Alpha-feto Protein (AFP): Structure, Function and Diagnostic Significance

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ABSTRACT:

Alpha-fetoprotein (α-fetoprotein, AFP) is a glycoprotein of MW 69,000 and belongs to the albumin class of protein. The AFP genes are located at chromosome 4 in humans and the coding sequence of 15 exons segregated by 14 introns. It is an onco-development protein, first identified in 1960s as a major protein in conditions related to hepatic carcinoma. Since then, the clinical utility of AFP as a tumor marker is now established in several pathological conditions, ranging from embryonal development defects, such as spina bifida in infants malignant conditions such as Hepatoblastoma, hepatocellular carcinoma and germ cell tumors. The present review describes the discovery of AFP with historical background, biology of its existence, structure specification and functional properties as well as diagnostic significance in malignant and chronic conditions.

Key words: AFP, α-feto protein, tumor marker, hepatocellular carcinoma, HCC
Short Title: Structure and Function of AFP

INTRODUCTION:

Alpha-fetoprotein (α-fetoprotein, AFP) is a large serum glycoprotein, belonging to the intriguing class of onco-development protein (1). Generally designated as tumor marker, AFP has attracted considerable attention since its discovery in late 1960s. It was recognized as an important blood component, having specific diagnostic utility after it was shown that the change in its serum concentration during pregnancy is the sign of numerous embryonic disorders such as spina bifida (1-4). Likewise, elevation of its level upto pathological range in adults correlates with the appearance of several malignant and chronic conditions, such as hepatocellular carcinoma (HCC) and chronic lever disease, respectively (1,2,5-8). The present review describes the discovery of AFP with some historical perspectives, its structure, regulation and function and clinical utilities in diagnosis of malignancies and related pathological conditions.

DISCOVERY OF AFP:

AFP was first identified in human fetal seca in 1956 on a paper electropherogram by two scientists, Bergstrand and Czar (9). However, the role of this specific protein remained unknown for several years besides confirmation of its presence in human body (1). In 1960s, two scientists, G.I. Abelev and N.L. Lazarevich, found an antigen in hepatoma cells of mouse that was comparatively absent in liver, blood and other tissue of normal mice (2). During the course of investigation of antigenic maturation of mouse liver, it was primarily established that this antigen is related to feto-specific serum protein (2,10). In proceeding years, it was demonstrated that an embryonic specific alpha-globulin is present in serum of patients suffering from HCC (11), which evidently confirmed by several studies carried out by G.I Abelev during late 1960s (12,13). Moreover, Abelev and coworkers established that AFP is also associated with teratoblastoma of testis and germ cell tumor (GCT) of ovaries (14,15). Late 1960s and early 70s, brought the confirmation and elaborated version of clinical and diagnostic importance of AFP, especially in monitoring surgery and chemotherapy in GCT (15-16). Another significant aspect of AFP was discovered by Brock and Sutchiff (17). They observed elevated level of AFP in amniotic fluid of patients having defects in fetal neural tubes. Later, it was also demonstrated that during fetal malformation, AFP level increase significantly (18), hence giving a yet another dimension to AFP significance as a tool to diagnose inborn defects.

Along with the research on diagnostic utility of AFP, several highly sensitive methods for AFP detection were also established (16,19), of which Radio-immuno assay (RIA) was found to be most accurate during early analytical strategies (19). However, development of advance
technologies and the concept of economical factor and stability gave way to ELISA, which was immediately taken as sensitive and more specific method to detect normal and pathological levels of AFP.

Presently, nearly three decades after discovery of AFP as a useful marker for HCC, GCT and fetal abnormalities, and the near past and present advances in its sensitivity and specificity (20-23), in addition to the investigation of structure-function relationship and isoforms (1,24), the research is still in progress to suggest more functional activities of AFP, receptor interaction, regulatory mechanism, and a possible input of all aspects in clinical application and diagnoses (1,2,24).

STRUCTURE AND FUNCTION:

**Synthesis:** The synthesis of AFP is initiated with embryonal hemopoiesis in the yolk sac in early fetal life (2), such that it became a major component of embryonal serum in mammals. It is stated that the amount of AFP in early fetal life is higher than the initial level of serum albumin (1,2), which is dominant protein in late fetal life and adult serum. In late stages of fetal development, embryonal hemopoiesis is replaced by fetal hemopoiesis and the synthesis of AFP and other serum proteins is transferred to the liver (2,12,16,25-27). However, the tissue distribution of AFP in early human embryogenesis has not be defined (7). Nonetheless, studies showed that AFP is expressed in the hepatic diverticulum at “26 d” post-ovulation and also expressed in the endoderm of GIT and yolk sac at this age. In addition, it is also expressed in the mesonephros and transiently in the developing pancreas (7,28).

Research on the association of AFP with fetal development revealed that concentration of AFP remains at lowest levels, which is around 0.1 mg/ml, in newborn human (15,29). However, a short-term increase takes place when liver injury is followed by regeneration (29,30). But still, none of the confirm relationship has been established between the nature of association of AFP and fetal development as such, that either it is functional or regulatory (2).

**Structure:** AFP is present in all mammals with a significant degree of homology, but with distinct immunological cross-reactivity (31). It is glycoprotein with approximately 4% carbohydrate moiety represented by one oligosaccharide residue. The protein fraction consisting of one polypeptide chain of 609 amino acids (Fig 1), arranged in three domains, with a MW of 69,000 (32). AFP is very similar to serum Albumin in its structure and physicochemical properties, by the only difference is the absence of carbohydrate moiety in albumin (2). The amino acid sequence of most of the mammalian AFP and its precursors have been determined and found to posses around 600-690 amino acid residues (49). Several monoclonal antibodies have been developed against AFP, which are used not only to identify the presence of AFP, but also to detect sub-fractions in native forms (2,33-35). Recently disease-specific isoforms of AFP have also been identified with the help of iso-electric focusing (24). It was noted that HCC-associated AFP isoforms represent a group of glycoproteins whose carbohydrate structure are mono-sialylated; where as those associated with benign liver disease and non-seminomatous germ cell tumors are di-sialylated forms (24).

**Function:** Functionally, AFP has a very high affinity for polyunsaturated fatty acids (PUFAs), which is unique property, not known in other albumin class of proteins (2,36). This clearly indicates that AFP absorbs PUFAs from maternal
circulation and transports it into embryonal tissue, which are unable to produce PUFAs (37). Additionally, AFP has also demonstrated to enter certain embryonal and actively proliferating tissue, such as malignant tumors (38), thus relating its significance and embryonal cycles. Research also showed that AFP has high specificity for estrogens (39), especially in rats and mice, therefore providing the hypothesis that AFP protects embryonal tissue from maternal estrogens (32,40), and its target actions (64,65). Immuno-suppressive activity has also been demonstrated in several studies, which is not due to AFP molecule alone but due to the conjugated PUFAs (32,41,42).

REGULATION OF AFP SYNTHESIS AT CELLULAR AND GENETIC LEVELS:

Cellular level: Regulation of AFP synthesis at cellular and genetic levels is the most significant area of clinical utility (2). The first site of AFP synthesis is the earliest differentiated embryonic structure of yolk sac visceral endoderm. The synthesis is regulated by intracellular interaction and manifested in immuno-histochemical analysis of fetal tissue and evaluation of AFP presence in tissue cultures (43,45). AFP is demonstrated to be produced by all hepatocytes in liver bud, with the early onset of liver formation during fetal life (2,44,47). AFP continues to express in human fetuses up to second half of pregnancy. The expression ceased with formation of definitive liver plates. However, the inhibition of expression appears to be reversal in most of the hepatocytes (2). Several studies demonstrated that chemical injury (intoxication, alcohol), hepatoxicity (viral infection) or malignant proliferation (carcinoma), cause reappearance of AFP in many layers of the affected hepatic areas (4748). Immuno-histochemical and electron microscopic analysis revealed that presence of AFP synthesis in reversibly repressed under intracellular interaction, and can be reversed under similar condition, but due to a more aggressive initiation.

Genetic level: The AFP gene has been cloned and sequenced together with its regulatory region. Fig 2 represents the complete mRNA and DNA sequence of AFP gene with a with a total of 2098 and 1083 bases, respectively (49,50). As stated earlier AFP gene belongs to albumin gene family together with serum albumin, a-albumin, vitamin D binding protein genes and localized in the same region of chromosome 4.
Several factors have been identified; among them are the NFI, API, the glucocorticoids, hormone-receptor complex (estrogen receptor) and the liver specific hepatic nuclear factor (e.g C/EBP, HNF1, HNF3) [54]. The hepatic nuclear factor, HNF3, belongs to the category of "master genes" and regulate the expression of liver-specific genes, including serum albumin and AFP [55]. Studies emphasized that the transcriptional factor, not only act as initiators during AFP regulation, but also act as transcriptional inhibitors, depending on the condition in the presence of other transcription factors [2,56].

CLINICAL SIGNIFICANCE:

General Consideration: Forty years after the discovery of AFP, its estimation remains a useful test for clinicians, oncologists and physicians involved in the management of patients with fetal defects (spina bifida), hepatic malignancies (HCC, hepatoma, Hepatoblastoma), hepatic infections (HCV, HBV) and cancers of pancreas and germ cell [2,20,21,57]. Serum AFP level is also used in monitoring response to therapy [57]. It has been determined that AFP is specific marker of embryonal carcinoma [2,16,58]. However, AFP level is correlated with the presence and amount of yolk sac visceral endoderm (YSVE) elements in that particular tumor [16,58]. Likewise, tumor of testicular or ovarian localization and retroperitoneal tumors of embryonic origin can equally be malignancies, mainly germ cell [26,56], pediatric Hb [25,26,30], HCC and in rare case of GIT tumors [25]. An important field of AFP diagnostic application is fetal malformation, primarily neural tube defects, such as anencephaly and spina bifida [18,60]. An AFP test is generally recommended for differential diagnosis as well as monitoring of surgery and chemotherapy in GCT, Hb and HCC [26].

Hepatoma and hepatocellular carcinoma: Pediatric Hb is a distinct type of cancer, differentiated from HCC by the presence of hepatoblast like cells similar to embryonal liver parenchymal cells [26]. In Hb, the specificity of AFP is 90% [26], which means that, Pediatric Hb is zero positive for AFP in almost all cases with AFP level greater than 1000 mg/ml. In HCC, however, its specificity is up to 70-80%, probably due to AFP production in poorly differentiated tumors [2]. However, a vast majority of basic as well as clinical studies strongly suggests that AFP is one of the best diagnostic entities for HCC of both cirrhotic and non-cirrhotic origin [6,8,20,21,22].

Germ cell tumor, pancreaticoblastoma, hepatoid adenocarcinoma of endometrium: AFP in GCT provides
marked specificity to differentiate embryonal cancer and yolk sac tumor from seminoma, dysgerminoma and stromal cell tumor [2,26,32]. Local tumor invasion, in case of pancreaticoblastoma, cause a rise in AFP level, as reported by a recent study [28]. Presence of AFP variants, detected by iso-electric focusing (IEF), was also noted [28]. Chemotherapy induce 95% fall in AFP level and depict the progress of treatment. Another recently reported study indicates the importance of AFP in diagnosing a uterine corpus cancer as hepatoid adenocarcinoma of endometrium [23].

**Conclusion:** This review covers the topic of discovery, structure, chemistry, function and diagnostic application of AFP, one of the most significant marker for clinical disorders and malignancies, specially related to development process in fetus and liver, germs cells and pancreas in adults. But despite all the information about structure and biology, and advancement in determination of its clinical utility, AFP as a component still remains to be thoroughly investigated for receptor integration, transcriptional regulation and intercellular expression and expression.

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