt) or with (75mg/kg b.wt) at days 7, 8, 9 and 10 of age were significantly different from each other and from the corresponding control and other treated group (F=221, P=0). Figure (2) showed the percentage increase of in arm length, where dose (25mg/kg b.wt) lies above the control while doses (50mg/kg b.wt) and (75mg/kg b.wt) respectively lie below control. Moreover, the correlation of arm length increase is inversely proportionate with dose. As the dose increases the arm length percentage change decreases.

The Mean length of **leg** of treated pups of group II at days 8, 9, 10 were 11.6±0.9, 11.9±0.8 and 12.3±0.8 mm respectively. These were significantly less than the corresponding control values of 12.7±2.4 mm for day 8, 13.6±2.7 mm for day 9 and 14±3.24 mm for day 10. The mean leg length of treated pups of group III at days 5, 6, 7, 8, 9 and 10 was significantly different from the corresponding control and other treated groups (F=116, P=0). The Correlation of the percentage increase in leg length with dose showed a negative relation in which r=-0.86. However the dose of (25mg/kg b.wt) lies above control while other doses (50mg/kg b.wt) and (75mg/kg b.wt) lie below control.

Mean **foot length** of treated pups of group III at days 6, 7, 8, 9 and 10 was significantly different from the corresponding control and other treated groups (F=69, P=0). Table 2 below shows the comparison between the control and group III. Percentage increase in foot length was negatively correlated with doses (r=-0.7).

Table (2) Comparison of foot length in mm of control pups with those of group IV

Age of pups (days)	Control	Group III (75mg/kg b.wt)
6	12.6± 1	11± 0.6*
7	13.5± 1.34	11.3± 0.7*
8	14.3± 1.7	11.8± 0.9*
9	15.2± 2	12.5± 1.2*
10	16± 2.3	13.6± 0.5*

* Significant difference.

Comparison of mean length of **tail** (mm) of pups of control and four groups treated with different doses of acetaldehyde has failed to yield any significant differences. And correlation analysis of the percentage increase in tail length resulted in poor positive *r*-value of 0.2.

Brain measurements and Purkinje cells count

The weight, length and breadth of the whole brain of the control pups and group II were observed and compared to each other using t-test for analysis of significance and are shown in table 3. The observed mean weight of treated group's brains was more than that of the control with P<0.001. Length and Breadth of the brains of treated pups were also more than that of the control.

Table 3. Comparison of means of weight, length and breadth of brains of pups of both the control and group II.

	Weight (gm)	Length (mm)	Breadth (mm)
Control	0.82±0.09	15.7±0.9	11.7±0.5
Group II (50mg/kg b.wt)	0.88±0.08	16.4±0.7	12±0.4
P-value	0.0014	0.000	0.0042
t-value (student t test)	3.32	4.3	2.96

The mean number of purkinje cells in mm^2 of cerebellum of control pups was found to be 5.75±0.96 and that of group II was 5.6±0.42. Though control pups' cerebella contain slightly more purkinje cells than treated group the statistical analysis of this deference failed to find any significance (t=0.42, P=0.7).

Discussion

Results of previous experiments elsewhere designed to investigate the role of acetaldehyde have been contradictory. They have provided evidence that supports and refutes the idea that acetaldehyde is responsible for the teratogenic effects observed in fetal alcohol syndrome.[11] The results of the present study indicated without doubt that injecting pregnant mothers with high doses of acetaldehyde caused significant mortality and anomalies of pups and the growth of the survived pups was also affected. The doses of 50, 75mg/kg b.wt yielded shorter limbs and the body of treated pups was generally shorter than the control. While the dose of (100mg/kg b.wt) was proven to be very toxic to fetuses. These teratogenic effects of acetaldehyde at dose 50mg/kg b.wt was previously found and the short crown rump measurement was one of the commonest deformity seen in FAS.[12,13] Thus we are able to confirm that this effect is due to action of higher dose of acetaldehyde. However, whither the ethanol may or may not exert the same effect directly is yet to be examined. Controlled experiments in which both ethanol and acetaldehyde are administered separately should clarify the differences between the direct and indirect action of alcohol. It has been found here that the ef fect of acetaldehyde on deformity of limbs is dose dependent and this confirm earlier reports.[14]

Low dose of acetaldehyde (25 mg/kg b.wt) failed to exert any ill effects and in some cases induced an increase in somatometric measurements such as arm length. These positive actions of small doses of acetaldehyde await further confirmation. It may be attributed to the success of defense mechanisms against a harmful material at low doses.

It has been postulated that ethanol through its derivative the acetaldehyde has a constant dose-effect on the development of the central nervous system. The results, which are presented here, failed to prove any neuropathological effects of acetaldehyde. The design of these experiments was based on a single dose treatment at day 14. Other experimental design and modules were more successful such as cell culture techniques which might be used to examine the effects of acetaldehyde, both independently and in conjunction with ethanol. This *in vitro* technique revealed some neurotoxicity of acetaldehyde independent of ethanol[11] However, *in vivo* experiments and whole body approaches are more relevant to the clinical presentation of FAS. Therefore it is recommended that a similar study to the one presented here may be repeated. But with injecting multiple doses at early stage or sustaining a continuous treatment of mother by low dose of acetaldehyde to reach a conclusive result to either refute or confirm the neurotoxic properties of acetaldehyde

Correspondence: PO Box 17192 Benghazi, Libya. Email: osamatashani@yahoo.com

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Vol 21 No (2), PP100-102

Congenital dyserythropoietc anaemia: variability in presentation

Amina Mohamed Beayou; Nureddin A .Daghman; Amal Alfakre; Faiza Ramadan Mohamed.

Department of paediatric Faculty of Medicine Garyounis University.

الملخص

يعتبر فقر الدم الخلقي الناتج عن سوء تكوين الكريات الحمراء (CDAs) مجموعة متباينة من الأمراض الوراثية نادرة الحدوث. وهناك أربعة أنواع مكتشفة: الأول منها يتميز بضخامة الكريات ووجود جسور صبغية بين النوى وتغيرات أرومة ضخمة في الكريات. النوع الثاني: أرومة حمراء متعددة النويّة وهي التي تعطى ناتج موجب في اختبار المصل الحامضي-HEMPAS وهو قد يكون ثنائي النويّة أومتعددة النوية مع وجود مستضدات غير طبيعية للخلايا الحمراء. النوع الثالث: يتميز بأرومة عملاقة ومتعددة النوية. النوع الرابع: مشابه للنوع الثاني ولكن مع ناتج سالب لاختبار المصل الحمضي.نقدم هنا 3 حالات منفصلة من هذا المرض من النوع الثاني اكتشفت في مستشفي الفاتح لطب وجراحة الأطفال في بنغازي مبينين بذلك مدى تنوع هذا المرض.

Introduction

Congenital dyserythropoietic anaemias (CDAs) are a heterogonous group of a relatively rare inherited disorders characterized by ineffective erythropoiesis and morphological abnormalities of red cell precursors that is pathognomonic for the disease[1]. Four types are recognized[2]: type I which is characterized by macrocytosis, internuclear chromatin bridges, and megaloblastic changes; type II, also known as hereditary erythroblastic multinuclearity with positive acid-serum test (HEMPAS), is characterized by binuclearity, multinuclearity, and the presence of abnormal red cell antigens; type III is characterized by gigantoblasts and multinuclearity; type IV resembles type II but with negative acid serum test. Families with other variants that do not fall into these categories have also been reported[2,3] and variations within the types is a well recognized phenomenon[4]. Here we present three unrelated cases of CDA type II diagnosed at AL-Fateh Childrens' Hospital in Benghazi who typically represent the heterogeneity of this variant.

Cases

The three unrelated patients are a product of first degree consanguinity. The diagnosis was based on clinical presentation and bone marrow findings. All patients tested positive for Ham's test, which is performed as described else where[5], and therefore they are categorized as CDA type II or HEMPAS. During the course of their illness, the patients demonstrated variability in various aspects of the disease, see Table 1.

The three patients had mild jaundice: serum bilirubin ranged from 2 to 3 mg/dl with the indirect component ranged from 1.5 to 1.9 mg/dl. Normal liver enzymes. Mild hepatosplenomegaly-about 2 to 3

Vol 21 No (2), PP100-102
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cm below costal margins. Normocytic normochromic erythrocytes with baso-philic stibling. Marrow erythroid hyperplasia with predominant binuclear erythroblasts in addition to less than 10% mul-tinuclear cells, see Figure 1. High iron content as shown by special marrow iron stains, see Figure 2. Normal Hb electrophoresis. Increased levels of LDH. Ham's test tested positive with number of random sera but not with the patient's own serum. None had gall stones. Serum ferritin in the three patients was 915, 800, and 700 ng/ml respectively (normal range is 30-400 ng/ml for males and 15-150 ng/ml for females).

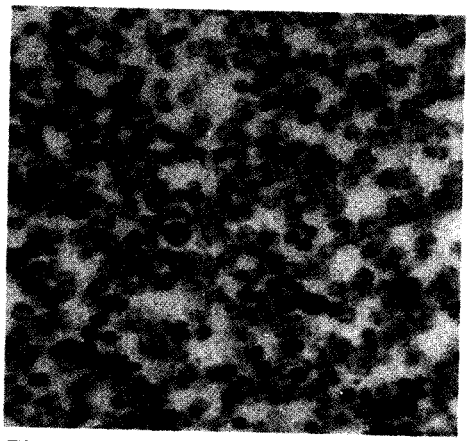

Fig1. Bonemarrow wright stain x40 show erythroblast binuclearty and multinucleartiy.

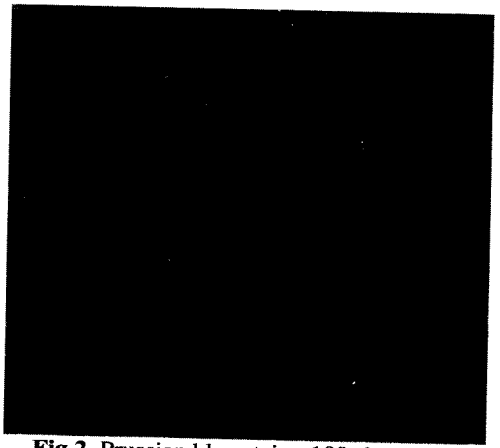

Fig 2. Prussian blue stain x100 show excess Free iron in erythroblasts.

Table 1: Clinical features of the cases

Cases	Year of birth	Age at presentation	Age at diagnosis	Hb* range since presentation	Family history.	Blood requirements
1	1993.	Neonate	5 year.	4-8 g/dl	2 siblings died.	Every 2-3 months
2	1991.	5 month.	1year.	7.5-8.5 g/dl	Negative	Every 2-3 years
3	1990.	4 year	4 .5 year.	9-12 g/dl.	Negative	NO transfusion.

*Hb: Haemoglobin.

Vol 21 No (2), PP100-102
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Discussion

The three cases presented here show variations in respect to age at presentation, severity of anaemia, requirement of blood transfusions, and family history. Though only one of our patients is transfusion dependent, ferritin levels were considerably high in all cases. Iron over load is a common complication[6] regardless of frequency of blood transfu-sion, most probably due to enhanced gastrointestinal absorption. Delay in diagnosis is not infrequently encountered in this condition. Despite being a congenital disorder diagnosis often delayed to adult life or later[7]. Interestingly significant delay, more than five years, was noted in only one of our cases. Some patients may display a thalasaemia-like picture[8]. Indeed one of our cases was labeled as thalasaemia and the true diagnosis of CDA was revealed only recently by bone marrow examination. Type II CDA, which is caused by defects in the enzymatic glycosylation of membrane proteins[9], is the most common of all types. The serological finding, positive Ham's test, characterizes this condition and being negative with patient's own serum distinguishes this disorder from paroxysmal nocturnal haemoglobinuria. Response to interferon, though partial, was reported in patients with type I CDA[10] but not for those with type II who may benefit from splenectomy to reduce blood requirements. The presented cases in this report add to the literature an example of variations within the same type of CDA.

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مجلة قاريونس الطبية تصدر موقتا بشكل نصف سنوي وتتناول العلوم الطبية . كل مجلد يحوى عددين احدهما فى أين النار والآخر فى ناصر

السعر	العملة المحلية	العملة الاجنبية مع رسوم البريد
اشتراك سنوى	16 دينار ليبى	12 دولار
نسخة واحدة	8 دينار ليبى	06 دولار
25 مستخلص من البحث	20 دينار ليبى	15 دولار

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