STUDY ON FREE LIVING AMOEBAE (ACANTHAMOEBA) IN ASSOCIATION WITH CONTACT LENSES

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Abstract
In Benha locality, free living amoebae (FLA) were isolated from 60% of 100 soft daily wear contact lenses, obtained from 50 contact lens wearers showing an improper lens care system and having problems with their lenses. Acanthamoeba and Naegleria species represented 63.3% and 36.7% of the isolated FLA strains, respectively. The isolated FLA in the present study were non-pathogenic as proven by concanavalin A-induced agglutination test and experimental animal pathogenicity test. Proper lens care system is essential in patients using contact lenses.

Introduction
Among the numerous free living amoebae (FLA) of soil and water habitats, certain species belonging to two genera, Acanthamoeba and Naegleria are facultative parasites of man. Human infections with amoeba of this type were first recognized by Fowler and Carter (1965), who reported that Naegleria fowleri is the major cause of primary amoebic meningoencephalitis, and the disease was named by Butt (1966). A more chronic type of amoebic encephalitis named granulomatous encephalitis is caused by Acanthamoeba (Martinez, 1977). Page (1967 a) reported that Acanthamoeba polyphaga is a human pathogen causing keratitis and uveitis. Key et al. (1980) reported a case of keratitis due to Acanthamoeba castellani. Margo (1987) isolated Acantham-
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Acanthamoeba cysts and trophozoites from both the primary host cornea and failed corneal graft in 41-years old women suffering from unhealed corneal ulcer. Acanthamoeba is being reported with increasing frequency as a pathogen responsible for chronic stromal keratitis and ulceration in contact lens wearers (Randy et al., 1986). Approximately 85% of Acanthamoeba keratitis cases are caused by contact lens wear, commonly in association with the use of non-sterile home made saline and cold disinfection methods (Stehr-Green et al., 1989). In these cases, Acanthamoeba has been cultured from soft contact lenses (Cohen et al., 1987), from solutions in contact lens case, from distilled water and from saline solutions (Moore, 1988).

The present work was a trial to isolate FLA (Acanthamoeba) from contact lenses of contact lens wearers, having eye complaint with their lenses in Benha locality and also to examine the pathogenicity of the isolated strains.

Material and Methods

Contact lenses from 50 contact lens wearers, using soft daily wear contact lens, attending contact lens clinic in Benha University hospital and complaining from eye redness and discomfort were taken and washed in sterile page's saline. A complete ophthalmological examination and detailed discussion regarding the employed contact lens care regime for each patient was done. All patients showed an improper care system.

Contact lens samples were cultivated on non nutrient agar previously seeded with Escherichia coli (E.coli) at 37°C and 43°C. Cultures were examined by direct microscopic examination and flagella-tion test, 24 and 48 hours later for identification of the isolated FLA. (Page, 1967b). Each of the isolates was subjected to concanavalin A-induced agglutination test (Trissele et al., 1978). Isolates were cultivated axinically in Chang's fluid medium at 37°C and 43°C. Organims were washed several times in phosphate buffer sa-
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Trisselle D., Martinez P. A., Delatorre M. and De Saurez E. p. (1978) : Surface properties of Entamoeba, increased rates of human erythrocyte phagocytosis in


Fig. 3: Light microscopy to demonstrate the general shape of the trophozoite and spiky acanthabodia of Acanthamoeba (x 200).

Fig. 4: Light microscopy to demonstrate the general shape of the cyst of Acanthamoeba with angular double wall (x 400).

Fig. 5: Acanthamoeba infected mice: Meninges showed mild congestion (H and E x 150).

Fig. 6: Acanthamoeba infected mice: Brain showed mild lymphocytic infiltration (H and E x 150).
Discussion

The present work was done on contact lens wearers using soft daily wear contact lenses in Benha locality. On using non-nutrient agar seeded with E. coli, free living amoebae (FLA) were isolated from 60 (60%) out of 100 soft contact lens samples of 50 contact lens wearers suffering from eye redness and discomfort. Majority of cases gave history of improper lens care. Identification of the isolates was done by direct microscopic examination and flagellation test. Naegleria species with rounded lobopodia of the trophozoites, rounded single wall of the cyst and positive flagellation test were identified from 22 (36.7%) out of 60 positive samples, while Acanthamoeba species with small spiky pseudopodia of the trophozoite, angular double wall of the cyst and negative flagellation test were identified from 38 (63.3%) out of 60 positive samples. It is noticed that Acanthamoeba isolates are more than Naegleria isolates in association with contact lens wear. The previous data are in agreement with Moore et al. (1985) and Mar-
onstration of amebic organisms. Naheed et al. (1993) isolated Acanthamoeba polyphaga from contact lens cases and from corneal scrapings of Acanthamoeba keratitis patient which was associated with the use of disposable soft contact lenses.

Proper lens care system is essential in patients using contact lenses. Leluan et al. (1991) reported that amoeba were detected in 5% of lens storage cases and that lens case contamination on account of improper care may be seen in up to 25% of subjects. The effectivity of contact lens disinfection systems against Acanthamoeba was tested by the percent kill rate of cysts 24 hours and one week after incubation. The kill rate percentage ranged from 94.4% to 1.4% and heat disinfection is offered as an effective option for killing Acanthamoeba (Conner et al. 1991). Gohn et al. (1989) reported that the omission of surfactant clearing and a rub and rinse step results in the accumulation of environmental foreign debris on the contact lens, which can potentially disrupt the epithelial barrier. Following exposure of the contact lens to the organism, Acanthamoeba cysts and trophozoites can immediately adhere to the surface. Suboptimal lens clearing and inadequate disinfection may result in the increased persistence of cysts and trophozoites on the lens surface.

Concanavalin A-induced agglutination test and animal pathogenicity test were done to clear-out the pathogenicity of the isolated strains. As regards concanavalin A-induced agglutination test, no agglutination occurred with Naegleria strains up to the dilution of 100 μg/ml, while Acanthamoeba strains showed no agglutination up to the dilution of 400 μg/ml. This is in agreement with Stevens and Kaufman (1979), meaning that the isolated strains, Naegleria and Acanthamoeba are most probably non pathogenic. Pathogenic variants of Naegleria fowleri produces fatal meningoencephalitis in mice after intranasal inoculation and histopathological studies of brain specimens showed diffuse
subarachnoid haemorrhage, necrosis of the olfactory lobes and trophozoites were detected on brain tissue (Carter, 1970). Pathogenic strains of Acanthamoeba produced fatal meningoencephalitis in experimental mice, when inoculated intranasally (De-Jonckheere, 1980). In the present study histopathological examination of the brain tissue of mice inoculated intranasally with the isolated Naegleria strains, 15 days post-infection showed no cysts or trophozoites but only mild lymphocytic infiltration. Acanthamoeba infected mice survived up to the end of observation days (15 days) and histopathological examination of the brain specimens revealed mild meningeal congestion and mild lymphocytic infiltration, but no granulomatous reaction. From these results, it is clear that the isolated FLA. strains, from studied contact lenses either Naegleria or Acanthamoeba are non pathogenic strains.

References


Carter R. F. (1970) : Description of a Naegleria spp. isolated from two cases of primary amoebic meningoencephalitis and the experimental pathological changes induced by it. J. pathol., 100: 217-244.


Table 2: Concanavalin A-induced agglutination test.

<table>
<thead>
<tr>
<th>Isolated strains</th>
<th>Serial dilution of concanavalin A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>400µg/ml</td>
</tr>
<tr>
<td>Naegleria</td>
<td>+ Ve</td>
</tr>
<tr>
<td>Acanthamoeba</td>
<td>- Ve</td>
</tr>
<tr>
<td>Control</td>
<td>- Ve</td>
</tr>
</tbody>
</table>

Table 3: Histopathological changes in brains of survived mice (pathogenicity test)

<table>
<thead>
<tr>
<th>Group strain</th>
<th>Number of brain specimens</th>
<th>Meninges</th>
<th>Histopathological changes</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>lymphocytic infiltration</td>
<td>Granulomatous reaction</td>
<td>Necrosis</td>
</tr>
<tr>
<td>Naegleria</td>
<td>20</td>
<td>Mild</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Acanthamoeba</td>
<td>20</td>
<td>Mild congestion</td>
<td>Mild</td>
<td>-</td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Fig. 1: Light microscopy to demonstrate the general shape of the trophozoite and rounded lobopodia of Naegleria (x 200).

Fig. 2: Light microscopy to demonstrate the general shape of the cyst of Naegleria with rounded single wall (x 400).
line (PBS), ph 7.2. Equal volumes of amoebae suspension and concanavalin A (Supplied by Sigma) of known concentration in PBS, ph 7.2 (Serial dilutions 400, 200, 100, 50, 25 and 12.5 ug/ml) were mixed in the wells of microtitre agglutination plates, covered and incubated at 37°C. Equal volumes of PBS alone and organisms suspension were mixed together as a control. Plates were examined by naked eye and microscopically for agglutination.

Animal pathogenicity test (Culbertsoni et al., 1968) was carried out on sixty laboratory bred albino swiss mice, 6-8 weeks old of both sexes with an average weight of 20 gm each. The isolated FLA. strains were cultivated axini-cally in Chang's fluid medium at 37°C and 43°C (De. Jonckheere, 1977). Each mice was infected through intranasal instillation route of amoebic suspension in 0.02 ml Chang's medium (20.000 trophozoites). The first group (20 mice) were inoculated with Naegleria strains, and the second group (20 mice) were inoculated with Acanthamoeba strains, while the third control group (20 mice) were inoculated with sterile Chang's medium. Mice were dissected fifteen days post infection and brain specimens were collected for histopathological examination.

Results
The results are shown in tables (1-3) and figures (1-6).

Table 1 : Isolation and identification of FLA. from contact lenses.

<table>
<thead>
<tr>
<th>Eye complaint</th>
<th>Number of patients</th>
<th>Number of contact lenses</th>
<th>Non-nutrient agar cultures positive for FLA.</th>
<th>Direct microscopic and flagellation test</th>
<th>examination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td>Naegleria No.</td>
</tr>
<tr>
<td>Discomfort</td>
<td>23</td>
<td>46</td>
<td>24</td>
<td>52.1%</td>
<td>8</td>
</tr>
<tr>
<td>Redness</td>
<td>27</td>
<td>54</td>
<td>36</td>
<td>66.7%</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100</td>
<td>60</td>
<td>60%</td>
<td>22</td>
</tr>
</tbody>
</table>

BIOCHEMICAL AND HISTOLOGICAL CHANGES IN EXPERIMENTAL FATAL ANAPHYLACTIC SHOCK

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Abstract

Anaphylactic shock was induced in guinea-pigs by sensitizing them with egg albumin through intramuscular injection of a single dose of 150 mg and then challenging them with a second intraperitoneal (i.p.) or intramuscular (i.m.) dose of the protein 4 weeks later. The animals which received the i.p. injections, developed the reaction rapidly within 7 minutes and died within 15 minutes (rapid shock). While those which received the i.m. injections developed the reaction more slowly within 20 minutes and died within about 35 minutes (slow shock). Blood for biochemical assays was taken immediately after death by cardiac puncture and tissue samples were excised from the lungs, heart, kidneys, brain, liver, spleen, adrenals and stomach for serotonin assay and histological studies.

Serotonin content of the stomach wall was significantly decreased both in the rapid and slow shock groups, but in the lungs it was significantly decreased only in the slow shock group. Plasma Serotonin was significantly elevated in both shock groups. Plasma cortisol was low in the rapid shock group, while it was within normal level in the slow shock group. High values of serum catecholamines and IgE were estimated in both shock groups.

The histological findings were similar in both shock groups. The most striking finding was observed in the lungs which were inflated and pale with congestion of pulmonary vessels. The alveoli were distended and
had thin walls (acute emphysema) with eosinophilic infiltration of the bronchial walls and alveolar septa. Acute renal tubular necrosis, inter-myocardial hemorrhage and brain oedema were also observed in some animals. No histological changes could be detected in the liver, spleen adrenals and stomach.

Thus, high plasma serotonin, serum IgE and total catecholamine levels as well as acute emphysema combined with bronchial eosinophilia could be considered as an important criteria in the diagnosis of death resulting from anaphylaxis.

**Introduction**

Anaphylaxis may be a cause of sudden death, and because of its accidental character such cases are sent for medicolegal investigation to determine the cause of death, its circumstances and possible liability (Hirvonen and Huttunen, 1984). Anaphylactic deaths may be rapid, and are preceded by symptoms and signs suggestive of hyperacute bronchial asthma or delayed for several hours with predominant nervous symptoms such as coma or with symptoms of circulatory failure. Such deaths are not likely to leave distinct or specific signs in the body and usually the only finding is pulmonary oedema, and the diagnosis being mostly based on anamnnesis, indicative signs such as atopic eczema or bronchial asthma and on the exclusion of other causes (Gordon and Shapiro, 1982).

However, biochemical parameters are becoming increasingly important in establishing a cause of death or in confirming the results of a necropsy and histopathology (Fischer and Petty, 1977). Anaphylactic reaction leads to release of at least 4 major chemical entities; histamine, serotonin, plasma kinins and slow-reacting substances. Thus histamine or serotonin measurement could serve as a postmortem test for anaphylactic death since they do not disappear from the serum within the first two days postmortem. Catecholamines and hydrocorticoids are also released as a countermeasure in anaphylactic shock, and these hormones are also measurable after death (Hirvonen and Huttunen,
1984). The level of immunoglobulin E (IgE) in serum is also useful in the study of allergic and anaphylactic reactions (Stefanini, 1979).

Hence, this work was conducted to study some biochemical parameters (serotonin, cortisol, total catecholamines and IgE) and histological changes which may be helpful in the diagnosis of fatal anaphylactic shock.

**Material and Methods**

Thirty adult male guinea pigs weighing 627-735gm were divided into three groups, each comprising 10 animals. Animals of the first two groups were sensitized with egg albumin intramuscularly at a dose of 150mg as a single injection (Hirvonen and Huttunen, 1984). Four weeks later, anaphylactic shock was induced with a second intraperitoneal dose of 150 mg egg albumin for the first group and an intramuscular dose of 200 mg of the same protein for the second group. The third non-sensitized group served as control and the animals were injected with saline and were killed by decapitation.

Blood was drawn by cardiac puncture immediately after death for the assay of serotonin, cortisol, total catecholamines and IgE.

Serotonin in blood and tissues was measured by the method of Weissbach (1965), by extracting the amine from alkaline solution into butanol and re-extracting it back into dilute acid and the developed fluorescence is measured spectrophotofluorometrically at a wave length of 550 mm. Serum cortisol was measured by enzyme linked immunosorbent assay using "UBI" Magiwel kit according to the method of Spark (1971). Total catecholamines was estimated by the method of Von Euler and Folding (1955). Serum IgE was measured by radial immunodiffusion according to the method of Batty and Torrigiani (1976).

The lungs (without inflation), heart, kidneys, brain (cerebrum, cerebellum and brain stem), liver, spleen. adrenals and stomach were dissected out. Specimens of these organs were fixed in neutral formal saline and 7 microns thickness sections were prepared and stained with haematoxylin and eosin for histological examination.

The Student's t test was utilized for the statistical analysis of the biochemical data.
Results

Guinea-pigs that received the second injection of the protein intraperitoneally developed the symptoms rapidly (rapid shock) within 7 minutes in the form of twitching of the nose, jerking of the limbs, dyspnoea and loss of consciousness and died after about 15 minutes. However, the animals which received the second injection intramuscularly developed the reaction more slowly (slow shock) within 20 minutes and died in about 35 minutes.

Biochemical Results

The results of plasma, stomach and lung serotonin are summarized in table (1). Plasma serotonin was significantly elevated in the rapid shock group than that of the control or slow shock groups. Serotonin content of the stomach wall was significantly lower in both shock groups as compared to the control group, but in the lungs it was significantly decreased only in the slow shock group.

The results of serum IgE, total catecholamines and cortisol are summarized in table (2). Serum IgE and total catecholamines were increased significantly in both shock groups. Plasma cortisol decreased significantly in the rapid shock group only.

Histological Results

The lungs of the 1st two groups appeared inflated and pale and microscopically, the alveoli were distended with thin interalveolar septa and intercommunication between the alveoli with rupture of some septa forming spurs (Fig. 1). Pulmonary vessels were congested (Fig.2) and there was eosinophilic infiltration in the bronchial walls and interalveolar septa (Fig.3). On the other hand, lungs of the control group appeared collapsed with intact interalveolar septa (Fig.4).

The kidneys in both shock groups showed necrosis of the tubular epithelium (Fig.5) as compared to the normal control picture (Fig. 6). The heart showed hypere mia with occasional intermyocardial hemorrhag (Fig.7), compared to the control picture (Fig.8). The brain showed hyperemia and mild oedema in some animals of the slow shock group but the neuron showed no signs of degeneration (Fig.9) compared to the control picture (Fig.10). The liver, spleen, adrenals and stomach in both shock groups showed no change from the control picture.
Fig. (1) : A photomicrograph of a section in the lung of a guinea pig after death from anaphylactic shock showing distended alveoli, thin interalveolar septa with rupture of some septa. Hx and E x100.

Fig. (2) : A photomicrograph of a section in the lung of a guinea pig after death from anaphylactic shock showing congestion of a pulmonary vessel. Hx and E x100.
Fig. (3): A photomicrograph of a section in the lung of a guinea pig after death from anaphylactic shock showing eosinophilic infiltration in the inter-alveolar septa. Hx and E. x1000.

Fig. (4): A photomicrograph of a section in the lung of a control guinea pig showing collapsed alveoli with intact inter-alveolar septa. Hx and E. x100.
Fig. (5): A photomicrograph of a section in the kidney of a guinea pig after death from anaphylactic shock showing necrosis of tubular epithelium.

Fig. (6): A photomicrograph of a section in the kidney of a control guinea pig showing normal tubular epithelium. Hx and E. x400.
Fig. (7): A photomicrograph of a section in the heart of a guinea pig after death from anaphylactic shock showing intermyocardial hemorrhage. Hx and E. x400.

Fig. (8): A photomicrograph of a section in the heart of a control guinea pig showing normal myocardium. Hx and E. x400.
Fig. (9) : A photomicrograph or a section in the brain of a guinea pig after death from anaphylactic shock showing brain oedema. Hx and E. x400.

Fig. (10) : A photomicrograph of a section in the brain of a control guinea pig showing normal appearance. Hx and E. x400.
Table 1 Serotonin values in the Plasma, stomach and lungs of guinea pigs after death from anaphylactic shock.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>PLASMA ug/ml</th>
<th>STOMACH ug/g wet tissue</th>
<th>LUNGS ug/g wet tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>± SD</td>
<td>± SE</td>
</tr>
<tr>
<td>Control</td>
<td>0.70</td>
<td>± 0.010</td>
<td>± 0.010</td>
</tr>
<tr>
<td></td>
<td>0.0051</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rapid shock</td>
<td>3.60</td>
<td>± 0.076</td>
<td>± 0.009</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>118.078</td>
<td>32.907</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Slow Shock</td>
<td>1.34</td>
<td>± 0.021</td>
<td>± 0.01</td>
</tr>
<tr>
<td></td>
<td>0.0066</td>
<td>76.659</td>
<td>40.249</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>S.</td>
<td>S.</td>
</tr>
</tbody>
</table>

N. B.: Values represent means of 10 animals
S: Significant difference from corresponding control values.
N. S.: No significant difference from corresponding control values.
Table 2: Cortisol total catecholamines and IgE levels of guinea pigs after death from anaphylactic shock.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>CORTISOL ug/ml</th>
<th>TOTAL CATECHOL AMINE Pg/dl</th>
<th>SERUM IgE ug/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Mean 9.57 ± 1.01</td>
<td>315.92 ± 32.92</td>
<td>11.95 ± 2.63</td>
</tr>
<tr>
<td></td>
<td>SD ± 0.30</td>
<td>± 10.43</td>
<td>± 0.083</td>
</tr>
<tr>
<td>Rapid shock</td>
<td>Mean 7.33 ± 1.32</td>
<td>396.40 ± 39.37</td>
<td>35.36 ± 6.02</td>
</tr>
<tr>
<td></td>
<td>SD ± 0.42</td>
<td>± 12.45</td>
<td>± 1.9</td>
</tr>
<tr>
<td></td>
<td>SE ± 4.262</td>
<td>4.956</td>
<td>11.269</td>
</tr>
<tr>
<td></td>
<td>t &lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Slow Shock</td>
<td>Mean 9.92 ± 1.06</td>
<td>372.92 ± 21.23</td>
<td>23.12 ± 3.96</td>
</tr>
<tr>
<td></td>
<td>SD ± 0.34</td>
<td>± 6.71</td>
<td>± 1.25</td>
</tr>
<tr>
<td></td>
<td>SE ± 0.756</td>
<td>4.839</td>
<td>7.430</td>
</tr>
<tr>
<td></td>
<td>t &gt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>N.S.</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

N. B.: Values represent means of 10 animals
S: Significant difference from corresponding control values.
N. S.: No significant difference from corresponding control values.
Discussion

Anaphylactic shock is an extreme reaction to allergy or hypersensitivity which occurs when an antigen combines with an antibody fixed to tissues. Anaphylaxis represents a wide spectrum of reactions, ranging from only mild distress to true respiratory insufficiency and cardiovascular collapse (Barkim and Burrington, 1987). In the present study, there was also a difference in the type of reaction depending on the route of administration of the second dose of the antigen. The reaction started immediately and the animals died within few minutes (15 minutes) when the antigen was injected intraperitoneally but when given intramuscularly, the reaction was milder and the animals died after 35 minutes. This may be attributed to the assumption that the intramuscular route did not allow the blood concentration of antigen to rise high enough for a full-scale liberation of histamine or serotonin (Hirvonen and Huttunen, 1984).

In the present study, serotonin was markedly increased in the plasma of animals which had died from rapid shock and to a lesser degree in those which had succumbed slowly. This increase may be attributed to the release of serotonin into the blood from stomach wall and lungs, which are rich in mast cells, since serotonin content of both organs was lowered. This was concordant with the results previously reported by Hirvonen and Huttunen (1984).

Serum IgE and Catecholamines were high in both shock groups. The role of IgE in allergy and anaphylaxis is well established; immunoglobulins combine with basophils and mast cells and sensitize them for antigen-induced release of histamine, serotonin and other vasoactive substances (Stefanini, 1979). The role of IgE in anaphylaxis is also emphasized by the predominant respiratory and gastrointestinal symptoms of the syndrome as abundant IgE is formed by plasma cells in the lymphatic tissues of the respiratory and gastrointestinal mucosa (Ishizaka and Ishizaka, 1970). High values of catecholamines, especially adrenaline, had been observed previously in anaphylactic shock (Hamberger et al., 1980), an effect which was not due to the stress of injection since control animals were subject-
ed to the same stress.

In the present study, cortisol values were low in the rapid shock group. Hirvonen and Huttunen (1984) reported that the release of cortisol under stress is usually rapid, but it is possible that it may be inhibited or retarded in anaphylactic shock or it may be immediately bound to mast cells in the tissues to prevent further liberation of histamine or serotonin by stabilizing the membranes damaged by the antigen-antibody reaction.

The histological changes detected in the animals which died from anaphylactic shock were similar in both the rapid and slow cases. The most prominent and constant change was the inflation of the lungs where the alveoli were markedly distended with rupture of the interalveolar septa, a picture of acute emphysema and indicating broncho-constriction. Eosinophilic infiltration was another finding which points to anaphylaxis. Similar changes have been found also in anaphylactic shock in apes (Smedegard et al., 1980), rabbits (Walter et al., 1961) and humans (Stefanini 1979). Broncho-constriction has been attributed to the high levels of serotonin and histamine (Goadby and Little, 1978).

From the present study it may be concluded that, postmortem diagnosis of acute anaphylactic shock could be achieved by the assay of plasma serotonin, serum catecholamines and IgE since high values were detected in both shock groups. This diagnosis could be confirmed by the histological findings in the lungs, kidney, heart and brain.

References


Fischer R.S. and Petty C.S. (1977): Forensic Patholo-


THE SIGNIFICANCE OF P53 AND P 170 EXPRESSION IN HEPATOCELLULAR CARCINOMA


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Abstract

Expression of p53 and P170 was investigated in 27 hepatocellular carcinomas (HCC) by means of immunohistochemistry. p53 expression was demonstrated in 10 out of 27 HCC (37%) with the incidence increasing in proportion to the histological grading of malignancy, thus 7.7% of well differentiated, 54.5% of moderately differentiated and 100% of poorly differentiated lesions were positive. The p53 expression had no statistically significant correlation with clinicopathological parameters including, tumour stage, liver cirrhosis, serum titres of alpha-feto protein (AFP), hepatitis B surface antigen (HBs Ag) and survival time.

Our results indicate that p53 expression takes place in late stage of hepatocarcinogenesis and is not related to genetic changes induced by hepatitis B virus (HBV) which are considered as an early event.

Expression of P-glycoprotein (P170) in HCC is associated with a shorter survival time, thus it can be considered as an independent marker of poor prognosis.

Introduction

Hepatocellular carcinoma is one of the most common human cancers world wide. The development of HCC probably occurs in multiple steps and is influenced by multiple factors. A striking correlation exists between areas where HCC is common and where HBV infection is endemic or exposure to dietary aflatoxins is highly intake, especially in South East Asia and Subsaharan Africa (Hasia et al., 1992).
gene located on the short arm of chromosome 17 (Isobe et al., 1986). Its coded product is a 53 KDa nuclear phosphoprotein, which is believed to regulate entry into and progression through the normal cell cycle (Mercer et al., 1984). The wild type p53 protein inhibits cell proliferation, and loss of this activity leads to a neoplastic transformation of the cells (Finlay et al., 1989). The mutated p53 protein has a longer half-life than the wild type protein and is able to complex with it, thus inactivating it. Therefore, it can be detected by standard immunohistochemical methods (Chang et al., 1991).

It is now widely accepted that genetic alterations affecting the p53 gene are among the most common changes that occur during malignant progression of several types of cancer, including HCC (Bartek et al., 1991). Recent immunohistochemical and molecular hybridization studies have shown that the prevalence of p53 positive HCC, was significantly higher in patients with serological markers of viral infection than in patients without these markers and hepatitis B virus infection is considered the most important risk factor for the development of HCC (Skopelitou et al., 1996).

P-glycoprotein is a 170 KDa membrane bound glycoprotein encoded by the multidrug resistance (MDRI) gene and functions as an ATP dependent pump propelling drugs and cytostatics out of the cell cytoplasm (Dietel, 1991). Expression of P-170 has been studied in HCC, which acquire drug resistance after chemotherapy by induced overexpression of MDRI and its P-glycoprotein (Soini et al., 1996).

This work aims to study the p53 protein and P170 expression in HCC to determine their relationship to type, grade, stage and other clinicopathological parameters.

**Materials And Methods**

**Patients:**

This prospective study included 27 cases of inoperable HCC, selected from the out patient clinic of National Cancer Institute, Cairo University. Clinical examination, laboratory investigations in the
form of serum alphafetoprotein (AFP) and hepatitis B surface antigen (HBsAg) and liver needle biopsy or wedge biopsy was obtained from all cases. The patients have been divided into two groups, the first group consisted of 18 cases received palliative symptomatic treatment and the other one consisted of 9 cases who received 4 cycles of intra-hepatic chemoembolization using platinol 100 mg. with one month interval inbetween each two consecutive cycles. The patients have been followed up. For controls, samples from 5 normal liver cases and 5 hepatitis B cases were obtained.

Pathology:

The biopsy specimens were immediately fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 microns, mounted on poly-l-lysine coated glass slides and stained with haematoxylin and eosin. Sections were examined for histological typing according to the criteria of W.H.O. (Gibson and Sobin, 1978), and for the presence of liver cirrhosis. The grade of the tumours was determined as suggested by Edmondson and Steiner (1954). Staging was done according to TNM classification which was supported by findings of clinical examination, x-ray, and ultrasonography (Spiessl et al., 1992). HBsAg in liver tissue were detected by orcein stain according to the method of Shikata et al., (1974). Cases with hepatitis B used as positive control. HBsAg appeared as dark purplish brown intracytoplasmic inclusions against a beige colour of the cytoplasm.

Immunohistochemistry:

Immunohistochemical staining for p53 protein was done employing dewaxed and rehydrated paraffin sections of routinely fixed tissue samples. Endogenous peroxidase was inactivated by 3% hydrogen peroxide for 10 minutes followed by 3 washes. 5 minutes each, with phosphate buffer saline (PBS), and tissue sections were incubated with 500 ml. of antigen retrieval solution in a microwave at 400 W for 15 minutes, washing in distilled water and in PBS and then incubated overnight at 4°C with the prediluted mouse monoclonal anti-p53 protein antibody (BioGenex, San Roman, California, USA). These sections then washed
twice for 5 minutes each, with PBS and incubated for 20 minutes at room temperature with an anti-
mouse biotinylated secondary antibody. Slides were washed twice for 10 minutes with PBS, incubated
for 30 minutes at room temperature with preformed streptavidin-
biotin peroxidase complex (ABC) (BioGenex), and washed with PBS
for 5 minutes. Diaminobenzidine (DAB) was used as the chromogen.
Then Meyer's haematoxylin was used as counterstain. A breast
cancer biopsy previously stained to be strongly positive for p53 pro-
tein was used as a positive control and the same biopsy processed
with replacement of the primary antibody by non immune serum
served as a negative control in each staining series. Cells positive
for p53 protein were identified by the presence of dark brown intra-
nuclear staining.

For P170 immunohistochemical staining, the same steps were
done using the prediluted mouse monoclonal anti P170 antibody
multidrug resistance (MDRI) gene as a primary antibody (BioGenex).
Normal liver biopsy served as positive control and negative controls
consisted of replacement of the primary antibody by non immune
serum. P170 staining reaction was considered positive when there is
brown cell membrane reaction and/or granular cytoplasmic
brown staining.

**Results**

The 27 cases studied were 21 cases male (77.8%) and 6 cases fe-
male (22.2%). Male to female ratio was 7: 2. The age of the patients
ranged from 12 years up to 70 years (the mean age was 52.8
years). The cases were classified into 4 histological types: 8 (29.7%)
acinar, 12 (44.4%) trabecular, 6 (22.2) compact and 1 case (3.7%)
sclerosing. These cases were graded according to the degrees of cel-
lar differentiation into 3 grades: 13(48.2%) of grade I, 11 (40.7%) of
grade II and 3 (11.1%) of grade III.

The cases were classified into 4 stages: 8 (29.7%) of
stage I, 10 (37%) of stage II, 3 (11.1%) of stage III, and 6 (22.2%)
of stage IV. Liver cirrhosis was demonstrated in 23 cases
(85.1%). AFP was elevated above 20ng/ml. in the sera of 21 cases
(77.7%). HBsAg was detected in
HBsAg in the tissue section by orcein stain was detected in 18 cases (66.6%) of HCC which appears as purplish brown cytoplasmic granules in hepatocytes of liver tissue adjacent to the tumour (Fig. 1). Tumour cells did not show cytoplasmic staining of HBsAg except in 8 cases. All the cases which were positive for HBsAg in the serum showed HBsAg in liver tissue adjacent to HCC by orcein stain.

According to the follow up data, the cases of this study were divided into 3 groups:

**group I**: Less than 6 months survival and consisted of 21 cases (77.8%).

**group II**: 6-12 months survival and consisted of 5 cases (18.5%).

**group III**: More than 12 months survival and consisted of one case (3.7%).

So that, 26 cases (96.3%) died within 1 year.

Association of p53 expression with clinicopathological parameters is presented in table I.

From these results p53 was detected immunohistochemically in the nuclei of 10 cases (37%) (Fig. 2&3). Normal liver tissue and liver tissue adjacent to tumour did not show any nuclear staining. 18 cases (66.6%) showed brown granular cytoplasmic staining in liver tissue. These cases were the same cases which showed positive HBsAg in orcein stained sections. Also, it is found that p53 protein expression had no statistically significant correlation with clinicopathological parameters except histological grade.

Membrane bound Positivity for P170 was observed in all cases of HCC (100%), together with granular cytoplasmic brown staining in 88.8% of the cases (Fig. 4). In addition P170 immunoreation was observed as a perinuclear staining in 5 cases. Normal liver tissue of control cases showed mild P170 expression in cell membrane and cytoplasm of hepatocytes near the portal tracts and strong expression in cells lined bile ducts. Being
expressed in all cases. P170 could not be related to any of the studied parameters. However the short survival of all cases involved in this study suggests the existence of a direct parallel relation between P170 expression and short survival.

Table 1: Association of p53 expression with clinicopathological parameters.

<table>
<thead>
<tr>
<th>Clinicopathological Parameters</th>
<th>Number of cases</th>
<th>p53 expression</th>
<th>Significance</th>
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<tbody>
<tr>
<td></td>
<td>Number</td>
<td>% Positive</td>
<td>% Negative</td>
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<tr>
<td>AFP</td>
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<td>&lt;20 ng/ml</td>
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<tr>
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<td>13</td>
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<tr>
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<td></td>
<td></td>
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<td>13</td>
</tr>
<tr>
<td>Negative</td>
<td>9</td>
<td>4</td>
<td>5</td>
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<tr>
<td>Survival time</td>
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<tr>
<td>1-6 months</td>
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<td>7-12 months</td>
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<tr>
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</table>

N.S. = not significant
S = significant
p = group
Fig. 1: Liver cells showing Dutra's haematoxylin- eosin-stained nuclei (X 400).

Fig. 2: HCC (fetal liver pattern showing positivity of p53 in more than 75% of the nuclei (ABC X400)).

Fig. 3: HCC (compact pattern showing high intensity of placental alkaline phosphatase in more than 75% X 400).

Fig. 4: HCC (peripheral pattern showing strong cell membrane and intracytoplasmic granular immunostaining of P57 (ABC X400)).
Discussion

Hepatocellular carcinoma is one of the common visceral tumours all over the world, it has a high prevalence rate in Subsaharan Africa and South East Asia. The Middle East including Egypt is an area of intermediate prevalence. It is associated with many aetiological risk factors (Okuda and Okuda, 1991).

HBV infection is one of the major aetiologic factors of HCC (Okuda and Okuda, 1991; Barwick and Rosai 1996).and HBsAg was demonstrated in the sera of 55.5% of our cases and in the tissue of 66.6% of cases by orcein stain. This higher incidence of demonstration in liver tissue may be due to the high sensitivity of orcein in detection of HBsAg. This result agrees with that of Agina (1989) who concluded that orcein stain is of good sensitivity for detection of HBsAg and of no false positive results. The Incidence of serum HBsAg carriers in HCC cases in this study was 55.5%.It is lower if compared with the 90% incidence of HBsAg carriers in HCC from high endemic areas e.g. Mousambique as proved by Okuda and Okuda (1991). This result confirms the classification of Egypt among countries of intermediate rate of HBV related HCC.

We found that serum AFP was increased in the sera of 21 cases (77.7%).This result agrees with that done by Huang et al. (1992) who detected increased level of serum AFP in 75% of HCC in their series. Also we found that 66.6% of cases showed HBsAg in tissue sections. This suggesting a direct relation between increased level of serum AFP and HBV infection, both are major causes of chronic liver damage and associated with HCC. This result is parallel with that of Lee et al. (1991). who related moderate increase in serum AFP to chronic liver damage associated with HCC.

p53 protein was detected in 10 out of 27 cases (37%) of HCC involved in this study, and we found a significant relation between p53 expression and the degree of cellular differentiation . This result is in accordance with the results of previous studies (Ng et al., 1995: Nagao et al., 1995), who concluded that p53 expression in HCC is
related to the degree of cellular differentiation. The expression of p53 in all cases (100%) of grade III HCC indicates that p53 mutation may be related to tumour aggressiveness and it may be a late event in hepatocarcinogenesis. This results agree with that of Nagao et al. (1995), and Skopelitou et al. (1996). Also it is supported by the work of other workers who found that p53 is a late event in colorectal carcinoma (Losi et al., 1996), and in ovarian carcinoma (McManus et al., 1996).

Because HCC is aetiology related to HBV which may be integrated into the hepatocyte genome causing several genetic changes. p53 mutation have been studied in relation to HBsAg, but we failed to find a relation between HBsAg and p53 mutation (p53 was expressed in 33.3% of HBsAg positive cases and 44.4% of HBsAg negative cases). This indicates that p53 mutation occurs independent on HBV infection and this agrees with the work of Ng et al. (1995) who failed to find a significant correlation between HBsAg positive cases and p53 mutation and this may be due to the genetic mutations induced by HBV occurs early in the process of hepatocarcinogenesis, while p53 mutation is a late event.

No relation could be detected between p53 expression and both tumour stage and survival. So that, p53 could not be considered as a relevant prognostic marker. This is supported by Shieh (1993), who concluded that p53 in HCC is not a useful prognostic marker, although p53 expression is considered as useful prognostic marker in breast cancer (Thor et al., 1992).

In contrast to the above finding, we found that the cell membrane bound glycoprotein P170 showed a strong parallel relation between short survival of all cases in this study and the overexpression of P170 in all cases. Survival was less than 6 months in 77.8%, between 6-12 months in 18.5% and more than 12 months in 3.7% of cases. Thus P170 may be a valuable prognostic indicator in HCC. This agrees with Huang et al. (1992), who detected P170 in 90% of inoperable HCC, while overexpression of P170 in surgically treated HCC was detected in 40%
of the series of Isshika et al. (1993), and in 65% of the series of Soini et al. (1996). These results are confirmed by the work of Sinicrope et al. (1994), who found that P170 expression is related to disease free interval and survival after surgical excision of breast and colon carcinoma.

The overexpression of P170 in all cases (100%) of this study, make it very difficult to find a relation between P170 expression and any other clinicopathological parameters involved in this study.

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Ng, I. O., Lai E.C., Chan A.
Ragaey R. Fahmy et al...


FAMILIAL BREAST CANCER IS DIFFERENT

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and Benha faculty of Medicine*, Egypt

Abstract

Twenty - one breast cancer patients with positive family history were examined for biological and pathological features and the presence of p53 protein to determine the cardinal features of familial breast cancer.

Familial breast cancer cases were characterized by: (1) early age onset (all cases are younger than 35 years); (2) high incidence of bilateral and multiple primary tumours (9.5% and 33.3% respectively); (3) higher rates occurred in first - degree relatives and in patients with multiple affected relatives (24% and 29% respectively); (4) 50% of infiltrating ductal carcinoma cases had prominent intraductal component; (5) Medullary carcinoma was the most specified type of infiltrating ductal carcinoma (20%); (6) higher incidence of lymph node metastases and blood vessel invasion (71.4%); and (7) it had a significantly high incidence of p53 protein expression (71.4%).

The p53 immunohistochemical demonstration was significantly related to young age, patients with several affected relatives, large tumour size, positive vascular invasion, high histologic grade and stage, but not related to lymph node status.

These findings indicate more genetic instability in familial breast cancer which can be used to estimate the probability that a carrier of a p53 germline mutation may develop breast cancer at a given age.

Introduction

Breast cancer is the most common female malignancy in Egypt (Sherif and Ibrahim, 1987). In most industrialized countries, one female in 10 will be diagnosed with invasive carcinoma of the breast during the course of her lifetime and approximately 40% of these patients will eventually die of the disease (Paterson, 1996). Most breast cancer cases are be-
Nadia M. Mokhtar and Ragaey R. Fahmy

lieved to be sporadic (nonhereditary) in origin. There is now unequivocal evidence that approximately 5% of breast cancer cases may be due to an inherited predisposition (Newman et al., 1988).

A family history of breast cancer is a well known risk factor for the disease. The risk to a woman with a first-degree relative with breast cancer is generally reported to be two to three times higher than the risk to a woman with a negative family history (Kelsey and Hildreth, 1983).

Anderson (1971), found that familial breast cancer is more prone to premenopausal women, tumour bilaterality, and multiple primary malignancies. He also found that, the first-degree relatives of familial breast cancer cases had higher rates of breast cancer than first-degree relatives of unselected series of breast cancer patients.

Attempts to determine whether there is an association between histology and a family history of breast cancer have not yielded any conclusive findings. In their review, Mulcathy and platt (1981), reported that the weight of evidence suggests that individuals with a medullary form of breast cancer are more likely to have a positive family history of the disease.

Recent developments in genetic epidemiology have provided statistical method called segregation analysis. The application of this method to breast cancer has confirmed the suspected autosomal dominant mode of inheritance in selected high-risk pedigrees (Go et al., 1983).

Three major predisposition genes, denoted BRCA1, BRCA2, and p53 have now been cloned and characterized to varying extents. Together, they account for the great majority of all heritable breast cancer segregating in large multiple-case families (Black, 1994). The third gene, p53, was discovered in an investigation into the hereditary basis of Li-Fraumeni syndrome (LFS), a rare familial cancer disorder featuring excessive clustering of premenopausal breast cancer in combination with childhood sarcoma, leukemia and brain, lung and adrenal cortical carcinomas (Birch, 1994).
The p53 gene is located on the short arm of chromosome 17 and it encodes a 53 KDa nuclear phosphoprotein involved in the control of cell proliferation (Baker et al., 1989). The wild-type p53 protein induces growth arrest, which occur at the G1 phase of the cell cycle, and accordingly p53 is a negative regulator of the cell cycle. p53 is also involved in DNA repair and cell death through apoptosis, so that, it can be considered as a guardian of the genome (Kastan et al., 1991). Recent studies have shown that p53 in its wild form may act as a tumour suppressor gene (Levine et al., 1991).

Immunohistochemical studies have shown that the detection rate of mutated p53 protein in breast cancer range between 13% and 53.5%, but the clinical significance of these findings is still disputed. Some analyses have indicated that overexpression of p53 protein is an independent prognostic factor (Thor et al., 1992), whereas others have failed to demonstrate such a relationship (Lipponen et al., 1993; Pietilainen et al., 1995).

The aim of the present study is to determine whether there is a difference in the pathobiology of breast cancer in females with positive family history in relation to p53 expression.

Materials and Methods

Patients:
This study included 21 cases of breast cancer with positive family history from a private laboratory. One case had 4 relatives, 2 cases with 3 relatives, 3 cases with 2 relatives and 15 cases with one relative with breast cancer. All cases were premenopause in the age range of 22 - 35 years old. 9 cases had bilateral and multicentric lesions.

Pathology:
The surgical specimens were fixed in neutral buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with haematoxylin and eosin. Sections were examined for histological type, grade, pathological stage, intraductal component and lymph node status. Typing of the tumours was done according to W.H.O. criteria (Azzopardi et al., 1982). Histological grading of the tumours was determined accord-
ing to modified Bloom and Richardson criteria (Elston and Ellis, 1991), and histological staging of the tumours was done according to the modified form of T.N.M. classification (Hartmann, 1984).

Immunohistochemistry:

Immunohistochemical staining for p53 protein was done employing dewaxed paraffin sections of routinely fixed tissue samples, then were pretreated in a microwave 700 W. for 5 minutes. The section were incubated for 18 hours at 4°C with the prediluted D07 monoclonal anti-p53 protein antibody (Biogenex, USA), and was followed by biotinylated rabbit anti-mouse immunoglobulin antiserum (Secondary antibody), followed by streptavidin peroxidase was the detection system. The chromogen used was diaminobenzidine (DAB) substrate (universal kit was from Biogenex, USA). Finally, the Meyer's haematoxylin was used as counterstain. A breast cancer biopsy previously shown to be strongly positive for p53 was used as a positive control and the same biopsy processed without the primary antibody served as a negative control in each staining series.

Scoring of p53 protein expression:

Cells positive for p53 were identified by the presence of dark brown nuclei, and the pattern of expression was graded as follow:

- Negative: 0 - 10% Positive nuclei
- grade 1 (weak +ve): 10 - 20% positive nuclei
- grade 2 (Moderate +ve): 20 - 50% positive nuclei
- grade 3 (strong +ve): > 50% positive nuclei

Results

The 21 cases studied were 4 cases, medullary carcinoma (Fig. 1), one case tubular carcinoma, one case intraductal carcinoma and 15 cases infiltrating ductal carcinoma not otherwise specified (NOS). Age was between 22-35 years (mean 29.2 years). Ten out of 20 cases of infiltrating ductal carcinoma (50%) showed prominent intraductal component (Fig. 2). 2 cases (9.5%) had a tumour bilaterality and 7 cases (33.3%) had multiple primary tumours, all these 9 cases were below 30 years old. 5 cases (24%) had first-degree affected relatives and 6 cases (29%) had multiple affected rela-
Fifteen out of 21 cases (71.4%) were positive for p53 expression. 3 cases were of grade I, 3 cases were of grade II and 9 cases were of grade III immunopositivity (60% of positive cases showed strong immunoreaction).

All the medullary carcinoma gave a strong positive immunoreaction with anti-p53 protein antibody (Fig. 3). The case of inductal carcinoma showed only a weak immunoreaction. The case of tubular carcinoma was negative for p53 protein, while 10 out of 15 cases (66.7%) of infiltrating ductal carcinoma NOS showed positive immunostaining (Fig. 4). The p53 positive staining was nuclear, although in some of the sections weak cytoplasmic positivity was also detected.

All clinical data and pathological criteria are summarized in Table I in relation to p53 immunoreaction.

From the above results, p53 positive nuclear staining was related to younger age, more number of affected relatives, large tumour size, positive vascular invasion, high histological grade and stage, but not related to lymph node status.

Table 1: P53 expression in relation to clinical data

<table>
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<th>Number of cases</th>
<th>P53 immunoreaction</th>
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<td>Age</td>
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<td>gr. II</td>
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<td>Number of affected relatives</td>
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Table 2: P53 expression in relation to pathological criteria

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<tr>
<td>grade III</td>
<td>5</td>
<td>4</td>
<td>4</td>
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<tr>
<td>Pathological stage</td>
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<td>1</td>
<td>1</td>
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<tr>
<td>stage I</td>
<td>2</td>
<td>1</td>
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<tr>
<td>stage II</td>
<td>4</td>
<td>2</td>
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<tr>
<td>stage III</td>
<td>1</td>
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<tr>
<td>stage IV</td>
<td>1</td>
<td>1</td>
<td>1</td>
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</tbody>
</table>

N.B. There is no histological grading in medullary carcinoma and intraductal carcinoma.

Fig. 1: Medullary carcinoma of the breast showing malignant cells surrounded by thin scanty stroma rich in lymphocytes.
(Hx. & E. x 200).

Fig. 2: Intraductal carcinoma of the breast as a prominent component of the tumour (Hx. & E. x 100).
Fig. 3: Medullary carcinoma of the breast showing strong nuclear immunopositivity for p53 protein.
  (anti-p53 protein x 200).

Discussion

The association of a family history of breast cancer with an increased risk of breast cancer has been well documented. This study had shown that the breast cancer with positive family history is clearly different in both pathological and biological features than the breast cancer occur in women with negative family history.

In the present study it is found that familial breast cancer occur in young women (all the patients were younger than 35 years), with high incidence of bilateral and multiple primary tumours (9.5% and 33.3% respectively) and higher rates in first degree relatives (24%). These results are in accordance with that study done by Anderson (1971).

In this study it is shown that 50% of infiltrating ductal carcinoma had prominent intraductal component and 4 cases out of 20 (20%) of the infiltrating ductal carcinoma are medullary carcinoma and these figures are higher than that occurring in normal popula-
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These results are in parallel with that found by Mulcathy and Platt (1981), who reported that patients with medullary carcinoma are more likely to have a positive family history of the disease.

In the present study it was found that 71.4% of cases of familial breast cancers showed positive lymph node metastases and blood vessel invasion.

Less is known about the association between breast cancer with positive family history and p53 gene abnormalities, which were the most common genetic abnormality in breast cancer (Porter et al., 1992). In the present study the abnormal p53 protein was detected in 71.4% of cases. This incidence is higher than the previous studies (Thor et al., 1992; Lipponen et al., 1993). This discrepancy is because this study was performed on patients who had a positive family history, but other studies were done on sporadic cases of breast cancer. This high incidence of p53 positivity may indicate genetic instability in familial breast cancer (Walker et al., 1996), and p53 gene mutation may be an important carcinogenesis in familial breast cancer (Kinoshita et al., 1995).

In our series, the rate of p53 positivity in medullary carcinoma was 100% which is higher than that observed by Karameris et al. (1995). Also, the rate of p53 positivity in infiltrating ductal carcinoma not otherwise specified was 66.6% which is higher than all previous studies (Thor et al., 1992; Lipponen et al., 1993), and this may indicate more genetic instability of familial breast cancer.

In the present study, cases with young age and several affected relatives were related to overexpression of p53. There is also increase in p53 protein overexpression with larger tumours, higher histopathologic grade, higher histopathologic stage and positive vascular invasion. These results are in line with several previous reports (Thor et al., 1992; Martinazzi et al., 1993; Pietilainen et al., 1995; Horne et al., 1996). This may be due to more genetic instability and mutations of the p53 gene in familial breast cancer ascribing an unfavourable progno-
sis for p53 positive breast cancers.

This current study gave no relation between p53 protein overexpression and lymph node status. This result is against that of Allred et al. (1993), who found a significant prognostic importance of p53 overexpression and node negative tumours. This may be due to our limited series of patients.

The p53 gene is probably one of the genes responsible for inherited susceptibility to various cancers particularly breast cancer. In our study, positive family history breast cancer exhibited more characteristic genetic changes than did negative family history cancers and p53 gene abnormalities appear to play an important role in carcinogenesis of familial breast cancer.

Bihan and Bonaiti-pellie (1994) found that a carrier of a p53 germ-line mutation develops a given malignant tumour at a given age so that, we can consider p53 gene mutation a breast cancer risk factor that is carried by individuals at high risk (as case with positive family history, especially in first-degree relative, with a bilateral or multiple breast masses appear before age of 35 years). So, screening first-degree relatives of patients with breast cancer for p53 gene mutation may help in identifying high risk individuals.

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Breast carcinomas occurring in young women (<35 years) are different. Br. J. Cancer 74: 1796: 1800.
EFFECT OF PARACETAMOL ON GASTRIC EROSIONS INDUCED BY INDOMETHACIN IN RATS

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Departments of Forensic Medicine & Toxicology, pathology* and Pharmacology**, Benha and Zagazig Faculties of Medicine, Egypt.

Abstract

Gastric mucosal injury induced by application of indomethacin, as a nonsteroidal anti-inflammatory drug (NSAID), is a well-documented phenomenon. This study examined the effect of acetaminophen (paracetamol) on indomethacin-induced gastric erosions. One hundred adult albino rats of both sexes, weighing 200-250 gm. were divided into two main groups, including a group of 10 rats acts as a control. The first group was to study the effect of a single oral dose of indomethacin, paracetamol and both drugs on the gastric mucosa, and the second group was to study the effect of repeated administration of each drug and both drugs together. The animals were killed and the histopathological changes of the stomach were examined microscopically.

Single therapeutic dose of indomethacin was found to induce multiple gastric erosions which were more marked after repeated administration of the drug. Single dose or prolonged administration of paracetamol were followed by very minimal changes in the gastric mucosa. Simultaneous administration of both drugs was found to reduce the pathological lesions induced by indomethacin alone, whereas the pretreatment with paracetamol one hour before a single oral dose of indomethacin did not affect its erosive activity. Post-treatment with paracetamol reduces the erosive effect of indomethacin either in a single dose or after repeated administration. The pathogenesis of gastric erosions and ulcers was believed to be due to prostaglandin inhibition. Indomethacin was known as a potent inhibitor of prostaglandin synthesis, whereas...
paracetamol is a very weak inhibitor of prostaglandin production.

It is also found that paracetamol had a protective action against the erosive effect of indomethacin and this appears to be mediated by mucosal regeneration, probably resulting from increased production of mucosal growth factors.

So, the use of paracetamol as antipyretic and analgesic drug is recommended instead of indomethacin, or they are administrated simultaneously with or after indomethacin when there is a possibility of gastric mucosal injury, especially in prolonged treatment.

Introduction

Indomethacin is one of the most commonly used NSAIDs, introduced in 1963 for the treatment of rheumatoid arthritis and related disorders including acute gout. It has a prominent anti-inflammatory and analgesic antipyretic properties similar to those of the salicylates (Abramson and Weissmann, 1989). Clinical trials of indomethacin as an anti-inflammatory agent have been reviewed by Felson et al. (1992) and Edmonds et al. (1993).

These trials have demonstrated that the drug relieves pain, reduces swelling and tenderness of the joints, and decreases the duration of morning stiffness. The estimation of its potency relative to salicylates vary between 10 and 40 times higher (Brooks and Day, 1991).

Although it is used widely and is effective, the high incidence of severe side effects associated with long-term administration and its toxicity, often limits the use of indomethacin (Lewis and Furst, 1987; Borda and Koff, 1992). Like many other NSAIDs, it induces gastrointestinal complications consist of single or multiple visible erosions and ulcers of the entire upper tract due to damage of the epithelial lining of gastric mucosa sometimes with perforations and haemorrhage. Occult blood loss may lead to anaemia, and diarrhea may occur associated with ulcerative lesions of the bowel (Gabriel et al., 1991; Graham et al., 1993; Korman, 1995; Scarpignato
Acetaminophen (Paracetamol) which is the active metabolite of phenacetin, has a potent central analgesic and antipyretic activity as great as that of the parent compound (Moore et al., 1995; Higgins, 1996). It is devoid of renal and haematological toxicity in contrast to indomethacin, which induces hematopoietic reactions as neutropenia, thrombocytopenia and impaired platelet function, and has a nephrotoxicity (Reynolds et al., 1989). Through endoscopic studies, paracetamol has been found to be free from gastric side effects (Lanas et al., 1995). Ivey and Sertree (1976) reported that large analgesic doses of paracetamol induce minimal structural changes in normal human gastric mucosa. Porter (1996) reported that the combination of acetaminophen with aspirin, on a fasting stomach, significantly decreases the incidence of gastric lesions compared with aspirin alone.

The present work studied the effect of paracetamol on other NSAIDs. indomethacin-induced gastric erosions after single and repeated administration by histopathological examination of the stomach.

Materials and Methods

One hundred adult albino rats of both sexes, 200-250 gm. body weight, were used in this study. The drugs used were indomethacin in the therapeutic dose (9 mg/kg body weight) and paracetamol in a therapeutic dose (135 mg/kg body weight).

A group of 10 rats were left without medication, only given the standard diet and water, to be used as a control group. The remainder rats were divided into two main groups (A and B).

Group (A): to study the direct effect of a single oral dose of indomethacin, paracetamol and of both drugs on the gastric mucosa. The rats of this group were subdivided into five subgroups (1-5) each included 10 rats. Food was prevented for 24 hours before medication, and water was freely given. Subgroup (1) the rats were given indomethacin, those of subgroup (2) were given paracetamol. Subgroup (3) the animals were...
Subgroup (8), was given indomethacin for 2 weeks and left for 1 week without treatment, the gastric erosions reduced in size, the congestion and haemorrhage became mild and the mucosa showed mild regenerative changes (by normal regeneration process of the gastric mucosa).

Subgroup (9), the therapeutic dose of indomethacin was administered for 2 weeks, then followed by the therapeutic dose of paracetamol for another one week. The gastric mucosa showed minimal loss of superficial mucosal cells and very minute erosions, the epithelium adjacent to the damaged area infiltrated by mild inflammatory cells. The mucosa showed moderate regenerative changes, and the erosions were repaired without the formation of granulation tissue (Fig. 6).

Table 1: The effect of Indomethacin and Paracetamol on gastric mucosa

<table>
<thead>
<tr>
<th>Groups</th>
<th>Subgroups</th>
<th>Congestion</th>
<th>Haemorrhage</th>
<th>Inflammatory cell infiltration</th>
<th>Superficial cell atrophy and loss</th>
<th>Erosions</th>
<th>Regeneration of erosions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
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<tr>
<td>Group (A)</td>
<td>Single dose</td>
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<tr>
<td>1. Indomethacin</td>
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<td></td>
<td>multiple small shallow erosions</td>
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<td>2. Paracetamol</td>
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<td>3. Indomethacin + paracetamol simultaneously</td>
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<tr>
<td>4. Paracetamol one hour after Indomethacin</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>multiple small shallow erosions</td>
<td></td>
</tr>
<tr>
<td>5. Paracetamol one hour after Indomethacin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>reduced in size</td>
<td></td>
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<tr>
<td>Group (B)</td>
<td>repeated doses</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>6. Indomethacin for 2 weeks</td>
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<td></td>
<td></td>
<td></td>
<td>multiple large deep erosions</td>
<td></td>
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<tr>
<td>7. Paracetamol for 2 weeks</td>
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<tr>
<td>8. Indomethacin for 2 weeks then paracetamol for 1 week without treatment</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>reduced in size</td>
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<tr>
<td>9. Indomethacin for 2 weeks then paracetamol for 1 week</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>very small and minute erosions</td>
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</table>

- = very minimal  
+ = mild  
++ = moderate  
+++ = severe
Fig. 1: The stomach of rat from a control group shows normal gastric mucosa. (Hx. & E. X 100)

Fig. 2: The antral gastric mucosa of rat. 4 hours after a single dose of indomethacin, shows small shallow mucosal erosions and mild inflammatory cells infiltration in the lamina propria. (Hx. & E. X 100).

Fig. 3: The fundic gastric mucosa, 4 hours after simultaneous administration of both indomethacin and paracetamol, shows chemical gastritis. The mucosal cells show minimal exfoliation and few inflammatorily cells infiltration in the lamina propria. (Hx. & E. X 100).

Fig. 4: The antral gastric mucosa of rat pretreated with paracetamol one hour before indomethacin, shows the typical indomethacin-induced erosions. (Hx. & E. X 100).
Fig. 5: The antral gastric mucosa of rat treated with indomethacin for 2 weeks shows large deep mucosal erosion and moderate number of inflammatory cell infiltration. (Hx. & E. X 100).

Discussion

In the present study, the stomach of control rats showed very minimal exfoliation and desquamation of the superficial cells of the gastric mucosa. This observation could be considered as a normal aging process, and it is in agreement with the finding of Croft (1977).

The damaged effect of indomethacin on the gastric mucosa was previously reported by many authors (Tordjman et al., 1995; Cook et al., 1996). In the present study, gastric mucosal erosions and exfoliation of the surface cells occurred 4 hours after indomethacin administration in a single therapeutic dose of 9 mg/kg body weight; this erosive effect became more marked after given the same dose of the drug for two weeks. Cook et al. (1996) reported that the potential difference (PD) that is maintained across healthy gastric mucosa was increased in rat
stomach after injury by a single dose of NSAID and accompanied by early superficial mucosal damage during the first 30 - 60 minutes after a single dose; the time course of deep and total damage of the mucosa more closely matched the alterations in PD. Also, Scarpignato et al. (1995) had been observed that the disruption of the gastric mucosal barrier by the so-called "barrier breakers" such as ethanol and NSAIDs, namely indomethacin, was associated with an increase in gastric potential difference, and there was a good correlation between the degree of histological damage and changes in gastric potential difference.

The present study showed that paracetamol in single therapeutic dose, produced no microscopic erosions, and its chronic administration for two weeks caused very minimal structural changes in the gastric mucosa. Also, the simultaneous administration of it with indomethacin caused only chemical gastritis but no any gastric erosions was observed i.e. paracetamol reduced the pathological effects produced by indomethacin alone. However, given of paracetamol one hour before indomethacin was found to have no effect on the gastric injly induced by indomethacin. While its administration. one hour after a single dose. or one week after prolonged treatment of indomethacin. appeared to reduce the incidence of gastric erosions significantly. NSAIDs induce the release of reactive oxygen metabolites which inhibit prostaglandin synthesis in animal models, and this may contribute to the gastroduodenal injury. This result was confirmed by McAlindon et al. (1996), in human volunteers. gastric injury was assessed endoscopically .and the effect on prostanoids was determined by measuring the antral prostaglandin E2 (PGE2) synthesis. Indomethacin was found to be a potent inhibitor of the prostaglandin -forming cyclooxygenase (COX1, COX2) (Meade et al., 1993; Mitchell et al., 1993).

Masferrer et al. (1994) and Tordjman et al. (1995) found that the reduction of PGE2 synthesis by indomethacin resulted from an enzymatic inhibition associated with a slight decrease of COX2
protein level. By endoscopical examination of the gastric mucosa carried by Murray et al. (1996), they confirmed that the total number of gastric erosions, both haemorrhagic and non-haemorrhagic, were judged by the gastric mucosal PGE2 values: suppression of PGE2 production by NSAIDs was associated with significantly higher mucosal damage.

On the other hand, paracetamol is a weak inhibitor of both PGE2 biosynthesis and COX enzymatic activity (Tordjman et al., 1995). There is a good correlation between the anti-enzyme activity of these drugs and their anti-inflammatory effect. Therefore, indomethacin is antipyretic, analgesic and anti-inflammatory, while paracetamol is antipyretic and analgesic but is only weak anti-inflammatory drug. The variations in the sensitivity of enzymes in the target tissues may be an important factor for such differences (Clissold, 1986; Vane and Botting, 1987; Battistini et al., 1994; Pellissier et al., 1996).

The protective and regenerative actions of paracetamol against the erosive effect of indomethacin, which is more than the normal regenerative process of gastric mucosa, can be explained by the studies of Brzozowski et al. (1996) and Romano et al. (1996), who concluded that paracetamol mediate the mucosal regeneration of the gastric erosions occurred by prolonged use of indomethacin and aspirin, and this probably resulting from increased luminal and mucosal contents of transforming growth factor alpha (TGF-alpha) and epidermal growth factor (EGF) and excessive expression of their receptors.

Because the paracetamol protects the gastric mucosa against irritation induced by indomethacin, therefore it is preferable to use it as antipyretic and analgesic instead of indomethacin, or used simultaneous with or after indomethacin when there is a possibility of gastric mucosal injury, especially for a long period of treatment.

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