ADVERSE EFFECTS OF MOSQUITO COIL SMOKE ON LUNG, LIVER AND CERTAIN DRUG METABILISHING ENZYMES IN MALE WISTAR ALBINO RATS

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SUMMARY

The adverse effects of chronic 8-hour daily inhalation of mosquito coil smoke for 6 weeks were investigated in male Wistar albino rats. Specific serum and urine biochemical parameters and tissue morphology were used as indices of toxicity. Activities of specific isozymes of microsomal monooxygenase (MFOs) were used to assess the potential effects of inhalation of mosquito coil smoke on drug metabolism. The animals that inhaled mosquito coil smoke had significantly elevated (23%) serum bilirubin ($p=0.015$) but lower (16%) albumin levels ($p=0.009$). Serum creatinine and urinalysis data were comparable to control levels. Gross pathology and histopathological studies revealed severe lung damaged evidenced by increased lung wet weight (26%), interstitial oedema, bronchopneumonia and emphysema in the coil smoke-exposed rats. Inhalation of mosquito coil smoke did not affect kidney cells but fatty infiltration and proliferation of liver cells were observed. Mosquito coil smoke inhalation significantly inhibited hepatic pentoxyresorufin O-deethylase (PROD) activity by 27%, lung PROD and p-nitrophenyl hydroxylase (PNPH) activities by 43% and 48%, respectively, and kidney ethoxyresorufin-O-deethylase (EROD) activity by 37% ($p=0.002$). These are consistent with the observed 33% increase in pentobarbital-induced sleeping time in the mosquito coil smoke exposed group. These findings indicate that inhalation of mosquito coil smoke induced selective pulmonary and hepatic damage accompanied by inhibition of drug metabolising enzymes in the rats. It is possible that chronic inhalation of mosquito coil smoke could have significant health implications for humans.

Keywords: Toxicity, mosquito coil, smoke, male rats, enzymes.

INTRODUCTION

Mosquito coil is used extensively in tropical and subtropical areas as an insect repellent or insecticide. It contains allethrin, the active substance, which is a synthetic analogue of the natural pyrethrum insecticides obtained from the flower heads of the plant *Chrysanthenim cinerariafolium* known to act by immobilizing the insect through poisoning of its nervous system[1]. Most mosquito coils sold in Ghana contain 0.1-0.3% allethrin (Okine LKN et al. unpublished survey).

Studies in female albino rats exposed to mosquito coil smoke indicated that, there were signs of toxicity to liver and lung but none to the kidney[2]. It has been suggested that the toxicity of mosquito coil smoke is caused by its combustion products such as sub-micron particles coated with heavy metals, allethrin and a wide range of organic vapour like phenol, o-cresol, benzene and toluene[2,3]. Chronic exposure of high doses of allethrin (50-200 mg/kg/day for two years) produced signs of toxicity such as increased liver and kidney weights and adverse morphological changes in liver tissue in female rats but not in dogs[4,5]. There was however, no observation of carcinogenic or teratogenic and/or developmental effects in exposed rats[4,5].

Chemical-induced organ toxicity may manifest as tissue or organ damage and/or derangement of cellular metabolism culminating in cell death and subsequently, organ failure[6,7]. Certain chemicals may exert their adverse effects through modulation of tissue microsomal mixed function oxidases (MFO) that metabolise endogenous substances like steroid hormones and xenobiotics leading to drug

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interactions and possible toxicological consequences[1,8,9].

In this paper, we report on the toxic effects of mosquito coil smoke inhalation on male rats and on some isozymes of the MFO in these animals. This is in view of the fact that, most studies on the toxicity of mosquito coil smoke inhalation have been conducted in female rats, and there is no readily available empirical data on the effects of mosquito coil smoke inhalation on MFO, which are responsible for the metabolism of important endogenous substances and xenobiotics.

MATERIALS AND METHODS

Chemicals and Reagents

Mosquito coils containing 0.3% allethrin were obtained from MIA Co. Ltd., Indonesia. Ames Reagent Strips for Urinalysis were purchased from Bayer-Sankyo Co. Ltd., Japan. NADPH, pentoxyresorufic, ethoxyresorufin, p-nitrophenol and resorufin were obtained from Sigma Chemicals Co., St. Louis, MO, USA. Bilirubin, albumin and creatinine reagent kits were purchased from Randox Laboratories Ltd., Co. Antrim, UK. Folin-Ciocalteau reagent was obtained from Hopkins-Williams, Essex, England. All other chemicals were obtained from Fluka Chemicals, Buchs, Switzerland or BDH Chemicals Ltd., Poole, UK.

Animals and Treatment

Adult male Wistar albino rats weighing about 250g obtained from the Animal Facility, Korle Bu Teaching Hospital, Korle Bu, Accra Ghana. The animals were housed in stainless steel cages and fed with pelleted food from GHAFCO Ltd. (Tema, Ghana) and distilled water *ad libitum*. The rats were divided into groups of five animals. Three groups of animals served as controls while three others were assigned as test groups. The test animals were exposed to mosquito coil smoke produced by burning two mosquito coils for 8hr/night, 7days/week for 6 weeks, in a partially ventilated room of size 4m x 3.5m x 3.5m. The control animals were kept in a room of similar ventilation and size without mosquito coil smoke for the period of time. One set of control and test animal groups were used for tissue morphological studies. Another set of control and test animal groups were used for plasma biochemical analyses, urinalysis and selected tissue MFO activity studies. A third set of control and test animal groups were used for pentobarbital sleeping time determination. The body weights of control and test animals were determined at baseline and at various time intervals during the course of the study.

Blood and Urine Sampling

Blood samples were collected from all animals prior to commencement of the studies and also at termination (6 weeks). The blood samples, collected into eppendorf tubes, were placed on ice and allowed to clot and centrifuged at 4,500g for 5 minutes to obtain serum. The serum was separated and stored at -20°C for biochemical analyses.

Serum Biochemical Analyses and Urinalysis

Serum bilirubin, albumin and creatinine were determined spectrophotometrically (Shimadzu UV-190, Japan) with commercially available kits (Randox Laboratories Ltd., Co. Antrim, UK). Urinalysis was performed on spot urine samples. The test strip was wetted with sample of urine and the colour changes compared with those of a standardised scale to give a semi-quantitative measure of protein, pH, glucose and blood in the urine.

Pentobarbital-induced Sleeping Time and Tissue Microsomal Enzyme Assays

Each animal from the third set of control and smoke-inhaled animal groups underwent the pentobarbital-induced sleeping time test[10]. For microsomal studies, each animal from the second set of control and smoke-inhaled animal groups was weighed at termination and euthanized by cervical dislocation. The kidneys, lung and liver of each animal were excised, blotted and weighed. The different organs from each animal were separately minced and homogenised and microsomes prepared by standard methods[11,12]. The microsomal pellets were each suspended in 2 ml of storage buffer made up of Na_2HPO_4/NaH_2PO_4 buffer pH 7.6, containing KCl (0.15M), EDTA (1mM) and glycerol (10%), and stored at -40°C for protein and enzyme assays. Protein determination was done on day of tissue enzyme assay by the Lowry method[13], using bovine serum albumin as standard following which the protein content of each microsomal preparation was adjusted to 1.0 mg/ml and the activities of p-nitrophenol hydroxylase (PNPH), ethoxyresorufin-O-deethylase (EROD) and pentoresorufin-O-deethylase (PROD) were determined[10].

Histology

One animal each from the first set of control and mosquito coil smoke-inhaled animals was euthanized by cervical dislocation at baseline and two animals each from each treatment group thereafter at 3 and 6 weeks. Lungs, liver and kidneys were excised and fixed in formaldehyde and dehydrated with alcohol. The tissues were cleared with xylene and impregnated with paraffin wax and sections

cut and stained with haematoxylin and eosin, and mounted on slides for light microscopic examination[14].

Statistical Analysis

One-way analysis of variance was conducted to determine statistical significance. The 0.05 level of probability was used as the criterion of significance in all instances. All statistical tests were performed with Jandel SigmaStat. Statistical Software Version 2.0 (1992-95).

RESULTS

There were no significant differences in the mean body weights between smoke-inhaled and control animals over the initial 2 week period of study. However, by week 5 there was a significant decrease ($p<0.042$) in the mean body weight of the smoke-inhaled animals compared to controls (results not shown).

The effects on inhalation of the mosquito coil smoke on some organ weights at termination are shown in Table 1. When compared to controls, lung wet-weight of smoked-inhaled animals increased (26%) significantly ($p=0.038$). Kidney weights of the smoke-inhaled animals decreased by 8% ($p=0.062$) but there was no change in liver weight.

Table 2 Effects of mosquito coil smoke inhalation on serum biochemical parameters at termination[a]

Serum parameter	Control	Test
Albumin (g/l)	50.7±0.46	42.2±2.46 (84)*
Bilirubin (μmol/l)	9.05±0.41	11.1±0.52(123)**
Creatinine (μmol/l)	172±3.60	179±2.80 (104)

[a]For details of animal treatment see Material and Methods section.
Values are means ± SEM of n=5
Value in parenthesis represents percent of control.
*Value significantly different from control; p=0.009
**Value significantly different from control; p=0.015.

The effects of mosquito coil smoke inhalation on monooxygenases activities in lung, liver and kidneys at termination are show in Table 3. Liver and lung PROD activities were significantly lower in animals exposed to mosquito coil smoke (27% and 43%, respectively) compared to control levels ($p=<0.001$). Kidney EROD activity in smoke-inhaled animals decrease by 37% ($p=0.002$). Similarly, only coil smoke exposed lungs showed a significant reduction (48%) in PNPH activity ($p=<0.001$). Pentobarbital-induced sleeping time was increased (33%) significantly ($p<0.034$) in the smoke-inhaled animals (255±18 min) compared to controls (192 ± 17min).

The morphological changes in control lungs, livers and kidneys and those from animals exposed to mosquito coil smoke are represented in Figures 1-3. The results indicated that there was Clara cell hyperplasia and thickening of the bronchiolar epithelial wall as well as inflammatory response in alveolar areas with alveolar septa thickening and hypercellularity in the smoke-inhaled animals at weeks 3 and 6 post-exposure. However, lung morphology of control animals at weeks 3 and 6 (results not shown) were similar to those of baseline controls, which showed normal alveolar areas and Clara cells lining a normal bronchiolar epithelial wall (Figure 1a, b & c)).

Table 1 Effects of mosquito coil smoke inhalation on organ weights at termination[a]

	Organ wet weight			
	Actual		% Body weight	
Organ	Control	Test	Control	Test
Lung	1.60 ± 0.07	2.01± 0.15 (125.6)*	0.57 ± 0.02	0.72 ± 0.05 (126.3)*
Liver	7.80 ± 0.30	0.09 ± 0.29 (103.7)	2.83 ± 0.11	2.91 ± 0.09 (102.8)
Kidney	1.32 ± 0.04	1.22 ± 0.03 (92.4)	0.48 ± 0.02	0.44 ± 0.01 (91.6)

[a]For details of animal treatment see "materials and methods".
Values are means ± SEM of n=5.
Value in parenthesis represents percent of control
*Value significantly different from control; p=0.038

Table 2 shows the effects of mosquito coil smoke inhalation on some serum biochemical parameters at termination. Compared to controls, serum albumin decreased (16%) significantly ($p=0.009$) in the smoke-inhaled animals. Conversely, serum albumin in these animals showed a significant increase (23%) ($p=0.015$). There was however no differences in urine pH, glucose and protein levels or presence of blood between the two groups (data not shown).

Table 3 Effects of mosquito coil smoke inhalation on tissue microsomal mono-oxygenases activity at termination[a]

Enzymes Parameter	Enzyme specific activity (nmol/min/mg protein)					
	Liver		Lung		Kidney	
	Control	Test	Control	Test	Control	Test
PROD	0.79 ± 0.03	0.58±0.02(73%)*	0.65±0.04	0.37±0.03(57%)*	0.37±0.02	0.42±0.01(114%)
EROD	0.30 ± 0.01	0.27±0.02(90%)	0.31±0.07	0.34±-.07(110%)	0.56±0.04	0.35±0.02(63%)[&]
PNPH	0.70 ± 0.08	0.59±0.10(84%)	0.33±0.02	0.17±0.02(52%)[$]	0.96±0.10	0.71±0.10(74%)

[a]For details of animal treatments see "materials and method"
PNPH=p-Nitrophenol hydroxylase; PROD = Pentoresorufin-O-deethylase; EROD=Ethoxyresorufin-O-deethylase.
Values are means ± SEM of n=5
Value in parenthesis represents percent of control.
*Value significantly different from liver or lung control; p=<0.001
[&]Value significantly different from kidney control; p=0.002
[$]Value significantly different from lung control; p=<0.001.

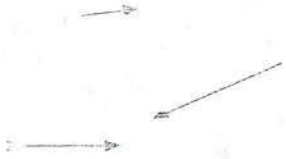

Figure 1a

Figure 1b

Figure 1c

Figure 1 Histological appearance of lung tissue of control animals showing normal alveolar areas (1) and Clara cells (2) lining normal bronchiolar epithelial wall (3), and animals exposed to mosquito coil smoke for three and six weeks showing inflammatory response, septa thickening and hypercellularity and consolidation in alveolar areas (1), Clara cell hyperplasia (2), thickening of bronchiolar epithelial wall (3) and interstitial oedema (4). Magnification x 132.

Figure 1a

Figure 2b

Figure 2c

Figure 2 Histological appearance of liver tissue of control animals showing normal hepatocytes, and animals exposed to mosquito coil smoke for three and six weeks

showing gross morphological changes characterised by cell proliferation and fatty infiltration of cells (1). Magnification x66.

Smoke exposed animals at weeks 3 and 6 showed gross morphological changes of liver characterised by proliferation and fatty infiltration of cells when compared to baseline controls (Figure 2a, b, & c).

There were no observable differences in kidney morphology of control and smoke-inhaled animals by week 6 (Figure 3a & b).

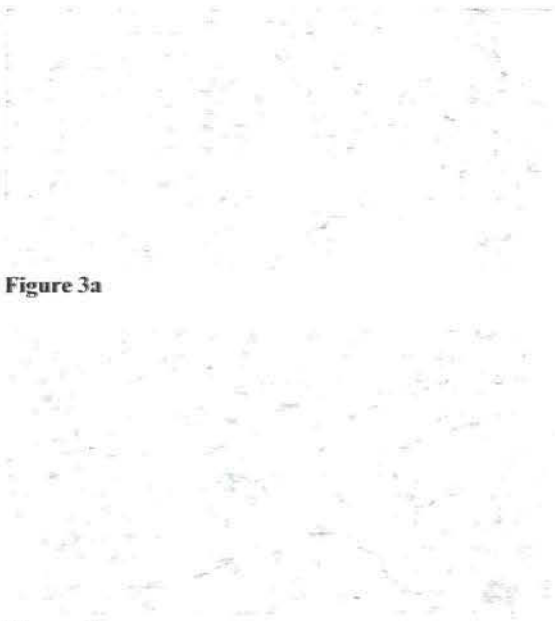

Figure 3a

Figure 3b

Figure 3 Histological appearance of kidney tissue of control animals (a) and animals exposed to mosquito coil smoke for six weeks (b) showing no differences in appearance of cells between the two treatment groups. Magnification x132.

Liver and kidney morphology of control animals at week 3 and 6 (results not shown) were similar to baseline controls.

DISCUSSION

Previous studies using female Wistar albino rats exposed to mosquito coil smoke showed that the smoke caused morphological changes in lung; and changes in some lung and plasma biochemical parameters but none in the kidney[2,3]. In the present study we investigated the effects of mosquito coil smoke on serum bilirubin, albumin and creatinine levels in male Wistar albino rats, and determined the effects of the smoke on tissue microsomal mono-oxygenase activities, which are likely to affect the metabolism of other xenobiotics and hence cause drug interactions.

Some biochemical indices used in this study to assess hepatotoxicity are elevated levels of serum bilirubin and reduced levels of serum albumin and/or globulins. These and others like elevated activities of enzymes like aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) are used as indices of hepatic damage or dysfunction[6]. Albumin, which is involve in the transport of substances like drugs and lipids[6] is synthesised by the liver. Therefore, the significantly lower level observed in the mosquito coil smoke inhaled rats would suggest that exposure to the coil smoke decreased the protein biosynthetic activity of the liver. This could affect capacity of serum protein-mediated transport of various substances, and may explain the fatty infiltration of some hepatocytes. The reasons for the elevated bilirubin levels cannot be explained at this time since RBC counts and direct and indirect bilirubin levels were not determined. However, it is known that some possible causes of increased serum bilirubin levels are the impairment of the biliary excretory system or an increased red blood cell haemolysis[6].

Morphological studies of the lung, liver and kidney in the male rat indicate that while the smoke did not affect the kidneys it caused damage to the lungs and liver as observed previously in female rats by others[2]. However, unlike the previous studies where signs of lung toxicity were observed at 60 days, lung and liver toxicity in the present study manifested as early as 21 days. This may be due to sex differences or to differences in experimental conditions such as the number of coils burn/day, the number of days the animals were exposed to the mosquito coil smoke/week, the size of room and the degree of room ventilation used in each study. A comparative study between male and female rats under identical experimental conditions are currently under investigations and may help to confirm sex differences in the expression of organ toxicity induces by mosquito coil smoke.

The lung tissue of smoke-inhaled rats in the study, showed Clara cell hyperplasia, thickening of the bronchiolar epithelial wall, alveolar septal thickening and hypercellularity, and consolidation in alveolar areas after 3 and 6 weeks of exposure (Figure 1a,b, &c), which are indicative of toxic lung insult. These were accompanied by signs of pulmonary oedema, fibrosis, emphysema and bronchopneumonia, as well as increase in mean lung

volved was usually the maxilla(Figure 1). In the case involving the frontal bone (Figure 2), expansion of the lesion into the orbit resulted in proptosis and downwards displacement of the eye.

cystic areas. With ossifying and cementifying fibroma, there was usually a clearly demarcated area of patchy radioluscency (Figure 3), appearing cystic at times. Forty-four (84.6%) of the fifty-two

Table 3 Site distribution of fibro-osseous lesion in the facial bones of 52 patients

Lesion	Mandible	Maxilla	Frontal bone	Total
Fibrous dysplasia	3 (18.75%)	12 (75.0%)	1 (6.25%)	16 (100%)
Ossifying fibroma	28 (87.5%)	4 (12.5%)	0	32 (100%)
Cementifying fibroma	3 (75.0%)	2 (25.0%)	0	4 (100%)
(Fibro-osseous lesions)	34 (65.4%)	17 (13.7%)	1 (1.9%)	52(100%)

Pain was not usually a significant factor, though it was present in some cases. This was often due to secondary infection arising from ulceration on the indented surfaces of the intra-oral expansion of the lesion where there was impingement of the teeth from the opposing jaws.

Figure 1 Fibrous dysplasia affecting the left maxilla in a 14-year old male patient. Note the upward displacement of left eye from expansion of the lesion into the orbit.

Figure 2 Photograph of a case of fibrous dysplasia of the frontal bone in a 12-year old male patient.

Radiographs largely exhibited a dense mass often with 'ground glass' appearance with ill-defined margins in the case of fibrous dysplasia. When it involved the maxilla, the lesion often expanded to obliterate the sinus on the affected side. In the mandible, the central portions of the largely 'ground glass' appearance often exhibited micro-

cases studied were correctly disgnosed by clinical and radiological finding while the remaining eight cases or 15.4% were diagnosed clinically as either osteoma or fibroma. In the list of differential diagnosis, benign osteoma topped with the highest index, followed by myxoma and ameloblastoma in the order.

Figure 3 Radiograph of the mandible affected with ossifying fibroma. Note the clearly demarcated area of patchy radioluscency in the left body of the mandible

Treatment

Of the 52 cases studied over the period, only 38 were treated and followed up by one of the authors; hence only the treatment of these patients is analysed in this paper. All the 38 patients were treated conservatively under general anaesthesia via an intra-oral approach. Teenage or younger patients presenting with fibrous dysplasia were often persuaded to defer surgery until they were about 21 years old, when skeletal growth was presumed to have ceased. In all such cases, surgery consisted of excising the enlarged diseased bone by paring it down and reshaping it to resemble that of the contra-lateral side. Where considerable bleeding was encountered during the surgery, the diseased bone was usually pared slightly lower than the normal side to allow for compensation by any organising haematoma following wound clo-

sure. Both ossifying fibroma and cementifying fibroma were essentially treated by curettage. Large volumes of normal saline were often used to wash the wounds thoroughly before closure. It was usual to achieve complete haemostasis before applying pressure dressing to minimize any haematoma formation. No drains were ever inserted.

Follow-up and results of treatment

The patients were usually hospitalized for periods ranging from 3 to 10 days post surgery. Review regimen started on the morning after surgery, and thereafter weekly for a month. The period was then extended to monthly for three months and then twice a year for as long as the patients could keep appointments. Most patients did not keep appointments beyond one year. Fourteen patients were followed-up beyond one year with one case of ossifying fibroma and another of fibrous dysplasia recurring nine years and eight months respectively after surgery. The recurrent lesion of fibrous dysplasia was larger and much more aggressive than the initial condition and thus necessitated complete removal of the affected maxillary bone, but sparing the palatal mucosa.

DISCUSSION

The overall incidence of fibro-osseous lesions of 2.4% of all head and neck tumors is comparable to that found in the literature[1-3]. In this study, ossifying fibroma was the commonest lesion forming in the 61.5% of cases. This is different from studies from advanced countries that found more fibrous dysplasia (67%)[1,2,11]. The reason for this difference in the incidence of the various types of lesions is not clear. The study showed a slight female preponderance even though the literature, from studies on larger samples, indicates equal incidence for males and females[10,11]. This finding is however, comparable to that reported by Ramsey et al (1968), who according to Killey et al[8], in their series of 47 patients, found 26 female and 21 male. The peak age for all lesions is reported as 25-35years[10]. This is higher than 11-20 years seen in this study and may be due to either the small number of cases reported on in this study, or possibly, to population differences.

The commonest site for fibrous dysplasia was the maxilla and the commonest site for cementifying fibroma and ossifying fibroma was the mandible. The main presentation of unilateral facial bone swelling is as reported by other studies[1-4]. There were no bilateral lesions seen. Though there are poly-ostotic forms of fibro-osseous lesions that may even have endocrine components[4-6], there was no such case in this study. Although the lesions may present difficulties with diagnosis, a combination of clinical findings and radiological appearances gave a high correlation with histological diagnosis. It is clear however that the best chance of correctly characterizing the lesions was a combination of clinical findings, radiological and histological appearances.

Fibro-osseous lesions are known to be benign and all who discuss it are virtually unanimous in recommending conservative treatment[7]. In view of the fact that areas of fibroma dysplasia continue to enlarge during the period of general skeletal growth it is advisable to defer surgery until growth has ceased[8]. Though, according to Killey et al[8], Seward (1970), reported cases where the lesion continued to enlarge after this time, no such observation was made here. In this series, most of the patients sought treatment only for cosmetic reasons, as there was very little disturbance of function. It was therefore easy to persuade most patients presenting with fibrous dysplasia to defer surgery until they were about 21 years old, when skeletal growth was presumed to have ceased.

This, in our view, accounted for the low rate of recurrence. In fact only one of the two cases that recurred in this survey was fibrous dysplasia. His deformity was so cosmetically unacceptable that earlier surgery was required. The patient was therefore, prior to the surgery, alerted of a possibility of recurrence. Indeed, the tumour recurred in eight months and required excision of the entire affected maxillary bone. There have been reports of malignant changes occurring in some cases of fibrous dysplasia, especially when radiated. No radiation was carried out in any of the patients studied, and there was no malignant change encountered in the series.

The ossifying fibroma and cementifying fibroma seldom recur and malignant degeneration has not been reported. For this reason, the sacrifice of large segments of bone is contraindicated. Cryotherapy has been reported to be very effective in treating tumours lying adjacent to or within bone, and has been effectively used in treating ossifying fibroma[7]. This facility is lacking in this hospital, and as such was never used. All the patients in this series were treated by simple curettage via intraoral approach. This method was largely satisfactory with only one case of recurrence after a period of nine years. In all the patients, there was never the need to sacrifice or damage either the inferior alveolar or infra-orbital nerves during treatment.

CONCLUSION

Fibro-osseous lesions are benign conditions with similar histological appearances but different

clinical behaviour in patients. When surgical treatment is carried out on them at an early age, though both ossifying fibroma and cementifying fibroma seldom recur, in view of the fact that areas of fibrous dysplasia continue to enlarge during the period of general skeletal growth, it may recur with disastrous consequences. Their successful management therefore depends largely on the establishment of accurate clinical diagnoses aided by extensive investigation and careful interpretation of radiographs and medical, family and dental history. Most patients seek treatment for cosmetic reasons therefore, unless there is a significant disturbance of function, it is advisable to defer surgery until the cessation of general skeletal growth.

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