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RESEARCH

The impact of mitotane therapy on serum-free proteins in patients with adrenocortical carcinoma

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Abstract

Introduction: Adrenocortical carcinoma (ACC) is a rare malignancy of the adrenal cortex. Whilst surgery is the preferred treatment, adjunctive therapy with mitotane may be offered post-surgically to minimise the risk of recurrence or, in the absence of surgery, to attenuate progression.

Aim: The objective was to evaluate the effects of mitotane treatment on serum protein concentrations in patients treated for ACC with mitotane therapy and compare this to patients with other adrenal neoplasms and a normal pregnant cohort.

Methods: Serum cortisol, thyroid function tests, adrenocorticotrophic hormone (ACTH), cortisol-binding globulin (CBG), thyroxine-binding globulin (TBG), gonadotrophins and androgens were measured on plasma and serum samples. Thirty-five patients with ACC were included, and mitotane levels were noted to be sub-/supra-therapeutic. Data were tested for normality, reported as mean ± S.D., and compared to other two cohorts using paired-sample *t*-test with a 5% *P*-value for significance and a 95% CI.

Results: Patients on mitotane therapy had a higher mean serum CBG concentration compared to the adrenal neoplasm group (sub-therapeutic: 79.5 (95% CI: 33.6, 125.4 nmol/L), therapeutic: 85.3 (95% CI: 37.1–133.6 nmol/L), supra-therapeutic: 75.7 (95% CI: −19.3, 170.6 nmol/L) and adrenal neoplasm: 25.5 (95% CI: 17.5, 33.5 nmol/L).

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Negative correlations between serum cortisol and CBG concentration were demonstrated within the supra-therapeutic plasma mitotane and adrenal neoplasm groups.

Conclusion: Patients with ACC and therapeutic plasma mitotane concentrations had higher serum CBG concentrations compared to those with adrenal neoplasms or pregnant women, and higher serum cortisol. Whilst there was no direct correlation with cortisol and mitotane level, the negative correlation of cortisol with CBG may suggest that the direct effect of mitotane in increasing cortisol may also reflect that mitotane has a direct adrenolytic effect.

Keywords: mitotane; adrenocortical carcinoma; serum-free proteins; cortisol-binding globulin; thyroid-binding globulin;

Introduction

Adrenocortical carcinoma (ACC) is a rare malignancy of the adrenal cortex with an annual incidence of between 0.5 and 2.0 cases per million (1). Whilst most cases of ACC are sporadic, typically occurring in the fifth and sixth decades of life and occurring more commonly in females, there are some genetic predispositions to ACC, including Li–Fraumeni, Beckwith–Wiedemann, and Lynch syndromes (2). Around 50–60% of patients with ACC present with clinical features of steroid hormone excess, with hypercortisolism accounting for 50–70% of these cases (2). Signs and symptoms resulting from the mass compressive effects in the abdomen, such as abdominal or flank plain and appetite loss, are also commonly reported (3). Less frequently reported are generalised symptoms of cancer such as cachexia, night sweats and fever (3).

The prognosis of ACC is generally poor, with a median overall survival of 3–4 years (2). Currently, the only curative treatment is surgery. The current best standard practice is surgery, mostly open rather than laparoscopic, involving complete adrenalectomy in localised or locally advanced cases (stages I–III) (3). Previous reports have suggested that the removal of local lymph nodes may assist in minimising the risk of metastasis (4).

Adjunctive therapy with mitotane or other systemic cytotoxic agents may be offered to those post-surgery to minimise the risk of recurrence and in those with inoperable disease and/or metastatic disease. Despite the ongoing use of mitotane, its mode of action remains poorly understood, and data surrounding its optimal administration are conflicting (5, 6, 7).

Mitotane is an adrenolytic agent: current knowledge suggests that it initially binds to mitochondria in adrenocortical cells by interacting with mitochondrial proteins, including CYP11A1 and CYP11B1. This, in turn, results in both the inhibition of steroidogenesis and disruption of mitochondrial function by the generation of reactive metabolites, resulting in cellular necrosis (8).

The European Society of Endocrinology (ESE) Clinical Practice Guidelines on the management of ACC in adults recommend monitoring mitotane blood levels every 3–4 weeks initially until the target therapeutic range is obtained (i.e. 14–20 mg/L) (9). Typically, a daily dose between 2 and 10 g is required to achieve this. However, the dose given depends on how well the drug is tolerated, as there are several commonly reported adverse effects of mitotane, including diarrhoea, vomiting and weight loss.

Administration of mitotane may also result in biochemical changes, including a marked increase in hormone-binding globulins and adrenal insufficiency (12, 13, 14, 15, 16). Specifically, measurement of total serum cortisol may not accurately reflect the glucocorticoid status of the patient and may lead to unrecognised adrenal insufficiency. Consequently, the altered binding protein profile and heightened risk of adrenal insufficiency lead to patients on mitotane therapy frequently being offered glucocorticoid replacement therapy (2).

Whilst the existing literature quite clearly demonstrates the impact of mitotane on steroids and hormone-binding proteins, to date, no studies have specifically related these to the plasma concentration of mitotane, focussing solely on the administered dose and disregarding the duration of mitotane administration. Concomitant medications that may skew results have also not been considered. Subsequently, we considered that further research was required to characterise the effects of mitotane more fully within its plasma concentration range, both therapeutic and non-therapeutic.

In this study, we, therefore, evaluated the effects of mitotane therapy on serum protein concentrations in patients with ACC and directly compared these to patients with an adrenal neoplasm (AN) not receiving mitotane and pregnant women at a tertiary referral service for ACC in London. The comparison between patients with an AN not receiving mitotane and pregnant women allowed us to draw conclusions between mitotane and the oestrogenic effects of pregnancy on binding globulins.

Methodology

A total of 35 retrospectively evaluated patients who were either being investigated for an adrenal mass ($n = 25$)

or pregnant women undergoing endocrine assessment (n = 10) in the period between April 2019 and June 2020 were included in the analysis (Table 1). Serum cortisol, full thyroid function tests (TSH, free T4 and free T3), adrenocorticotrophic hormone (ACTH), cortisol-binding globulin (CBG), thyroxine-binding globulin (TBG), gonadotrophins, DHEAS, testosterone and plasma EDTA ACTH were collected using BD Vacutainer during the same venepuncture. Samples were stored at −80°C until analysis. All samples were taken at 09:00 h after an 8 h fast in accordance with the Programmed Investigations Unit protocol at King's College Hospital. All our patients on mitotane were on 20–20–10 mg (50 mg per day) hydrocortisone therapy.

Assays and proteins

ACTH, DHEAS and androstenedione were measured using the Siemens Immulite 2000 immunoassay. The limit of quantification (LoQ) for ACTH, DHEAS and androstenedione were 5 ng/L, 0.4 µmol/L and 1.1 nmol/L, respectively.

Cortisol, fT4, TSH, testosterone and gonadotrophins were measured using Siemens ADVIA Centaur. The LoQ for cortisol, fT4 and TSH were 3 nmol/L, 1.3 pmol/L and 0.005 mIU/L, respectively.

Mitotane concentrations were obtained by LYSOSAFE Service, HRA Pharma, using a standardised HPLC method with an LoQ below 1 mg/L.

Thyroid-binding globulins in serum were measured in duplicate using an automated solid-phase competitive chemiluminescent enzyme immunoassay (Immulite 2000, Siemens, UK). Grossly lipaemic or haemolysed samples were removed. The LoQ of the assay was 29.6 nmol/L, and there was no reported cross-reactivity with similar analytes. Intra-assay and inter-assay coefficient of variation (CV%) at 68.8 nmol/L were 16.2% and 21.2%, and at 1330.1 nmol/L were 6.1% and 10.7%, respectively.

A manual ELISA method was used to quantify cortisol binding globulin in serum, which was a non-competitive sandwich immunoassay (BioVender, Brno, Czech Republic). There was no reported cross-reactivity of the assay. Intra-assay CV at 43.2 µg/mL and at 73.28 µg/mL were 1.2% and 2.2%, and inter-assay CV at 34.7 µg/mL and 39.59 µg/mL were 6.8% and 7.3%, respectively.

Table 1 Total group demographics and ACC characteristics for patients seen at King's College London Hospital between April 2019 and June 2020. Staging for ACC is based on American Joint Committee on Cancer (AJCC) and ENSAT staging system (10, 11).

Baseline characteristics	Results
Diagnosis	
ACC on mitotane	13
Median age (years) (IQR)	63.0 (46, 72.5)
Racial background	
Caucasian white	22 (62.9%)
Southeast Asian	5 (14.3%)
Black Afro-Caribbean	4 (11.4%)
Other	1 (2.9%)
Missing	1 (2.9%)
Gender	
Male	6 (46.2%)
Female	7 (53.8%)
Stage (AJCC and ENSAT staging system)	
Stage I	0
Stage II	0
Stage III	6 (46.2%)
Stage IV	7 (53.8%)
Adrenal neoplasm without mitotane	12
Median age (IQR)	60 (49.75, 70.25)
Sex	
Male	4 (33.3%)
Female	8 (66.6%)
Pregnant	10
Median age (IQR)	32.5 (30.75, 40)

Inclusion criteria

Inclusion in the analysis was based on whether an individual was investigated for a malignant adrenocortical neoplasm treated with mitotane, or age- and gender-matched number of patients with ANs not treated with mitotane under the Endocrinology team, or pregnant women who had an assessment of thyroid function and serum cortisol. None of the patients with ACC had Cushing's syndrome clinically or biochemically. Patients included in the analysis were seen in the period between April 2019 and June 2020.

Statistical methods

IBM SPSS Statistics version 27 was used for statistical analysis, whilst GraphPad Prism version 9.3.1 was utilised for graph illustrations. Data considered as continuous are summarised using mean plus S.D., or median with the interquartile range (IQR) (Q1, Q3) if not normally distributed. Normality of distribution was explored using Kurtosis.

Categorical data are summarised with a percentage of individuals within each category.

Results are viewed as parametric if within −1 and +1. The Mann–Whitney U test was used to assess statistical difference when the outcome was continuous and non-parametric. In cases of more than two independent groups for the same variable, Kruskal–Wallis test was used. The Pearson's chi-squared test was used to assess for statistical significance in two or more independent groups if the outcome variable is binary. Pearson's correlation test was used to assess for correlation between two continuous variables, unless there were less than ten samples within the variable, or the outcome

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variable was non-parametric, in which case, Spearman's rank was used. Whilst the values are essentially arbitrary, $r < 0.4$ was regarded as weak, 0.40–0.69 as moderate and $r > 0.7$ as strong. Statistically significant values are those with a $P < 0.05$.

Ethics

Patient consent was not required for this retrospective project using anonymised data associated with direct clinical care. GAfRec approval was obtained from King's College Hospital R&D Department (GAfRec-Endo202). The study was registered with clinicaltrials.gov (study ID: NCT05344027).

Results

Twenty-five patients recieved a diagnosis of an AN under the care of the Endocrinology Department at King's College Hospital between April of 2019 and June 2020. Of the 25 patients, 13 had an ACC without Cushing's syndrome, and all were on mitotane therapy. The remaining 12 patients had benign ANs and were not receiving mitotane. Benignity was defined histologically. Additionally, ten pregnant women were included in the analysis to compare changes occurring to binding proteins in pregnancy with those resulting from mitotane therapy. The median age of the individuals on mitotane with ACC was 63 years (46, 72.5), with a range of 24–85 years. The median age of individuals with an AN but not on mitotane therapy was matched at 60 years (49.75, 70.25), ranging from 27 to 73 years. The median age of pregnant women was 32.5 years (30.75, 40), ranging from 19 to 41 years.

Mitotane concentrations

Thirteen patients with ACC were on mitotane therapy at the time of assessment. The target therapeutic range for mitotane is 14–20 mg/L (8). Of the ACC patients, four (30.8%) had a serum mitotane concentration below, six (46.3%) within and three (23.0%) above the target therapeutic range.

ACTH

Twenty-four patients had their plasma ACTH concentrations measured (4, 6, 3, 11 and 0 from sub-therapeutic, supra-therapeutic mitotane plasma concentration range groups with ACC, AN and pregnant women groups, respectively).

Whilst the median plasma ACTH concentration within the therapeutic mitotane plasma range appears considerably higher compared to the other groups (42.5; IQR = 5.0, 116.3 ng/L), no statistically significant results were found between them ($P > 0.05$).

Cortisol concentration

Figure 1 shows that the ACC group within the therapeutic plasma mitotane concentration range had the highest mean serum cortisol concentration (889.8 ± 451.2 nmol/L). However, no statistically significant results were found between the different groups ($P > 0.05$). According to our pathology labs, reference values for cortisol at 09:00 h range between 133 and 537 nmol/L (06:00–10:00 according to Roche gen 2 assay).

TSH concentration

Thirty patients had their plasma TSH concentration assessed (4, 6, 3, 7 and 10 individuals from the sub-therapeutic, supra-therapeutic plasma mitotane concentration in patients with ACC, patients with AN and pregnant group).

Figure 3 demonstrates that the pregnant cohort had a higher median plasma concentration of TSH compared to other groups (median value: 1.73, IQR: 1.22, 2.24 mIU/L). All groups were found to have serum TSH concentrations within the reference range, except for patients in the supra-therapeutic plasma mitotane

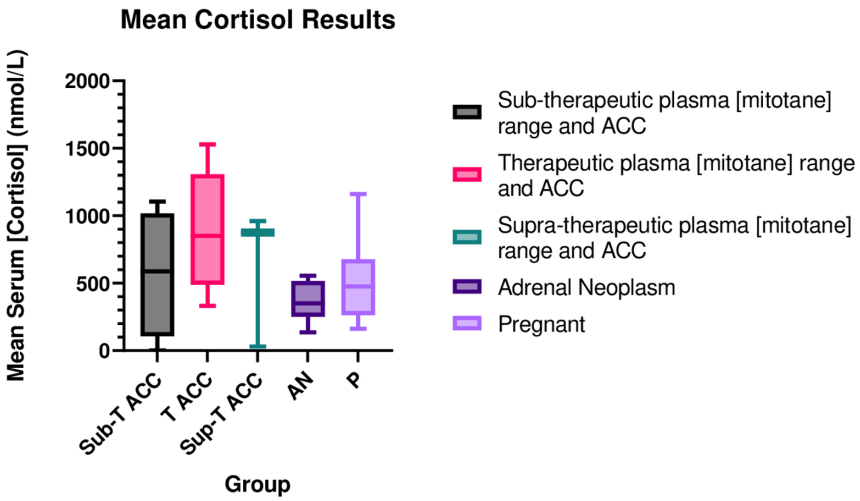

Figure 1

Mitotane plasma concentration correlation with serum cortisol concentration per group.

group, where significantly low TSH was observed . No statistically significant differences were found between the different groups.

Free thyroxine concentration

Figure 4 highlights that the median serum fT4 concentration is reduced with therapeutic and supra-therapeutic plasma mitotane concentrations. Patients with ANs and pregnant women had higher fT4 concentration compared to patients within the supra-therapeutic or therapeutic mitotane groups.

Thirty patients had their serum fT4 concentrations assessed (4, 6, 3, 7 and 10 individuals from sub-therapeutic, supra-therapeutic plasma mitotane range, AN and pregnant group).

Further analysis showed that ACC groups with supra-therapeutic or therapeutic mitotane concentrations had a mean fT4 concentration below the reference range.

Different fT4 concentrations were observed among patients in the AN group when compared with supra-therapeutic mitotane plasma concentration groups (AN: 14.9 (IQR = 12.5, 22.1), supra-therapeutic ACC: 9.6 (2.9), $P = 0.02$ and therapeutic: 10.6 (8.7, 11.3 pmol/L), $P = 0.004$). FT4 concentrations observed in the sub-therapeutic mitotane plasma concentration group were significantly higher compared to the supra-therapeutic or therapeutic mitotane plasma concentration range groups (sub-therapeutic ACC: 14.0 (IQR = 13.7, 18.1), $P = 0.017$, 0.005 respectively.

DHEAS

Twenty-two patients had their DHEAS serum concentrations measured during the period (n = 4, 6, 3 and 9 in sub-therapeutic, therapeutic, supra-therapeutic and AN, respectively). The median results are the same among those with an ACC on mitotane, regardless of plasma mitotane concentration range. The results were found to be 1.5× higher in the AN group.

DHEAS concentrations were similar among the different groups ($P > 0.05$).

Testosterone

Seventeen patients had their testosterone concentration measured: 9 (52.9%) were male (n = 2, 2, 2 and 3 for sub-therapeutic ACC, therapeutic ACC, supra-therapeutic ACC and AN, respectively). Eight (47.0%) were female (n = 1, 3 and 4 for sub-therapeutic ACC, therapeutic ACC and AN, respectively). No statistically significant results were found among groups ($P > 0.05$) in both sexes.

CBG concentration

Figure 2 demonstrates that patients with ACC on mitotane had a higher mean serum CBG concentration, compared to pregnant women or patients with ANs. The highest mean CBG concentration was found in the therapeutic plasma mitotane concentration range (85.3 ± 46 ng/mL).

Patients with ANs had a lower mean CBG concentration compared to all subgroups of patients with ACC on mitotane ($P < 0.05$).

Negative correlations were observed between circulating CBG and cortisol within all the groups, apart from the sub-therapeutic mitotane group and pregnant women ($r = 0.60$, $P = 0.40$; $r = 0.790$, $P = 0.007$, respectively). A strong negative correlation was

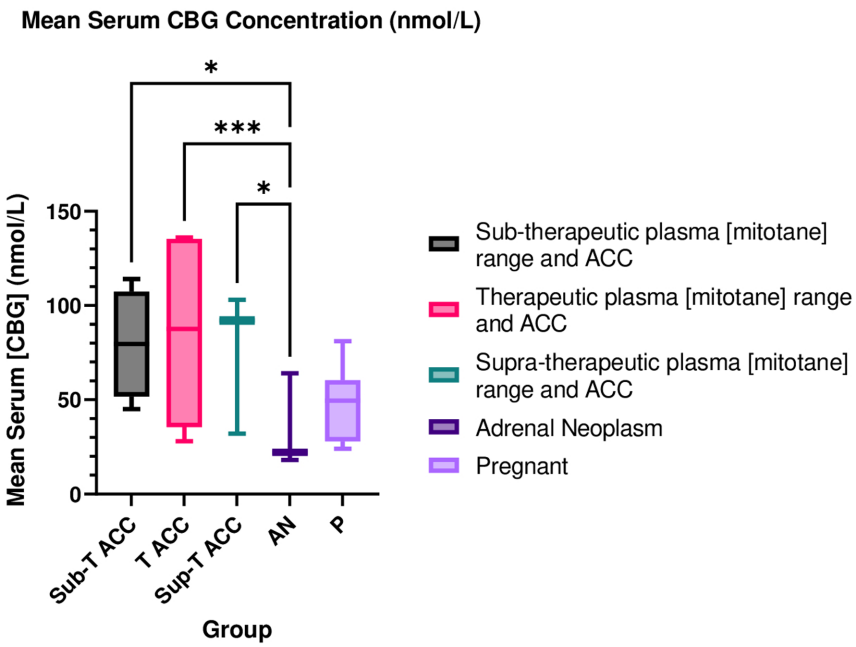

Figure 2

Median serum CBG concentration correlation to plasma mitotane concentration (*$P < 0.05$, ***$P < 0.01$).

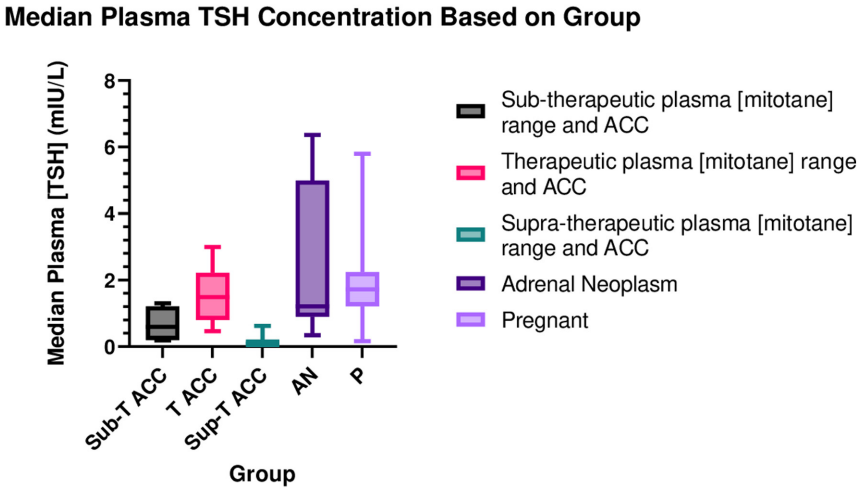

Figure 3

Median plasma TSH concentration correlation to plasma mitotane concentration per group.

demonstrated within the supra-therapeutic mitotane plasma concentration range (r = −1.00).

Negative correlations between cortisol and ACTH were observed among the sub-therapeutic and supra-therapeutic plasma mitotane concentration groups (r = −0.949, P = 0.051; r = −0.866, P = 0.333, respectively). Positive correlations were demonstrated within therapeutic mitotane dose and AN groups (r = 0.337, P = 0.461 and r = 0.042, P = 0.903, respectively).

TBG concentration

TBG concentrations (n = 4, 6, 3, 12 and 10 for sub-therapeutic, therapeutic, supra-therapeutic plasma mitotane concentration ranges, AN and pregnant female cohort, respectively) are shown in Fig. 5.

A slight reduction in TBG concentration with higher plasma mitotane concentrations was noted. The cohort of pregnant women had the highest mean plasma TBG concentration compared to all the other groups (791.6 ± 177.8 nmol/L). Patients with an AN had a significantly lower mean TBG concentration compared to groups with sub-therapeutic mitotane plasma concentration and pregnant women (AN: 365.7 nmol/L (95% CI: 276.9, 454.5), sub-therapeutic: 676.3 nmol/L (95% CI: 507.8, 844.7), P = 0.027 and pregnant: 791.6 nmol/L (95% CI: 664.4, 918.8), P < 0.01). Patients with ACC in the therapeutic and supra-therapeutic mitotane concentration subgroups had numerically higher TBG concentrations comparing them to patients with AN though not reaching statistical significance.

All the groups had a mean plasma TBG concentration above the reference range (150–360 nmol/L).

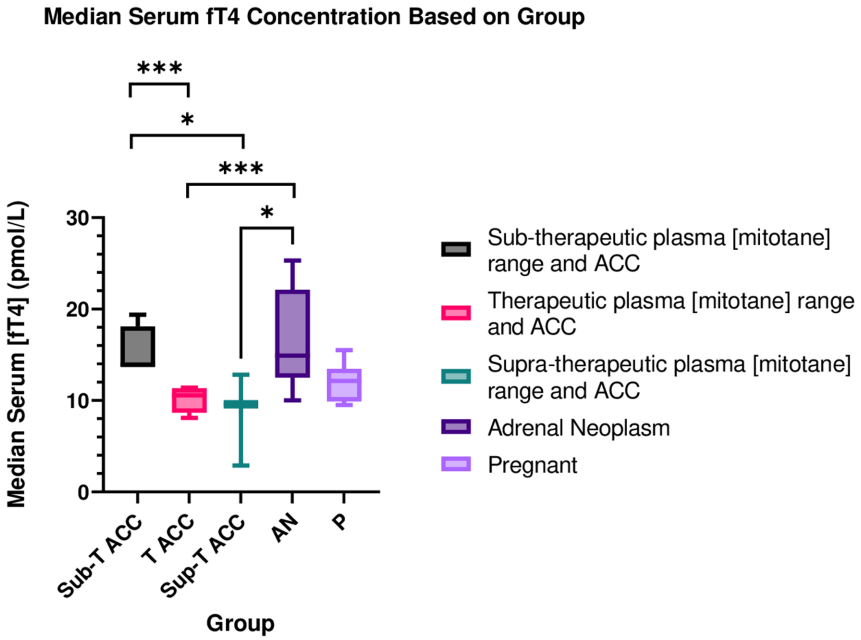

Figure 4

Median fT4 concentration per patient group (*P < 0.05, ***P < 0.01).

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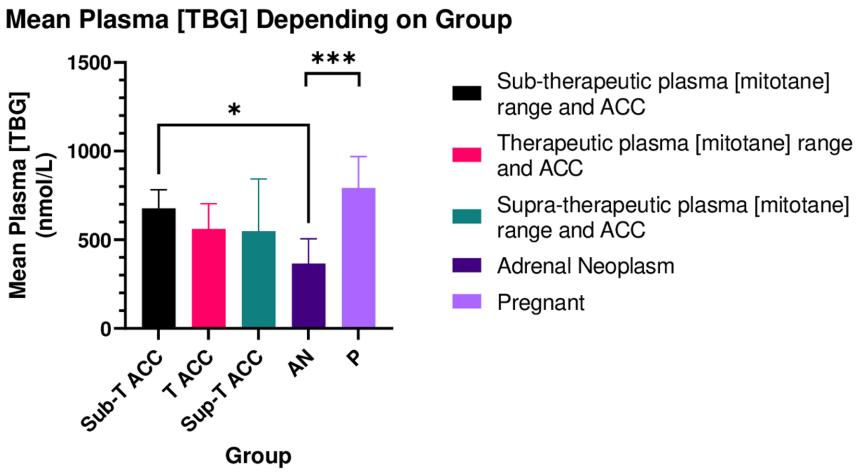

Figure 5

Mean plasma TBG concentration per group (*P < 0.05, ***P < 0.01).

When looking at the relationship between fT4 and TBG, negative correlations were found among all groups, apart from the supra-therapeutic plasma mitotane concentration group; in which a strong positive correlation was found (r = 1.00). A strong negative correlation was only found within the sub-therapeutic groups (r = −0.949, P = 0.051).

Indices of free hormone fraction

Indices of free hormone fractions for thyroxine and cortisol were measured to better understand the differences of total hormones concentrations and free hormones fractions. No statistically significant results were found in the indices of free hormone fractions for either thyroxine or cortisol between the different groups.

Discussion

Patients with an ACC within the therapeutic plasma mitotane concentration group had the highest mean serum cortisol concentrations compared to the other groups. These patients were also on 20–20–10 mg hydrocortisone therapy daily (50 mg per day), double the dose of what is usually administered in cases of adrenal insufficiency. Other studies have demonstrated reduced plasma cortisol concentration with mitotane administration and the duration of its use (5, 12). In our cohort of patients on mitotane, hydrocortisone was offered as part of a block-and-replace strategy.

Similarly, when looking at plasma CBG concentrations, individuals with an ACC and therapeutic mitotane plasma concentration range had the highest mean CBG concentration compared to the other groups. Mitotane therapy groups, regardless of their plasma concentration ranges, had a statistically higher mean plasma CBG concentration compared to those with a benign AN (Fig. 3). Similar results have been demonstrated in previous work, such as that by Alexandraki (2010) (5), in which CBG concentrations were positively correlated to plasma mitotane concentrations. These results are observed, as mitotane administration results in increased hepatic production of CBG, subsequently pharmacologically increasing total cortisol concentration.

Because of the drug-induced increase in CBG concentration, total serum cortisol concentration is of little to no clinical utility when evaluating hypothalamic–pituitary–adrenal (HPA) axis capacity in patients on mitotane (13).

The relationship between cortisol and CBGs was assessed in patients on mitotane therapy; the rank revealed a negative correlation between the CBG and cortisol concentration within all mitotane groups, except for the sub-therapeutic mitotane plasma concentration group (r = 0.600, P = 0.40).

A strong negative correlation between CBG and cortisol was found within the supratherapeutic mitotane plasma concentration range group (r = −1.00). Whilst this is an expected result, the small sample size for this group should be taken into account when interpreting these results.

Negative correlations were also demonstrated between ACTH and cortisol concentrations within all groups, contrary to therapeutic mitotane plasma concentration and benign AN groups. Low ACTH concentration may be because of mitotane therapy, hydrocortisone use or a combination of both. Positive correlations between ACTH and cortisol observed in the therapeutic mitotane plasma concentration and benign AN groups may reflect under replacement with hydrocortisone, despite the high measured cortisol concentrations or even the effects on signalling. Similarly, the increase observed in cortisol, which contrasts to previous work, is the effect of hydrocortisone replacement therapy, rather than endogenous production. Despite the high doses of hydrocortisone administered, CBG concentrations remained high in the mitotane groups.

When investigating the effects of mitotane on TSH, no statistically significant differences were found among the groups (Fig. 4). Similar findings have been established in previous published work, such as that by Russo (7).

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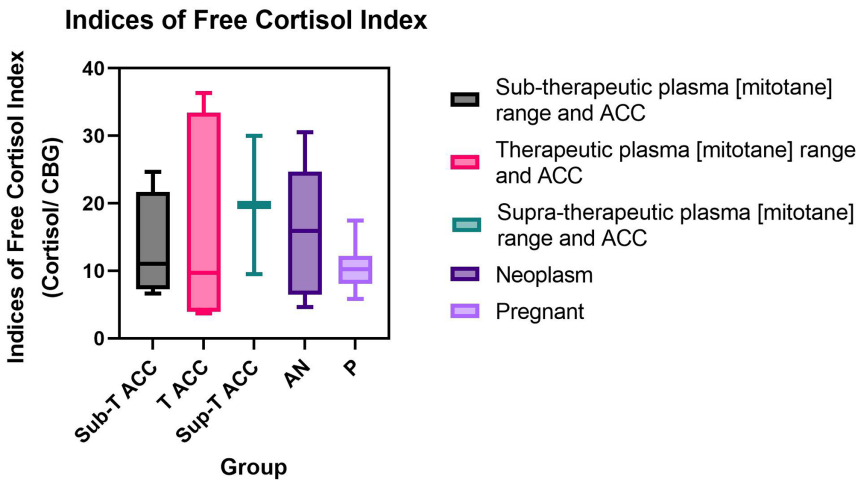

Figure 6

Free cortisol fraction index.

However, statistically significant results were found when comparing the median fT4 serum concentrations. Patients within the AN group had a higher median serum fT4 concentration compared to the supra-therapeutic and therapeutic plasma mitotane range groups (Fig. 5). Further, the supra-therapeutic and therapeutic mitotane plasma concentration range groups had a statistically lower median serum fT4 concentration in comparison to the sub-therapeutic plasma mitotane concentration group (Fig. 5). These results were expected, as central hypothyroidism has been recognised as a side effect of mitotane therapy, meaning that whilst the TSH concentration may appear within the reference range, the fT4 concentrations are generally low. Poirier (14) concluded that induction of mitotane therapy had been associated with a central hypothyroidism prevalence of 73.3%. This accentuates the importance of regular thyroid function investigations to assess individuals' needs for levothyroxine therapy (7, 14).

The pregnant women group had the highest mean plasma TBG concentration compared to the other groups. This is an expected phenomenon occurring during pregnancy, in which a rise in binding globulins is observed. Individuals on mitotane therapy, regardless of plasma concentration, had a higher mean plasma TBG concentration compared to those with a benign AN. However, statistically significant results were observed only between the sub-therapeutic plasma mitotane concentration range and benign AN groups. Previous work has also observed a rise in plasma TBG concentrations along with mitotane therapy.

A negative correlation was found among fT4 and TBG within all the groups, except the supra-therapeutic plasma mitotane concentration range group. This suggests that as the concentration of TBG increases, fT4 concentration decreases, and as a result, there is less fT4 converted into active fT3 for physiological functioning. Therefore, there is an increased likelihood of reliance on levothyroxine replacement to manage the adverse effects of mitotane (15).

No statistically significant results were found among the groups when exploring the median serum DHEAS concentrations. Previous data had reported a progressive decline in DHEAS concentrations with mitotane therapy

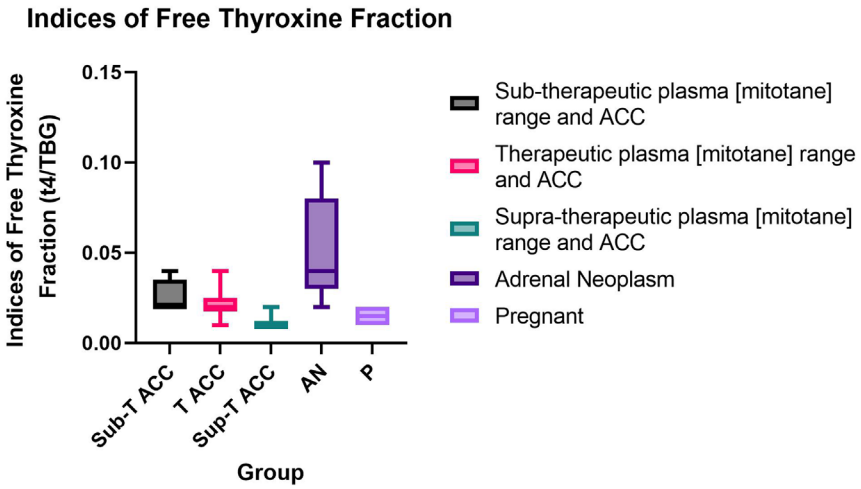

Figure 7

Free thyroxine fraction index.

(16). This could be because there is an induction in 16-hydroxylation in mitotane administration; albeit the changes may not necessarily reflect the decrease in DHEAS but rather its conversion into another DHEA species, which may not cross-react with the DHEAS immunoassay. Further reasoning could involve the duration of mitotane use, and thus, it would be beneficial to see the extent of mitotane therapy administration duration effect on the concentration of DHEAS.

Similarly, no statistically significant differences were found among the groups when evaluating testosterone concentrations. These results differ to those published previously (16). This could be related to the duration of mitotane use, sex, age and race of the individuals included in the different papers.

Finally, no statistically significant results were found between the groups when comparing the indices of free hormone fractions (Figs. 6 and 7). This suggests that mitotane does not affect the binding capacity of hormones to their respective binding proteins.

Limitations of this study include the retrospective nature and small sample size, but this can be partially justified through the rarity of this disease in the first place. Conversely, similar findings have been implicated in previous research, increasing the reliability of our findings. With a restricted sample size, having enough data from the lab to assess for statistically significant differences became less manageable. Often, the number of individuals in each of the groups assessed for a certain protein was sparse. Additional problems experienced concerning the data provided included that some of the protein measurements were not diluted enough to give us the actual values, although we feel this would not significantly impact the final conclusions. This included proteins such as DHEAS and androstenedione, for which the median values for those on mitotane, regardless of plasma concentration range, are the same.

Conclusions

Our study demonstrates a statistically higher mean plasma CBG concentration for all patients treated with mitotane, regardless of their mitotane plasma concentration ranges, when compared to patients not on treatment. This has been previously reported by others and suggests that total serum cortisol concentration cannot reliably reflect HPA axis integrity in patients with ACC treated with mitotane. Our study additionally looked at the effects of mitotane therapy on several hormones beyond the HPA axis comparing them to individuals with benign ANs and a group of pregnant women. We provide further evidence demonstrating that mitotane therapy is associated with significantly lower serum fT4 concentrations in comparison to patients not on treatment. This may be suggestive of central hypothyroidism in patients with ACC on mitotane and highlights the importance of considering replacement therapy for thyroid function in patients receiving mitotane, which can be often neglected. Further research, including large prospective multicentre studies with a longer follow-up period, is needed.

Declaration of interest

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Author contribution statement

ML performed data analysis and wrote the manuscript, RR reviewed the final version of the manuscript and carried out assay analysis, RPV carried out assay analysis, supervised the project and reviewed the final version of the manuscript, LG reviewed the final version of the manuscript and carried out assay analysis, JL reviewed the final version of the manuscript and carried out assay analysis, DRT reviewed the final version of the manuscript and carried out assay analysis, FLT reviewed the final version of the manuscript, PR reviewed the final version of the manuscript, EE reviewed the final version of the manuscript, ABG reviewed the final version of the manuscript, SJBA treated patients and reviewed the final version of the manuscript and GKD treated patients, supervised the project and reviewed the final version of the manuscript.

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