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Original Article

Paroxetine augments while naloxone abolishes the analgesic effect of paracetamol in acute nociceptive pain in mice

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الباروكسيتين يعضد بينما النالوكسون يلغى التأثير المسكن للباراسيتامول ضد الألم الفورى فى الفئران

د. محمد رأفت، د. وليد المالكى، د. محمد أحمد
قسم الأدوية والسموم ـ كلية الصيدلة ـ جامعة أم القرى ـ مكة المكرمة ـ المملكة العربية السعودية

الملخص العربي

بعد مئة عام من تصنيعه ما زالته عمل دواء الباراسيتامول مثيرة للجدل، حيث ترجح بعض الدراسات السابقة علي الجرذان أن التأثير المسكن لهذا الدواء ربما يستغل المسارات الهابطة في الجهاز العصبي المركزي المثبطة للإحساس بالألم والتي توظف مواد السيروتونين و الإندورفينات كوسائط لنقل الإشارة العصبية. والغرض من هذه الدراسة هو استكشاف هذا الإفتراض في الفئران وذلك باستخدام دواء "نالوكسون" الغالق لمستقبلات الافيونيات و دواء "باروكسيتين" المثبط لعملية استعادة السيروترنين داخل الألياف العصبية لهذه المسارات .

وقد تم تقسيم الحيوانات إلى مجموعتين رئيسيتين، كل لتجربة منفصلة، و قسمت كل مجموعة إلي ثلاث مجموعات فرعية، وظفت المجموعة الفرعية الأولي في كلي التجربتين كمجموعة ضة . والمجموعة الثانية فى كليهما تم حقنهما بالباراسيتامول في الغشاء البريتوني بجرعة 200 مجم لكل كجم. وبالنسبة للمجموعة الفرعية الثالثة، ففي التجربة الأولي تم مناولة الفئران عقار باروكسيتين بالفم بجرعة 20 مجم لكل كجم لمدة سبعة أيام متتالية قبل حقنها بالباراسيتامول، وفي التجربة الثانية تم حقنها بدواء نالوكسون بجرعة 5 مجم لكل كجم قبل حقنها بالباراسيتامول بثلاثين دقيقة. و في يوم التجربة تم قياس التأثير المسكن للباراسيتامول باستخدام إختبار السطح الساخن.

أظهرت النتائج أن التأثير المسكن للباراسيتامول تم تعضيده بواسطة دواء باروكسيتين، وظهر ذلك علي هيئة أمتداد لفاعلية الباراسيتامول لمدة اطول من تلك الملاحظة فى المجموعة الضابطة. بينه ألغي تماما دواء نالوكسون التأثير المسكن للباراسيتامول. وعليه فإن هذه النتائج تعضد الملاحظة السابقة في الجرذان وهي أن التأثير المسكن للباراسيتامول يشمل تنشيط المسارات الهابطة في الجهاز العصبي المركزي المثبط للإحساس بالألم، والتى تستخدم السيروتونين و الإندورفينات كوسائط لتوصيل الإشارات العصبية في ألياف هذه المسارات.

الكلمات الدالة:
باراستيمول- باروكسيتين - نالوكسون - فئران

ABSTRACT

Objectives: The mechanism(s) of analgesic action of paracetamol (acetaminophen; N-acetyl-p-aminophenol) remains controversial. Previous studies on rats suggest that the antinociceptive action of paracetamol might involve the central descending inhibitory pain pathways recruiting both a serotoninergic and an opioidergic system. This study explores this issue in mice using paroxetine, the most potent selective serotonin re-uptake inhibitor, and the nonselective opioid pure antagonist naloxone.

Animals were divided into two main groups for two separate experiments, each subdivided into 3 subgroups. In both experiments; the first group served as control, the second group received paracetamol (200 mg/kg, i.p). In one experiment, the third group received paroxetine (20 mg/kg p.o for 7 days) before paracetamol. In the other experiment, animals of the third group were pretreated with naloxone (5 mg/kg, i.p) 30 min before paracetamol. The antinociceptive effect of paracetamol was tested using the hot plate test.

Paracetamol displayed a significant antinociceptive activity that was augmented by pretreatment with paroxetine as was shown by maintenance of its effect beyond that shown by paracetamol alone. On the other hand, pretreatment with naloxone abolished paracetamol's antinociceptive activity in the hot-plate test.

These results extend the previous observation in rats that the antinociceptive effect of paracetamol involves activation of a central descending pain inhibitory pathway with serotonin and opioidergic peptides being potential mediators recruited.

Keywords*: Paracetamol, Paroxetine, Naloxone, Mice*

INTRODUCTION

More than 100 years after its synthesis, the mechanism of analgesic action of paracetamol (acetaminophen; N-acetyl- p-aminophenol) remains controversial. Postulated mechanisms[1,2], including inhibition of cyclooxygenase isozymes, have been inadequate [3]. Its inhibitory activity on the synthesis of prostaglandin is more evident on cyclo-oxygenase 1 than on cyclooxygenase 2 [4], both peripherally and within the CNS, even though the exact antinociceptive mechanism of action of this drug is still not completely clear[5]. Its biochemical properties, such as its weak inhibitory activity on the synthesis of peripheral prostaglandins, its low plasma-protein binding, its liposolubility and its ability to cross the blood-brain barrier suggest a central activity, which has been reported in several studies both in animals[6] and in humans[7].It has been postulated that this central effect might be linked to the ability of paracetamol to inhibit central cyclo-oxygenase [2,5]. On the other hand, it has been demonstrated that tissue cyclooxygenase in rat brain homogenates is not inhibited in doses of paracetamol up to 100 mg/kg[8]. Thus, the inhibition of cyclo-oxygenase may not be solely responsible for the central analgesic effect of non steroidal anti-inflammatory drugs (NSAIDs)[9].

There is evidence to suggest that the serotonergic system may play a role in the antinociceptive mechanism of NSAIDs[10] and of paracetamol[11]. There was considerable evidence supporting a role for 5-hydroxytryptamine (5-HT) in the modulation of nociceptive thresholds. Studies have shown that 5-HT plays an important role in the descending inhibitory pathway of pain transmission from

brainstem to the spinal cord. Descending pain pathways originate in brainstem nuclei, the hypothalamus and the cortex and interact with afferent fibers, interneurons and projecting neurons in the dorsal horn of the spinal cord. They are multiple and their stimulation leads to inhibitory effects in most studies [12, 13, 14]. The neurotransmitters involved in these descending controls are serotonin, noradrenalin, dopamine and opioids[15]. 5-Hydroxy-tryptamine, applied iontophoretically to dorsal horn neurons does reduce the nociceptive responses of these neurons. Alloui and colleagues [16] showed that the 5-HT_3 receptor antagonist, tropisetron, injected intrathecally, abolished the antinociceptive effect of paracetamol in an inflammatory pain model in rats. Most of the authors reported that 5-HT_3-receptor activation had an antinociceptive action [17, 18, 19, 20], while few showed an involvement of 5-HT_2 receptor subtype [21] or 5-HT_1subtype[22].

It has been also proposed that other neurotransmitter systems, including opioidergic pathways, may be involved in the central analgesic effect of this class of drugs[2]. Raffa and co-workers [23] have discovered that the analgesic effect of acetaminophen involves recruitment of endogenous opioid pathways that lead to antinociceptive spinal-supraspinal "self-synergy". They also demonstrated a synergistic enhancement of acetaminophen's antinociceptive action by spinal administration of phentolamine[24], implicating an interaction between descending endogenous opioid pathways and spinal sites. On the other hand, a recent clinical study on human volunteers that naloxone does not inhibit paracetamol antinociception, suggesting no significant implication of the opioid system in paracetamol mechanism of action[25].

The study of the impact of modulating the serotonergic and opioidergic systems on the analgesic activity of paracetamol, therefore, might throw some light on the complex antinociceptive activity of this widely used drug. Accordingly, we decided to conduct a study on both neurotransmitter systems, serotonergic and opioidergic, to gain further insight into the mechanism of the analgesic action of paracetamol.

The purpose of this study was twofold. First, to evaluate the impact of enhancing the central serotoninergic neurotransmission by the most potent selective serotonin reuptake inhibitor, paroxetine[26, 27], on the antinociceptive effect of paracetamol in the hotplate test, hence the clinically relevant potential drug interaction between therapeutic doses of both paracetamol and this selective serotonin reuptake inhibitor is highlighted. Second, to find out whether naloxone, the opiate receptors pure antagonist, was able to modify or prevent the antinociceptive effect of paracetamol in the same analgesimetric test, thus ruling out the potential involvement of endogenous opioid polypeptides in mediating the analgesic effect of this widely used medicine.

MATERIAL AND METHODS

I. Animals

Adult albino mice weighing 25 - 30 g of either sex were used in our study. They were purchased from the animal facility of the pharmacology department, College of Pharmacy, King Abdul-Aziz University. The animals were housed in cages kept under constant environmental and nutritional conditions throughout the period of investigation. They were allowed a free access to water and diet consisting of standard chow.

II. Drugs

Paracetamol was obtained from Sigma-Aldrich Company, USA. Paroxetine hydrochloride was obtained from GlaxoSmithKline Company. Naloxone HCl was obtained from Hikma Pharmaceuticals, Amman, Jordan. Drugs were freshly prepared in aqueous solution in a concentration adjusted so that the volume administered is 0.1ml/10 g animal weight.

III. Experimental design and treatment protocol

The animals were divided into two sets, dedicated each for a separate experiment. Each set was subdivided into three groups, consisting each of 10 mice.

a. Paroxetine experiment

Animals in Group 1 (served as normal control) as well as Group 2 were orally administered normal saline, at the same volume of the drug, for one week. In Group 3, paroxetine was daily administered by oral gavage in a dose of 20 mg/kg [28] for one week. At the end of the experiment day (on day 7), all the animals were subjected to the hotplate test to determine the baseline withdrawal latency (see below). Thereafter, animals in group 1 were intraperitoneally (i.p) injected with normal saline, while in group 2 and group 3, animals were i.p injected with paracetamol (200 mg/kg)[29] one hour after receiving the last oral dose of normal saline or paroxetine. Exactly after 15 min, the hotplate test was started as described below.

b. Naloxone experiment

On the experiment day, all the animals were subjected to the hotplate test before receiving any treatment to determine the baseline withdrawal latency (see below). Thereafter, animals in Group 1 were i.p injected with normal saline and served as control, while in group 2, animals were i.p injected with paracetamol (200 mg/kg). Animals in group 3 where pretreated with naloxone (5 mg/kg, i.p)[30] 30 min before paracetamol injection. Exactly 15 min after, the hotplate test was started as described below.

IV. Hot-plate test

The central antinociceptive activity of paracetamol was evaluated by using a modified hot plate test following the method of Lavich *et al*.[31]**.** This test measures the complex response to an acute, noninflammatory, nociceptive input and can be considered a good model for studying central antinociceptive activity[32.]

Animals were placed individually onto a hot plate with temperature fixed at 55±0.5°C (Harvard Apparatus Ltd., Kent, UK). Exposure to heat was continued till the animal shows withdrawal response in the form of hind paw licking, shaking or lifting or jumped off. To minimize tissue damage, a cut-off time (removing from the plate) of 30 seconds was adopted. The withdrawal latency was defined as the time period between the moment when the animal was placed on the hot plate surface and the moment when the animal licked, shaked or lifted any of its hind paws or jumped off to avoid thermal pain. The baseline latency (pretreatment value) was determined just before paracetamol or saline injection. The withdrawal latency was again determined at 15, 30, 45, 60, 75, 90, 105 and 120 min after. The prolongation in the withdrawal latency was taken as an index for the antinociceptive effect of paracetamol.

V. Data analysis

All values in this study are expressed as mean ± standard error of the mean (M±SEM). Data were analyzed by one-way analysis of variance (ANOVA). When variation among groups was found significant, Tukey-Kramer multiple comparisons test was carried out to compare between groups. Differences were considered significant when p value was < 0.05.

RESULTS

Effect of paroxetine

As shown in figure1, the normal (pretreatment) withdrawal latency of control mice was 13.25 ± 0.62 sec. After saline injection, this value showed insignificant variation along the whole experimental period. After injection of paracetamol, the withdrawal latency was gradually and significantly prolonged, starting 15 min after injection, reaching a maximum of 26.63 ± 0.86 seconds after 90 min. However, it started to decline back to

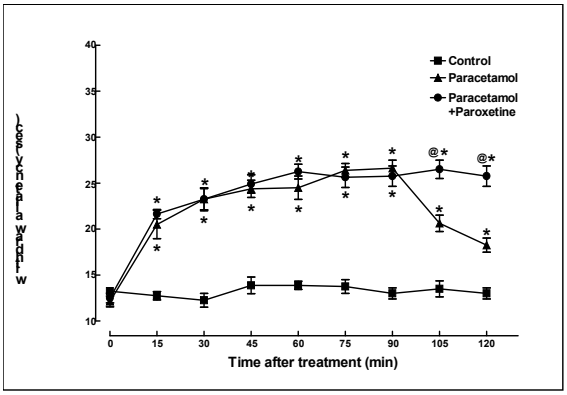

Figure 1: Antinociceptive effect of a single dose of paracetamol (200 mg/kg, i.p) in normal mice and in mice pretreated with paroxetine (20 mg/kg, p.o for 7 days). Values are represented as means ± S.E. of 8-10 separate experiments.
* Significantly different from respective control values at P<0.05.
@ Significantly different from respective paracetamol values at P<0.05.

Effect of naloxone

As shown in figure2, the normal (pretreatment) withdrawal latency of control mice was 8.88 ±1.13 sec. After saline injection, this value showed insignificant variation along the whole experimental period. After injection of paracetamol, the withdrawal latency was gradually and significantly prolonged, starting 30 min after injection, reaching a maximum of 23.13 ±0.7sec. after 75 min. However, it started to decline back to reach 10.88 ±1.37 sec. at the end of the evaluation period (120 min).

In naloxone-pretreated animals; the effect of paracetamol was reversed such that no antinociceptive effect was observed at any of the evaluation time points.

In paroxetine-pretreated animals, the effect of paracetamol did not quantitatively differ from that in paracetamol-only treated group. However, at the time where its effect started to fade in the paracetamol-only treated group (105 min), the antinociceptive effect of paracetamol continued at the same level, achieving a value of 25.75 ± 1.11 sec. at the end of the test period. It could be concluded that pretreatment with paroxetine potentiated the antinociceptive effect of paracetamol during the late phase of its action, leading to prolongation of its effect for at least 30 min.

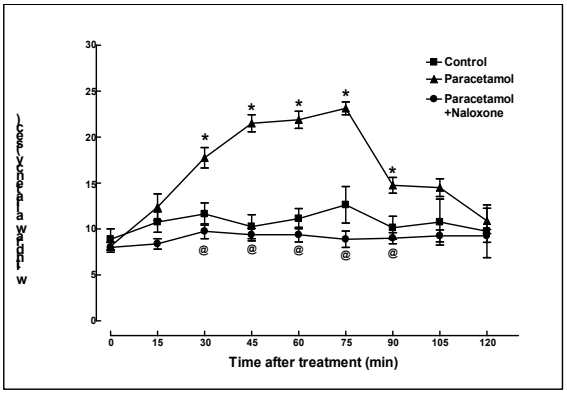

Figure 2: Antinociceptive effect of a single dose of paracetamol (200 mg/kg, i.p) in normal mice and in mice pretreated with naloxone (5 mg/kg, i.p 30 min before). Values are represented as means ± S.E. of 10 separate experiments.
* Significantly different from respective control values at P<0.05.
@ Significantly different from respective paracetamol values at P<0.05.

DISCUSSION

In the present investigation, the antinociceptive effect of paracetamol against thermal pain was evident shortly after i.p. injection, manifested as prolongation in the withdrawal latency 15-30 min after injection. This effect reached a maximum after 75-90 min, thereafter started to fade gradually. Paracetamol is often classified in the group of aspirin like[33] or NSAIDs-like drugs [34]. However, it does not share the same profile both in terms of therapeutic activities and side effects. This seems to be due, at least in part; to the inhibition of the synthesis of PGs [34].These

marked differences suggest that its mechanism of action may differ. In vitro, paracetamol weakly inhibits COX, compared to several NSAIDs [35] Clinical experiments have shown that therapeutic doses of paracetamol failed to reduce 6-keto-PGF1α urinary excretion[36] or PGE2 synovial fluid levels[37]. Also, Vane[38] demonstrated that paracetamol weakly inhibits peripheral COX but has a more potent effect on the centrally located enzymes. This limited inhibition of COX, especially of peripheral COX, led several authors to propose a central mechanism of action of paracetamol[19, 39]. Such a hypothesis is in line with the ability of paracetamol to cross the blood brain barrier both in rats [40] and humans[19], and with its efficacy maintained in animal pain models after central administration[16] and in models devoid of any inflammation and only sensitive to centrally acting drugs[41]. Some neurobiochemical hypotheses have been proposed for this centrally mediated effect since paracetamol reduces behavior induced by intrathecally injected substance P or N-methyl-D-aspartate (NMDA)[42]. Involvement of endogenous opioids[29, 32] and of another variant of COX 1 (COX 3) located in the CNS as a crucial enzyme inhibited by the drug[43]. An inhibitory effect of paracetamol on a COX1 variant (COX3) has been described by Botting[44]. However, the author stated that this enzyme would be involved in the resolution of inflammation, i.e. in a late phase after carrageenan administration, which excludes the involvement of such a mechanism in the ‘‘rapid’’ antinociceptive effect of paracetamol observed here. Hence, systemically administered paracetamol acts differently from aspirin and NSAIDs and independently of peripheral PG synthesis and of any anti-inflammatory effect.

There are already reports of the central actions of paracetamol in a variety of pain models [22, 32, 45] or of its actions at a spinal level [39, 46, 47]. These reports have also linked the actions of paracetamol to a descending 5-HT pathway [39, 46, 48]. Control of analgesia is performed by the descending inhibitory pathways in the central nervous system. The key part of this descending system is the periaqueductal grey area (PAG) which receives inputs from different brain regions and is assumed to be a gate in control of nociception, especially in the dorsal horn. PAG mainly stimulates the nucleus raphe magnus (NRM) and some fibers in the spinal cord, which form synaptic connections on dorsal horn interneurons. 5-HT is the major transmitter both at these synapses and the pathway from the NRM to the substantia gelatinosa of the dorsal horn[25]. Activation of this pathway inhibits transmission specifically in nociceptive pathways [49]. The 5-HT3 receptors located in the dorsal horn of the rat spinal cord have been shown to mediate an antinociceptive effect[50]. Alloui and his colleagues[46] demonstrated a spinal antinociceptive action for paracetamol that was reversed by the 5-HT_3 receptor antagonist, tropisetron. The augmentation of the antinociceptive action of paracetamol in mice being treated with paroxetine observed in this study may further highlights the involvement of 5-HT in this action and gives further insight into this postulation. Several authors have demonstrated a serotoninergic involvement in the antinociceptive effect of paracetamol[11, 16, 21, 48, 51, 52].

Since the antidepressant mechanisms of SSRI drugs are attributed to an increase in the amount and action of serotonin in the synaptic gap due to its serotonin re-uptake inhibitory effect on the presynaptic site[26, 27], extension of this effect to the descending serotoninergic spinal pathways would be conceivable as a mechanism of potentiation and/or prolongation of the analgesic effect of paracetamol. Indeed, Duman and co-workers[30] demonstrated that the 5-HT_3 receptors antagonist, ondansetron inhibits the antinociceptive effect of paroxetine, while the 5-HT_2 receptors antagonist ketanserin could not. This finding suggests

a contribution of 5-HT_3 receptors rather than 5-HT_2 types, to the antinociceptive action of paroxetine. In conclusion, both paracetamol and paroxetine antinociception implicate the descending inhibitory serotoninergic pathway in their effect, with 5-HT_3 subtype being the receptor involved. Our results, thus, would be compatible with a mechanistic scheme, which involved a central site of action of paracetamol, with algesia being devoid of a peripheral inflammatory component. The potential clinically relevant drug interaction between this widely used analgesic and SSRIs might warrant investigation on human volunteers.

In our study, the reversal of the antinociceptive action of paracetamol in mice being treated with naloxone supports the involvement of endogenous opioids in this action and gives further insight into this postulation. The results of the present study confirm that opioidergic system was engaged in the mechanism of paracetamol action. This observation is in agreement with results obtained by Pini *et al*[52] who also noted that the antinociceptive effect of paracetamol was reversed by nonselective opioid receptor antagonist naloxone in the hot-plate test in rats. Some studies have indicated that some NSAIDs exert a central opioid receptor-mediated effect[53], although the exact mechanism has not been fully elucidated. Indeed, indirect action on opioid receptors with release of endorphins or enkephalins has already been proposed for diclofenac[54] and ketorolac[55]. On the other hand, our result is not in line with that of Pickering *et al*,[25] who observed that naloxone does not inhibit paracetamol antinociception, in human volunteers, suggesting no significant implication of the opioid system in paracetamol mechanism of action. However, the authors attributed this apparent lack of effect to a matter of the power of their study, being carried out on only12 healthy male volunteers.

Possible interaction of paracetamol with naloxone binding sites has been investigated. Competition experiments demonstrated that paracetamol, though with low affinity, competes for [3H] naloxone binding sites[32]. This indicates that paracetamol may behave like morphine regarding not only its analgesic effect but also its action on μ-receptors. The authors suggested a dose-related effect in which paracetamol may bind directly to opioid receptors only at high concentrations. It is, however, hard to believe that paracetamol acts directly on opioidergic receptors since Pelissier and co-workers[11] were unable to demonstrate paracetamol affinity for these receptors *in vitro*. It may be, therefore, suggested that paracetamol activates opioidergic system indirectly via still unknown mechanism or mechanisms. In this regard, it has been suggested that paracetamol indirectly activate opiate receptors that in turn may increase 5-HT levels, at least in the cerebral cortex and in the pons, thus provoking an analgesic effect[47]. Indeed, in the mechanism of action of paracetamol, a 5-HT-mediated antinociception is of interest because central 5-HT activation potentiates the effect of opioids, as observed in rats[56] and humans[57]. On the other hand, it has been shown that naloxone blocks the increase in 5-HT levels in the brain induced by paracetamol[32]. These potentially regulatory and interactive mechanisms between 5-HT and opioid transmission in nociception are supported by the finding that the analgesic effect of paracetamol depends on an intact 5-HT neurotransmission and is antagonized by the opioid antagonist naloxone [32]. Noteworthy, morphine induces changes in the serotoninergic system similar to those obtained with paracetamol, which are also reversed by naloxone. Thus it may be hypothesized that paracetamol, in acting on opiate receptors, may release 5-HT that provokes an analgesic effect. This is supported by many findings which indicate that 5-HT takes part in the complex nociceptive pathways and plays a pivotal role in antinociception[58]. In conclusion, these data provide further evidence for a

central antinociceptive effect of PARA antagonized by naloxone, which suggests that this activity may involve the opioidergic pathways which in turn activate the serotonergic system.

REFERENCES

1. Walker JS. NSAID: an update on their analgesic effects. Clin. Exp. Pharmacol. Physiol. 1995; 22: 855-860.

2. Björkman R. Central antinociceptive effects of non-steroidal anti-inflammatory drugs and paracetamol. Acta Anaesthesiol Scand. 1995; 39 (Suppl. 103): 2- 44.

3. Warner TD, Vojnovic I, Giuliano F, Jime´nez R, Bishop-Bailey D, Mitchell JA. Cyclooxyge-nases 1, 2, and 3 and the production of prostaglandin I2: investigating the activities of acetaminophen and cyclooxygenase-2-selective inhibitors in rat tissues. J Pharmacol Exp Ther. 2004; 310: 642-647.

4. Vane JR, Botting RM. A better understanding of anti-inflammatory drugs based on isoforms of cyclooxygenase (COX-1 and COX-2). Adv Prostaglandin Thromboxane Leukot Res. 1995; 23:41-48.

5. Clissold S P. Paracetamol and Phenacetin. Drugs. 1986; 32: suppl. 4: 46-59.

6. Carlsson KH, Monzel W, Jurna I. Depression by morphine and the non-opioid analgesic agents metamizol (Dipyrone), lysine acetylate and paracetamol, of activity in rat thalamus neurons evoked by electrical stimulation of nociceptive afferents. Pain. 1988; 32: 313-326.

7. Chen ACN, Chapman CR. Aspirin analgesia evaluated by event related potentials in man: possible central action in brain. Exp Brain Res. 1980; 39: 359-364.

8. Abdel-Alim MS, Sjoqvist B, Anggard E. Inhibition of prostaglandin synthesis in rat brain. Acta Pharmacol Toxicol. 1978; 43: 266-272.

9. Malberg AB, Yaks, TL. Antinociceptive actions of spinal anti-inflammatory agents on the formalin test in the rat. J Pharmacol Exp Ther. 1992; 263: 136-146.

10. Sandrini M, Vitale G, Dondi M, Pini LA. Effects of acetylsalicylic acid on serotonin brain receptor subtypes. Gen Pharmacol. 1995; 26: 737-41.

11. Pelissier T, Alloui A, Caussade F, Dubray C, Cloarec A, Lavarenne J, Eschalier A. Paracetamol exerts a spinal antinociceptive effect involving an indirect interaction with 5-hydroxytryptamine3 receptors: in vivo and in vitro evidence. J Pharmacol ExpTher.1996; 278:8-14.

12. Willer JC, Roby A, Le Bars D. Psychophysical and electrophysiological approaches to the pain-relieving effects of heterotopic nociceptive stimuli. Brain. 1984; 107: 1095-1112.

13. Price DD, McHaffie JG. Effects of heterotopic conditioning stimuli on first and second pain: a psychophysical evaluation in humans. Pain.1988 ;34: 245-252.

14. Talbot JD, Duncan GH, Bushnell MC. Effects of diffuse noxious inhibitory controls (DNICs) on the sensory-discriminative dimension of pain perception. Pain.1989; 36: 231-238.

15. Julien N, Marchand S. Endogenous pain inhibitory systems activated by spatial summation are opioid-mediated. Neurosci Lett. 2006; 401: 256-260.

16. Alloui A, Pelissier T, Cloarec A, Lavarenne J, Eschalier A. Tropisetron inhibits the antinociceptive effect of intrathecally administered paracetamol and serotonin. Fundam. Clin Pharmacol. 1996; 10: 406-407.

17. Glaum SR, Proudfit HK, Anderson EG. Reversal of the antinociceptive effects of intrathecally administered serotonin in the rat by a selective 5-HT3 receptor antagonist. Neurosci Lett.1988; 95: 313-317.

18. Glaum SR, Proudfit HK, Anderson EG.5-HT3 receptors modulate spinal nociceptive reflexes. Brain Res. 1990, 510:12-6.

19. Bannwarth B, Netter P, Lapicque F, Gillet P, Pe´re P, Boccard EJ, Eschalier A. Paracetamol exerts a spinal antinociceptive effect involving an indirect interaction with 5-Hydroxytryptamine3 receptors: in vivo and in vitro evidence. J Pharmacol Exp Ther. 1996; 278: 8-14.

20. Bardin L, Schmidt J, Alloui A, Eschalier A. Effect of intrathecal administration of serotonin in chronic pain models in rats. Eur J Pharmacol. 2000; 409: 37-43.

21. Srikiatkhachorn A, Trasu N, Govitrapong P. Acetaminophen induced antinociception via central 5-HT 2A receptors. Neurochem Int. 1999; 34: 491-498.

22. Graham GG, Scott KF. Mechanism of action of paracetamol. Am J Ther. 2005; 1:46-55.

23. Raffa RB, Stone Jr, DJ, Tallarida RJ. Discovery of "self-synergistic" spinal/supraspinal antinociception produced by acetaminophen (paracetamol). J Pharmacol Exp Ther. 2000; 295: 291- 294.

24. Raffa RB, Stone Jr, DJ, Tallarida RJ. Unexpected and pronounced anti-nociceptive synergy between spinal acetaminophen (paracetamol) and phentolamine. Eur J Pharmacol. 2001; 412: R1- R2.

25. Pickering G, Moustafa F, Desbrandes S, Cardot JM, Roux D, Dubray C. Paracetamol and opioid pathways: a pilot randomized clinical trial. Fundamental Clin Pharmacol. 2011(Ahead of print).

26. Bourin M, Fiocco AJ, Clenet F. How valuable are animal models in defining antidepressant activity? Hum Psychopharmacol. 2001; 1: 9-21.

27. Richelson E. Where are all the novel antidepressants? Curr Opin Investig. Drugs. 2001; 2: 256-8.

28. Takeuchi T, Owa T, Nishino T, Kamei C. Assessing anxiolytic-like effects of selective serotonin reuptake inhibitors and serotonin-noradrenaline reuptake inhibitors using the elevated plus maze in mice. Methods Find Exp Clin Pharmacol. 2010; 32:113-21.

29. Rezende RM, França DS, Menezes GB, dos Reis WG, Bakhle YS, Francischi JN. Different mechanisms underlie the analgesic actions of paracetamol and dipyrone in a rat model of inflammatory pain. Br J Pharmacol. 2008; 153:760-768.

30. Duman NE, Kesim M, Kadioglu M, Yaris E, Kalyoncu NI, Erciyes N. Possible involvement of opioidergic and serotonergic mechanisms in antinociceptive effect of paroxetine in acute pain. J Pharmacol Sci 2004; 94:161-165.

31. Lavich TR, Cordeiro RS, Silva PM, Martins MA. A novel hot-plate test sensitive to hyperalgesic stimuli and non-opioid analgesics. Braz J Med Biol Res. 2005; 38:445-51

32. Pini LA, Vitale G, Ottani A, Sandrini M. Naloxone-reversible anti-nociception by paracetamol in the rat. J Pharmacol ExpTher. 1997; 280: 934-940.

33. Ferreira SH. Prostaglandins, aspirin like drugs and analgesia. Nature 1972; 240: 200-203.

34. Insel, PA. Analgesic– antipyretic and anti-inflammatory agents and drugs employed in the treatment of gout. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Goodman
Gilman A. (Eds.). Goodman and Gilman's The Pharmacological Basis of Therapeutics, 1996, 9th edn. McGraw-Hill, USA, pp: 617- 657

35. Mitchell JA, Akarasereenont P, Thiemermann C, Flower RJ, Vane JR. Selectivity of non steroidal anti-inflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. Proc Natl Acad Sci. 1994; 90:11693-11697.

36. Seppala E, Laitinen O, Vapaaltalo H. Comparative effects of acetyl-salicylic acid, indomethacin and paracetamol on metabolites of arachidonic acid in plasma and urine in man. Int J Clin Pharmacol Res. 1983; 4: 265-269.

37. Bippi H, Frohich JC. Effects of acetylsalicylic acid and paracetamol alone and in combination on prostanoid synthesis in man. Br J Clin. Pharmacol. 1990; 29: 305-310.

38. Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin like drugs. Nat New Biol. 1971; 231: 232-235.

39. Bonnefont J, Chapuy E, Clottes E, Alloui A, Eschalier A. Spinal 5-HT1A receptors differentially influence nociceptive processing according to the nature of the noxious stimulus in rats: effect of WAY-100635 on the antinociceptive activities of paracetamol, venlafaxine and 5-HT. Pain. 2005; 114:482-490.

40. Courade J, Caussade F, Martin K, Besse D, Delchambre C, Hanoun N, Hamon C, Eschalier A, Cloarec A. Effect of acetaminophen on monoaminergic system in the rat central nervous system. Naunyn Schmiedebergs Arch Pharmacol. 2001; 364:534-537.

41. Carlsson KH, Jurna I. Central analgesic effects of paracetamol manifested by depression of nociceptive activity in thalamic neurons of the rat. Neurosci Lett. 1987; 79: 339- 343.

42. Björkmann R, Hallman KM, Hedner J, Hedner T, Henning M. Acetaminophen blocks spinal hyperalgesia induced by NMDA and substance P. Pain. 1994; 57: 259-264.

43. Botting R, Ayoub SS. COX-3 and the mechanism of action of acetaminophen / paracetamol. Prostaglandins Leukot Essent Fatty Acids. 2005; 72: 85-87.

44. Botting, RM. Mechanism of action of paracetamol: Is there a cyclooxygenase 3? Clin Infect Dis. 2000; Suppl 5: S202-S210.

45. Bonnefont J, Courade JP, Alloui A, Eschalier A. Antinociceptive mechanism of action of paracetamol. Drugs. 2003; 63:1-4.

46. Alloui A, Chassaing C, Schimidt J, Ardidi D, Dubray C, Cloarec A. Paracetamol exerts a spinal, tropisetron-reversible, antinociceptive effect in an inflammatory pain model in rats. Eur J Pharmacol. 2002; 443: 71-77.

47. Raffa RB, Walker EA, Sterious SN. Opioid receptors and paracetamol (acetaminophen). Eur J Pharmacol. 2004; 503: 209-210.

48. Sandrini, M., Vitale, G., Ottani, A., Pini, L.A. The potentiation of analgesic activity of paracetamol plus morphine involves the serotoninergic system in rat brain. Inflamm Res. 1999; 48: 120- 127.

49. Fields HL, Basbaum AI. Central nervous system mechanisms of pain modulation. In: Wall PD, Melzack R, editors. Textbook of pain. 3rd ed. Edinburgh: Churchill Livingstone; 1994. Pp: 243-257.

50. Sasaki M, Ishizaki K, Obata H, Goto F. Effects of 5-HT2 and 5-HT3 receptors on the modulation of nociceptive transmission in rat spinal cord according to the formalin test. Eur J Pharmacol. 2001; 424: 45-52.

51. Tjolsen A, Lund A, Hole K. Antinociceptive effect of paracetamol in rats is partly dependent on spinal serotonergic systems. Eur J Pharmacol. 1991; 193: 193-201.

52. Pini L.A, Sandrini M, Vitale G. The antinociceptive action of paracetamol is associated with changes in the serotoninergic system in the rat brain. Eur J Pharmacol. 1996; 308: 31-40.

53. Vanegas H, Tortorici V. Opioidergic effects of nonopioid analgesics on the central nervous system. Cell Mol Neurobiol. 2002; 22:655-61.

54. Sacerdote P, Moza G, Mantegazza P, Panerai AE. Diclofenac and pirprofen modify pituitary and hypothalamic beta-endorphin concentrations. Pharmacol Res Commun. 1983; 17: 679-684.

55. Domer F. Characterization of the analgesic activity of ketorolac in mice. Eur J Pharmacol. 1990; 177:127-135.

56. Baraldi M, Poggioli R, Santi M, Verogoni AV, Bertolini A. Antidepressantsand opiates interactions: pharmacological and biochemical evidences. Pharmacol Res Commun. 1983; 15: 843-857.

57. Bentley, K. C. and Head, T. W.: The additive analgesic efficacy of acetaminophen,1000 mg, and codeine, 60 mg, in dental pain. Clin Pharmacol Ther. 1987; 42: 634-640.

58. Malmgren R. The central serotoninergic system. Cephalalgia. 1990; 10:199-204.

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Original Article

Accuracy Of Fine Needle Aspiration Cytology Of Thyroid Swellings in Comparison of Histopathology : Experience from King Faisal Hospital, Makkah, Kingdom of Saudi Arabia.

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مدى دقة وتوافق الفحص الخلوى بارتشاف الابرة الرفيعة (العينة الخزعية) في تشخيص اورام الغدة الدرقية مقارنة بالفحص النسيجي: خبرة من مستشفى الملك فيصل، مكة المكرمة، المملكة العربية السعودية.

ا
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الملخص العربي:

المقدمة: تعد اورام الغدة الدرقية من الحالات الشائعة التي يتم فحصها بعيادات الجراحة الخارجية وهناك كثير من الفحوصات لتقييم اورام الغدة الدرقية قبل الجراحة ومن اكثرها شيوعا الفحص الخلوى بارتشاف الابرة الرفيعة (العينة الخزعية) والتي تعتبر الفحص الذهبي والمسح المبدئ لتشخيص اورام الغدة الدرقية وذلك لسهولته ورخصه ونتائجه الجيدة والذي اثبتته سلسلة من الدراسات والبحوث

الهدف : تهدف هذه الدراسة لمعرفة وتحديد مدي دقة وكفاءة الفحص الخلوى بارتشاف الابرة الرفيعة (العينة الخزعية) في تشخيص اورام الغدة الدرقية مقارنة بالفحص النسيجي

الطريقة : لقد تمت دراسة ملفات ونتائج فحوصات 106 مريض بقسم الحراحة بمستشفى الملك فيصل، مكة المكرمة والذين اجري لهم الفحص الخلوى بارتشاف الابرة الرفيعة (العينة الخزعية) قبل العملية الجراحية لتشخيص اورام الغدة الدرقية ثم تمت مقارنة النتائج بالفحص النسيجي بعد الجراحة لكل المرضي في الفترة من يناير 2007 وديسمبر 2011

النتائج : اظهرت نتائج الدراسة ان الفحص الخلوى بارتشاف الابرة الرفيعة (العينة الخزعية) في تشخيص اورام الغدة الدرقية قبل الجراحة بين ان 81 (76.4 %) حالة من بين 106 اورام حميدة و 8 (7.5 %) اورام سرطانية و 17 حالة (16%) غير محددة بينما الفحص النسيجي اظهر ان 91 حالة (85.8 %) اورام حميدة و 15 (14.2 %) اورام سرطانية وقد دللت الدراسة على الحساسية العالية للعينة الخزعية لأورام الغدة الحميدة بينما الأورام السرطانية لهذا الفحص تحتاج لدعم وفحوصات أكثر دقة للتأكد.

الخلاصة : ان الفحص الخلوى بارتشاف الابرة الرفيعة (العينة الخزعية) في تشخيص اورام الغدة الدرقية عالي الحساسية للأورام الحميدة وغير محدد في كثير من الاورام السرطانية وقد أظهرت هذه الدراسة نسبة حساسية 53.33% ,و نسبة الدقة 89.01% , والقيمة التنبؤية الايجابية 44.44% و القيمة التنبؤية السالبة 92.05%

الكلمات الدالة : اورام الغدة الدرقية ـ الفحص الخلوى بارتشاف الابرة الرفيعة (العينة الخزعية) – فحص النسيج الخلوي.

ABSTRACT

Objectives: The aim of this study was to determine the efficacy and accuracy of FNA cytology in diagnosis of thyroid swellings versus histopathology result in our surgical unit.
Methods: Between January 2007 - December 2011. A total of 106 patients who underwent thyroid surgery at King Faisal Hospital, Makkah and who had preoperative FNAC were enrolled in this retrospective study. The preoperative FNAC results were compared with definitive histological diagnosis following thyroid surgery. Fine needle aspiration cytology was performed using aspirate techniques on each thyroid swelling. The cytological sample was assessed by a single centre consultants pathologist and was classified as, non-neoplastic, neoplastic, suspicious or indeterminate. The histology were classified as non-neoplastic (benign), neoplastic (malignant).
Results: Out of 106 patients included in this study fine needle aspiration cytology analysis revealed 81(76.4%) non-neoplastic, 8 (7.5%) neoplastic and 17 (16%) suspicious aspirates (indeterminate). Histological analysis showed 91 (85.8%) benign, 15 (14.2%) malignant specimens. Fine needle aspiration cytology had a sensitivity , specificity and accuracy rate of ?%, respectively for diagnosing thyroid malignancy. Our results indicate that FNAC is very useful in the diagnosis of thyroid pathology specially benign conditions. However, complete histopathological analysis is essential to distinguish suspicious conditions.
Conclusion: FNAC is safe and cost-effective diagnostic modality in the investigation of thyroid pathology pre-operatively and plays a useful role in planning the surgical management of thyroid nodules and still not decisive for suspicious / undetermined cases. However, results must be interpreted with the clinical picture in mind. The suspicious and indeterminate results prove to be an area of uncertainty often resolved by diagnostic surgical resection and tissue cytology.
Keywords: Thyroid swellings; FNA; Accuracy, histopathology

INTRODUCTION

Thyroid swellings are common clinical findings among patients in surgical outpatient clinic. Have a reported prevalence of 4-8% of the adult population [1,2,3]. Most of the thyroid swellings turned out to be benign and approximately 5-10% are anticipated to be malignant [4] .The problem in clinical practice is to distinguish reliably the few malignant tumours from the many harmless nodules so that a definitive pre-operative tissue diagnosis of malignancy allows planning of appropriate surgery and relevant patient counseling.

There are several tests, such as high resolution ultrasonography, radioisotope scanning and FNA biopsy have been used for evaluation of thyroid swellings before proceeding to thyroid surgery. Studies have demonstrated that among all these diagnostic modalities, Fine-needle

aspiration cytology (FNAC), as practiced today is an interpretive art with histopathology as its scientific base [1]. The FNAC was first reported by Martin and Ellis in 1930 [4,] and then in 1960 Swedish perfected the concept and in 1970s it gained acceptance in the UK and the USA[5,6.] Now, FNAC is practiced world-wide and has become a critical step in the evaluation and screening test for diagnosis of thyroid swellings[6,7]. It is well established out-patient procedure used in the initial diagnosis of thyroid swellings. It is simple, cost effective screening test, readily repeated, and quick to perform in the outpatient clinics with excellent patient compliance.

The limitations include false negative results, false positive results and a proportion of FNA results that are not obviously benign or malignant and fall into the indeterminate or suspicious group. Published data showed diagnostic accuracy of FNAC varies between different series. [7,8.]

Objective:

The aim of this study was to determine the efficacy and accuracy of FNAC in diagnosis of thyroid swellings in our surgical department and compare and matching, accuracy, sensitivity, specificity, positive predictive value and negative predictive value of the results in various thyroid swellings in correlation with histologic diagnosis.

MATERIAL AND METHODS

In this study retrospectively we evaluated 106 FNA performed between January 2007-December 2011, at department of surgery, King Faisal Hospital Makkah, KSA.. FNA were performed on all patients presented with palpable thyroid swellings in the surgical outpatient clinics and subsequently underwent a thyroid surgery and concurrent postoperative histopathologic tissue diagnoses were compared to estimate the accuracy of FNAC. The data was collected from medical records. All FNACs were carried out by consultant pathologist in tertiary hospital laboratory. FNAC and histology specimens were analyzed by a consultant pathologist same centre. FNAC results were classified, *interpreted and analyzed accordingly. The lesions were categorized into benign, suspicious/undeterminate and neoplastic conditions. The cytological findings were compared with those of histopathology wherever available.* The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of FNAC in diagnosing thyroid swellings was calculated. Statistical analysis was done by using SPSS software.

RESULTS

From 2007-2011 a total of 106 aspirates of thyroid swellings with subsequent thyroid surgery were identified, 12 (11.3%) were male and 94 (88.7%) were females. Age of the patients ranged from 22 to 68 years characteristics of the patients were shown in Table 1,
cytological and histological results is shown in table 2 for diagnosis of thyroid diseases. The study showed that FNAC most common lesion was benign 81 (76.4%), and 8 (7.5%) cases were malignant and 17 (16 %) were undetermined lesions. In comparison with histopathology of post operative tissue results, the benign lesions were 91 (85.8%), and malignant cases were 15 (14.2%). Among the 106 cases of histopathological results of FNAC correlations were available, the sensitivity, specificity, negative predictive value and positive predictive value in this study as shown sensitivity of 53.33%, specificity of 89.01%, NPV of 92.05%, PPV of 44.44%. Table3. This FNAC results have been compared with the corresponding

Category	FNAC	P.O*
Benign	81	91
Malignant	8	15
Suspicious/undeterminate	7	0
Adenomatous goitre	2	0
Hashimoto's	3	0
Follicular adenoma	2	0

histological diagnosis including indeterminate FNAC sample with the final results of post operative tissue examination Table2. In our study the final histopathological tissue examination revealed no suspicious/indeterminate cases and all the seven cases of indeterminate of FNAC, which has been reported as adenomatous goiter, hashimoto's and follicular adenoma has been proved in the final histopathology malignant Table2 Table4.

Table 1 : Patients characteristics

Characteristics	Total patients(n =106)
Age (in years)	
20-29	**9**
30-39	**38**
40-49	**43**
50-59	**14**
> 60	**2**
Sex	
Male	**12**
Female	**94**
Duration of complaints	
<6 months	**7**
6-24 months	**24**
2-3 years	**26**
>3 years	**49**
Treatment history	
Yes	**32**
No	**74**

Table-2: Nature of thyroid swellings in FNAC and histopathology (number of patients n =106)

Thyroid lesion	FNAC	Histopathology
Begnin	81 (76.4 %)	91 (85.8 %)
Malignant	8 (7.5%)	15 (14.2 %)
Undetermined	7 (6.7%)	None (0 %)

Table-3: Statistical analysis for FNAC indices results

Indecis	FNAC
Sensitivity.	53.33 %
Specificity	89.05 %
NPV	92.05 %
PPV	44.44%

Table 4: Benign or suspicious neoplastic lesions diagnosed by FNAC and their comparison with histopathological diagnosis

P.O. = Postoperative histopathology.

DISCUSSION

Thyroid enlargement, whether diffuse or nodular, leads to a big range of investigations, mainly to rule out the possibility of a neoplasm or toxicity. FNAC is the first line of investigation and other investigations like ultrasonography, thyroid function test, thyroid scan and antibody levels are done subsequently for appropriate management [9]. The sensitivity of thyroid FNAC ranges from 78-92 % and its specificity from 74-99%.[10,11,12].

In our study the sensitivity was 53.33% and specificity 89.01% . This shows that FNAC is more specific than sensitive in detecting thyroid benign cases . The diagnostic accuracy for cytologic diagnosis for malignant cases was matching and comparable with other studies.[9,13,]

Fine needle aspiration cytology is regarded as the gold standard initial investigation in the diagnosis of thyroid swellings. The technique is safe simple and quick with a low complication

rate. FNAC has been shown to have similar or higher sensitivity and accuracy levels than frozen section examination [14,15.] In our study FNAC has shown lower sensitivity and accuracy levels than post operative tissue diagnosis. There is a group of lesions which overlap benign and malignant features, 17 cases labeled as undetermined in FNAC while in histopathology were clearly distinct. Cytological diagnosis of follicular adenoma versus carcinoma is not possible on FNAC and diagnosis is dependent on histological assessment for capsular/vascular invasion. Another limitation of thyroid FNAC is the large number of inadequate aspirates. Published data suggest inadequate sample ranges between 9-31% [16,17]. In the published data,

the sensitivity, specificity and accuracy of thyroid FNAC in detecting malignancy ranges from 84-86%, 52-86% [18,19], respectively. In our study sensitivity rate was low compared to others as three out of four (75%) 'indeterminate' FNAC results were later found to have malignancy on histological examination. Our positive and negative predictive values are comparable with published data is higherr than published data [20,21].

CONCLUSION

From this study, it can be concluded that FNAC is simple, very useful and readily available tool for evaluating thyroid swellings. It is safe and cost-effective diagnostic modality in the investigation of thyroid pathology pre-operatively and plays a useful role in planning the surgical management of thyroid nodules. However, results must be interpreted with the clinical picture in mind. The suspicious and indeterminate results prove to be an area of uncertainty often resolved by diagnostic surgical resection.

REFERENCES

1. Oertel YC. "Fine-needle aspiration and the diagnosis of thyroid cancer," Endocrinology and metabolism ; Clinics of North America 1996: 25 ,1: 69–91

2. Ali Rizvi SA, Husain M, Khan S, Mohsin M. A comparative study of fine needle aspiration cytology versus non-aspiration technique in thyroid lesions. Surgeon 2005, 4:273-276.

3. Ylagan LR, Farkas T, Dehner L. Fine needle aspiration of the thyroid: a cytohistologic correlation and study of discrepant cases.Thyroid 2004; 14: 35-41

4. 5. Martin HE, Ellis EB. Biopsy by needle puncture and aspiration.Ann Surg 1930;92:169–81.

5. Lewis CM, Chang KP, Pitman M, Faquin WC,Randolph GW. Thyroid fine needle aspiration biopsy: variability in reporting . Thyroid 2009; 19: 717-723

6. Wienke JR, Chong WK, Fielding JR, Zou KH, Mittelstaedt CA. Sonographic features of benign thyroid nodules. Interobserver reliability and overlap with malignancy American Institute of Ultrasound in Medicine. J Ultrasound Med 2003;22:1027-31.

7. Stojadinovic A, Peoples GE, Libutti SK, Henry LR, Eberhardt J, Howard RS. Development of a clinical decision model for thyroid nodules. BMC Surg 2009;9:12.

8. Morgan JL, Serpell JW, Cheng MS. Fine-needle aspiration cytology of thyroid nodules: how useful is it? ANZ J Surg 2003;73:480-3.

9. De Micco C, Zoro P, Garcia S et al. Thyroid peroxidase immunodetection as a tool to assist diagnosis of thyroid nodules on fine needle aspiration biopsy. Eur J Endocrinol 1994;131:474-9.

10. Ko HM, Jhu IK, Yang SH et al. Clinicopathologic analysis of fine needle aspiration cytology of the thyroid. A review of 1,613 cases and correlation with histopathologic diagnoses. Acta Cytol 2003;47:727- 32.

11. Bista M, KC Toran, Regmi D, Maharjan M, Kafle P, Shrestha S. Diagnostic accuracy of fine needle aspiration cytology in thyroid swellings. J Nepal Health Res Counc 2011;9:14-6.

12. Guhamallick M, Sengupta S, Bhattacharya NK et al. Cytodiagnosis of thyroid lesions - usefulness and pitfalls: A study of 288 cases. J Cytol 2008;25:6-9.

13. Mahar SA, Husain A, Islam N. Fine needle aspiration cytology of thyroid nodule: Diagnostic accuracy and pitfalls. J Ayub Med Coll Abbottabad 2006;18:26-9.

14. Sclabas GM, Staerkel GA, Shapiro SE, et al. Fine-needle aspiration of the thyroid

and correlation with histopathology in a contemporary series of 240 patients. Am J Surg 2003 ; 186: 702-10.

15. Baloch ZW, Sack MJ, Yu GH, Livolsi VA, Gupta PK. Fine needle aspiration of thyroid: an institutional experience. Thyroid 1998, 8:565-569.

16. Burch HB, Burman KP, Reed HL, Buckner L, Raber T, Owenbey JL. Fine needle aspiration biopsy of thyroid nodules: determinants of insufficiency rate and malignancy yield at thyroidectomy. Acta Cyto. 1996, 40:1176-1183.

17. Gharib H, Goellner JR, Johnson DA. Fine needle aspiration cytology of the thyroid: a 12 year experience with 11000 biopsies. Clin Lab Med.1993, 13:699-709.

18. Holleman f, Hoekstra JB, Ruitenberg HM. Evaluation of fine needle aspiration cytology in the diagnosis of thyroid nodules. Cytopathology. 1995, 6:175-186.

19. Cap J, Ryska A, Rehorkova P, Hovorkova E, Kerekes Z, Pohnetalova D. Sensitivity and specificity of the fine needle aspiration biopsy of the thyroid: clinical point of view. Clinical endocrinology 1999, 51:509-515.

20. SahinM, Sengul A, Berki Z, Tutuncu NB, Guvener ND. Ultrasound-guided fine-needle aspiration biopsy and ultrasonographic features of infracentimetric nodules in patients with nodular goiter: correlation with pathological findings. Endocr Pathol2006;17:67–74. CrossRefMedline

21. CaiXJ, Valiyaparambath N, Nixon P, Waghorn A, Giles T, Helliwell T. Ultrasound-guided fine needle aspiration cytology in the diagnosis and management of thyroid nodules. Cytopathology2006;17: 251–256. CrossRefMedline

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Original Article

P53 and *P63* as Associated Molecular Markers in Breast Cancer in Saudi Arabia Patients

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رابطة الواسمات الجزيئية مع الدرجات والمراحل المختلفة لسرطان الثدي. بالمملكة العربية السعودية

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الملخص العربي:

الهدف : هذه الدراسة تهدف الي التحقق و تقييم قيمة التعبير الوراثي في الجينات p63 و p53 في سرطان الثدي . وبالإضافة إلى ذلك، لتحديد رابطة هذه الواسمات الجزيئية مع الدرجات والمراحل المختلفة لسرطان الثدي. بالمملكة العربية السعودية
الطريقة :تم جمع عينات الأنسجة 26 الذين يعانون من سرطان الثدي .تم تنفيذ أسلوب المناعية الصبغيه للكشف عن البروتين p53 و .p63 وكان التعبير الجيني عن P53 كان أيجابيا في 15.3٪ من مجموع العينات في حين أن التعبير الجيني عن p63
النتائج : كان ايجابيا في ٪ 19.1 من العينات. ووجدت الدراسة أن الجينات p63 و p53 كان إيجابيا في حالة سرطان الثدي ولا سيما في الفئة العمرية 30-60 سنة ومع المرحلة الثانيه والثالثه من المرض .
الخلاصة : الدراسه الحاليه تبين ان التعبير الجيني ل p63 و P53 لا يرتبطان معا مما يوحي بأن p63 يمكن أن يعمل بشكل غير مباشر على أنه الجين الورمي وتثبيط عمل P53 مما يفسر فرضية ارتباط p63 مع مؤشرات أخرى عديدة من المضاعفات الخطيرة.

ABSTRACT

Objectives:. The aim of the study is to evaluate and investigate the prognostic value of *p53* and *p63* genes in breast cancer in Saudi patients.
Method and Materials: A total of 26 tissue samples from men and women suffering from breast cancer were collected. Immunohistochemistry method was performed to detect *p53* and *p63*.
Results: The expression of *p53* was positive in15.3% of the total cases of samples while the expression of *p63* was positive in 19.1% of samples. The study found that the *p53* and *p63* genes was positive in case of breast cancer, especially within the age group 30-60 years old and with grade II, III.
Conclusion, *p53* is rarely co expressed with *p63*, suggesting that *p63* could act indirectly as an oncogene by inhibiting *p53*. This hypothesis could also explain why *p63* correlated with several other indicators of poor prognosis.

Keywords*: Breast cancer, p53, p63, immunohistochemistry, prognosis, Saudi Arabia.*

INTRODUCTION

The global burden of cancer has more than doubled in the past 30 years and breast cancer is the most common cancer among women worldwide (Lord, et al., 2007). It was reported by World Health Organization (WHO) that 636,000 and 514,000 incident cases occurred in developed and developing countries during 2008, respectively.

Breast cancer, one of the most frequent and deadly cancers in women, has been recognized as a heterogeneous disease in terms of natural history, genetic alteration, histopathological features, gene expression profile, and response to treatment in individual patients (Lakhani and Ashworth, 2001; Quackenbush, 2006; Sotiriou and Piccart, 2007; Stingl and Caldas, 2007).

Male breast cancer is a rare condition, accounting for only about 1% of all breast cancers. The American Cancer Society estimates that in 2008, about 1,990 new cases of breast cancer in men will diagnose and that breast cancer will cause approximately 480 deaths in men in comparison with more than 40,000 women die of breast cancer each year. Most cases of male breast cancer is detected in men between the ages of 60 and 70, although the condition can develop in men of any age (Fattaneh et al., 2003). The causes of the breast cancer in male are the same in female (Borgen et al., 1992; Fentiman et al., 2006).

P53, located at 17p13.1, was the first gene identified as a mutant in human tumors. Its

normal protein product participates in regulation of the cell cycle and in apoptosis. Mutations of *p53* have occurred in 17–40% of sporadic breast cancers examined, (Coles et al., 1992) and most of them are missense mutations concentrated in a core region that encodes the sequence-specific DNA-binding. Mutant forms of *p53* protein interfere with the growth-suppressing effects of wild-type *p53*, indicating that the gene product is actually a tumor suppressor (dominant negative). Many investigators have examined mutations of *p53* in detail and have correlated them with the prognosis and with the sensitivity to anti-tumor drugs. A statistically significant association has been noted between *p53* mutations that occur in conserved domains, and poor prognosis. As *p53* mutations are found most frequently in advanced breast

cancers, it appears that aberrant *p53* is involved in the progression stages of such tumors (Nagahata et al., 2002).

P63 belongs to the family of transcription factors that also includes *p53* and *p73*. All members of the family have three highly conserved domains: a transactivation domain (TA), a DNA binding domain (DBD) and an oligomerization domain (OD), *p63* and p73 have an additional protein interacting domain at their c-terminus known as sterile alpha motif (SAM) (Graziano and De Laurenzi, 2011).

Several studies have attempted to correlate *p63* expression with prognosis but further work is required to obtain a clear picture of the value of this gene as a prognostic marker. A correlation of *p63* expression with increased features of poor prognosis has been reported (Garcia, et al., 2007), indeed expression increases from grade IIIa to grade IIIb–IV breast carcinomas (Ribeiro-Silva et al., 2003) and correlates with nuclear pleomorphism, a known feature of aggressive tumors (Thike et al., 2010). Moreover in one study it was reported that *p63* is negative in cancers smaller than 2 cm, but its expression increases with tumor size (Ribeiro-Silva et al., 2003). Conversely well differentiated tumors over expressing *p63* are associated to a good prognosis (Hanker, et al., 2009). It is possible that expression of *p63* in cells of different origin has a different phenotype or more likely that the isoforms expressed by the different tumors are different and result in a different outcome. Indeed one could imagine that while basal like tumors express the oncogenic ΔN isoforms, more differentiated tumors express the tumor suppressor TA form. Hopefully future studies investigating differential isoform expression will clarify this point and allow the use of *p63* as a prognostic marker.

The use of immunohistochemical staining has been a major part of the routine diagnostic procedure in various malignancies, and recent studies have reported a relationship between immunohistochemistry (IHC) profiles of various types of breast carcinomas and molecular taxonomic classification (Ginestier, et al., 2002; Makretsov, et al., 2004; Nielsen, et al., 2004; Jacquemier, et al., 2005).

The current study was designed to identify the association of molecular markers (*p63* and *p53*) with breast cancer in different grades and stages in both men and women in Saudi Arabia.

MATERIAL AND METHODS

This study was performed at King Abdul-Aziz university hospital in histopathology laboratory. Samples were collected from Al-noor hospital, Alawi Tunsi hospital, King Abdul-Aziz hospital, Al Hada, and Al Taif military hospital. A total of 26 samples were collected. Samples studied belong to different age and sex. The questionnaire form included: file number, age, gender, diagnosis, stage and grade of cancer.

Samples preparation

Formalin-fixed, paraffin-embedded tissue blocks from 26 patients with breast carcinoma. The immunohistochemistry assay was performed on 4-5 µm sections were prepared and placed on positively charged slides where tumor sections were de waxed by placing in a glass jar containing xylene for 30 minutes with regular 10 minutes interval shaking. Slides were hydrated through a series of different ethanol concentrations (100%, 90% (v:v), and 70% (v:v)) one minute at each concentration. Slides were then rinsed in running tap water for 1 minute. All tissue sections were placed in a plastic microwavable container with 250 ml 10

µm citrate buffer (*pH* 6.0), which was heated at full power (750 watts) for 5 minutes. After 5 minutes, approximately 50 ml distilled water was added to substitute the evaporated water and continued heating for another 5 minutes at full power (750 watts). After the heating step, the container and tissue sections were placed at room temperature for 20 minutes to cool down in the same citrate buffer solution prior to rinsing in running tap water followed by transferring step where slides transferred to the DAKO Autostainer.

Immunohistochemistry

The current study used DakoCytomation immunohistochemistry which is refers to the Universal EnVision Doublestain System permits the simultaneous demonstration of two antigens within one specimen by double immunoenzymatic staining. IHC used to show whether or not the cancer cell has *p53-p63* proteins in it.
Immunohistochemistry was then carried out using the DakoCytomation EnVision™ Detection Kit (DakoCytomation Ltd, US), according to the manufacturer's instructions using a semi-automated staining system (Autostainer, DakoCytomation Ltd., USA).

Immunohistochemical Procedure

Slides were initially rinsed with 2.5 µl tris buffered saline (TBS) (*pH* 7.6), followed by addition of the primary antibodies (Table -1-), which were diluted using antibody diluents solution (DAKO), incubated for 30 minutes, and rinsed with 2.5 µl TBS buffer. Endogenous peroxidase in the tissue sections were blocked by addition of 2.5 µl peroxidase blocking solution (DAKO) and incubated for 5 minutes. Slides were rinsed with 2.5 µl TBS buffer followed by addition of 2.5 µl Alkaline phosphatase labelled polymer, which was biotinylated goat anti-mouse and anti-rabbit immunoglobulin (ChemMate™ Detection Kit, Peroxidase/DAB, Rabbit/Mouse, DakoCytomation, USA), incubated for 30 minutes before rinsed again with 2.5 µl TBS buffer. Two point five microliters of labelled polymer reagent, which is streptavidin peroxidase, HRP, was added to the tissue sections before incubation for 30 minutes. Slides were rinsed with 200 µl TBS buffer before 2.5 µl substrate-chromogen solution,(DAB) (ChemMate™ Detection Kit, DakoCytomation, USA) was added and incubated for 5-15 minutes. Slides were then removed from the Autostainer and rinsed in running tap water for 2 minutes followed by a counterstain step using haematoxylin (code S3309), by dipping the slides once in haematoxylin. Slides were rinsed in running tap water until the solution was clear. Slides were placed 10 times into a bath of 0.037 mol/L ammonia water , then rinsed in tap water, followed by dehydration through a series of different concentrations of ethanol (70% (v:v), 90% (v:v) and 100%) one minute for each concentration before immersed in xylene for three minutes. Slides then were mounted under cover slips with Glycergel and observed by light microscopy.

Table -1-: Breast cancer markers used in this study and their condition.

Protein	Catalog #	Isotype	Epitope	Company	Clone	Dilution factor
p63	sc-8431	mouse IgG_{2a}	1-205 (h)	Santa cruz biotechnology inc.	4A4	x100
p53	sc-47698	mouse IgG_{2b}	1-45 (h)	Santa cruz biotechnology inc.	DO-7	x100

RESULTS:

Our results are summarized in the Tables 2 and 3. The expression of *p63* was positive in 19.1% of the total cases of breast cancer and the expression of *p53* was positive in 15.3% while 34.4% of all cases expressed *p63* or *p53*.
The results were showed in Table 2 the relationship between the age and the percentage of expression on *p53* and *p63*

in breast cancer patients. The result confirmed the expression of *p53* and *p63*in age group 30-50 in men and women. The results showed that expression of *p63* was found in 50-70 age groups.

Table -2- showed the relationship between the age and the percentage of expression on *p63 and p53* in breast cancer patients.

Age	% of expression *p63*	% of expression *p53*	Total %
Less than30	3.8	3.8	7.6
30-50	11.5	11.5	23
50-70	3.8	0	3.8
Total %	19.1	15.3	34.4

Table 3 explained that the expression of *p63* and *p53* in different types of breast cancer. The result showed that expression of *p63* and *p53* were found in grade II and III of breast cancers. Also, the result showed expression of *p53* was more than *p63* in grade II in breast cancer patients in Saudi Arabia.

Table -3- showed the relationship between the grade and the percentage of expression on *p53* and *p63* in breast cancer patients.

Grade	% of expression *p63*	% of expression *p53*	Total %
I	**0**	**0**	**0**
II	**3.8**	**7.6**	**11.4**
III	**3.8**	**3.8**	**7.6**
Total %	**7.6**	**11.4**	**19**

Image analysis of biomarker expression showed that normal terminal-duct lobular unit with *p63* expressing myoepithelial cells, which served as positive internal control as shown in figure A. Figures B-F, Strong and diffuse expression of *p63* in metaplastic carcinomas. *P63* Expression in Invasive Carcinomas of the Breast, *p63* was expressed in the nuclei of myoepithelial cells of normal ducts and lobules adjacent to the carcinomas, which also served as internal positive controls in all cases. *P63* was negative in all the other invasive ductal, lobular and mixed ductal and lobular carcinoma as shown in Figure 1.

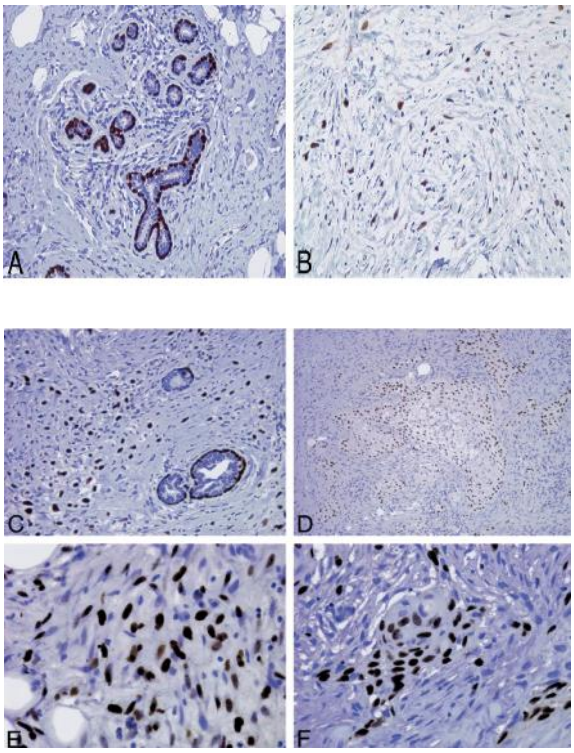

Figure -1-: (A) Normal terminal-duct lobular unit with *p63* expressing myoepithelial cells. (B-F) Strong expression of *p63* in metaplastic carcinomas. The image analysis also explained Basaloid squamous cell carcinoma. The *p63* immunohistochemical staining demonstrating diffuse nuclear staining as clarify in

Figure -2-

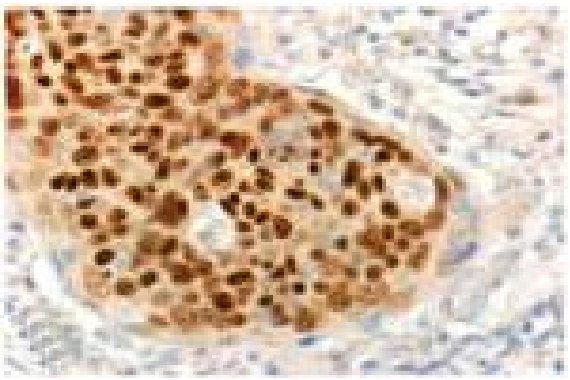

Figure -2-: *p63* immunohistochemical staining demonstrating diffuse nuclear staining. Basaloid squamous cell carcinoma.

The detection of mutation by using p53 biomarker showed that *p53* mutation is the most common genetic abnormality found so far in human cancer,

and in breast cancer *p53* mutation/alteration is seen in 15.3% of breast carcinomas Figure -3-.

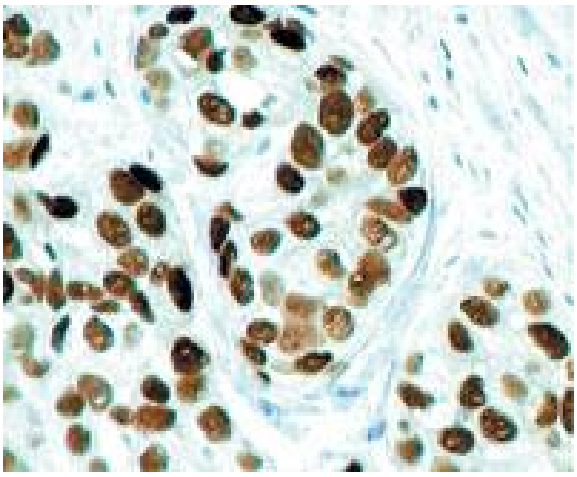

Figure -3-: *p53* mutation in breast cancer

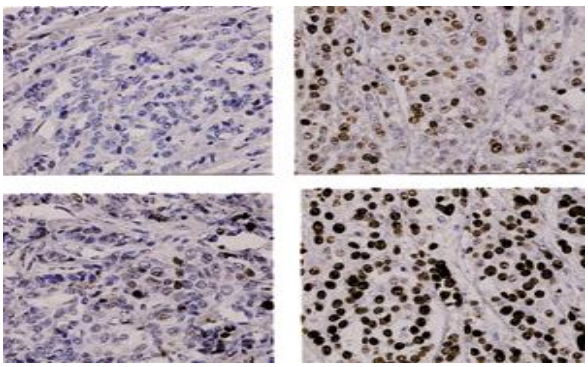

Figure -4-: four sections of different breast cancers stained with DO-7 to detect *p53* protein.

DISCUSSION

Cancers occur when a there is an increase of genetic mutations in critical genes—those that control cell growth and division or the repair of damaged DNA—allow cells to grow and divide uncontrollably to form a tumor. In most cases, these genetic changes are acquired during a person's lifetime and are present only in certain cells. These changes, which are called somatic mutations, are not inherited. Less commonly, gene mutations inherited from a parent increase the risk of developing cancer. In people with these inherited genetic changes, additional somatic mutations in other genes must occur for cancer to develop.

According to change in breast tissue; breast disease is originating from breast tissue, most commonly from the inner lining of milk ducts or the lobules that supply the ducts with milk. Cancers originating from ducts known as ductal carcinomas; those originating from lobules are known as lobular carcinomas. It may be benign, as in fibro adenoma, or it may be malignant, in which case it is termed breast cancer (Schuetz et al., 2006).

This study is to evaluate the prognostic value of the expression of *p63* and *p53* genes in breast cancer. In addition, to identify the association of molecular markers (*p63* and *p53*) with breast cancer in different grades and stages were performed on women and men attending in main hospitals in the western region of Saudi Arabia.

In the current study, molecular markers of *p63* and *p53* genes were evaluated in breast cancer in Saudi Arabia. In 26 samples we found *p63* was express in (19.1%) more than *p53* (15.3%) of breast cancer. In compared with other study in carcinomas, *p63* was expressed only in poorly differentiated ductal carcinomas (11.76%) of cases whereas *p53* was expressed in (21.17%) of carcinomas (Ribeiro-Silva et al., 2003). Since their initial identification *p53* homologues *p63* and *p73* have been expected to play a role in cancer development due to their close homology to *p53*, notoriously one of the most mutated genes in cancer. However soon after their discovery the awareness that these genes were rarely mutated in cancer seemed to indicate that they did not play a role in its development. However a large number of data collected in the following years indicated that altered expression rather than mutation could be found in different neoplasia and play a role in its biology. In particular *p63* due to its fundamental role in epithelial development seems to play a role in a number of tumors of epithelial origin (Graziano and De Laurenzi, 2011).

According to the results obtained, *p53* expressed in 3.8% axillaries lymph node, 3.8% of intraductal axillaries lymph node, 3.8% of ductal carcinoma, 7.6% of phyllodes tumor, 7.6% of invasive duct carcinoma (IDC), and 3.8% of DCIS. Comparing with the results published by Rudasa and others in 1997, they used 121 carcinomas and found 19% was lobular in situ carcinomas (LCIS), 61.2% was intra ductal carcinomas (DCIS) and 19.8% was minimal invasive carcinomas (Rudasa et al., 1997). The variation between their results and our results obtained may be due to the amount of samples size, as our samples size was 26 samples and they used 121 samples. Also it can be due to the types of carcinomas samples used.

The expression of *p63* was found in 3.8% of axillaries lymph node, 7.6% of invasive ductal carcinoma (IDC), 3.8% of ductal carcinoma in situ (DCIS), 3.8% of lobular carcinoma in situ (LCIS), and no phyllodes tumor and this agreements with previous study (Koker et al., 2004).

The expression of *p63* in myoepithelial cell which, positivity more than 80% and this agreements with previous studies (Hsiao et al., 2010).

The literature strongly suggests that *p63* is necessary for normal development of epithelial organs and may be essential for the maintenance of a stem cell population in various epithelial tissues, beings marker of reserve cells. *P63* expression in normal breast and in metaplastic carcinomas.

Conclusions

In conclusion the *p63* is a specific myoepithelial cell marker in normal breast tissue and is expressed in a minority of breast carcinomas, being seen only in grade III,II ductal carcinomas. In ductal carcinomas, malignant *p63*-positive cells have an immunophenotype similar to that of myoepithelial cells, suggesting that these cells originate from a primary progenitor cell that underwent divergent differentiation to ductal and myoepithelial cells during clonal expansion. Our study argues against a direct role in mammary tumorigenesis. However, *p53* is rarely coexpressed with *p63*, suggesting that *p63* could act indirectly as an oncogene by inhibiting *p53*. This hypothesis could also explain why *p63* correlated with several other indicators of poor prognosis.

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REFERENCES

1. Borgen PI; Wong GY; Vlamis V; Potter C; Hoffmann B; Kinne DW. Current management of male breast cancer. Annals of surgery 1992; 215(5), 471-477.
2. Coles C; Condie A; Chetty U; Steel CM; Evans HJ; Prosser J. *p53* mutations in breast cancer. Cancer Res 1992; 52(19): 5291–5298.
3. Fattaneh A; Tavassoli; Devilee, P. World Health Organization Classification of Tumours: Pathology & Genetics; Tumours of the breast and female genital organs 2003 (Vol. fifth): IARC Press International Agency for Research on Cancer.
4. Fentiman IS; Fourquet A; GN H. Male breast cancer. Lancet (2006); 18;367(9510): 595-604.
5. Garcia S; Dales JP; Charafe-Jauffret E; Carpentier-Meunier S; Andrac-Meyer L; Jacquemier J; et al. Poor prognosis in breast carcinomas correlates with increased expression of targetable CD146 and c-Met and with proteomic basal-like phenotype, Hum. Pathol. 38 (6) (2007) 830–841.
6. Ginestier C; Charafe-Jauffret E; Bertucci F; Eisinger F; Geneix J; Bechlian D; et al. Distinct and complementary information provided by use of tissue and DNA microarrays in the study of breast tumor markers. Am J Pathol 2002;161:1223e33.
7. Graziano V; De Laurenzi V. Role of *p63* in cancer development. Biochimica et Biophysica Acta (2011); 1816: 57–66.

8. Hanker L; Karn T; Ruckhaeberle E; Gaetje R.; Solbach C; Schmidt M; et al. Clinical relevance of the putative stem cell marker *p63* in breast cancer, Breast Cancer Res. Treat. 2009; 122: (3) 765–775.
9. Hsiao Y; Su Y; Tsai2 H; Mason J; Chou M; Man Y. Increased invasiveness and aggressiveness in breast epithelia with cytoplasmic *p63* expression. Inter J Bio Scie.2010; 6 :428-442.
10. Jacquemier J; Ginestier C; Rougemont J; Bardou VJ; Charafe-Jauffret E; Geneix J;et al. Protein expression profiling identifies subclasses of breast cancer and predicts prognosis. Cancer Res 2005; 65:767e79.
11. Koker MM; Meryem M; Kleer C.. *p63* expression in breast cancer: a highly sensitive and specific marker of metaplastic carcinoma. Ame J of Sur path, 2004; *28*(11): 1506-1512.
12. Lakhani SR; Ashworth A. Microarray and histopathological analysis of tumours: the future and the past? Nat Rev Cancer 2001; 1:151e7.
13. Lord SJ; Lei W; Craft, P; Cawson JN; Morris I; Walleser S; Griffiths A; Parker S; Houssami N. Eur J of Can. 2007;43 (13), 1905–1917.
14. Makretsov NA; Huntsman DG; Nielsen TO; Yorida E; Peacock M; Cheang MC; et al. Hierarchical clustering analysis of tissue microarray immunostaining data identifies prognostically significant groups of breast carcinoma. Clin Cancer Res 2004; 10: 6143e51.
15. Nagahata T; Kosaka N; Shimizu M; Emi M. Molecular Diagnosis for Breast Cancer. Breast cancer, 2002, 45(6): 265–270,
16. Nielsen TO; Hsu FD; Jensen K; Cheang M; Karaca G; Hu Z; et al. Immunohistochemicaland clinical characterization of the basal-like subtype of invasive breast carcinoma. Clin Cancer Res 2004; 10:5367e74.
17. Quackenbush J. Microarray analysis and tumor classification. N Engl J Med. 2006; 354: 2463e72.
18. Ribeiro-Silva A; Leandra N; Ramalho Z; Garcia S; Zucoloto S. (2003). The Relationship Between *p63* and *p53* Expression in Normal and Neoplastic Breast Tissue. Arch Path & Lab Med, 127(3), 336-340.
19. Rudasa M; Neumayera R; Gnantb MFX; Mittelböckc M; Reiner R. *p53* protein expression, cell proliferation and steroid hormone receptors in ductal and lobular in situ carcinomas of the breast. Eur J of Can,1997; *33*(1): 39-44.
20. Schuetz CS; Bonin M; Clare SE; Nieselt K; Sotlar K; Walter M; Fehm T; Solomayer E; Riess O; Wallwiener D; Kurek R; Neubauer HJ. Progression-specific genes identified by expression profiling of matched ductal carcinomas in situ and invasive breast tumors, combining laser capture microdissection and oligonucleotide microarray analysis. Cancer Res. 2006; 66(10):5278-86.
21. Sotiriou C; Piccart MJ. Taking gene-expression profiling to the clinic: when will molecular signatures become relevant to patient care? Nat Rev Cancer 2007; 7:545e53.
22. Stingl J; Caldas C. Molecular heterogeneity of breast carcinomas and the cancer stem cell hypothesis. Nat Rev Cancer 2007; 7:791e9.
23. Thike AA; Cheok PY; Jara-Lazaro AR; Tan B; Tan P; Tan PH. Triple-negative breast cancer: clinicopathological characteristics and relationship with basal-like breast cancer. Mod Pathol 23 (1) (2010) 123–133.

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Original Article

Outcome Of Pediatric Hydronephrosis Diagnosed By Ultrasound

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تحديد المخرجات من تشخيص مرض استسقاء حوض الكليه الولادى بواسطة الموجات فوق الصوتية عند الاطفال

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الملخص العربي:

الهدف :إن الغرض من هذه الدراسة هو تحديد النتائج المكتشفه عند الرضع والاطفال اللذين تم تشخيصهم بمرض استسقاء حوض الكلية عن طريق الموجات فوق الصوتية.
خطوات البحث: تم دراسة 46 رضيع وطفل اقل من 11 سنه تم لديهم تشخيص استسقاء حوض الكلية في الفترة مابين أبريل 1995إلى أبريل 2000، وجرى تقييم نتائج متابعتهم خلال فتره لا تقل عن 12 شهرا.
النتائج : تم تحديد46 طفلا32 من الذكور:14 من الإناث متوسط اعمارهم عند تشخيص استسقاء حوض الكلية ± 1.10 0.27سنة وتم تشخيص 58٪ من المرضى عند الولادة .28طفلا كان الاستسقاء من جانب واحد 60.8)٪ (من الحالات، وعلى الأقل كان الاستسقاء فى حوض الكلية بنسبه معتدلة أو شديدة في 43.4) 20٪ (من الكلى المتضررة .وبما لايقل عن 22طفلا 46من 47.8) ٪ (طفلا اختفي الاستسقاء من عندهم خلال فترة المتابعه ،بينما استمر الاستسقاء عند 24 طفلا 52.1٪ .
الخلاصة : أن أهم ميزة نحصل عليها من معرفة الأطفال المصابين باستسقاء ولادى فى حوض الكلى عند اجراء فحص الموجات فوق الصوتية هي القدرة على التقييم في الوقت المناسب لبدء تحديد شدة الاصابه وبدايه المتابعه ومنع التدهور التدريجي في وظيفة الكلى

ABSTRACT

Objectives: The purpose of this study was to determine the outcome of infants with a history of hydronephrosis diagnosed by ultrasound.

Methods: In a five-year period from April 1995 to April 2000, 46 infants and children < 11 years of age with diagnosis of neonatal hydronephrosis were evaluated and followed for at least 12 months.

Results: 46 children were identified (32 male: 14 female). The mean age at diagnosis of hydronephrosis was 1.10 ± 0.27 years and 58% of the patients were diagnosed at birth. The hydronephrosis was unilateral in 28 (60.8%) of cases, and hydronephrosis was at least moderate or severe in 20 (43.4%) of affected kidneys.
At last follow-up, the abnormality had resolved in 22 out of 46 (47.8%) available patients, 24 (52.1%) had persistent hydronephrosis.

Conclusion: We conclude that the most important advantage of knowing that the children has ultrasound findings of persistent hydronephrosis is ability to begin timely evaluation to identify severity of hydronephrosis and prevent progressive deterioration of renal function.

Keywords: *Hydronephrosis, ultrasound, outcome*

INTRODUCTION

Thyroid Hydronephrosis is one of the common anomalies detected during fetal ultrasound evaluation. [1] counseling for antenatal hydronephrosis has gained popularity and more and more parents attend these clinics to find out the outcome. [2] .Infants with neonatal hydronephrosis can have a range of abnormalities including ureteropelvic junction obstruction (UPJO), ureterovesical junction obstruction, megacystis megaureter, or vesicoureteral reflux (VUR).

The second and third entities are fairly uncommon and, therefore, pediatric patients who have hydronephrosis and a normal voiding cystourethrogram (VCUG) are presumed to have UPJO. This abnormality occurs in approximately 1 in every 2,000 live births and accounts for approximately half of the cases of prenatal hydronephrosis.[3,4]

In most cases, neonatal hydronephrosis and presumed UPJO gradually resolves without surgical

intervention. There is a strong correlation between the Society of Fetal Urology (SFU) grading of hydronephrosis and the likelihood of spontaneous resolution: Grade I resolves in approximately 50% of patients, and grades II, III, IV hydronephrosis resolve in 36%, 16%, and 3% of cases, respectively.[3]

However, in current practice, there is little information about the natural history of hydronephrosis and the time for resolution, in antenatal ultrasound screening for pregnant women. Moreover, there are few guidelines regarding frequency of follow-up visits and referral to urology for more extensive study such as performance of renal radionuclide scans and possible decision for surgery in children with hydronephrosis who are

cared for by general pediatricians and pediatric nephrologists. Additionally, the value of clinical markers such as birth history, urinary tract infection (UTI), and severity of the hydronephrosis as a guidance in predicting patients likely to require urological evaluation has not been adequately studied.

The aim of the study is to assess the outcome of fetal hydronephrosis, segregate them into simple groups so that the milder ones could be favorably counseled. At the same time, patients with more serious type could be informed about the importance regarding regular follow-up, prompt evaluation and timely intervention whenever necessary. The data from this study can be useful for the physicians involved in the counseling of these parents pre and postnatal.

Objective:

The aim of this study was to determine the efficacy and accuracy of FNAC in diagnosis of thyroid swellings in our surgical department and compare and matching, accuracy, sensitivity, specificity, positive predictive value and negative predictive value of the results in various thyroid swellings in correlation with histologic diagnosis.

MATERIAL AND METHODS

The study was conducted in all pediatric patients followed by one pediatric nephrologist with different presentation at different ages diagnosed with neonatal hydronephrosis at our institution between 1995 and 2000 and followed by the Division of Pediatric Nephrology.

The clinical data were collected on pre-approved data collection sheets, the information
was identified, and the sheet linking the study number with the individual patient was stored
in a secure location.
The project was reviewed and approved by the Institutional Review Board.
A database maintained at the Division of Nephrology, which includes patient gender, age, and chief complaint was scanned to identify patients with "UPJ obstruction," "hydronephrosis," or "obstructive uropathy." The review of charts with these three diagnostic terms was done to ensure complete ascertainment of patients with hydronephrosis and presumed UPJO.

Children were included in this review if they had: (1) hydronephrosis without hydroureter detected in a postnatal ultrasound and a negative VCUG; or (2) hydronephrosis without hydroureter in the absence of UTI and absence of a documented negative VCUG because of parents refusal of the study.

The standard recommendation at this institution is to perform a VCUG in any newborn or infant under 3 months of age with confirmed hydronephrosis. In addition, patients had tqaqqqqo be followed for a minimum of 12 months to be eligible for inclusion in the study.
Cases were classified as mild, moderate, or severe hydronephrosis on the basis of the findings of their initial ultrasonographic examinations using the classification criteria of Mandell.[15]

The following data were recorded for patients: date of birth, gender, presence of prenatal hydronephrosis, gestational age/birth weight, history of perinatal complications or UTI, severity of hydronephrosis, age at diagnosis, unilateral or bilateral disease, serial ultrasounds, and outcome, namely resolution of hydronephrosis, persistent hydronephrosis, or referral to urology. The time from diagnosis to each outcome was then calculated by month.

Severity of hydronephrosis of each renal unit was recorded based on the radiology report as mild, moderate or severe. Outcome was recorded as improving, worsening, stable, or resolved.

Statistical Methodology

Frequency tables (number, percent) were mainly calculated for all the measurements

Comparability tests were measured in the study using chi-square test for categorical variables, like sex, etc. and t-test for continuous variables, like age levels. Significance was detected at p value < 0.05

RESULTS

Patients Demographics:

Table (1) shows the sample characteristics of hydronephrosis cases:

The total 46 patients, 26 (56%) had mild hydronephrosis, while 20 (43%) had moderate and severe hydronephrosis, with average age at diagnosis 1.10 ± 0.26 years range from 0.0 to 6 years, 32 (70%) were male patients and 14 (30%) were female patients. Serum creatinine average was 38.58± 2.39 micromoles range from 20 to 29 micromoles and serum creatinine average follow up was 51.74± 9.82 micromoles range from 23 to 223 micromoles.

		Overall	Mild	Moderate and severe	P Value
Distribution		46	26 (56%)	20 (43%)	
Age (years)	Mean ± SE Range	1.10 ± 0.26 0.0 - 6	1.46 ± 0.40 0.0 - 6	0.63 ± 0.28 0.0 – 4	0.0981
Sex (%)	Male Female	32 (70%) 14 (30%)	20 (43%) 6 (13%)	12 (26%) 8 (17%)	0.2162
Serum Creatinine (micromoles)	Mean ± SE Range	38.58 ± 2.39 20 – 90	39.16 ± 2.63 20 – 73	37.78 ± 4.48 20 – 90	0.792
Serum Creatinine in following up (micromoles)	Mean ± SE Range	51.74 ± 9.82 23 – 223	38.54 ± 3.31 23 – 66	86.90 ± 21.57 24 – 233	0.1961

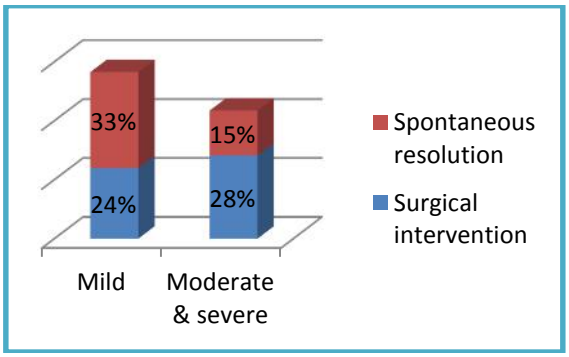

Figure 1 showed outcome of hydronephrosis.
Mild hydronephrosis patients had 26 patients and only 11 (24%) required surgery. Moderate and severe hydronephrosis patients had 20 patients and 13 (28%) required surgery. The difference in outcome between the groups was statistically not significant (P value= 0.1267).

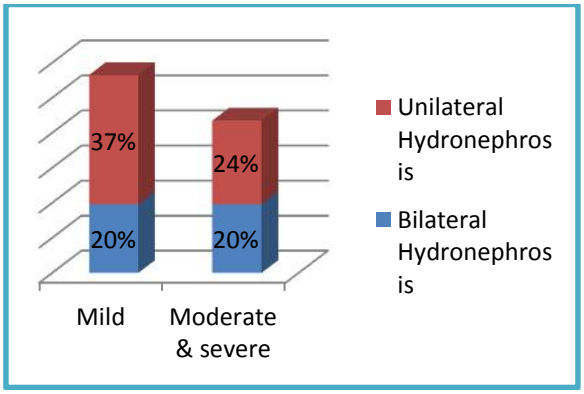

Figure 2 showed 18 (40%) had bilateral hydronephrosis, ureteric dilatation or bladder wall thickening in mild hydronephrosis and in moderate and severe hydronephrosis, while 28 (60%) had isolated unilateral hydronephrosis in mild, moderate and severe hydronephrosis with no significance difference between both P value=0.4744.

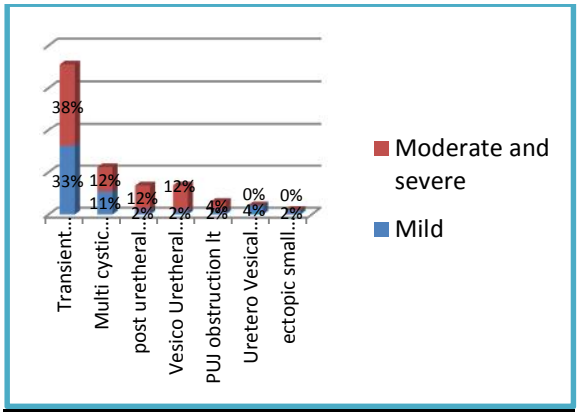

Figure 3 showed outcome of hydronephrosis
Transient hydronephrosis recorded 15/26 patients in mild hydronephrosis patients and 10/20 patients in moderate and severe patients followed by Multi-cystic dysplastic kidney (MCDK) recorded 5/26 in mild patients and 3/20 in moderate and severe patients, Vesico uretheral reflux (VUR) and Uretero vesicle junction obstruction 1/26 in first group and 3/20 in second group. There was no significant difference between both groups in diagnosis (P value= 0.1714).

DISCUSSION

The term of hydronephrosis although is clearly descriptive, it does not by itself mention the underlying etiology such as uretero pelvic junction obstruction, vesico ureteric reflux, posterior urethral valve, etc. For a treating physician, in addition to the etiology, it is also essential to know the natural history of the disease. On the other hand, for the parents, obstetricians, pediatricians, and urologists who are involved in the antenatal counseling, it would be easy if the fetal hydronephrosis outcomes are explained in a clear and simple way based on the sonographic findings rather than the potential differential diagnoses.

Extensive use of prenatal ultrasound has led to an increased rate of diagnosis of neonatal hydronephrosis, of which UPJO is the most frequent cause.[4] The postnatal imaging and management of children with hydronephrosis and presumed UPJO is debated due to the high number of cases that resolve without intervention.

This study showed that more than half of cases of hydronephrosis detected in the neonatal period or early childhood resolved spontaneously over 30 months. While the remaining of these patients had transient worsening at some time point, they all ultimately resolved. This would underestimate the rate of resolution of hydronephrosis and likely lend further support to our conservative recommendations.

The initial severity of the lesion at the time of diagnosis and presence of the hydronephrosis at birth were the only two factors that predicted failure to resolve. The recent time period (1997–2000) of our study reflects the inclusion of children who had prenatal sonograms and reflects the outcomes of hydronephrosis under current standards of antenatal care when the majority of women receive prenatal sonograms regularly during pregnancy.

To our knowledge, this study represents the largest cohort of patients with hydronephrosis followed exclusively by a medical service. In children with hydronephrosis, the question of timing of follow-up imaging studies and their management has significant implications for routine pediatric health care. This study provides support for the practice of monitoring children with hydronephrosis for a longer period of time without mandatory early intervention or referral to urology.

Twenty-three percent of neonates followed for 6 years in one study[5] and 7% of neonates with unilateral hydronephrosis followed for 5 years in another ultimately required surgical intervention.[6,7]

In our study, only 48% resolved out of the total 46 caseses. Mild hydronephrosis patients had 26 patients and only 11 (24%) required surgery. Moderate and severe hydronephrosis patients had 20 patients and 13 (28%) required surgery. Furthermore, a few of the children that ultimately resolved had transient worsening, which is interesting although in those children with hydronephrosis that worsens need urgent referral and surgical evaluation. In children with posterior urethral valves, it is suggested that ultrasound imaging be done every 4 months for the first year of life.[8]

Our data support the need for less frequent ultrasound examinations in children with hydronephrosis
secondary to presumed UPJO. We suggest that in the absence of severe disease or renal parenchyma thinning, the physician can choose to follow the children with serial sonography every 6–12 months for the first 2–3 years of life and observe for improvement. Most children who do require pyeloplasty usually do so within the first 2 years of life.[9]

We have characterized the patients as having hydronephrosis due to presumed UPJO. The later entity requires confirmation with nuclear medicine studies. Thus, a 99mTc-Mag3 renal radionuclide scan with furosemide washout is often part of the assessment of children with moderate to severe hydronephrosis.[10,11]

A large meta-analysis by Sidhu et al. showed that Grade I-II hydronephrosis was about 5 times more likely to resolve as compared to higher severity lesions.[12] Similarly, in our study, hydronephrosis that was mild to moderate resolved in 32% of patients, compared to 15% of children with moderate to severe hydronephrosis.

This supports the claim that severity of hydronephrosis predicts failure of the lesion to resolve. However, Onen and colleagues found that even with more severe lesions, two-thirds of patients still do not require surgery.[13]

In our study, children were referred both immediately after birth and later in life for evaluation of their hydronephrosis. This could potentially influence our results because the follow-up time period may be different physiologically in terms of kidney development from the time of birth compared to that same interval later in life.

Our aim is to focus on the role of general pediatricians and pediatric nephrologists
in the care of children with hydronephrosis. The outcomes of those managed by urologists would be the subject of an independent study.

CONCLUSION

The results suggest that prolonged monitoring is suitable for the majority of children with neonatal hydronephrosis. Our study provides an approach for primary care physicians and pediatric nephrologists to manage hydronephrosis by allowing a longer observation period for spontaneous resolution in following-up ultrasounds.

REFERENCES

1. Blyth B, Snyder HM, Duckett JW. Antenatal diagnosis and subsequent management of hydronephrosis. J Urol 1993;149:693-8

2. Gunn TR, Mora JD, Pease P. Antenatal diagnosis of urinary tract abnormalities by ultrasonography after 28 weeks' gestation: Incidence and outcome. Am J Obstet Gynecol 1995;172:479-86.

3.Dillon HK. Prenatally diagnosed hydronephrosis: the Great Ormond Street Experience. BJU. 1998;81(Suppl 2):39–44.

4. Lim DJ, Park JY, Kim JH, et al. Clinical characteristics and outcome of hydronephrosis detected by prenatal ultrasonography. J Korean Med Sci. 2003;18:859–62.

5. DiSandro MJ, Kogan BA. Neonatal management. Role for early intervention. Urol Clin North Am. 1998;25:187–97.

6. Ransley PG, Dhillon HK, Gordon I, et al. The postnatal management of hydronephrosis diagnosed by prenatal ultrasound. J Urol. 1990;144:584–7.

7. Koff SA, Campbell KD. The nonoperative management of unilateral neonatal hydronephrosis: Natural history of poorly functioning kidneys. J Urol. 1994;152:593.

8. Apocalypse GT, Oliveira EA, Rabelo EA, et al. Outcome of apparent ureteropelvic junction obstruction identified

by investigation of fetal hydronephrosis.Int Urol Nephrol. 2003;35:441–8.
9. Moslehi J, Herndon CD, McKenna PH. Posterior urethral valves presented at birth despite normal prenatal ultrasound scans. Urology. 2001;157:1178.
10. Ulman I, Jayanthi VR, Koff SA. The longterm followup of newborns with severe unilateral hydronephrosis initially treated nonoperatively. J Urol. 2000;164(3 Suppl 1):787–9.
11. Fefer S, Ellsworth P. Prenatal hydronephrosis. Pediatr Clin N Am. 2006;53: 429–47.
12. Elder JS. Antenatal hydronephrosis. Pediatr Clin N Am. 1997;44:1299–321.
13. Sidhu G, Beyene J, Rosenblum ND. Outcome of isolated antenatal hydronephrosis: a systematic review and meta-analysis. Pediatr Nephrol. 2006;21: 218–24.
14. Onen A, Jayanthi JR, Koff SA. Long-term follow-up of prenatally detected severe bilateral newborn hydronephrosis initially managed nonoperatively. J Urol. 2002;168:1118–20.
15. Mandell J. Prenatal diagnosis and treatment of obstructive uropathies. Probl Urol 1990; 4:547–554.

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Original Article

Clinical And Ultrastructural Evaluation Of Topical Application Of Vitamin E In Chronic Gingivitis Associated With Removable Partial Dentures

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التقييم السريري و الميكروسكوبي للتأثير الموضعي لفيتامين هـ في علاج التهاب اللثة المزمن المصاحب للأطقم الجزئية المتحركة.

د. هنادي لمفون ـ استاذ مساعد د. مها محمود – استاذ مساعد ـ د. هنادي فانسي ـ استاذ مساعد ل –كلية طب الاسنان جامعة ام القري-مكة المكرمة- السعودية.

الملخص العربي:

الهدف : تهدف الدراسة لتقييم التأثير الموضعي لفيتامين هـ كمضاد للأكسدة في علاج التهاب اللثة المزمن المصاحب للأطقم الجزئية المتحركة.
الطريقة : تم تطبيق هذا البحث على أربعه عشر موضعا لالتهاب اللثة المزمن في عشرة أشخاص ممن يستخدمون الأطقم الجزئية المتحركة و يعانون من التهاب اللثة تم تقسيمهم كالأتي: المجموعه الأولى تم علاجها بازاله الرواسب الجيرية و الكحت اللاجراحى مع أضافه فيتامين هـ كماده مضادة للأكسدة على هيئه زيت موضعي أما المجموعة الثانية فقد تمت معالجتها بازاله الرواسب الجيرية و الكحت اللاجراحى فقط كما تم تقييم المجموعتين اكلينيكيا و ميكروسكوبيا باستخدام المجهر الألكترونى .
النتائج : دلت نتائج هذا البحث على أن استخدام فيتامين هـ قد أدت إلى تحسن ذو دلالة أحصائية فى حالة اللثة أكلينيكيا من حيث درجة قياس التهاب اللثة و معامل الرواسب الجيرية. وقوى هذه النتائج الفحص تحت المجهري لأنسجة اللثة حيث أدى استخدام المادة المستخدمة فى البحث الي زيادة ترابط خلايا النسيج الخلوى للثة مع تحسن فى أغشية الأنوية و الميتوكوندريا.
الخلاصة: بناء علي النتائج السابقة فانه يوصى بإضافة المواد المضادة للأكسدة مثل فيتامين هـ إلى العلاج التقليدي لالتهاب اللثة المزمن المصاحب للأطقم الجزئية المتحركة حيث انه يسرع في تحسين حالتها إكلينيكيا ويمنع من تطور المرض .

ABSTRACT

Objectives: Objectives: To evaluate the clinical and biological effects of topical application of vitamin E as an antioxidant therapy in chronic gingivitis associated with removable partial dentures.
Methods: Fourteen chronic gingivitis involved sites were enrolled for this study, they were divided into two groups. Group 1: comprised seven chronic gingivitis involved sites managed by mechanical debridement with topical application of vitamin E , and Group II: comprised seven chronic gingivitis involved sites managed by mechanical debridement only. Moreover, a biopsy(1x1mm) was taken from the interdental papillae of the selected sites of both groups to be examined using the transmission electron microscope.
Results: Clinical results showed significant improvement in clinical parameters (plaque index and gingival index) in both studied groups. The magnitude of improvement was in favor to group I. A statistically significant difference was observed between group I and group II only in relation to the gingival index. The histological results obtained from the present study revealed that: In group II, abnormal changes in the gingival keratinocytes and fibroblasts especially the nuclear membrane, desmosomal junction, and mitochondria. While in group I marked improvement was observed following the topical application of antioxidant therapy.
Conclusion: The topical application of vitamin E improves the gingival tissues clinically and ultra structurally and it is recommended to be used as an adjunctive treatment in chronic gingivitis in patients wearing removable partial dentures.

Keywords*: dentures, gingivitis, vitamin E, antioxidants.*

INTRODUCTION

Chronic gingivitis is an inflammatory disorder caused by substances derived from microbial plaque accumulating at or near the gingival sulcus; all other suspected local and systemic etiologic factors either enhance plaque accumulation or retention, or enhance the susceptibility of the gingival tissue to microbial attack.[1]

It was suggested that insertion of removable partial dentures creates the potential for quantitative and qualitative changes of plaque formation on the remaining teeth that is representative by proliferation of spiral organisms. Thereby there is an increased risk for development of gingivitis and periodontitis.[2,3]

Multiple molecular players are involved in gingivitis and periodontitis, among them is reactive oxygen species(ROS) which is postulated to have a role in both the bacterial and host mediated pathway of tissue damage[4].

ROS is the end result of the reduction of molecular oxygen which is required for all mammalian cells to obtain energy. This reduction process is accompanied by a large free energy release that gives rise to free radicals and/or ROS [5].

ROS has been adopted to include molecules such as hydrogen peroxide, hypochlorous acid and single oxygen. They cause tissue damage by a variety of mechanisms including DNA damage, and lipid peroxidation. Moreover it exerts protein damage of gingival hyaluronic acid and proteoglycans, it also stimulates pro-inflammatory cytokine release by monocytes and macrophages[6].

Whilst most ROS have extremely short lives, they can cause substantial tissue damage. It is therefore not surprising that the body contains a number of protective antioxidant mechanisms, whose specific role is to remove harmful oxidants or ROS as soon as they form, or to repair damage caused by ROS in vivo[7].

Antioxidant defense mechanisms are located in both water or aqueous part of our body. Among the major antioxidants are vitamin E, ubiquinol and various carotenoids derived from dietary sources[8].

Vitamin E is the collective name for a group of fat-soluble compounds with distinctive antioxidant activities. Vitamin E is found naturally in some foods, added to others, and available as a dietary supplement. It was investigated that vitamin E might help prevent or delay the chronic diseases associated with free radicals by neutralizing free radicals that cause oxidative cellular damage.[9]

Moreover, vitamin E exhibits anti-inflammatory properties which may limit inflammation-induced tissue destruction. As an antioxidant, vitamin E may protect lysosome membranes leading to a decrease in histamine and serotonin from mast cells during inflammation. [10]

Studies [11-12] indicated that in chronic gingivitis and periodontitis ROS overproduction can also be induced by periodontal pathogens that may induce collagen and periodontal cell breakdown . It was also suggested that when ROS are scavenged by antioxidants there can be a reduction of collagen degradation.[13] .

In another study it was found also that the ROS not only affect the progression of chronic gingivitis, but they are also proved to increase the severity of inflammation in peri-implant tissues.[14]

Recently, in 2008,[15] the relationship between ROS and apoptosis in normal human keratinocytes and fibroblasts was studied ultrastructurally and it was reported that they can induce apoptotic cell death in form of chromatin condensation, plasma membrane blebbing and rounding up of cells in primary normal keratinocytes and fibroblast.

Thus the role of antioxidants as a defense agent against the free radicals released by the mitochondria is no longer a point of debate; however, further studies are needed to detect their clinical and biological effect on the gingival and periodontal tissues in chronic periodontitis.

The aim of the present study was to evaluate the clinical and ultrastructural effect of the topical application of vitamin E in gingival tissue of chronic gingivitis patients with removable partial dentures.

MATERIAL AND METHODS

I. Patients' selection

A total of fourteen matched chronic gingivitis sites from ten patients were chosen from the outpatient clinic of Oral Medicine and Periodontology department, Faculty of Dentistry, Alexandria University, whose age ranged from 32-56 years with a mean age of42 years. Informed consent was obtained from each subject prior to the study.

The patients were chosen on the basis of:

1- Wearing removable partial dentures with at least one year with chronic gingivitis related to the abutment teeth. [16]

2- Systemically healthy, nonsmoker males and with no history of any drug therapy in the 3 months preceding time of research.

II. Materials

Vitamin E antioxidant 400mg capsules *was used in the present study.

III. Methods

Each patient was subjected to a thorough clinical dental examination including

plaque index PI [17,18], gingival index GI. [19]

After base line examination all patients were given oral hygiene instructions and they were randomly divided into two groups:

Group 1: Mechanical debridement consisting of supragingival scaling was performed using hand instruments and ultrasonic scalers in addition to topical application of400mg vitamin E * twice daily for a period of six weeks.

Group II: Managed only by mechanical debridement. All clinical parameters were taken prior to treatment and 6weeks following treatment. All patients were given oral hygiene instructions and placed on strict maintenance recall visits during which oral hygiene was reviewed. The data was collected, tabulated and statistically analyzed by Mann-Whitney test.

Histological study:

A biopsy(1x1mm) was taken from the interdental papillae of the selected sites of both the test and control sites fixed in 2% glutaraldehyde solution and 2% formaldehyde and immediately in 5M sodium phosphate buffered at pH 7.3. Then the tissues were cut into pieces `1-2mm^2, rinsed in cacodylate buffer for one hour and post fixed for two hours in 1% osmium tetroxide buffered with 0.15M sodium phosphate at pH7.3. After this post fixation, the tissues were rinsed in buffer, dehydrated in ethanol and embedded in Epon Araldite.[20]

Semi thin sections were stained with methylene blue and examined with the light microscope. Ultrathin sections were cut and stained with alcoholic uranyl acetate and lead citrate. And then examined with Jeol electron microscope100cx.[21].

RESULTS

Clinical Results

Statistically significant improvement was observed in PI in both studied groups at the six weeks follow up period when compared to the base line values.

On comparing both treated groups, no significant difference was found in PI at various periods of follow up (Table I).

Table (1): Comparison between group I and group II in the different stages according to GI , PI.

		Base line		6 weeks	
		G II	**G I**	**G II**	**G I**
GI	**Mean ± SD**	2.29 ± 0.76	2.29 ± 0.76	1.43 ± 0.79	0.29 ± 0.49
	U (p)	24.500 (1.000)		6.500* (0.014)	
	Z (p)			1.604 (0.109)	2.392* (0.017)
PI	**Mean ± SD**	2.14 ± 0.69	2.00 ± 0.82	0.71 ± 0.76	1.00 ± 0.82
	U (p)	22.000 (0.728)		19.500 (0.493)	
	Z (p)			2.232* (0.026)	2.333* (0.020)

U: Mann-Whitney test between G I and G II in the different stages

Z : Z for Wilcoxon signed ranks test between base line and the other stages

* : Statistically significant at $p \leq 0.05$

Table (2):Comparison between the two studied groups according to % of change between base line and 6 weeks

	G II	**G I**	**U (p)**
GI	28.6 ± 40.50	88.10 ± 20.9	7.500* (0.025)
PI	35.80 ± 39.30	57.10 ± 61.90	23.000 (0.842)

U: Mann-Whitney test between G I and G II in the different stages

* : Statistically significant at $p \leq 0.05$

Histological Results

Group I

The gingival epithelium

The basal cells contained large nuclei; the nuclei were bound by evenly contoured nuclear membrane. The cell organelles were found around the nuclei. The basal lamina was distinct.

The keratinous cells were densely packed with tonofibrils, rounded empty spaces of various diameters occurred within the cytoplasm (fig.1,2)

The lamina propria

There was a subsidence of the inflammatory reaction in the connective tissue.

The fibroblast was found to be a typical secretory cell with fusiform nucleus, a abundant rough endoplasmic reticulum, mitochondria and numerous membrane bound vesicles located near the cell membrane. (fig. 3).

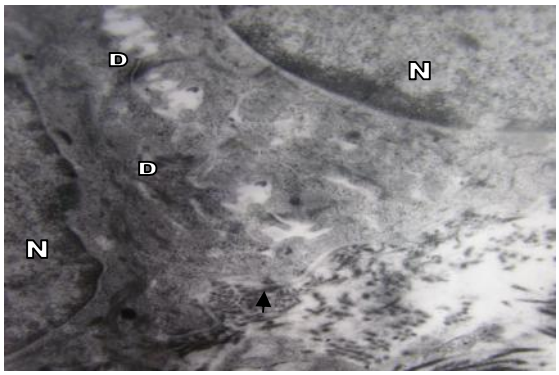

Fig (1) basal cells with more or less rounded nucleus (N). Note many desmosomal junctions (D) there is thick basal lamina (arrow)
X10000

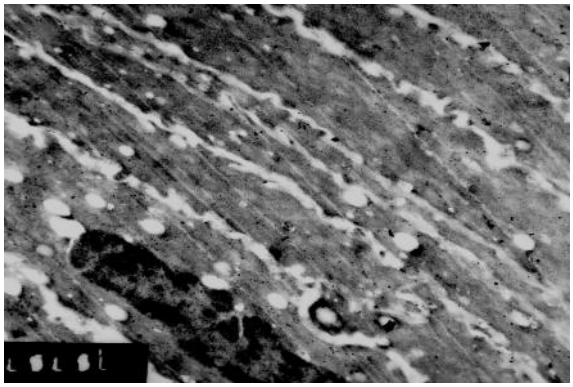

Fig (2) keratinous layer with dense tonofibrills. Granular cell (G). Note the interdigitation between the cell membranes. x 7500

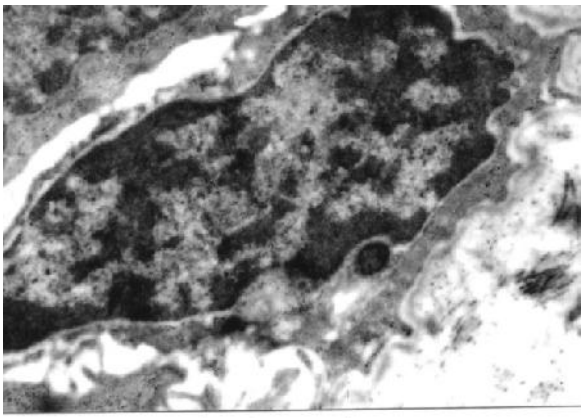

Fig (3) fibroblast in the Lamina propria shows well developed nucleus.& L.S.x14000

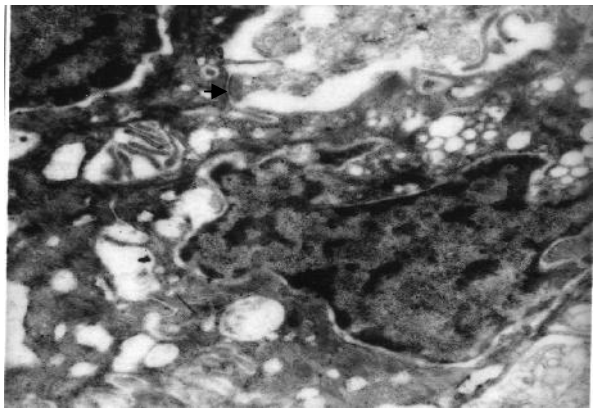

Fig (4) the basal cells with few desmosomal junction (D) and wide intercellular spaces (IS)filled with amorphous substance. The nucleus shows irregular outline. note the vacuoles (V)in the cytoplasm. X 10000.

Group II

The gingival epithelium

-The nuclei of the basal cells showed irregular outline with vacuolated cytoplasm, few desmosomal junctions could be seen in between the cells. The intercellular spaces were wide and filled with amorphous substance. The basal lamina was indistinct.

-The keratinous layer showed wide intercellular spaces filled with amorphous substance, no desmosomal junction was noticed. (fig.4,5)

The lamina propria

-The fibroblasts were found to show atypical appearance with irregular outline of the nuclei and vacuolated cytoplasm.

-The mitochondria were swollen and have floculant appearance. Many inflammatory cells were found in the lamina propria. The macrophages showed indented nucleus and many phagosomes in their cytoplasm (fig.6,7).

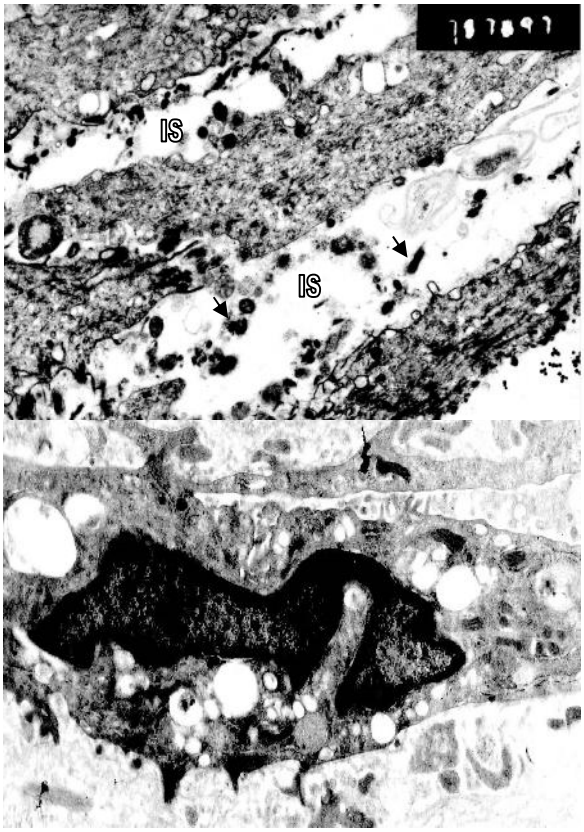

Fig (6) fibroblast in the lamina propria with irregular outline of the nucleus. The cytoplasm shows many vacuoles& Mitochondria X 7500.

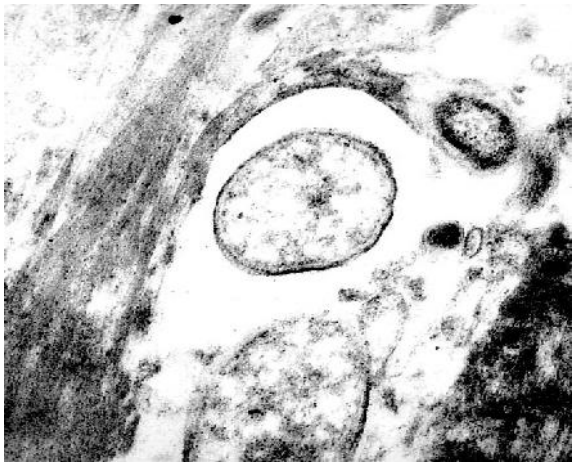

Fig (7) mitochondria with a floculant appearance (M). X 15000.

DISCUSSION

There has recently great interest in antioxidants for the treatment and prevention of disease. Vitamin E, as an important antioxidant, has received much research attention in the medical literature in the last several years[9,22].

A removable partial denture is a common treatment available for restoration of partially edentulous ridges. Many investigators have studied the effect of RPDs on gingival health. [23,24]Orr et al [25] reported an increase in gingival index after 21 days of constructing acrylic resin base plate, moreover, longitudinal studies indicate that RPDs have been associated with increased gingivitis, periodontitis, and abutment mobility.[26]

Studies [27,28] indicated that the reaction of the host inflammatory and immune responses to pathogenic species within the periodontal environment can generate reactive oxygen species within the adjacent tissues. Protection against such species is provided by antioxidants, many of which are released locally at sites of infection by inflammatory cells such as PMNs. It is likely that the local antioxidant system act in concert of total antioxidants capacity in gingival crevicular fluid and deficiencies in these systems could place individuals at increased risk of destructive periodontal diseases [29].In the current study, the antioxidant vitamin E was used topically as an adjunctive therapy to traditional nonsurgical debridement in sites with chronic gingivitis in patients with RPD. This therapeutic management was evaluated clinically and ultra structurally and compared to non-surgical debridement alone. The selected individuals of the present study were all systemically healthy and non-smokers. Studies indicated that smoking increases gingival inflammation in periodontal tissue of smoker periodontal patients that may occur due to the effect of smoking on the defense mechanism of the gingiva against free radicals causing exaggerated inflammatory response [30].The clinical results of the present study showed that plaque index exhibited reduced scores during the course of treatment and did not present statistically significant differences

between the two groups at various study periods. It appears that the reduced plaque scores was accomplished by the continuous reinforcement of home oral and denture hygiene measures and the professional dental care program during the whole study period. The results of the present study revealed also a statistically significant differences between group I and group II in relation to the gingival index at 6 weeks post treatment period. The significant decrease in GI may be attributed to the low PI, the increase of antioxidant level in gingival and periodontal environment and the effect of vitamin E on microbial environment in group I following the application of vitamin E.Okada et al [31] recommended that treatment with oral administration of antioxidant may restore the immune function in chronic periodontitis where the aggression of bacterial plaque is frequently enhanced by unfavorable regional defects in immune system. In the current study, the histological results of group II revealed wide intercellular spaces filled with amorphous substances, altered few desmosomal attachment and ill formed tonofilaments between the gingival keratinocytes. The fibroblasts showed incomplete nuclear membranes, and mitochondrial alteration with many autophagic vacuoles filled with collagen fibers indicating high rate of fibrin turn over. These histological features could explain the increased inflammatory condition as manifested by increase GI when compared with group I. On the other hand, the histological results obtained from group I revealed normal appearance of the basal cell with gingival epithelial cells packed with many tonofilaments, small intercellular spaces with many desmosomal junction. This was manifested clinically by firm shrinkage gingiva with no exudates (significant decrease in GI). The lamina propria showed many fibroblasts in the active stage of protein synthesis denoting the subsidence of inflammatory condition and the fibrotic appearance of the gingiva following application of vitamin E. Our result are in accordance to Sobaniec et al [32] who demonstrated destruction of alveolodental ligament in the of experimental ligature-induced periodontitis in rats . The authors suggested that these results may occur due to the decrease of basic antioxidant enzymes activity with simultaneous increase of the final products of lipid peroxidation. Moreover, Wadleigh et al [33] found that vitamin E is effective in the treatment of gingivostomatitis. A randomized, double blind placebo-controlled study was done to determine whether topical Vitamin E would be effective in healing mucositis. A total of eighteen adult patients receiving chemotherapy for various types of malignancies were included in this study. Six of the nine patients who received Vitamin E had complete resolution of their oral lesions compared with only one out of nine patients who received the placebo (p = .025). These results suggest that Vitamin E may be effective therapy in treatment of gingivostomatitis. On contrary to our results, Parish et al[34] found no beneficial effects from the therapeutic use of vitamin E to combat periodontitis. The authors conducted a study on the effect of vitamin E on the course of periodontitis in thirty-six adult male albino rats. The rats were divided into three groups of twelve and placed on test diets that either was deficient in, or contained adequate and high amounts of, vitamin E. A local irritant in the form of a stainless steel wire was placed around the maxillary left second molar of each animal as a collector of plaque and debris. Migration of the epithelial attachment, alveolar bone level, and numbers of inflammatory round cells were then evaluated on both sides of the maxilla. The results of this experiment indicate that a deficiency of vitamin E does not cause increased destruction of the periodontium in the presence of periodontitis. Moreover, no beneficial effects from the therapeutic use of vitamin E to combat periodontitis was found. The importance of antioxidants in

inflammatory tissues has been studied in vivo[35]. Degradation of collagen powder by experimental granulation tissue induced by cellulose sponges in the rat was monitored as the radioactivity excreted in urine. By administering pharmacological doses of both vitamin E and selenium subcutaneously and by injection into sponges implanted subcutaneously, this breakdown of collagen was reduced. Injections in the sponges also arrested the maturation of the granulation tissue. The results revealed that vitamin E and selenium are potential inhibitors of the free oxygen radicals from phagocytic inflammatory cells. It is therefore suggested that these radicals may play a role in the collagen destruction by granulation tissues, as in periodontal diseases.

CONCLUSIONS

1. The topical application of vitamin E in adjunct with supragingival debridement further improved GI and PI in chronic gingivitis associated with RPD.
2. The topical application of vitamin E might enhance resistance of gingival tissue destruction as manifested by the histological evidence.
3. Vitamin E has proved to be an easy method for topical application of antioxidant therapy.

RECOMMENDATIONS

1. Further studies are indicated in order to evaluate the use of antioxidant therapy in periodontitis.
2. The involvement of antioxidant material in a carrier system is recommended in order to achieve an effective duration and drug concentration of therapy.
3. Routine supplementary use of antioxidant therapy is recommended as therapeutic management or protection from destructive periodontal diseases.

REFERENCES

1- Page RC. Gingivitis .J Clin Periodontol 1989;13:345-359.
2- Bates JF, Addy M. Partial dentures and plaque accumulation .J Dent 1978;6(4):285-293.
3- Addy M, Bates JF. Plaque accumulation following the wearing of different types of removable partial dentures. J Oral Rehabil 1978;6:111-117.
4-Mantle D ,Wilkins RM, Preedy V. A novel therapeutic strategy for Ehlers-Donals syndrome based on nutritional supplements. Med Hypotheses 2005;64(2):279-283.
5- Waddington RJ, Moseley R, Embery G. Periodontal disease mechanisms. Reactive oxygen species: a potential role in the pathogenesis of periodontal disases. Oral Dis 2000;6:138-151.
6- Chapple ILC: Reactive oxygen species and antioxidants in inflammatory diseases . J Clin Periodontol 1997;24:287-296.
7- Halliwell B, Gutteridge J M C, Cross C E. Free radicals. Antioxidants and human disease: Where are we now? J Lab Clin Med 1992; 598-620.
8- Halliwell B. How to characterize biological antioxidants. Free Radical Research Communications 1990; 9:1-31.
9- Traber MG. Vitamin E. In: Shils ME, Shike M, Ross AC, Caballero B, Cousins R, eds. Modern Nutrition in Health and Disease. 10th ed. Baltimore, MD: Lippincott Williams & Wilkins 2006;396-411.
10-Singh U, Jialal I . Anti-inflammatory effects of alpha-tocopherol. Annals of the New York Academy of Sciences 2004;1031:195-203.
11-Altman LC, Baaker C, Fleckman P, Luchtel D, Oda D. Neutrophil-mediated damage to human gingival epithelial cells. J Periodontal Research 1992;27(1):70-79.
12- Aman B, Wijkander P, Hjerpe A. Reduction of collagen degradation in

experimental granulation tissue by vitamin E and selenium. J Clin Periodontology 1994;21(1):45-47.
13- Ellis S D, Tucci MA, Serio FG, Johnson RB. Factors for progression of periodontal diseases. J Oral Pathology Medicine 1998;27(3):101-105.
14-Tozum TF, Türkyilmaz I, Yamalik N, Tümer C, Kilinç A, Kilinç K, Karabulut E, Eratalay K. Analysis of the possible impact of inflammation severity and early and delayed loading on nitric oxide metabolism around dental implants. Int J Oral Maxillofac Imlants 2005;20(4):547-56.
15-Lukandu OM, Costea DE, Neppelberg E, Johannessen AC, Vintermyr OK. Khat(Catha edulis) induces reactive oxygen species and apoptosis in normal human oral keratinocytes and fibroblasts. Toxicol Sci 2008;103(2):311-24.
16- Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontol 1999; 4: 1.
17- Silness J, Löe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. Acta Odont Scand 1964; 22: 121.
18- Löe H. The gingival index, the plaque index and the retention system. J Periodontol 1967; 38: 610.
19- Löe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity, Acta Odont Scand 1963;21:533-551.
20-Luft JH. Improvement in epoxy resin embedding methods. J Bio Phys Biochem Cytol 1961 ;9:409-413.
21- Venable JH, Coggeshall R. A simplified lead citrate stain for use in electron microscope. J Cell Biol 1965;25:407-409.
22- Battino M, Ferreiro MS, Quiles JL, Stefano B ,Luciana L ,Pedro B. Alterations in the oxidation products, antioxidant capacity and lipid patterns in plasma of patients affected by Papillon-Lefevre eyndrome. Free Radic Res 2003; 37(6):603-609.
23- McHenry KR , Johansson OE , Christersson LA . The effect of removable partial denture framework design on gingival inflammation: A clinical model. The Journal of Prosthetic Dentistry 1992; 68(5) : 799-803.
24-Bergman B, Ericson G. Cross sectional study of the periodontal status of removable partial denture patients. J Prosthet Dent 1989;61:208-211.
25-Orr S ,Liden GJ, Newman HN. The effect of partial denture connectors on gingival health. J Clin Periodontol 1992;19(80:589-594.
26-Bergman B, Hugson A, Olsson CO. Periodontal and prosthetic condition in patients treated with removable partial dentures and artificial crowns. A longitudinal two-year study. Acta Adontol Scand 1971;29:621-638.
27-Kantarci A, Oyaizu K, Van Dyke TE. Neutrophil-mediated tissue injury in periodontal disease pathogenesis: Findings from localized aggressive periodontitis. J Period 2003;74(1):66-75.
28-Honda H, Domon H, Okui T, Kajita K, Amanuma R, Yamazaki K. Balance of inflammatory response in stable gingivitis and progressive periodontitis lesions. Clinical and experimental Immunology 2006;144(1):35-40.
29-Panjamurthy S, Manoharan S , Ramachandran CR. Lipid peroxidation and antioxidant status in patients with periodontitis. Cellular and molecular biology letters 2005;10(2):255-264.
30-Katsuragi H, Hasegawa A, Saito K. Distribution of metallothionein in cigarette smokers and non-smokers in advanced periodontitis patients. J Periodontol 1997;68:1005-1009.
31-Okada H. Phenotypic and functional characterization of peripheral blood T cells in adult periodontitis. J Periodont Res 1991;26:289-292.
32-Sobaniec H, Sobaniec-Lotowska ME. Morphological examinations of hard tissues of periodontium and evaluation

of selected processes of lipid peroxidation in blood serum of rats in the course of experimental periodontitis. Med Sci Monit 2000;6(5):875-81.

33- Wadleigh RG , Redman RS , Graham ML, Krasnow SH, Anderson A , Cohen MH .Vitamin E in the treatment of chemotherapy-induced mucositis. The American Journal of Medicine 1992;92(5):481-484.

34- Parrish JH, DeMarco TJ , Bissada NF. Vitamin E and periodontitis in the rat. Oral surgery oral medicine oral pathology 1977;44(20):210-218.

35- Asman B, Wijkander P, Hjerpe A. Reduction of collagen degradation in experimental granulation tissue by vitamin E and selenium. J of Clinical Periodontology 1994;21(1):45-47.

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A case report

Delayed encephalopathy after acute carbon monoxide poisoning:

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تأخر الاعتلال الدماغي الحاد بعد التسمم بأول أكسيد الكربون:

عبدالعزيز الخوتاني
أستاذ مساعد قسم طب الأطفال، كلية الطب، جامعة أم القرى جامعة

الملخص العربي

الملخص: هذا تقرير عن حالة طفل عمره خمس سنوات يشتبه في تعرضه لتسمم حاد بأول أكسيد الكربون أحضره رجال الشرطة في حالة إغماء إلى غرفة الطوارئ. لوحظ تحسن كبير في الحالة العصبية للمريض بعد العلاج المبدئي بـ100% أكسجين باستخدام التهوية الميكانيكية. بعد ثلاثة أيام من التحسن، ظهر على المريض اضطراب عصبي حاد مفاجئ يشير إلى اعتلال دماغي متأخر. تمت معالجة المريض مرة أخرى باستخدام 100% أكسجين باستخدام التهوية الميكانيكية وعلاجات مساعدة أخرى. تمت إزالة الأنبوب من المريض في اليوم العاشر و قد خرج المريض من المستشفى بعد أسبوعين. بعد ستة أشهر من معالجة المريض بواسطة العلاج الطبيعي المكثف والتأهيل والمتابعة تم تقريبا علاج المريض.

الكلمات الدالة: التسمم بأول أكسيد الكربون، اعتلال الدماغ المتأخر

ABSTRACT

Objective: This case report describes a five years old boy that was brought to the emergency room by police in an unconscious state with suspected acute carbon monoxide poisoning. After initial management with 100 % oxygen through mechanical ventilation, patient showed remarkable improvement in neurological status. Three days after initial recovery, he showed abrupt and profound neurological deterioration indicating onset of delayed encephalopathy. The patient was managed again by 100% oxygen through mechanical ventilation and other supportive measures. He was exubated on day 10th and discharge after two weeks. With intensive physiotherapy and rehabilitation, there was nearly complete recovery after six months follow up.

***Keywords*:** Carbon monoxide poisoning, delayed encephalopathy

INTRODUCTION

Carbon monoxide (CO) is a colorless, odorless, toxic gas produced as a by-product of incomplete combustion of carbon-based fuels. It is a major indoor pollutant in the developing world and an important cause of mortality worldwide [1, 2]. Major sources of CO are household fires, motor vehicles, heater appliances [3].

Due to varying presentation of CO poisoning, ranging from vague flu-like symptoms to profound central nervous system dysfunction and prominent neuropsychiatric manifestation, it has been called "the disease of thousand faces" [4].

CO binds reversibly to hemoglobin with an affinity 200-250 times that of oxygen, thereby blocking the capacity of hemoglobin to transport oxygen leading to oxygen debt and lactic acid accumulation. The brain and myocardium are more susceptible to hypoxia due to increased oxygen demand [5].

Majority of the patients of CO poisoning recover well without any complication with hyperbaric or high oxygen therapy. However the incidence of delayed neuropsychiatric sequelae is seen in 10-30% of these cases [6].

The clinical presentation of delayed encephalopathy is in the form of deterioration and relapse of cognitive ability, behavior movement after initial recovery from acute CO poisoning [7, 8]. Latent period between acute presentation and delayed encephalopathy may vary from few days to weeks [8].

The most common grey matter lesion is bilateral necrosis of the globus pallidus. Other parts like hippocampus or focal area of cortex may also be affected [8].

A case of delayed encephalopathy in five years old boy following CO poisoning is reported here. It was characterized by apparent recovery after acute poisoning, followed by an abrupt and profound neurological deterioration with a seemingly reversible course. Diffusion weighted magnetic resonance imaging was quite characterstic in our patient.

CASE REPORT

A five years old boy was brought to the Pediatrics Emergency unit of Alnoor Specialist Hospital on 20/8/1432 by police after evacuating him from a burning building. Parents after evacuation were admitted in the medical unit of other nearby hospital. It was not known how long the patient has been exposed to the toxic fumes before police team arrived. The source of the fire was short circuiting of electrical wires in the kitchen which happened late in the night when family members were sleeping. He was brought in

unconscious state (GC scale 8/15) with irregular breathing, poor peripheral pulses, delayed tissue perfusion and hypotension (BP 80/40 mm of Hg). His mucous membranes were pink. There was smell of smoke but no thermal injury or burn. Evaluation of central nervous system revealed an unconscious child with normal sized pupils reacting to light, head lag, generalized hypotonia, generalized hypereflexia, power of 2/5 in all the four limbs and bilateral positive babinski.

His initial arterial blood gas (ABG) showed pH: 7.10, PCO_2: 38 mm, PO_2:40 mm of Hg, O_2 saturation 60%, HCO_3: 12.8, SBE: – 16. He was immediately intubated and connected to mechanical ventilator with 100% oxygen. He was sedated with fantanyl and medozolam. He received bolus of normal saline and started on dopamine. He was put on broad spectrum antibiotics, prophylactic phenytoin and ranitidine. He was also started on dexamethasone for presumed brain edema. Repeat ABG within half an hour showed improvement, pH: 7.32, PCO_2: 35.6, PO_2: 68 mm, HCO_3: 17.8, SBE: -8. His tissues perfusion and BP also improved (110/70mm of Hg).

His other initial investigations revealed CPK: 3176 IU/L, CK MB 77 IU/L and LDH of 396 IU/L. His CBC, ESR, serum electrolytes, liver enzymes, blood sugar, urea and creatinine were essentially normal. Electrocardiogram upon admission showed normal sinus rhythm with no other abnormality. Skeletal survey did not show any fracture. Initial brain CT scan was normal. Due to absence of appropriate facilities, carboxyhaemoglobin (COHb) measurement could not be carried out.

There was remarkable progress in next 4 days. He regained consciousness. He was exubated and did well after extubation. His ABGs & vitals were maintained within normal range. He started moving upper limbs and lower limbs with good tone and reflexes.

On 6th day of admission, his condition deteriorated. He developed generalized tonic and clonic convulsions and became unconscious. Examination at this point revealed GC scale 8/15, BP 119/71, HR 110/m, RR 25/m, generalized hypotonia, hypereflexia, grade 2/5 power in all four limbs and bilateral positive babinski. There were no meningeal signs and pupils were bilaterally dilated but reacting to light. He was again shifted to ICU and ventilated with 100% O_2, PIP/PEEP 28/6. Because of his progressive neurological problems, further investigations were carried out. CSF analysis was done which showed RBC: 1000/mm WBC: 5/mm, protein 26mg%, sugar 116 mg%, & sterile culture.

MRI brain revealed swollen basal ganglion displaying high signal intensity on FLAIR and T2 WI. On diffusion WI, centrum semiovale displayed restricted diffusion. Considering the patient's history, this finding was suggestive of global ischemia due to CO poisoning.

He was continued on mechanical ventilation, started on IV phenbarbitone, dopamine. He showed remarkable improvement and exubated on 10th day. In-patient physiotherapy and intensive rehabilitation was started and he was discharged after 2 weeks. At the time of discharge he was fully oriented, responsive, moving all the four limbs but still could not sit in the bed. He was advised to continue physiotherapy at home.

On the last follow up in out-patient clinic after six months, he was able to ambulate without any assistive devices.

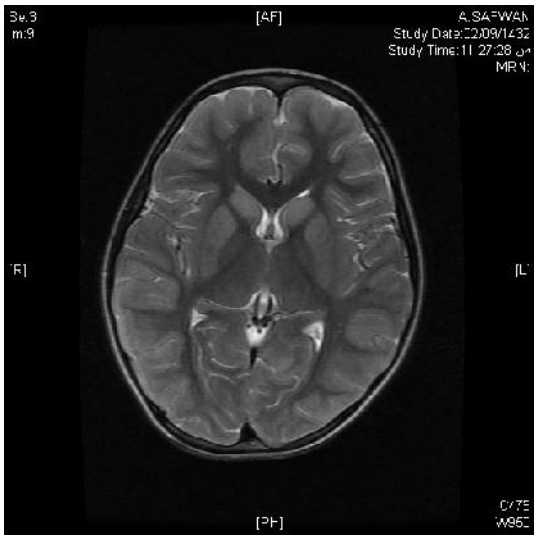

Figure 1

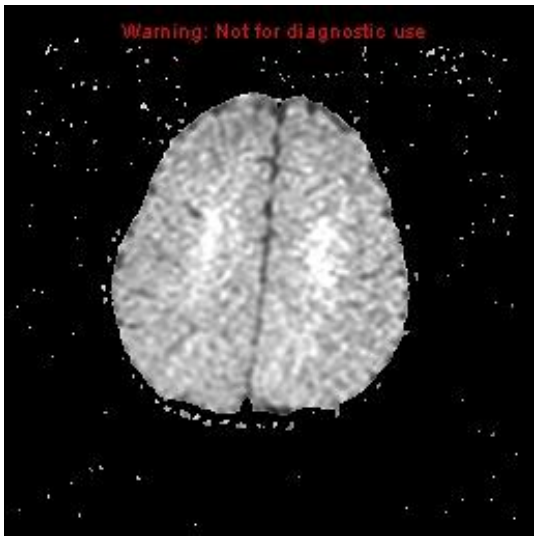

Figure 2

DISCUSSION

In the patient under report, positive history of exposure to fumes, convergence of symptoms and signs along with characterstic brain MRI findings satisfied the criteria for the diagnosis of CO toxicity, even in absence of blood COHb level. The studies conducted have shown significant correlation between severities of toxicities with neurological manifestation than with blood CO level, thus validating the importance of clinical judgment [9, 10]. However, it is important to determine blood CO level to confirm the diagnosis if facilities are available.

The classical presentation of delayed encephalopathy includes apathy, disorientation, amnesia, extra-pyramidal symptoms, incontinence, psychosis, global cognitive impairment, seizure, and coma [11].

Patients with delayed encephalopathy due to CO poisoning usually have a lucid period of few weeks before the appearance of a progressive decline in their neurocognitive functions [7, 8]. However in our patient the lucid interval was very short (3 days) and clinical presentation was profound in the form of seizure, coma and quadriplegia. The severe presentation in our patient may be because of his young age.

Functional imaging has been used to show globus pallidus, basal ganglion to be one of the most frequently injured areas during the acute stage of CO poisoning [8]. MRI in our patient revealed swollen basal ganglion displaying high signal intensity on FLAIR and T2 WI.

Recovery from delayed encephalopathy occurs in 50% -75% within one year [3, 12]. Risk factors predicting the onset of delayed encephalopathy in patients with acute CO poisoning is not so clear. Clinical status or carboxyhaemoglobin level on initial presentation could not predict the occurrence of delayed encephalopathy. Some investigators have shown a significant correlation between cerebral white matter changes on initial CT scan and the development of delayed encephalopathy in acute CO poisoning [13]. Brain MRI showing bilateral symmetric white matter hyperintensity (T2WI/ FLAIR) could be a good predictor of delayed encephalopathy in patient with acute CO intoxication [14]. Other imaging

technique like positron emission tomography and single photon emission tomography which detect the blood flow abnormality in affected area, might be more sensitive and better predictor of clinical course in patients with acute CO poisoning [15, 16].

The pathogenesis of the delayed encephalopathy of CO intoxication remains debatable; No single reason (e.g. hypoxia) alone is adequate enough to explain the varying presentations. Various theories have been proposed for this e.g. immuopathological damage, disturbance of dopaminergic and serotonergic functions, role of CO as an endogenous neurotransmitter[15].

Oxygen therapy including the use of hyperbaric oxygen has been for years the mainstay treatment of acute cases. Administration of 100% O_2 competitively displaces CO from its transport site of hemoglobin molecule, thereby decreasing the half life from 200-300 minutes to 60-90 minutes. Half life can be further shortened to 30 minutes by use of hyperbaric oxygen leading to quick removal of CO from the blood and thus preventing lipid peroxidation of the brain[5, 17]. Despite this convincing hypothesis, there are conflicting reports on the use of hyperbaric oxygen in acute CO poisoning.

A significant higher neurological morbidity was found in those treated with normobaric oxygen compared to those who received hyperbaric oxygen[18]. On the contrary, in a randomized controlled trial, Scheinkestel et al [19] compared the two different modalities and found poorer outcome in those treated with hyperbaric oxygen as compared to the group treated with normobaric oxygen.

CONCLUSIONS

This case report demonstrates that while treating the immediate complications of CO poisoning, clinician must be aware of delayed complications among survivors which usually occur few days to weeks after acute poisoning. Regular follow up and monitoring of neurocognitive functions of these patients is very critical to caution them and their families of this potential complication.

REFERENCES

1. Oslon KR. Carbon monoxide poisoning mechanisms, presentation and controversies in management. J Emerg Med 1984; 1:233-43

2. Mehta SR, Niyogi M, Kasthuri AS, Dubal U, Bindra S, Prasad D, Lahiri AK, . Carbon monoxide poisoning. J Assoc Physicians India 2001; 49: 622-5

3. Toungrat T, Niphon P. Delayed encephalopathy and cognitive sequelae after acute carbon monoxide poisoning. Report of a case and review of the literature. J Med assoc Thai 2009; 92: 1374-9

4. Fisher J, Carbon monoxide poisoning: a disease of a thousand faces. Chest 1999;115:322-3

5. Liebelt EL. Hyperbaric oxygen therapy in childhood carbon monoxide poisoning. Curr Opin Pediatr 1999; 11;259-64

6. Ernst A, Zibrak JD. Carbon monoxide poisoning. N Engl J Med 1998; 339:1603-8.

7. Hsiao CL, Kuo HC, Huang CC. Delayed encephalopathy after carbon monoxide intoxication- Long term

prognosis and correlation of clinical manifestation and neuroimages. Acta Neurol Taiwan 2004; 13:64-70

8. Allesandro S. Zagami, A, Keith Lethlean, Ross Mellick. Delayed neurological deterioration following carbon monoxide poisoning. MRI findings J Neurol 1993; 240:113-116

9. Myers RA, Britten JS. Are arterial blood gases of value in treatment decision for carbon monoxide poisoning? Crit Care Med 1989; 17:139-42

10. Messier LD, Myers RA. A neuropsychological screening battery for emergency assessment of carbon monoxide poisoned patients. J Clin Psychol 1991;47:675-84

11. SP Lam, S Y Y Fong, A K Wok, T Wong, Y K Wing. Delayed neuropsychiatric impairment after carbon monoxide poisoning from burning charcoal. Hong Kong Med J 2004; 10:428-31

12. Choi IS. Delayed neurologic sequelae in carbon monoxide intoxication. Arch Neurol 1983; 40:433-5.

13. Choi IS, Kim SK, Choi YC, Lee SS, Lee MS, Evaluation of outcome after acute carbon monoxide poisoning by brain CT. J Korean Med Sci 1993; 8:78-83

14. Otubo S, Shirakawa Y, Aibiki M, Nishiyama T, Maekawa S, Kikuchi K, et al. Magnetic resonance imaging could predict delayed encephalopathy after acute carbon monoxide intoxication. Chudoku Kenkyu 2007;20: 253-61

15. Hurley RA, Hopkins RO, Bigler ED, Taber KH. Applications of functional imaging to carbon monoxide poisoning. J Neuropsychiatry Clin Neurosci 2001;13:157-60.

16. Sesay M, Bidabe AM, Guyot M, Bedry R, Caille JM, Maurette P. Regional cerebral blood flow measurements with Xenon-CT in the prediction of delayed encephalopathy after carbon monoxide intoxication. Acta Neurol Scand Suppl 1996;166:22-7.

17. Tibbles PM, Edelsberg JS. Hyperbaric-oxygen therapy. N Engl J Med 1996;334:1642-8.

18. Hawkins M, Harrison j, Charters P. Severe carbon monoxide poisoning; Outcome after hyperbaric oxygen therapy. Brit Anaesth 2000;84;584-6

19. Scheinkestel CD, Bailey M, Myles PS, Jones K, Cooper DJ, Millar IL, Tuxen DV. Hyperbaric or normobaric oxygen for acute carbon monoxide poisoning: a randomized controlled clinical trial. Med J Aust 1999; 170;203-10

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Discussion
Emphasize the new and important aspects of the study and conclusions that follow from them. Do not

repeat in detail data or other material given in the introduction or the results section. Include in discussion section the implications of the findings and their limitations including implications for future research. Relate the observations to other relevant studies.

Conclusion
Link the conclusions with the goals of the study but avoid unqualified statements and conclusions not completely supported by data. State new hypothesis when warranted but clearly label them. Such

Acknowledgements
Persons who have contributed intellectually to the paper but whose contributions do not justify authorship may be named and the function or contribution described.

References
References should be cited in the Vancouver style in consecutive numerical order at first mentioned in the text and designated by the reference number in superscript. References appearing in a table or figure should be numbered sequentially with those in text.

> **Vancouver style of references:**
> Snowdon J. Severe depression in old age. Medicine Today. 2002 Dec;3(12):40-47.
>
> Skalsky K, Yahav D, Bishara J, Pitlik S, Leibovici L, Paul M. Treatment of human brucellosis: systematic review and meta-analysis of randomised controlled trials. BMJ. 2008 Mar 29;336(7646):701-4.

Illustrations
Illustrations should clarify and augment the text. The selection of sharp, high-quality illustrations is of paramount importance. Photographs including all types of images should be prepared as .jpg uncompressed files at a resolution of 300 dpi. Figures of inferior quality will not be acceptable.

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Original article: maximum 3000 words excluding title page and a structured abstract of 250 words and references with no more than three tables or figures and 40 references Short Reports / Short Communications / Special Communications / Case reports: maximum 1250 words excluding title page and an unstructured abstract of 150 words and references with no more than two tables or figures and 10 references. It should not have more than six authors

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Short Report: Abstract; Introduction; Patients Methods and Results; and Conclusion.

Special Communication: Abstract; Introduction; Methods and Results; and Conclusion.

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