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REVIEW ARTICLE

# Age Estimation by Using Aspartic Acid Racemization from Purified Elastin of Aorta

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## *ABSTRAK*

*Penganggaran umur dalam pemprofilan biologi adalah penting dalam pengenalpastian mangsa dalam bidang forensik. Penggunaan kaedah biokima yang dikenali sebagai peraseman asid aspartik dalam manusia adalah lebih jitu dan memberi ralat yang lebih kecil dalam pengaggaran umur. Semasa penuaan, asid amino dalam protein bertukar di antara format D dan L (peraseman) dan kandungan asid amino-D meningkat. Perubahan ini adalah selaras dengan penuaan. Peraseman asid aspartik digunakan secara lumrah kerana proses perasemannya yang paling cepat. Peraseman asid aspartik berlaku bukan sahaja pada gigi dan pelbagai tulang, tetapi juga pada struktur mudah lentur yang mengandungi banyak elastin. Sebagai contoh, ia berlaku di ligamen kuning kulit, parakima peparu dan aorta. Elastin merupakan protein penting yang terkandung dalam beberapa struktur dan ia merupakan kandungan penting dalam fiber elastik. Tambahan pula, elastin, dengan kadar metaboliknya yang rendah, adalah protein yang paling tahan lama di dalam badan. Kebelakangan ini, teknik penulenan protein untuk mendapatkan satu protein dalam aorta manusia telah dibangunkan. Terdapat korelasi yang baik di antara kadar peraseman (nisbah D/L) dan umur. Maka, peraseman asid aspartik dengan menulenkan elastin dari aorta merupakan penanda penganggaran umur. Ulasan ini memberi gambaran keseluruhan tentang peraseman asid aspartik, protein elastin, teknik penulenan berlandaskan peraseman dan penggunaan nisbah D/L daripada penulenan elastin dari arteri untuk penganggaran umur.*

*Kata kunci: umur, arteri, asid aspartik, elastin, penganggaran, sains forensik, peraseman*

## ABSTRACT

Age estimation is the one of biological profiles which plays an important role in identification in forensic field. The application of biochemical approach known

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as aspartic acid racemization in human provides more accuracy and less error of estimated age in age determination. During aging, amino acids within protein transform between D-and L-form (racemization), which results in increasing concentration of D-amino acids. This change of amino acids is related to aging. Aspartic acid has been generally applied in racemization because it has fastest rate of racemization. Racemization of aspartic acid not only takes place in teeth and many kinds of bone, but also can occur in the flexible structure which contain mostly elastin. For example, skin yellow ligament, lung parenchyma, and aorta. Elastin is essential protein which is comprised most composition of several structures and it is major content in the elastic fibers. Additionally, elastin is the longest lasting protein in the body with slow metabolic turnover rate. Recently, there is a purification technique to obtain only single protein in human aorta. There was a good correlation between degree of racemization (D/L ratio) and age. Therefore, aspartic acid racemization by purifies elastin from aorta is a one of indicator for age estimation. This review provides an overview of aspartic acid racemization, elastin protein, purification method bases on racemization and the application of D/L ratio by purified of elastin in artery for age estimation.

Keywords: age, artery, aspartic acid, elastin, estimation, forensic science, racemization

## INTRODUCTION

Identification of human remains has been a long practice in forensic practice (Franklin 2010). When an unknown person cannot be identified, the first step for general identification process concerns the biological profile which is assessed from sex, ethnicity, stature and age (Lynnerup 2013). The estimation of age at death is one of important work of any forensic investigator. Age estimation conduce to establish a biological profile to compare with any missing person in forensic circumstances (Cunha et al. 2009). Each individual of the similar chronological age may be has difference of morphological appearance of age related skeleton (Zioupos et al. 2014). Therefore, age estimation techniques depend on the variation methods and skill of the specialist (Dobberstein et al. 2010). However, there are still high accuracy methods in age estimation which complicate the procedure more. One of these methods which has been accomplished in age estimation is amino acid racemization.

## AMINO ACID RACEMIZATION

Amino acids are vital components in living organisms and are subunit structure of all proteins. The amino acid structure is composed of a carbon atom (C) which has four different groups include an amino group ($-NH_2$), a carboxyl group (-COOH), a hydrogen atom (-H), and side chain or Rgroup (-R). Side chain is different part of amino acids that depends upon the kind of

amino acids. There are twenty types of amino acids which are present in various tissues and bones. Amino acid is linked to one another with peptide bond and many amino acid molecules form a polypeptide (Johnson & Miller 1997). Most amino acids are present as two active isomers which are D (dextrorotary) and L (levorotary) forms. These forms are called enantiomers which are mirror images of each other (Robins et al. 2001). Amino acids are synthesis in the L-forms, but during life amino acid convert into mixture of the D- and L-forms. Thus, D-form of amino acids which accumulates during life has been related to aging process known as racemization (Bada 1982; McCudden & Kraus 2006). The degree of racemization of amino acid may be used for dating various fossil materials such as fossil shell (Hare & Abelson 1968), marine sediment (Kvenvolden 1973; Wehmiller & Hare 1971) and fossil bone (Bada 1985; Bada & Protsch 1973). In a previous study about amino acid in fossil reported that D-amino acid is derived from the change of L-amino acids in proteins. It was found the amount of racemization increased with age of the fossil and this is the first application of amino acid racemization in date events on the geological time scale (Bada & Protsch 1973).

## RACEMIZATION OF ASPARTIC ACID

Aspartic acid is one of the non-essential amino acids group in the organism that can be formed in the human. Moreover, this amino acid is the component of some nutrition which is taken into the body. Since aspartic acid has fastest rate of racemization among all amino acids, it is used for an estimator in age determination. Aspartic acid racemization is non-enzymatic reaction within stable proteins or long-lived proteins. There is accumulation of D-aspartic acids increased with age in human tissues and positive correlation was found between age and ratio of D/L aspartic acid. Thus, racemization of aspartic acid can be utilized for age indicator of individuals and also investigated protein aging. In addition, there was found that aspartic acid racemization process involve some pathological diseases (Bada et al. 1973; Jousse et al. 2004; Ritz-Timme & Collins 2002).

Pathway of aspartic acid racemization occurs in proteins via a succinimide intermediated (succinyl residue or Asu) and the chiral center of succinimide can be easily racemized. Succinimide intermediate can be formed from L-aspartic acid which converts into D-aspartic acid later (Robins et al. 2001). Succinimide intermediate is formed by nucleophilic attack process that begin from the nitrogen of the C-terminal peptide bond attract to the carbon atom of carboxyl group resulting in cyclization (connection together in the form of ring). The succinimide ring is sensitive to racemization due to their instability so racemization rapidly occurs in succinimide ring. It make this structure can be converted from L- form of aspartic acid to D-form of aspartic acid (McCudden & Kraus 2006). Therefore, rate of racemization

depends on the structure of amino acids within polypeptide that related to rapid accumulation of D-aspartic acids in proteins (Geiger & Clarke 1987; Radkiewicz et al. 1996; Ritz-Timme & Collins 2002).

## FACTORS TO RACEMIZATION

The previous study investigated amino acid racemization from dental enamel in human and the results showed that there was different degree of racemization between different types of teeth. Amino acid racemization in tooth molars is more rapid than tooth incisors. Different racemization may result from temperature. Since tooth molars are located at the back of the mouth, there is slightly higher temperature than other teeth which have cooler temperature (Ohtani et al. 2003). This indicates that the higher the temperature, faster is the racemization rate. In the preparation of sample before analysis step, the sample should be stored at low temperature (McCudden & Kraus 2006; Ohtani et al. 2005). There are studies on the influence of pH for aspartic acid racemization of age estimation in dentin. The researchers stored the teeth in many solutions with different pH condition consisting of acidic (pH 4), alkaline (pH 9) and distilled water, and dry conditions. It was found that the rate of racemization or the reaction rate constant was highest in a pH 9 solution, then distilled water, in pH 4 solution, and in dry condition, respectively. The estimated age from tooth dentin in different environments for 1 year showed that there was slight increase by 0.007 years in a dry condition, 0.1 years in pH 4 condition, and 0.2 in water. On the other hand, teeth dentin left in the pH 9 solution found that the estimated age increased by 0.6 years. Moreover, the teeth left in the pH 9 solution for 5 years showed increase of 3.2 years. It is suggested that in alkaline condition may be more influent to amino acid racemization than other conditions (Ohtani 1995a). Moreover, fixation of sample is one of important factors to amino acid racemization. Ohtani and colleagues studied age estimation from human teeth by using amino acid racemization related influence of fixative. They used the teeth from cadavers and stored teeth in different fixatives include 95% ethanol, 10% formalin solution or 10% neutral formalin fixative at different temperatures. The results showed rate of aspartic acid racemization was highest in 10% neutral formalin solution, then in 10% formalin solution, and in 95% ethanol, respectively. In addition, it was found that the teeth stored at 15˚C in all fixative almost did not influence the rate of racemization (Ohtani et al. 1997) and the previous study investigated the fractions of amino acids based on racemization in human dentin for age estimation. Total amino acid was divided into acid-insoluble collagen and acid soluble peptides and relationship between the amino acid racemization of different fractions and age were examined. The results showed that there was highest correlation in acid-soluble peptide of central incisor, followed by in total amino acids, and in acid-insoluble collagen, respectively

(Ohtani & Yamamoto 1991). Another study determined racemization of aspartic acid from femur. It was found that the ratio of D/L aspartic acids in acid-soluble peptide fraction was highest correlated in males and the acid-soluble peptide had the highest rate of racemization. Furthermore, the different sizes of bone powder could influence D/L ratios of amino acids and it was found that larger particles had lower D/L ratio of amino acids (Ohtani et al. 1998).

## HISTORY OF AGE ESTIMATION BY USING ASPARTIC ACID RACEMIZATION

Since aspartic acid is fastest racemization rates of the stable amino acids, this amino acid is usually applied in racemization for age determination. The previous preliminary studies results demonstrated amino acids such as aspartic acid are racemized substantially faster than other amino acids. Bada et al. (1973) first applied aspartic acid racemization on bone fossil samples. Then, there were studies on racemization of aspartic acid in tooth enamel by Helfman and Bada (Helfman & Bada 1975). The researchers found that the positive correlation between aspartic acid racemization ratios (D/L ratio) and age at death (r=0.921). This indicated that racemization of aspartic acids is sufficient rapidly to be detectable in both fossil and living human tissue.

Helfman and Bada (1976) determined change of D-aspartic acid related with age in human enamel and dentine. They reported a relationship between concentration of D-aspartic acid and age in these tissues and particularly in dentine which is better correlate than enamel (r=0.979). The previous study (Helfman et al. 1977) reported that aspartic acid racemization is a process which occurs in metabolically stable proteins of the mammalian body and amino acid within protein and are changed with increasing age. In addition, there were many studies the relationship between extent of amino acid racemization and age in human teeth (Ohtani 1995b; Shimoyama & Harada 1984). They found high correlation of racemization and age that was consistent with results of another study by Helfman et al. (1977). This suggested that amino acid racemization can be utilized for age estimation.

Racemization of amino acids is one of alternative methods which is highly accurate for the age estimation (Waite et al. 1999) and the teeth are the best organs for racemization based upon accuracy, simplicity, and time required. Nevertheless, depending on circumstances one can choose the organs such as bone, cartilage, and eye lens. These organs have lower correlate between age and racemization than teeth. Recently, there was report the techniques with amino acid racemization by using single protein. Even though the purification of protein is more complicate and need skills, the correlation between D/L ratios of amino acid and age is rather good for example, the purification of osteocalcin in bones and elastin in elastic structure (Ohtani & Yamamoto 2005).

## ELASTIN AND PURIFICATION

Elastin is vital protein which is comprised most composition of several elastic structures. For example, skin, lung, cartilage, and arterial wall in the human body. Elastin is major content in the elastic fibers that it is degenerate in older age and also reduces elasticity of structures which is important factors about the pathophysiology disorder of older individuals for example atherosclerosis condition and lung emphysema. Formation process of elastin is related to precursor known as tropoelastin which is cross-linked to polymer of elastin. Additionally, elastin protein is characterized by insolubility and it resists to chemical and physical effects i.e. proteolytic enzymes cannot be attacked to elastin. Elastin is the longest lasting protein in the body elastin and has a half-life of approximately 74 years. Additionally, elastin is well preserved after post-mortem for a long time. Elastin can be easily used for purification which is used to heat and alter condition of pH i.e. hot alkali being considered for purified elastin tissue (Mecham 2008). There has been a comparative method of purification to study differences in separation and emzyme digestion to take away non-elastin contaminants (Daamen et al. 2001).

## APPLICATION OF ASPARTIC ACID RACEMIZATION IN PURIFIED ELASTIN FOR AGE ESTIMATION

One of structure comprises of high elastin the yellow ligaments of the spine. There was study aspartic acid racemization in yellow ligament for age estimation. They determined degree of racemization by purified elastin from yellow ligaments and found that aspartic acid racemization tend to increase with age. The correlation between aspartic acid racemization and age was high in yellow ligament (r =0.96–0.99). This suggests that it can be used purified elastin from tissues as an indicator for age estimation which may be important method for identification of unknown cases. Elastin purification for age estimation is complex method but it should be promptly apply in forensic practice. Purified elastin from Lung parenchyma (intact lungs or lobes) were derived from 14 individuals who had attained widely ages (range 6-78 years). They determined racemization of aspartic acids by detecting elastin in lung and found high correlation between age and racemization (r = 0.98) and suggested that elastic fiber in other tissue is a distinct difference in turnover about metabolic activity of human proteins (Shapiro et al. 1991).

Aspartic acid racemization was examined in human skin (Ritz-Timme et al. 2003). The authors studied racemization base on purification of elastin. The results showed D/L ratio of aspartic acid increased with age and rate of racemization was rapidly. Furthermore, there was high correlation between degree of amino acid racemization and age (r =0.98) and suggested that the accumulation of aspartic acid occur to be ordinary in ageing elastin.

There was study aspartic acid

racemization in human aorta. They observed the accumulation of D-aspartic acids of elastin within arterial wall and found that amount of D-aspartic acids increased linearly with age from 3% in young individuals to 13% in the older individuals (mid 80 years). This result is inconsistent with the collagens of the human aorta (Powell et al. 1992).

Recently, aspartic acid racemization by purification of elastin from human aorta was examined. They used 68 samples of the abdominal aorta that aged between 6 weeks and 91 years and they also considered about the influence of different stages of atherosclerosis and putrefaction of aorta. The results showed aspartic acid racemization (D/L ratio) increased with age and found good correlation between D/L ratio of aspartic acids and age ($r = 0.90$) (Dobberstein et al. 2010) and suggests that aspartic acid racemization depend on kind of tissue in body.

## AMINO ACIDS ANALTICAL METHODS

There are general methods of chromatography for separate and quantify amino acids include gas liquid chromatography (GC) and high performance liquid chromatography (HPLC). The chromatographic system consists of three basic components. First is the mobile phase (liquid or gas which includes the sample mixture), second is the stationary phase (material packing or lining a column), and the last one is the detection system. The sample mixture is separated by interaction between the mobile and stationary phases under change of physical or chemical environment (Johnson & Miller 1997).

## CONCLUSION

The method of aspartic acid racemization provides more reliable and precise for age estimation. The accumulation of D-aspartic acids increases with age in human tissues and it was found that there was positive correlation between age and ratio of D/L aspartic acid. Besides the teeth and bone, aspartic acid racemization by purification of elastin from artery is a good alternative for age indicator. Elastin protein is resistant to chemical and physical effects like the proteolytic enzymes. Moreover, it is well preserved after death for a long time. In few cases where other tissues are not available, it may apply as single protein like elastin to analysis. Therefore, aspartic acid racemization using purified elastin from artery is one useful indicator in aging. Since the purification procedure is complicated method, takes a long time and needs more skills, this technique should be further developed in racemization for age estimation.

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