HUMAN FIBROID ARGINASE: PURIFICATION AND CHARACTERIZATION

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Abstract: Arginase activity has been reported to be high in various cancers and the possibility of the use of this enzyme as index of malignancy in cancerous tissues has being considered. This research work is aimed at finding out if arginase activity is also increased in fibroid and therefore could be used as index too. Human fibroid tissues were obtained with ethical permit from the State Hospital, Sokenu, Abeokuta, Ogun State. Arginase was isolated and purified to apparent homogeneity using ammonium sulphate precipitation, chromatograpy on DEAE-Sephacryl, Reactive Blue Agarose affinity gel andgel filtration on Biogel P-100. The enzyme had a specific activity of 1.712 U/mg, K_m of 47.62 mM and V_{max} of 2.27 µmolurea/ml/min. The apparent molecular and subunit molecular weight was estimated to be 104,000 Da and 34,000 Da, respectively. The activity of the enzyme was not affected by Ba^{2+}, Ca^{2+}, K^+, and Mg^{2+} but inhibited by Na^+, NH_4^+ and Hg^{2+}. The enzyme had an optimum temperature of 50°C and pH of 8.5. Result from this study showed that the activity of arginase from fibroid tissue is quite small compared to that from many benign and malignant cancers, therefore, cannot be used as bioindicator for fibroid tissue.

Keywords: Arginase, benign tumour, malignant tumour, fibroid tissue, index of cancer

Introduction

Arginase is a key enzyme of the urea cycle in the liver where it catalyzes the hydrolysis of L-arginine to form L-ornithine and urea completing the last step in the urea cycle which is a series of biochemical reactions in mammals during which the body disposes offs harmful ammonia (Ash, 2004; Munder, 2009). In mammals, there are two forms of arginase, the cytosolic arginase I which is most abundant in the liver and plays an important role in the ammonia detoxification and arginase II which is extrahepatic and found in tissues like kidney, brain, intestine, mammary gland, erythrocytes and skin (Gokmen *et al.,* 2010; Mashra and Mashra, 2017). Arginase II has been reported to be involved in the production of ornithine, a precursor of proline, glutamate and polyamines such as spermine and putresine, essential for cell proliferation and growth (Mori and Gotoh, 2004; Mumenthaler, 2008; Pegg, 2009; Abdulaali-Azeez *et al.,* 2016). Increased activity of arginase has been linked with various diseases such as asthma and lung infections (Maarsingh *et al.,* 2009), Sickle cell disease (Morris *et al.,* 2005), heart ailments such as atherosclerosis, myocordial infarction and ischemia (Steppan *et al.,* 2006), diabetes (Bjelakovic *et al.,* 2009; Wang *et al.,* 2014). Raised activity of arginase has also been implicated in the development and growth of various cancers (Erbas *et al.,* 2015; Pham *et al.,* 2016). Jamshidzadeh *et al.* (2001) reported higher arginase activity in eosophagus, stomach and lung cancer. High levels of arginase had also been detected in gastric cancer (Wu *et al.,* 1996).

Fibroids are the most common benign tumors in females and are typically found during the middle and later reproductive years (Asad-Ur-Rehman, 2014). African-Americans develop fibroids at younger ages than Caucasians and they tend to persist to menopause (Peddada *et al.,* 2008; Catherino *et al.,* 2011). Fibroids, particularly when small, may be entirely asymptomatic, symptoms depend on the location of the lesion and its size (Asad-Ur-Rehman, 2014). It affects a woman's quality of life, as well as her fertility and obstetrical outcomes. Fibroids affect approximately 35-77% of reproductive age-women (Day-Baird *et al.,* 2003; Ezzati *et al.,* 2009), although the real prevalence is much higher since many fibroids may be asymptomatic. Fibroids are present in 5-10% of infertile patients, and may be the sole cause of infertility in 1 – 2.4% (Donnez and Jadoul, 2002; Cook *et al.,* 2010). This work is aimed at determining the activity of arginase in fibroid tissue, in order to explore the high or low activity of the enzyme as an index of fibroid growth.

Materials and Methods

Materials

Manganese (ll) Chloride ($MnCl_2.4H_2O$), Sucrose, sodium chloride, phosphoric acid, p-Dimethylaminobenzaldehyde (Ehrlich reagent), ammonium sulphate, urea, ethanol, methanol and glacial acetic acid were obtained from BDH Chemical Limited, Poole, England. Potassium Chloride was obtained from May and Baker, Dagenham, England. Tris (hydroxymethyl) aminomethane (Tris base), Bovine Serum Albumin, Coomassie Brilliant Blue G-250 and R-250, SDS molecular weight markers (12,400 to 200,000) were obtained from Sigma Chemical Company, St. Louis, U.S.A. DEAE-Sephacryland Biogel P-100 were from Pharmacia Fine Chemical, Uppsala, Sweden. All other reagents were of analytical grades and obtained from the aforementioned companies. All solutions were prepared with glass-distilled water.

Methods

Collection and confirmation of tissue as Leiomyoma (fibroid)

The tissue mass used for this research work were collected from the State Hospital Sokenu, Abeokuta, Ogun State, Nigeria immediately after surgical operation into an ice bucket to preserve the enzyme and then taken to the histology laboratory. The pathologist prepared slides and confirmed the tissue mass as Leiomyoma (fibroid). After confirmation, the tissues were then taken to the laboratory and stored in a refrigerator until required.

Arginase assay and protein determination

Arginase activity was determined according to the method of Kaysen and Strecker (1973). The urea produced was estimated from the urea standard curve prepared by varying the concentration of urea between 0.1 M and 1.0 M. The unit of activity of arginase is defined as the amount of enzyme that will produce one micromole of urea per minute at 37°C. Protein concentration was routinely determined by the method of Bradford (1976) using bovine serum albumin (BSA) as standard.

Crude enzyme isolation

The frozen fibroid tissues were thawed at room temperature and 83 g of the tissue was then homogenized in 9 volumes

(v/w) of the homogenization buffer, 0.02 M Tris-HCl buffer, pH 7.5 containing 0.02 M $MnCl_2$ and 0.25 M sucrose. The homogenate obtained was stirred occasionally for one hour before being centrifuged at 10,000 rpm for 20 min. The supernatant was then decanted and the cellular debris was re-suspended in 2 volumes of the homogenization buffer, homogenized and centrifuged under the same conditions. The supernatants obtained were combined together and used as crude enzyme. An aliquot of the supernatant was taken and assayed for crude enzyme activity and protein concentration. The purification of human fibroid arginase was done using the under listed methods according to Reyero and Dorner (1975) with little modification.

Heat treatment

The combined supernatant was incubated at 60^0C for 5 min in a water bath with continuous swirling, cooled in an ice bucket and centrifuged at 10,000 rpm for 20 min. The supernatant was collected while the pellet was discarded. The supernatant was then assayed for arginase activity and protein concentration.

Ammonium sulphate precipitation

The supernatant from the heating step was brought to 70% ammonium sulphate saturation by the addition of appropriate amount of solid ammonium sulphate (472 g/l), which was added slowly with continuous stirring. This was left over night in the refrigerator and then centrifuged at 10,000 rpm for 30 min. The supernatant was discarded and the precipitate was then re-suspended in 0.02 M Tris-HCl pH 7.2 containing 0.02 M $MnCl_2$.

Chromatography on DEAE-Sephacryl

DEAE-Sephacryl was prepared according to the method described in the manufacturer (Pharmacia Fine Chemicals) manual and then packed into a column (2.5 × 40 cm). The ammonium sulphate precipitate was dialysed overnight against several changes of 0.02 M Tris-HCl pH 7.2 containing 0.02 M $MnCl_2$. The dialysate was layered on the DEAE-Sephacryl column that had been equilibrated with the same buffer. The column was washed with 150 ml of the buffer and 5 ml fractions were collected at a flow rate of 30 ml/hr. Elution was performed with a linear gradient of 0-1.0 M NaCl (150 ml). Protein concentration was monitored at OD_{280} and the peaks were assayed for arginase activity. The fractions containing arginase activity were pooled for further purification.

Affinity chromatography on reactive blue agarose

The resin was pre-treated by first washing with distilled water followed by equilibration with 0.02 M Tris-HCl buffer pH 7.2 containing 0.02 M $MnCl_2$ and then packed in a (2.5 × 10 cm) column. The enzyme solution from the preceding step was layered on the column. The column was washed with 30 ml of the same buffer. Two ml fractions were collected at a flow rate of 12 ml/hr. The column was eluted with 30 ml of 0.02 M Tris-HCl buffer, pH 7.2 containing 0.02 M $MnCl_2$ and 0.05 M arginine. Protein concentration was monitored at OD_{280} and the peaks were assayed for arginase activity. The fractions containing arginase activity were pooled for further purification.

Gel filtration on biogel P-100

Biogel P-100 was prepared by swelling 10 g of Biogel P-100 in 250 ml of distilled water for 2 h. The resin was packed into 2.5 × 90 cm column and equilibrated with 0.02 M Tris HCl buffer, pH 7.2 and containing 0.02 M $MnCl_2$. The pooled enzyme from the preceding step was then layered on the Biogel P-100 column. The column was washed with 300 ml of the same buffer and 5 ml fractions were collected at a flow rate of 30 ml/hr. Protein concentration was monitored at OD_{280} and the peaks were assayed for arginase activity. The fractions containing arginase activity were pooled for further analysis.

Polyacrylamide gel electrophoresis (PAGE)

Polyacrylamide gel electrophoresis in the absence of sodium dodecyl sulphate (SDS) was carried out according to the method described in the Pharmacia manual (Polyacrylamide gel electrophoresis laboratory technique. Revised edition, 1983) on 7.5% gel to ascertain the purity of the enzyme.

Molecular weight determination

The subunit molecular weight of the pure enzyme preparation was determined by SDS polyacrylamide gel electrophoresis in accordance with the procedure of Weber and Osborn (1975) on 10% gel rod using the phosphate buffer system, pH 7.2.

Apparent molecular weight determination

The molecular weight of the native enzyme was determined by gel filtration according to the method of Andrews (1967). The gel filteration was carried on a Biogel P-100 column (2.5 × 90 cm) previously equilibrated in 0.02 M Tris-HCl pH 7.2 containing 0.02 M $MnCl_2$. The void volume (V_o) of the column was determined by the elution volume of Blue Dextran. Bovine α-Chymotrpsinogen (Mr. 24,000 Da (4 mg/ml)), Oval albumin (45,000 Da (4 mg/ml)), Bovine serum Albumin (M_r 66,000 Da (5 mg/ml)), Creatinine phosphokinase (Mr. 88,000 Da (5 mg/ml) and Arginase (120,000 Da (4 mg/ml)) were used as molecular weight standards.

Determination of kinetic parameters (Km and Vmax)

The K_m and V_{max} of the enzyme was estimated by varying the concentration of arginine between 25 and 125 mM according to the method of Kaysen and Strecker (1973). Both K_m and V_{max} were them determined using double reciprocal plot of Lineweaver and Burk (1934).

Effect of cations on the enzyme activity

The method of Kaysen and Strecker (1973) was used to study the effect of various chloride metal ions on the activity of human fibroid arginase. The salts used are Ammonium Chloride (NH_4Cl), Barium chloride ($BaCl_2$), Calcium Chloride ($CaCl_2$), Magnesium Chloride ($MgCl_2$), Manganese Chloride ($MnCl_2$), Mercury Chloride ($HgCl_2$), Potassium Chloride (KCl) and Sodium Chloride (NaCl) at a final concentration of 0.01 M and 0.015 M. In each case, incubation was conducted at 37^0C for 30 min. Arginase activity was determined as previously described.

Effect of Temperature on the Enzyme Activity

The activity of arginase was assayed at temperature between 20 and 90^0C to investigate the effect of temperature on the activity of the enzyme. The assay mixture was first incubated at the indicated temperature for 10 min before initiating reaction by the addition of an aliquot of the enzyme, which had been equilibrated at the same temperature.

Effect of pH on the enzyme activity

The effect of pH on arginase activity was performed by assaying the enzyme at pH between 5 and 11. Buffers of different pH values were used for the analysis. Citrate buffer (pH 5-6), phosphate buffer (6.5-7.5), Tris buffer (8-9.5) and borate buffer (10-11).

Results and Discussion

The results of the various purification procedures were summarized in Table 1. The enzyme was stabilized with manganese chloride throughout the purification procedures. Fig. 1 shows the elution profile after ion-exchange chromatography on DEAE-Sephacryl. The enzyme was bound to the resin. The elution profile for the affinity chromatography on Reactive Blue Agarose gel is shown in Fig. 2 while the elution profile for the gel filtration on Biogel P-100 is shown in Fig. 3.

One protein band was observed after gel electrophoresis in the presence and absence of sodium dodecylsulphate (Figs. 4 and 5, respectively). The calibration curve on BiogelP-100 for native molecular weight determination is shown in Fig. 6

while the calibration curve of SDS-PAGE for subunit molecular weight determination is presented in Fig. 7.

The Lineweaver-Burk plot for the determination of kinetic properties (K_m and V_{max}) of the human fibroid arginase is as shown in Fig. 8 while Table 2 shows the summary of the effect of various metal ions on the activity of the arginase. Figs. 9 and 10 represent the temperature and pH profile for the determination of optimum temperature and pH, respectively.

Table 1: Summary of purification procedures

Fractions	Volume (ml)	Total Protein (mg)	Total Activity (U)	Specific Activity (Umg^{-1})	Purification Fold	Yield (%)
Crude extract	400	6360.0	400	0.063	1.1	100
Heating Step	160	2436.0	336	0.138	2.2	84
70% $(NH_4)_2 SO_4$	80	2142.2	320	0.149	2.4	80
DEAE-Sephacryl	38	368.6	304	0.825	13.1	76
Reactive Blue	30	203.0	288	1.413	22.4	72
Bio Gel P-100	20	118.0	202	1.712	27.2	51

Each procedure was carried out as described in the methods. The activity of the enzyme was measured by the rate of the formation of urea. One unit of activity of arginase is defined as the amount of enzyme that will produce one mol of urea per min at 37°C. Protein concentration was determined by the method of Bradford. The specific activity of the purified enzyme was 1.712 U/mg

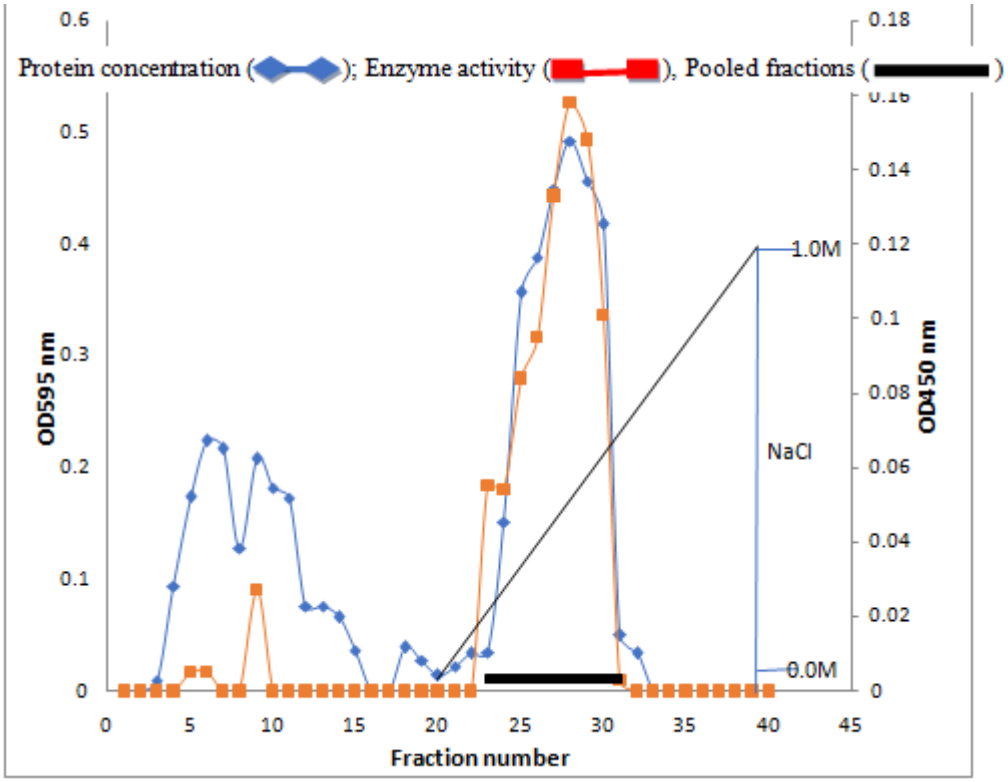

Fig 1: Elution profile after ion-exchange chromatography on DEAE-Sephacryl

The column was first washed with 150 ml of 0.02 M Tris HCl buffer, pH 7.2 and containing 0.02 M $MnCl_2$ and 5 ml fractions were collected at a flow rate of 30 ml/hr. Elution was performed with a linear gradient of 0-1.0 M NaCl (150 ml). Only one major activity peak was obtained, fractions within the peak area were pooled.

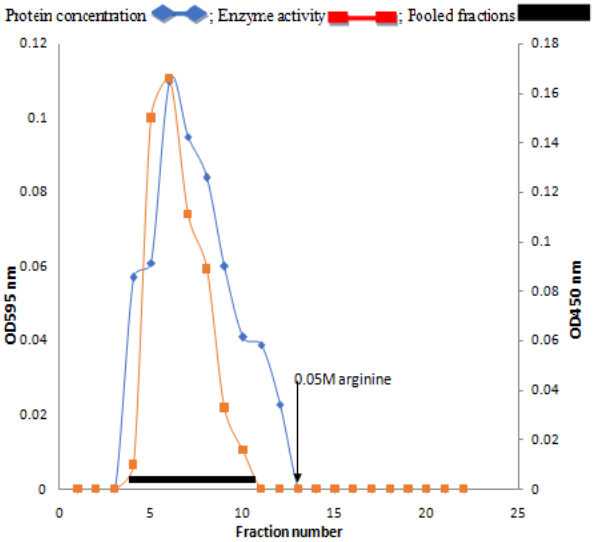

Fig 2: Elution profile after affinity chromatography on reactive blue agarose gel

The column was washed with 30 ml of of 0.02 M Tris-HCl buffer, pH 7.2 containing 0.02 M MnCl2. Two ml fractions were collected at a flow rate of 12 ml/hr. The column was then eluted with 30 ml of 0.02 M Tris-HCl buffer, pH 7.2 containing 0.02 M MnCl2 and 0.05 M arginine.
Only one major activity peak was also obtained, fractions within the peak area were pooled.

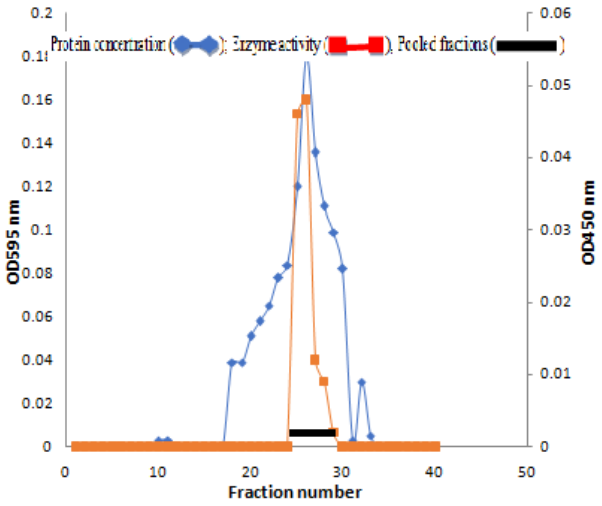

Fig 3: Elution profile after gel filtration on bio gel P-100

The column was washed with 300 ml of 0.02 M Tris-HCl buffer, pH 7.2 containing 0.02 M $MnCl_2$ and 5 ml fractions were collected at a flow rate of 30 ml/hr. Only one major activity peak was also obtained, fractions within the peak area were pooled.

Fig. 4: Photography of the polyacrylamide gel electrophoresis of human fibroid arginase in the absence of SDS

The PAGE was carried on a 7.5 % gel to ascertain the purity of the enzyme, only one protein peak was obtained.

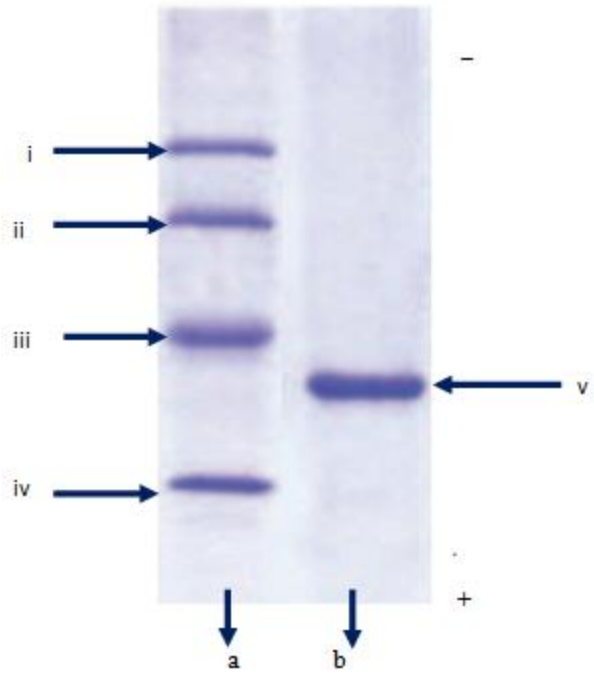

Fig. 5: Photography of the polyacrylamide gel electrophoresis of human fibroid arginase in the presence of SDS

The PAGE was carried on a 10 % gel to ascertain the purity of the enzyme, only one protein peak was obtained. The molecular weight of the standards are presented (i) α-amylase-200,000 Da (ii) yeast alcohol dehydrogenase (YAD)-150,000 Da (iii)Bovine serum albunin (BSA)- 66,000 Da (iv) Bovine Erythrocyte carbonic anhydrase (BECA)-29,000 Da (V) position of the protein band of human fibroid arginase. (a) Proteins standard (b)Purified enzyme

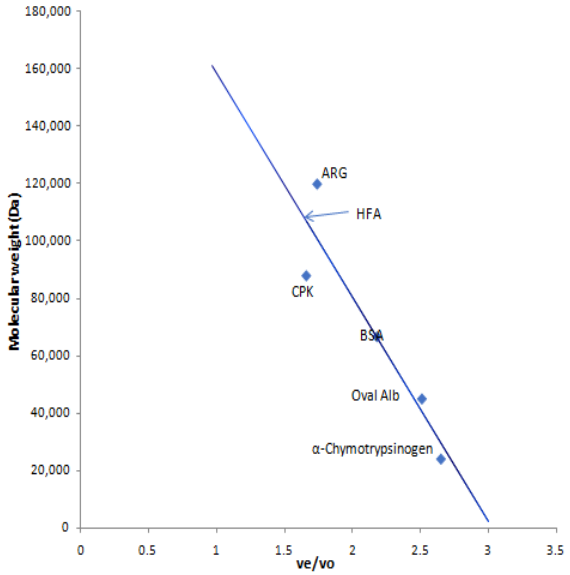

Fig. 6: The calibration curve on biogel P-100 for native molecular weight determination

The standard protein as indicated on the curve are; Arginase (Arg, Mw 120,000 Da), Creatitine phosphokinase (CPK, Mw 88,000 Da), Bovine serum albunin (BSA, Mw 66,700 Da), Oval albunmin (Mw 45.000 Da) and α-chymotrypsinogen (Mw 24,000 Da)

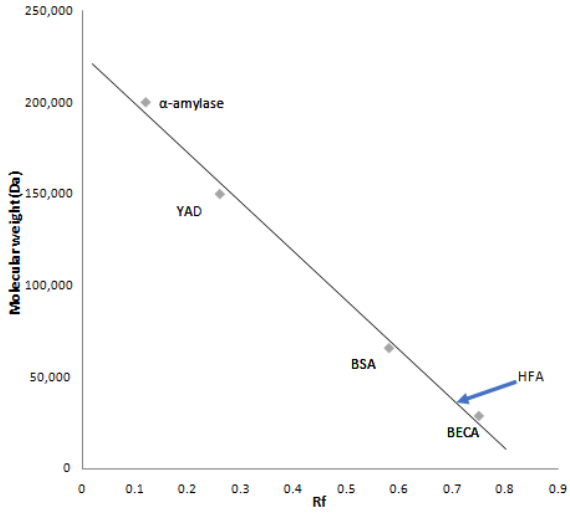

Fig. 7: The calibration curve of SDS-PAGE for subunit molecular weight determination

The standard protein as indicated on the curve are; α-amylase-200,000 Da, yeast alcohol dehydrogensae (YAD)-150,000 Da, Bovine serum albunin (BSA)-, 66,000 Da and Bovine Erythrocyte carbonic anhydrase (BECA)-29,000 Da

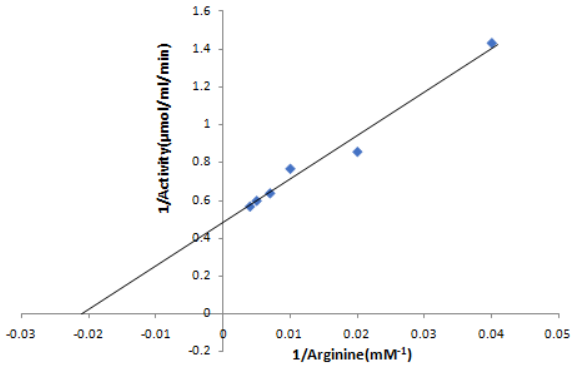

Fig. 8: Lineweaver-Burk plot for the determination of kinetic parameters

The double reciprocal plot for the determination of the kinetic properties of Human fibroid arginase was obtained by varying the concentration of arginine between 25mM and 225mM

Table 2: Effect of metal ions on the activity of human fibroid arginase

Metal ions	%Residual Activity	
	0.01 M	0.02 M
Control	100	100
Ca^{2+}	96	62
K^{+}	80	16
Ba^{2+}	119	44
Na^{+}	24	33
Sn^{2+}	61	83
Mg^{2+}	71	34
NH_4^{+}	39	8
Hg^{2+}	44	55
Mn^{2+}	172	181

The metal ions were tested at a final concentration of 0.01 M and 0.015 M as indicated. In each case, incubation was conducted at 37^0C for 30 min. Arginase activity was determined as previously described.

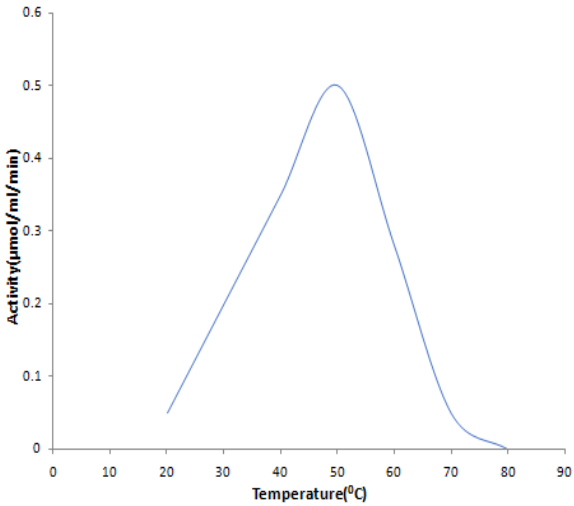

Fig. 9: Effect of temperature on enzyme activity

The activity of arginase was assayed at temperature between 20^0C and 90^0C, the assay mixture was first incubated at the indicated temperature for 10 min before initiating reaction by the addition of an aliquot of the enzyme, which had been equilibrated at the same temperature.

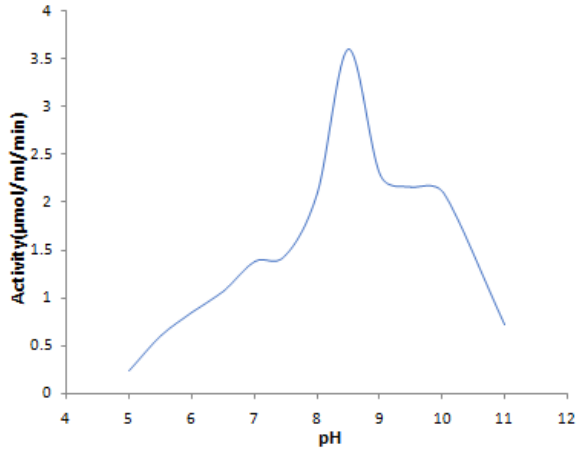

Fig. 11: Effect of pH on enzyme activity

The effect of pH on Human fibroid arginase activity was performed by assaying the enzyme at pH between 5 and 11.

Arginase isolated from human fibroid tissues was purified to homogeneity using ion-exchange chromatography on DEAE-Sephacryl, affinity chromatography on Reactive Blue Agarose gel and gel filtration on Biogel P-100. The procedures yielded a homogeneous arginase with specific activity of about 1.712 U/mg of protein starting with 83 g of sample and a yield of about 51%. Specific activity obtained for human fibroid arginase is lower than that reported by Gokmen *et al.* (2010) for benign and malignant skin cancer (10.32±3.93 and 20.55±12.47 U/mg, respectively). Gokman and Co-worker had earlier in 2001 reported a specific activity of 16.63±19.10 and 18.49 ±10.47 U/mg for basal cell and squamous cell skin cancer, respectively. Kaplan *et al.* (2012) reported 10.4±9.4 and 8.1±3.4 U/mg for esophageal carcinoma for patient with and without metastases, respectively. Mahmoud *et al.* (2009) reported specific activity of 20.15±1.5 U/mg for benign breast cancer and 35.61±6.5 U/mg for malignant breast cancer while Porembska *et al.* (2003) reported 31.0 U/mg for breast cancer. The specific activity reported by Jamshidzadeh *et al.*(2001) for esophagus, stomach and lung are 6.3±1.8, 6.2±2.1 and 4.5±1.7 U/mg, respectively. Lower arginase activities however have been reported from breast cancer (1.14±0.4 U/mg) by Jamshidzadeh *et al.* (2001), benign prostatic hyperplasia (0.56±0.008 U/mg) and prostatic carcinoma (1.16±0.45 U/mg) by Harris *et al.* (1983). Porembska *et al.* (2001) concluded in their work that increased arginase activity in serum and colorectal cancer was due to raised level of the cationic isoform (arginase II) of the enzyme. Arginase II gene has demonstrated significant up-regulation and down-regulation respectively in malignant compared to benign prostate tissue, whereas arginase I is more often present in cancer than benign samples (Reschner *et al.*, 2009). Arginase II expression is increased in most malignant thyroid tumors, but absent in benign lesions and normal tissues (Cerutti *et al.*, 2004). Arginase I was reported to be a sensitive and specific marker of benign and malignant hepatocytes (Yan *et al.*, 2010). Most reports have associated the increased arginase activity with the need for malignant cells to produce polyamines to sustain their rapid proliferation (Chang *et al.*, 2001).

The enzyme preparation was adjudged to be homogenous as shown by the presence of only one band in non SDS polyacrylamide gel electrophoresis at pH 7.2. The molecular weight (M_r) of the native enzyme from human fibroid arginase was estimated to be 104,000 Da by gel filtration on Biogel P-100 and subunit molecular weight of 34,000 Da by sodium dodecyl sulphate gel electrophoresis (SDS-PAGE) suggesting that human fibroid arginase is homotrimeric. This result corroborates the findings of Jenkinson *et al.* (1996) that estimated the molecular weight for arginases from ureotelic species to varies from 100,000 – 140,000 Da and that most of the ureotelic liver enzymes have been found to be trimetric in structure. Shivraj and Se (2016) reported a molecular weight of 118,000 Da for buffalo liver while arginase isolated from human liver arginase and human erythrocyte was reported to be 107,000 Da (Beruter *et al*., 1978) and 105,000 Da (Ikemoto *et al.*, 1989) respectively. The molecular weight of arginase from human fibroid tissue is slightly higher than that reported by Fayhaa *et al.* (2017) for human erythrocyte arginase (94,406 Da), Srivastava and Ratha (2013) for Indian air-breathing teleost, *Heteropneustesfossilis* arginase (96,000 Da), Mohamed *et al.* (2005) for *Fasciola gigantic* arginase (95,000 Da) and Ezima and Agboola (2007) for fruit bat liver arginase (80,000 Da). The subunit molecular weight of 34,000 Da for the human fibroid arginase compares very well with various reports. Rat liver arginase has a subunit molecular weight of 34,925 Da (Kawamoto *et al.*, 1987), human liver arginase of 34,732 Da (Haraguchi *et al.*, 1987), liver of tortoise (*Kinixyserosa*) 31,250±450 Da, (Okonji *et al.*, 2011), Indian air-breathing teleost, *Heteropneustesfossilis* arginase 48,000 Da (Srivastava and Ratha, 2012), buffalo liver 47,000 Da (Shivraj and Se, 2016).

The K_m of 47.62 mM for L-arginine obtained for human fibroid arginase is higher than that from Human type II arginase of 4.8 mM (Colleluori *et al.*, 2001), the buffalo liver arginase of 7.2 mM (Shivraj and Se, 2016), arginase AII of the liver of the air-breathing teleost, *Heteropneustesfossilis* of 5.25±1.12 mM (Srivastava and Ratha, 2013), *Fasciola gigantic* arginase of 6 mM (Mohamed *et al.*, 2005), fruit bat liver arginase of 17 mM (Ezima and Agboola, 2007), the native and highly active recombinant arginase from *Leishmaniaamazonensis* of 23.9±0.96 mM and 21.5±0.90 mM, respectively (da Silva *et al.*, 2008). However, this value is lower than that from tortoise liver arginase of 66 mM (Okonji *et al.*, 2011). The K_m value fall within the range of the uricotelic arginase from 40-200 mM (Jenkinson *et al.*, 1996) which are found mainly in extra hepatic tissues where they perform functions other than the urea cycle. The high K_m exhibited by human fibroid arginase could indicate that the enzyme performs function other than the hydrolysis of arginine into urea and ornithine, it may function in the homeostastis of arginine and in the production of ornithine, a biosynthetic precursor of proline (Cederbaum, 2004) which is essential in connective tissue formation.

Human fibroid arginase was sensitive to inhibition at 0.01 M of Na^+, NH_4^+ and Hg^{2+}, Sn^{2+} had slight inhibitory effect on the enzyme while Ca^{2+}, K^+ and Mg^{2+}had slight negative effect on the enzyme. Mn^{2+} activated the enzyme while Ba^{2+} stabilized the enzyme. Report from diverse researcher had shown that Mn^{2+} is the physiologic activator of arginase, the effect of other metal ion depends on the specie of the enzyme; Srivastava and Ratha (2013) showed that Mn^{2+} and Co^{2+} are effective activator of Indian air-breathing teleost, *Heteropneustesfossilis* arginase. In gill tissue of bivalve *Semele solida*, Mn^{2+}, Ni^{2+}, Cd^{2+} and Co^{2+} satisfy the metal ion requirement of the enzyme but inhibited by Zn^{2+} (Carvajal *et al.*,1994). Mohamed *et al.* (2005) reported that arginase from *Fasciola gigantic* arginase was activated by Mn^{2+}, where the other metals Fe^{2+}, Ca^{2+}, Hg^{2+}, Ni^{2+}, Co^{2+} and Mg^{2+} had inhibitory effects. In the case of buffalo liver enzyme, Mg^{2+} restored almost all the activity while Cd^{2+}, Ni^{2+} and Ca^{2+} restored about 40-50% of the original activity of the enzyme.

Fe^{2+} and Zn^{2+} completely inactivated the enzyme (Dabir *et al.*, 2005).

Human fibroid arginase showed its highest activity at 50ºC, this enzyme is more thermostable than that obtained from *F. gigantic* Arg II, buffalo liver with highest activity at 40ºC (Mohamed *et al.*, 2005; Shivraj and Se, 2016) and human erythrocyte with maximum activity at 45ºC (Fayhaa *et al.*, 2017). More thermostable arginases have been reported from fruit bat liver, tortoise liver with optimum temperature of 60ºC (Ezima and Agboola, 2007; Okonji *et al.*, 2011). Lavulo *et al*. (2001) reported an optimum temperature of 70ºC for wild type arginase from rat liver.

The effect of pH on human fibroid arginase was studied in the presence of Mn^{2+}; the optimum pH obtained was 8.5. This pH value is slightly lower than the optimum pH range of 9.0-9.5 for buffalo liver arginase, fruit bat liver arginase and tortoise liver arginase (Dabir *et al.*, 2005; Ezima and Agboola, 2007; Okonji *et al.*, 2011). Srivastava and Ratha (2013) reported an optimum pH of pH 10.5 for arginase AII of the liver of the air-breathing teleost, *Heteropneustes*while Fayhaa *et al.* (2017) reported highest activity at pH 10 for human erythrocyte.

Conclusively, the various results obtained from our study showed the presence of arginase in human fibroid arginase, this arginase is presumed to be an anionic isoform of arginase (arginase I) based on its binding to the DEAE-Sephacryl resin. The specific activity of arginase from fibroid tissue is quite small compared to that from some other afore mentioned benign and malignant tumors, therefore, arginase activity here cannot be used as an indicator of the presence of fibroid.

References

Abdulaali-Azeez N, Alshamaa SD & Hadi IA 2016. Comparison between tumor marker cea, ca19-9 and some marker enzymes (alkaline sphingomyelinase, cyclooxygenase-2, thymidylate synthase and arginase) in serum of colon cancer patients. *World J. Pharm. Pharmac. Sci*., 5(4): 438-460.

Andrews P 1967. Estimation of molecular weight of protein by Sephadex gel. *Biochem. J.*, 91: 222-233.

Asad-Ur-Rehman JI, Akhter S & Mamoon N 2014. Leiomyoma of renal pelvis. *.J. Coll. Phys. Surg. Pak*., 24(1): S57-59.

Ash DE 2004. Structure and functions of arginase. *J. Nutr*., 134: 2760-2764.

Beruter J, Colombo JP & Bachmann C 1978. Purification and properties of arginase from human liver and erythrocytes. *Biochem. J.*, 175: 449-454.

Bjelakovic G, Sokolovic D, Ljiljana S, Kocic G, Jevtovic T, Stojanovic I, IIic M, Bjelakovic LJ, Zivic S, Pavlovic D, Nikolić J & Basic J 2009. Arginase activity and magnesium levels in blood of children with Diabetes mellitus. *J. Basic. Clin. Physiol. Pharmacol.*, 20:319–334.

Bradford MM 1976. A rapid and sensitive method for the quantification of microgram quantities of protein, utilizing the principle of protein dye binding. *Anal. Biochem*., 72: 248-254.

Carvajal N, Torres C, Uribe E & Salas M 1994. Interaction of arginase with metal ions: studies of the enzyme from human liver and comparison with other arginases. *Comp. Biochem. Physiol. B.*, 112: 153–159.

Catherino WH, Parrott E & Segars J 2011. Proceedings from the national institute of child health and human development conference on the uterine fibroid. Research update workshop. Fertilsteril., 95: 9-12.

Cederbaum SD, Yu H, Grody WW, Kern RM, Yoo P & Iyer RK 2004. Arginases I and II: do their functions overlap? *Mol. Genet. Metab*., 81(1): S38–S44.

Cerutti JM, Delcelo R, Amadei MJ, Nakabashi C, Maciel RM, Peterson B, Shoemaker J & Riggins GJ 2004. A preoperative diagnostic test that distinguishes benign from malignant thyroid carcinoma based on gene expression. *J. Clin. Invest.*, 113: 1234-1242.

Chang CI, Liao JC & Kuo L 2001. Macrophage arginase promotes tumor cell growth andsuppresses nitric oxide-mediated tumor cytotoxicity. *Cancer Res*., 61: 1100–1106.

Colleluori DM, Morris Jr SM & Ash DE 2001. Expression, purification and characterization of human type II arginase. *Arch. Biochem. Biophy*., 389(1): 135-143.

Cook H, Ezzati M & Segars J 2010. The impact of uterine leiomyomas on reproductive outcomes. *Minerva. Ginecol.*, 62: 225–236.

da Silva E R, da Silva MF, Fischer H, Mortara RA, Mayer MG, Framesqui K, Silber AM & Floeter-Winte, LM 2008. Biochemical and biophysical properties of a highly active recombinant arginase from Leishmania (*Leishmania amazonensis*) and subcellular localization of native enzyme. *Mol. Biochem. Parasitol.*, 159(2): 104 – 111.

Dabir S, Dabir P & Somvanshi B 2005. Purification, properties and alternate substrate specificities of arginase from two different sources: Vigna catjang cotyledon and buffalo liver. *Int. J. Biol. Sci*., 1: 114 – 122.

Day-Baird D, Dunson D & Hill M 2003. High Cumulative incidence of uterine leiomyoma in black and white women: ultrasound evidence. *Am. J. Obstet. Gynecol*., 188: 100–107.

Donnez J & Jadoul P 2002. What are the implications of myomas on fertility? A need for a debate? *Hum. Reprod*., 17: 1424– 1430.

Erbaş H, Bal O & Çakır E 2015. Effect rosuvastatin on arginase enzyme activity and polyamine production in experimental breast cancer. *Balkan Med. J*., 32: 89 – 95.

Ezima EN & Agboola FK 2007. Purification and Characterization of fruit bat (*Eidolon helvum* Kerr) liver Arginase. Int. J. Biol. Chem., 1 (1): 11 – 20.

Ezzati M, Norian J & Segars J 2009. Management of uterine fibroids in the patient pursuing assisted reproductive technologies. *Women's Health*, 5: 413–421.

Fayhaa MK, Noor N & Essam FA 2017. Human erythrocyte arginase purification and characterization. *IOSR-JMCE*., 14(5): 40 – 60.

Gokman SS, Argit AC, Ayhan MS, Yorulmaz F & Gulen S 2001. Significance of arginase and ornithine in malignant tumor of the human skin. *J. Lab. Clin. Med*., 137: 340 – 344.

Gokmen SS, Kazezoglu C, Aygit AC, Yildiz R, Cakir B, Ture M & Gulen Ş 2010. Arginase and ornithine in human benign and malignant skin tumors. *Turk. J. Biochem*., 35(4): 319–324.

Haraguchi Y, Takiguchi M, Amaya Y, Kawamot, S, Matsuda I & Mori M 1987. Molecular cloning and nucleotide sequence of cDNA for human liver arginase. *Proc. Nat. Aca. Sci*., 84: 412-415.

Harris BE, Pretlow TP & Bradley EL Jr 1983. Arginase activity in prostatic tissue of patients with benign prostatic hyperplasia and prostatic carcinoma. *Cancer Res*., 43: 3008-3012.

Ikemoto M, Tabata M, Murachi T & Totani M 1989. Purification and properties of human erythrocyte arginase. *Ann. Clin. Biochem.*, 26: 547 – 553.

Jenkinson CP, Grody WW & Cederbaum SD 1996. Comparative properties of arginase. *Comp. Biochem. Physiol*., 114B(1): 107-132.

Kaplan I, Aydin Y, Bilen Y, Gen CF, Keles MS & Eroglu A 2012. The evaluation of plasma arginine, arginase, and

nitric oxide levels in patients with esophageal cancer. *Turk. J. Med. Sci*., 42(3): 403-409.

Kawamoto S, Amaya Y, Murakami K, Tokunaga F, Iwanaga S, Kobayashi K, Saheki T, Kimura S & Mori M 1987. Complete nucleotide sequence of cDNA and deduced amino acid sequence of rat liver arginase. *J. Biol. Chem*., 262: 6280-6283.

Kaysen GA & Strecker HJ, 1973. Purification and properties of arginase of rat kidney. *Biochem. J*., 133: 779-788.

Lavulo LT, Sossong TM Jr, Brigham-Burke MR, Doyle ML, Cox JD, Christianson DW & Ash DE 2001. Subunit-subunit interactions in trimeric arginase. Generation of active monomers by mutation of a single amino acid. *J. Biol. Chem*., 276: 14242-14248.

Lineweaver H & Burk D 1934. The determination of enzyme dissociation constants. *J. Am. Chem. Soc*., 56: 658-666.

Maarsingh H, Zaagsma J & Meurs H 2009. Arginase a key enzyme in pathophysiology of allergic asthma opening novel therapeutic perspectives. *Br. J. Pharmaco*., 158(3): 652 – 664.

Mahmoud AA, ElSaid SE, Mandou MA, Zakhary MM & Maximous DW 2009. Arginase Activity in Breast Cancer: is it a significant biomarker? *Bull. Pharm. Sci*., 32: 241-247.

Mashra S & Mashra R 2017. Mitochondrial membrane-bound activity of arginase is independent of nitrogen excretion pattern in ureogenic and non ureogenic vertebrates. *Ind. J. Expt. Biol*., 55: 74-78.

Mohamed SA, Fahmy AS, Mohamed TM & Hamdy SM 2005. Urea cycle of *Fasciolagigantica*: Purification and characterization of arginase. *Comp. Biochem, Physiol. Biochem. Mol. Biol.*, 142(3): 308-316.

Mori M & Gotoh T 2004. Supplement: Arginine metabolism: Enzymology, nutrition and clinical significance. *J. Nutr*., 134: 2820S-2825S.

Morris CR, Kato GJ, Poljakovic M, Wang X, Blackwelder WC, Sachdev V, Hazen SL, Vichinsky EP, Morris SM Jr& Gladwin MT 2005. Dysregulated arginine metabolism, hemolysis-associated pulmonary hypertension, and mortality in sickle cell disease. *JAMA*., 294(1): 81-90.

Mumenthaler SM, Yu H, Tze S, Cederbaum SD, Pegg AE, Seligson DB & Grody W 2008. Expression of arginase II in prostate cancer. *Int. J. Oncol.*, 32: 357 – 365.

Munder M 2009. Arginase: An emerging key player in the mammalian immune system. *Br. J. Pharmacol*., 158: 638–651.

Okonji RE, Agboola FK. & Afolayan A 2011. Tortoise (*Kinixys erosa*) liver arginase: Purification and characterisation. *Austr. J. Basic Appl. Sci*., 5(7): 259-275.

Peddada S, Laughlin S & Miner K 2008. Growth of uterine leiomyomata among premenopausal black and white women. *PNAS*., 105: 19887–19892.

Pegg AE 2009. Mammalian polyamine metabolism and function. *IUBMB Life*, 61: 880-894.

Pham T, Bordage S, Pudlo M, Demougeot C, Thai K & Girard-Thernier C 2016. Cinnamide derivatives as mammalian arginase inhibitors: Synthesis, biological evaluation and molecular docking. *Int. J. Mol. Sci*., 17(10): 1656.

Porembska Z, Luboinski G, Chrzanowska A, Mielczarek M, Magnuska J & Baranczyk-Kuzma, A 2003. Arginase in patients with breast cancer. *Clin. Chim. Acta*., 328: 105 – 111.

Porembska Z, Zabek J, Grabon W, Rahden-Staron I & Baranczyk-Kuzma A 2001. Arginase isoforms in human colorectal cancer. *Clin. Chim. Acta*., 301: 157-165.

Reschner A, Harlin H, Laven B, Eriksson F, Pisa P & Egevad L 2009. Expression of immunomodulating genes in prostate cancer and benign prostatic tissue. *Anal. Quant. Cytol. Histol*., 31: 74–82.

Reyero C & Dorner F 1975. Purification of arginase from human leukemic lymphocytes and granulocytes: Study of their physicochemical and kinetic properties. *Eur. J. Biochem*., 56: 137-142

Shivraj HN & Se WP 2016. Purification and characterization of buffalo liver L-arginase and its kinetic properties with dihydropyrimidine and metal ions. *Ind. J. Expt. Biol*., 54: 414 – 419.

Srivastava S & Ratha BK 2013. Unusual hepatic mitochondrial arginase in an Indian air-breathing teleost, *Heteropneustesfossilis*: Purification and characterization. *Comp. Biochem. Physiol. Part B: Biochem. & Mol. Biol*., 164(2): 133 – 141.

Steppan J, Ryoo S, Schuleri KH, Gregg C & Hasan RK 2006. Arginase modulates myocardial contractility by a nitric oxide synthase 1-dependent mechanism. *Proc. Natl. Acad. Sci*., 103: 4759-4764.

Wang S, Fang F, JinW, Wang X & Zheng D 2014. Assessment of serum arginase I as a type 2 diabetes mellitus diagnosis biomarker in patients.Exp. Ther. Med., 8(2): 585–590.

Weber K & Osborn M 1975. In: The *Proteins* (Neurath H & Hill R, Eds.) 3rd ed., 1, Press, New York, pp. 179-223.

Wu GW, Chung WN & Chi CW 1996. Immunohistochemical study of arginase in cancer of the stomach. *Virchows Arch.*, 28: 327-331.

Yan BC, Gong C, Song J, Krausz T, Tretiakova M, Hyjek E, Al-Ahmadie H, Alves V, Xiao SY, Anders RA & Hart JA 2010. Arginase-1: a new immunohistochemical marker of hepatocytes and hepatocellular neoplasms. *Am. J. Surg. Pathol*., 34: 1147-1154.